TRANSMEMBRANE PROTEASE, SERINE 6 (TMPRSS6) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

Related Applications

This application claims the benefit of priority to U.S. Provisional Application No. 63/179,607, filed on April 26, 2021, and U.S. Provisional Application No. 63/278,227, filed on November 11, 2021. The entire contents of each of the foregoing applications are 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 April 18, 2022, is named 121301_15420_SL.TXT and is 673,291 bytes in size.

Background of the Invention

TMPRSS6 (Transmembrane Protease, Serine 6), also known as matriptase-2, is a type II serine protease. It is primarily expressed in the liver, although high levels of TMPRSS6 mRNA are also found in the kidney, with lower levels in the uterus and much smaller amounts detected in many other tissues (Beliveau *et al.*, 2019, *Cell Chemical Biology 26*, 1559–1572). TMPRSS6 plays a key role in iron homeostatis *via* modulation of hepcidin expression. Hepcidin, a liver-derived peptide hormone, is known as a central regulator of systemic iron homeostasis, and its unbalanced production contributes to the pathogeesis of a spectrum of iron disorders. Hepcidin functions by blocking the absorption of dietary iron from the intestine, and the release of iron from macrophages and hepatocytes (Ganz T. 2011, *Blood*, vol. 117, 17, 4425-4433). Hepcidin gene expression can be stimulated in response to iron through BMP/SMAD-dependent signal transduction cascade mediated by the BMP-co-receptor hemojuvelin (HJV). TMPRSS6 inhibits BMP-mediated upregulation of hepcidin by cleaving the BMP co-receptor HJV, thus preventing BMP signaling, SMAD translocation to the nucleus, and hepcidin transcriptional activation, which causes downregulation of hepcidin levels (Finberg, K.E., *et al.*, 2010, *Blood* 115, 3817–3826; Wang, C.Y., *et al.*, 2014 *Front. Pharmacol.* 5, 114).

Therefore, inhibition of TMPRSS6 results in increased hepcidin levels, making it an attractive pharmacological target for disorders associated with iron overload and inappropriately low hepcidin or for disorders where iron restriction is desirable. Numerous disorders, such as thalassemias, hemochromatosis, and certain types of myelodysplastic syndromes (MDS), are associated with iron overload, a condition characterized by increased levels of iron. Iron overload can result in excess iron deposition in various tissues and can eventually lead to tissue and organ damage. In addition, iron restriction is desirable in certain disorders such as polycythemia vera.

Current treatments for disorders associated with iron overload and disorders where iron restriction is desirable (*e.g.* polycythemia vera) include phlebotomy or venesection, a treatment to remove iron-rich blood from the body; splenectomy; iron chelation therapy; and dieting. However,

these treatments are not always effective. Accordingly, there is a need in the art for alternative treatments for subjects having disorders associated with iron overload.

Summary of the Invention

The present invention provides iRNA compositions which affect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding Transmembrane protease, serine 6 (TMPRSS6). The TMPRSS6 gene may be within a cell, *e.g.,* a cell within a subject, such as a human subject. The present invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of a TMPRSS6 gene and/or for treating a subject who would benefit from inhibiting or reducing the expression of a TMPRSS6 gene, *e.g.*, a subject suffering or prone to suffering from a TMPRSS6-associated disorder, *e.g.,* an iron overload associated disorder and/or a disorder of ineffective erythopoiesis, such as thalassemia, *e.g.*, β-thalassemia, hemochromatosis, myelodysplastic syndromes (MDS), or polycythemia vera.

Accordingly, in an aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Transmembrane protease, serine 6 (TMPRSS6) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding TMPRSS6, and wherein the region of complementarity comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-7.

In one embodiment, the dsRNA agent comprises a sense strand comprising a contiguous nucleotide sequence which has at least 85%, *e.g.,* 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, nucleotide sequence identity over its entire length to any one of the nucleotide sequences of the sense strands in any one of Tables 2-7 and an antisense strand comprising a contiguous nucleotide sequence which has at least 85%, *e.g.,* 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, nucleotide sequence identity over its entire length to any one of the nucleotide sequences of the antisense strands in any one of Tables 2-7.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2-7 and an antisense strand comprising at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-7.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2-7 and an antisense strand comprising at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-7.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the sense strands in any one of Tables 2-7 and an antisense strand comprising at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-7.

In one embodiment, the dsRNA agent comprises a sense strand comprising or consisting of a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the sense strands in any one of Tables 2-7 and an antisense strand comprising or consisting of a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the antisense strands in any one of Tables 2-7.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of Transmembrane protease, serine 6 (TMPRSS6) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0, nucleotides from any one of the nucleotide sequence of nucleotides 187-210; 227-254;322-363; 362-390; 398-420; 404-429; 410-435; 439-461; 443-467; 448-474; 460-483; 466-488; 496-519; 519-542; 526-548; 557-593; 641-671; 652-676; 687-713; 725-762; 757-794; 886-908; 921-951; 956-987; 1051-1082; 1233-1269; 1279-1313; 1313-1341; 1327-1351; 1415-1439; 1447-1480; 1464-1486; 1486-1509; 1559-1589; 1571-1595; 1579-1609; 1707-1735; 1738-1764; 1806-1828; 1864-1886; 1934-1966; 1967-1991; 2008-2031; 2015-2043; 2042-2072; 2287-2311; 2297-2354; 2336-2361; 2360-2384; 2416-2438; 2481-2510; 2496-2527; 2526-2558; 2665-2693; 2693-2719; 2707-2729; 2799-2821; 2851-2874; 2971-2999; 2981-3006 and 3155-3195 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Transmembrane protease, serine 6 (TMPRSS6) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0, nucleotides from any one of the nucleotide sequence of nucleotides 230-252, 324-346, 560-578, 560-582, 2338-2360, 3163-3185, 3169-3191, and 3172-3194 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0, nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Transmembrane protease, serine 6 (TMPRSS6) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0, nucleotides from any one of the

nucleotide sequence of nucleotides 560-578, 2338-2360, and 3169-3191 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In some embodiments, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0, nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1556360, AD-1571158, AD-1571033, AD-1554875, AD-1571160, AD-1555117, AD-1554911, and AD-1556915.

In some embodiments, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0, nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1556360, AD-1571158, and AD-1571033.

In one embodiment, the dsRNA agent comprises at least one modified nucleotide.

In one embodiment, substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand comprise a modification; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

In one embodiment, all of the nucleotides of the sense strand comprise a modification; all of the nucleotides of the antisense strand comprise a modification; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxythimidine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), a nucleotide comprising a 2' phosphate, and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

In one embodiment, the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide,

a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), *e.g.,* Ggn, Cgn, Tgn, or Agn, a nucleotide with a 2' phosphate, *e.g.,* G2p, C2p, A2p or U2p, and, a vinyl-phosphonate nucleotide; and combinations thereof.

In some embodiments, the modified nucleotide comprises a short sequence of 3'-terminal deoxythimidine nucleotides (dT).

In some embodiments, the dsRNA agent further comprises at least one phosphorothioate internucleotide linkage. In some embodiments, the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages. In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand. In a related embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand. In another embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand.

The double stranded region may be 19-30 nucleotide pairs in length;19-25 nucleotide pairs in length;19-23 nucleotide pairs in length; 23-27 nucleotide pairs in length; or 21-23 nucleotide pairs in length.

In one embodiment, each strand is independently no more than 30 nucleotides in length.

In one embodiment, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

The region of complementarity may be at least 17 nucleotides in length; between 19 and 23 nucleotides in length; or 19 nucleotides in length.

In one embodiment, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides.

In one embodiment, the dsRNA agent further comprises a ligand.

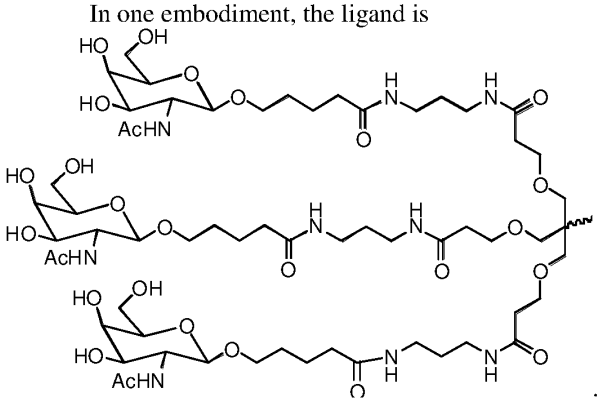
In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

In one embodiment, the ligand is conjugated to the 5' end of the sense strand of the dsRNA agent.

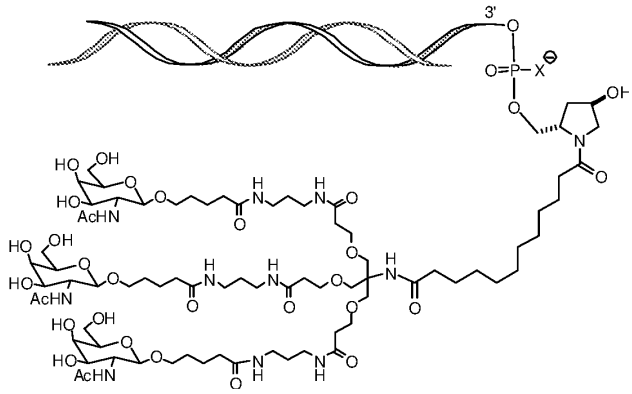
In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

In one embodiment, the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one embodiment, the ligand is



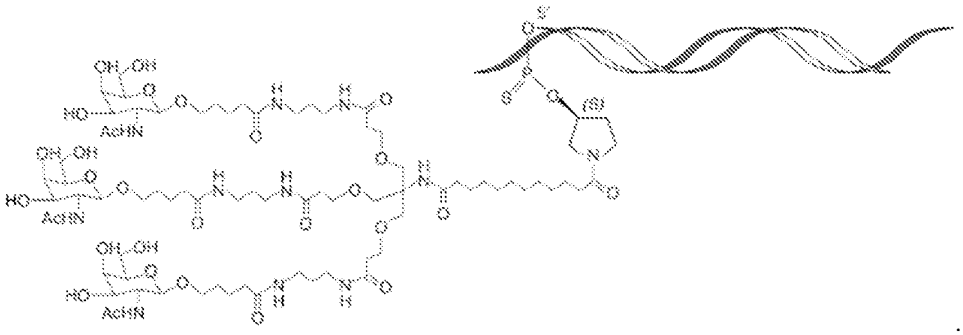
In one embodiment, the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

In one embodiment, the X is O.

In one embodiment, the dsRNA agent is conjugated to the ligand as shown in the following schematic



In one embodiment, the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand, *e.g.,* the antisense strand or the sense strand.

In another embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand, *e.g.,* the antisense strand or the sense strand.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. In one embodiment, the strand is the antisense strand.

In one embodiment, the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

In one embodiment, the sense strand comprises at least 17 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-GACGCCACGCAUGCUGUGUGU -3'(SEQ ID NO: 119).

In one embodiment, the sense strand comprises at least 19 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-GACGCCACGCAUGCUGUGUGU -3'(SEQ ID NO: 119).

In one embodiment, the sense strand comprises or consists of the nucleotide sequence of 5'-GACGCCACGCAUGCUGUGUGU -3'(SEQ ID NO: 119).

In one embodiment, the antisense strand comprises at least 17 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

In one embodiment, the antisense strand comprises at least 19 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

In one embodiment, the antisense strand comprises at least 21 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

In one embodiment, the antisense strand comprises or consists of the nucleotide sequence of 5'- ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-GACGCCACGCAUGCUGUGUGU -3'(SEQ ID NO: 119) and the antisense strand comprises the nucleotide sequence of 5'- ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

In one embodiment, the sense strand differs by no more than 3, e.g., 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'- gsascgccacGfCfAfugcugugugu-3' (SEQ ID NO:371) wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

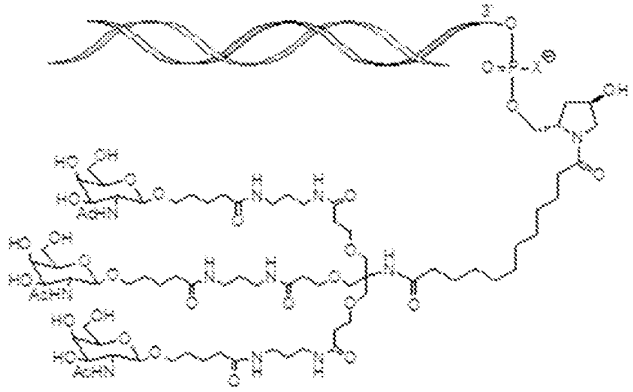
In one embodiment, the antisense strand differs by no more than 3, e.g., 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'- asdCsacdAcdAgcaudGcGfuggcgucsasc -3' (SEQ ID NO: 497), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein dA, dG, and dC are 2`-deoxyadenosine-3`-phosphate, 2`-deoxyguanosine-3`-phosphate, and 2`-deoxycytidine-3`-phosphate respectively; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-gsascgccacGfCfAfugcugugugu-3' (SEQ ID NO: 371) and the antisense strand comprises the nucleotide sequence of 5'- asdCsacdAcdAgcaudGcGfuggcgucsasc -3' (SEQ ID NO: 497), wherein a,

g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein dA, dG, and dC are 2`-deoxyadenosine-3`-phosphate, 2`-deoxyguanosine-3`-phosphate, and 2`-deoxycytidine-3`-phosphate respectively; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-gsascgccacGfCfAfugcugugugguL96 -3' (SEQ ID NO: 371) and the antisense strand comprises the nucleotide sequence of 5'- asdCsacdAcdAgcaudGcGfuggcgucsasc -3' (SEQ ID NO: 497), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein dA, dG, and dC are 2`-deoxyadenosine-3`-phosphate, 2`-deoxyguanosine-3`-phosphate, and 2`-deoxycytidine-3`-phosphate respectively; s is a phosphorothioate linkage, and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-gsascgccacGfCfAfugcugugugu -3' (SEQ ID NO: 371) and the antisense strand comprises the nucleotide sequence of 5'- asdCsacdAcdAgcaudGcGfuggcgucsasc -3' (SEQ ID NO: 497), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein dA, dG, and dC are 2`-deoxyadenosine-3`-phosphate, 2`-deoxyguanosine-3`-phosphate, and 2`-deoxycytidine-3`-phosphate respectively; and s is a phosphorothioate linkage, wherein the 3'-end of the sense strand is conjugated to the ligand as shown in the following schematic:



and, wherein X is O.

In one embodiment, the sense strand comprises at least 17 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-CCUUUGGAAUAAAGCUGCCUU -3'(SEQ ID NO: 844).

In one embodiment, the sense strand comprises at least 19 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-CCUUUGGAAUAAAGCUGCCUU -3'(SEQ ID NO: 844).

In one embodiment, the sense strand comprises or consists of the nucleotide sequence of 5'-CCUUUGGAAUAAAGCUGCCUU -3'(SEQ ID NO: 844).

In one embodiment, the antisense strand comprises at least 17 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

In one embodiment, the antisense strand comprises at least 19 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

In one embodiment, the antisense strand comprises at least 21 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

In one embodiment, the antisense strand comprises or consists of the nucleotide sequence of 5'- AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-CCUUUGGAAUAAAGCUGCCUU -3'(SEQ ID NO: 844) and the antisense strand comprises the nucleotide sequence of 5'- AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

In one embodiment, the sense strand differs by no more than 3, e.g., 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'- cscsuuugGfaAfUfAfaagcugccuu -3' (SEQ ID NO: 2095) wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

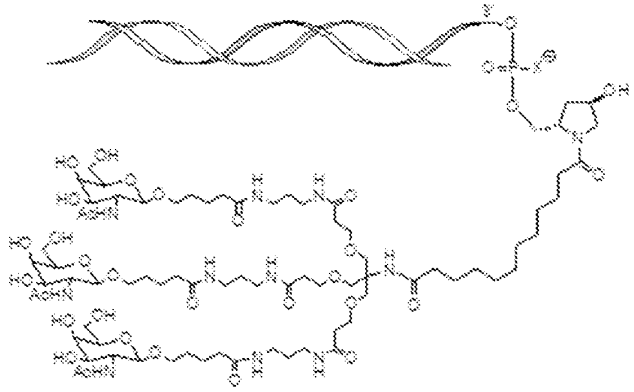
In one embodiment, the antisense strand differs by no more than 3, e.g., 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'- asAfsggdCa(G2p)cuuuauUfcCfaaaggsgsc -3' (SEQ ID NO: 2324), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein G2p is guanosine-2`-phosphate; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-cscsuuugGfaAfUfAfaagcugccuu -3' (SEQ ID NO: 2095) and the antisense strand comprises the nucleotide sequence of 5'- asAfsggdCa(G2p)cuuuauUfcCfaaaggsgsc -3' (SEQ ID NO: 2324), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein G2p is guanosine-2`-phosphate; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-cscsuuugGfaAfUfAfaagcugccuuL96 -3' (SEQ ID NO: 2095) and the antisense strand comprises the nucleotide sequence of 5'- asAfsggdCa(G2p)cuuuauUfcCfaaaggsgsc -3' (SEQ ID NO: 2324), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein G2p is guanosine-2`-phosphate; s is a phosphorothioate linkage, and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-cscsuuugGfaAfUfAfaagcugccuu -3' (SEQ ID NO: 2095) and the antisense strand comprises the nucleotide sequence of 5'- asAfsggdCa(G2p)cuuuauUfcCfaaaggsgsc -3' (SEQ ID NO: 2324), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are

2′-fluoro A, G, C and U respectively; wherein G2p is guanosine-2`-phosphate, s is a phosphorothioate linkage, and wherein the 3'-end of the sense strand is conjugated to the ligand as shown in the following schematic:



and, wherein X is O.

In one embodiment, the sense strand comprises at least 17 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-UCACCUGCUUCUUCUGGUU-3'(SEQ ID NO: 1686).

In one embodiment, the sense strand comprises or consists of the nucleotide sequence of 5'-UCACCUGCUUCUUCUGGUU-3'(SEQ ID NO: 1686).

In one embodiment, the antisense strand comprises at least 17 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of 5'-AACCAGAAGAAGCAGGUGA-3' (SEQ ID NO: 1790).

In one embodiment, the antisense strand comprises or consists of the nucleotide sequence of 5'- AACCAGAAGAAGCAGGUGA-3' (SEQ ID NO: 1790).

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-UCACCUGCUUCUUCUGGUU-3'(SEQ ID NO: 1686) and the antisense strand comprises the nucleotide sequence of 5'- AACCAGAAGAAGCAGGUGA -3' (SEQ ID NO:1790).

In one embodiment, the sense strand differs by no more than 3, e.g., 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'-UfcAfcCfuGfcUfuCfuUfcUfgGfsusUf -3' (SEQ ID NO: 1974) wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

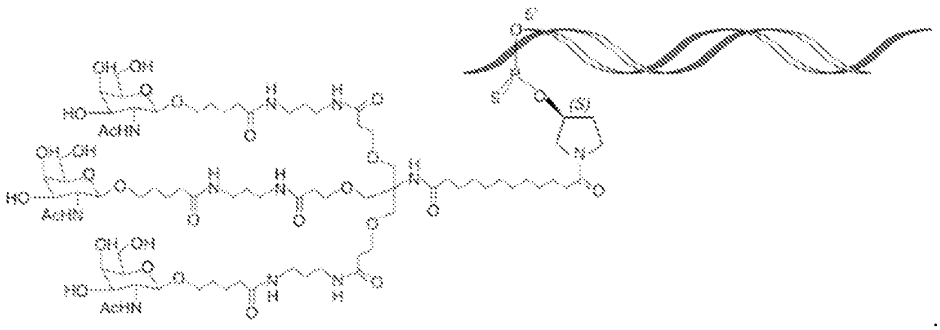
In one embodiment, the antisense strand differs by no more than 3, e.g., 0, 1, 2, or 3, modified nucleotides from the nucleotide sequence of 5'- asAfscCfaGfaAfgAfaGfcAfgGfusGfsa -3' (SEQ ID NO:), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-UfcAfcCfuGfcUfuCfuUfcUfgGfsusUf-3' (SEQ ID NO: 2203) and the antisense strand comprises the nucleotide sequence of 5'- asAfscCfaGfaAfgAfaGfcAfgGfusGfsa -3' (SEQ ID NO:), wherein a,

g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-Q191sUfcAfcCfuGfcUfuCfuUfcUfgGfsusUf -3' (SEQ ID NO: 1974) and the antisense strand comprises the nucleotide sequence of 5'- asAfscCfaGfaAfgAfaGfcAfgGfusGfsa -3' (SEQ ID NO: 2203), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; s is a phosphorothioate linkage, and Q191 is N-[tris(GalNAc-alkyl)-amidododecanoyl]-(S)-pyrrolidin-3-ol-phosphorothioate (p-C12-(GalNAc-alkyl)3).

In one embodiment, the sense strand comprises the nucleotide sequence of 5'-UfcAfcCfuGfcUfuCfuUfcUfgGfsusUf -3' (SEQ ID NO: 1974) and the antisense strand comprises the nucleotide sequence of 5'- asAfscCfaGfaAfgAfaGfcAfgGfusGfsa -3' (SEQ ID NO: 2203), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage, wherein the 5'-end of the sense strand is conjugated to the ligand as shown in the following schematic:



In another embodiment, the RNAi agent is a pharmaceutically acceptable salt thereof. "Pharmaceutically acceptable salts" of each of RNAi agents herein include, but are not limited to, a sodium salt, a calcium salt, a lithium salt, a potassium salt, an ammonium salt, a magnesium salt, an mixtures thereof. One skilled in the art will appreciate that the RNAi agent, when provided as a polycationic salt having one cation per free acid group of the optionally modified phosophodiester backbone and/or any other acidic modifications (*e.g.*, 5'-terminal phosphonate groups). For example, an oligonucleotide of "n" nucleotides in length contains n-1 optionally modified phosophodiesters, so that an oligonucleotide of 21 nt in length may be provided as a salt having up to 20 cations (e.g, 20 sodium cations). Similarly, an RNAi agents having a sense strand of 21 nt in length and an antisense strand of 23 nt in length may be provided as a salt having up to 42 cations (*e.g.*, 42 sodium cations). In the preceding example, where the RNAi agent also includes a 5'-terminal phosphate or a 5'-terminal vinylphosphonate group, the RNAi agent may be provided as a salt having up to 44 cations (*e.g.*, 44 sodium cations).

The present invention also provides cells containing any of the dsRNA agents of the invention and pharmaceutical compositions comprising any of the dsRNA agents of the invention.

The pharmaceutical composition of the invention may include dsRNA agent in an unbuffered solution, *e.g.,* saline or water, or the pharmaceutical composition of the invention may include the dsRNA agent is in a buffer solution, *e.g.,* a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof; or phosphate buffered saline (PBS).

In one aspect, the present invention provides a method of inhibiting expression of a Transmembrane protease, serine 6 (TMPRSS6) gene in a cell. The method includes contacting the cell with any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby inhibiting expression of the TMPRSS6 gene in the cell.

In one embodiment, the cell is within a subject, *e.g.,* a human subject, *e.g.,* a subject having a Transmembrane protease, serine 6 (TMPRSS6)-associated disorder, such as a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia.

In some embodiments, the TMPRSS6-associated disorder is β-thalassemia. In one embodiment, the TMPRSS6-associated disorder is β-thalassemia major. In another embodiment, the TMPRSS6-associated disorder is β-thalassemia intermedia. In some embodiments, the TMPRSS6-associated disorder is polycythemia vera.

In certain embodiments, the TMPRSS6 expression is inhibited by at least about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%. In one embodiment, inhibiting expression of TMPRSS6 decreases TMPRSS6 protein level in serum of the subject by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%.

In certain embodiments, contacting the cell with the dsRNA agent increases the expression of hepcidin by at least 50%, 60%, 70%, 80%, 90%, or 95%. In one embodiment, increasing expression of hepicidin increases hepicidin protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one aspect, the present invention provides a method of treating a subject having a disorder that would benefit from reduction in Transmembrane protease, serine 6 (TMPRSS6) expression. The method includes administering to the subject a therapeutically effective amount of any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby treating the subject having the disorder that would benefit from reduction in TMPRSS6 expression.

In another aspect, the present invention provides a method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in Transmembrane protease, serine 6 (TMPRSS6) expression. The method includes administering to the subject a prophylactically effective amount of any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in TMPRSS6 expression.

In certain embodiments, the disorder is a Transmembrane protease, serine 6 (TMPRSS6)-associated disorder, *e.g.,* a disorder associated with iron overload and/or a disorder of ineffective

erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermiedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia .

In some embodiments, the TMPRSS6-associated disorder is β-thalassemia. In one embodiment, the TMPRSS6-associated disorder is β-thalassemia major. In another embodiment, the TMPRSS6-associated disorder is β-thalassemia intermiedia. In some embodiments, the TMPRSS6-associated disorder is polycythemia vera.

In certain embodiments, administration of the dsRNA to the subject causes a decrease in the iron level, ferritin level and/or transferrin saturation level and/or a decrease in TMPRSS6 protein accumulation in the subject. In some embodiments, administration of the dsRNA to the subject causes an increase in the hemoglobin level and/or the hematocrit level in the subject.

In a further aspect, the present invention also provides methods of inhibiting the expression of TMPRSS6 in a subject. The methods include administering to the subject a therapeutically effective amount of any of the dsRNAs provided herein, thereby inhibiting the expression of TMPRSS6 in the subject.

In one embodiment, the subject is human.

In one embodiment, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

In one embodiment, the dsRNA agent is administered to the subject subcutaneously or intravenously.

In one embodiment, the methods of the invention include further determining the level of TMPRSS6 in a sample(s) from the subject.

In one embodiment, the level of TMPRSS6 in the subject sample(s) is a TMPRSS6 protein level in a blood, serum or liver sample(s).

In one embodiment, the methods of the invention include further determining the level of iron and/or hepcidin in a sample(s) from the subject.

In certain embodiments, the methods of the invention further comprise administering to the subject an additional therapeutic agent. In one embodiment, the methods of the invention further comprise administering an iron chelator, *e.g.*, deferiprone, deferoxamine, and deferasirox, to a subject.

The present invention also provides kits comprising any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, and optionally, instructions for use. In one embodiment, the invention provides a kit for performing a method of inhibiting expression of TMPRSS6 gene in a cell by contacting a cell with a double stranded RNAi agent of the invention in an amount effective to inhibit expression of the TMPRSS6 in the cell. The kit comprises an RNAi agent and instructions for use and, optionally, means for administering the RNAi agent to a subject.

The present invention also provide an RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agents of the present invention.

Brief Description of the Drawings

Figure 1 is a schematic depicting the study plan to determine the efficacy of the dsRNA agents disclosed herein *in vivo* in Cynomolgus monkeys.

Figure 2 is a graph showing the percent of serum TMPRSS6 mRNA remaining in Cynmologous monkeys (n=3 per group) subcutaneously administered a single 3 mg/kg or 10 mg/kg dose of the indicated dsRNA duplexes at Days 21, 22, 57, and 85 post-dose. TMPRSS6 mRNA levels are shown relative to control levels obtained from Cynmologous monkeys administered PBS as a control.

Figure 3 is a graph showing the plasma iron levels, as a percent of predose levels, in Cynmologous monkeys (n=3 per group) subcutaneously administered a single 3 mg/kg or 10 mg/kg dose of the indicated dsRNA duplexes at Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85 post-dose.

Figure 4 is a graph showing the percent transferrin saturation levels in Cynmologous monkeys (n=3 per group) subcutaneously administered a single 3 mg/kg or 10 mg/kg dose of the indicated dsRNA duplexes at Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85 post-dose.

Detailed Description of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a Transmembrane protease, serine 6 (TMPRSS6) gene. The gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the corresponding gene (TMPRSS6) in mammals.

The iRNAs of the invention have been designed to target the human Transmembrane protease, serine 6 (TMPRSS6) gene, including portions of the gene that are conserved in the TMPRSS6 orthologs of other mammalian species. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing properties and the specific target sites or the specific modifications in these iRNAs confer to the iRNAs of the invention improved efficacy, stability, potency, durability, and safety.

Accordingly, the present invention provides methods for treating and preventing a Transmembrane protease, serine 6 (TMPRSS6)-associated disorder, *e.g.*, a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a TMPRSS6 gene.

The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is up to about 30 nucleotides or less in length, *e.g.*, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-

21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a TMPRSS6 gene.

In certain embodiments, one or both of the strands of the double stranded RNAi agents of the invention is up to 66 nucleotides in length, *e.g.*, 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length, with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of a TMPRSS6 gene. In some embodiments, such iRNA agents having longer length antisense strands may, for example, include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

The use of iRNAs of the invention enables the targeted degradation of mRNAs of the corresponding gene (TMPRSS6 gene) in mammals. Using *in vitro* assays, the present inventors have demonstrated that iRNAs targeting a TMPRSS6 gene can potently mediate RNAi, resulting in significant inhibition of expression of a TMPRSS6 gene. Thus, methods and compositions including these iRNAs are useful for treating a subject having a TMPRSS6-associated disorder, *e.g.*, a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia.

Accordingly, the present invention provides methods and combination therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a TMPRSS6 gene, *e.g.*, a Transmembrane protease, serine 6 (TMPRSS6)-associated disease, such as a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a TMPRSS6 gene.

The present invention also provides methods for preventing at least one symptom in a subject having a disorder that would benefit from inhibiting or reducing the expression of a TMPRSS6 gene, *e.g.*, a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a TMPRSS6 gene as well as compositions, uses, and methods for treating subjects that would benefit from inhibition and/or reduction of the expression of a TMPRSS6 gene, *e.g.*, subjects susceptible to or diagnosed with a TMPRSS6-associated disorder.

I. Definitions

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

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

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

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

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

The term "at least", "no less than", or "or more" prior to a number or series of numbers is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 19 nucleotides of a 21 nucleotide nucleic acid molecule" means that 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

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

As used herein, methods of detection can include determination that the amount of analyte present is below the level of detection of the method.

In the event of a conflict between an indicated target site and the nucleotide sequence for a sense or antisense strand, the indicated sequence takes precedence.

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

As used herein, "Transmembrane protease, serine 6," used interchangeably with the term "TMPRSS6," refers to the type II plasma membrane serine protease (TTSP) gene or protein. TMPRSS6 is also known as matriptase-2, IRIDA (iron refractory iron-deficiency anemia),

transmembrane protease serine 6, type II transmembrane serine protease 6, and membrane-bound mosaic serine proteinase matriptase-2. TMPRSS6 is a serine protease Type II transmembrane protein of approximately 899 amino acids in length. TMPRSS6 contains multiple domains, *e.g.*, a short endo domain, a transmembrane domain, a sea urchin sperm protein/enteropeptidase domain/agrin (SEA) domain, two complement factor/urchin embryonic growth factor/BMP domains (CUB), three LDL-R class a domains (LDLa), and a trypsin-like serine protease domain with conserved His-Asp-Ser triad (HDS).

The sequence of a human TMPRSS6 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1755203660 (NM_153609.4; SEQ ID NO:1; reverse complement, SEQ ID NO: 2). The sequence of mouse TMPRSS6 mRNA can be found at, for example, GenBank Accession No. GI: 125656151 (NM_027902.2 ; SEQ ID NO:3; reverse complement, SEQ ID NO: 4). The sequence of rat TMPRSS6 mRNA can be found at, for example, GenBank Accession No. GI: 194474097 (NM_001130556.1 ; SEQ ID NO:5; reverse complement, SEQ ID NO: 6). The sequence of Macaca fascicularis TMPRSS6 mRNA can be found at, for example, GenBank Accession No. GI: 982272225 (XM_005567384.2; SEQ ID NO: 7; reverse complement, SEQ ID NO: 8). The sequence of Macaca mulatta TMPRSS6 mRNA can be found at, for example, GenBank Accession No. GI: 1622838152 (XM_015150283.2; SEQ ID NO: 9; reverse complement, SEQ ID NO: 10).

Additional examples of TMPRSS6 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, and the *Macaca* genome project web site.

Further information on TMPRSS6 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=TMPRSS6.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term TMPRSS6, as used herein, also refers to variations of the TMPRSS6 gene including variants provided in the SNP database. Numerous seuqnce variations within the TMPRSS6 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=TMPRSS6, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a TMPRSS6 gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodment, the target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a TMPRSS6 gene.

The target sequence may be from about 19-36 nucleotides in length, *e.g.*, about 19-30 nucleotides in length. For example, the target sequence can be about 19-30 nucleotides, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. In certain embodiments, the target sequence is 19-23 nucleotides in length,

optionally 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

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

"G," "C," "A," "T," and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.,* Table 1). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

The terms "iRNA", "RNAi agent," "iRNA agent,", "RNA interference agent" as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The iRNA modulates, *e.g.,* inhibits, the expression of a TMPRSS6 gene in a cell, *e.g.,* a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that interacts with a target RNA sequence, *e.g.,* a TMPRSS6 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). Thus, in one aspect the invention relates to a single stranded RNA (siRNA) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.,* a TMPRSS6 gene. Accordingly, the term "siRNA" is also used herein to refer to an iRNA as described above.

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

In certain embodiments, an "iRNA" for use in the compositions, uses, and methods of the invention is a double stranded RNA and is referred to herein as a "double stranded RNA agent," "double stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA", refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.,* a TMPRSS6 gene. In some embodiments of the invention, a double stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.,* an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, the majority of nucleotides of each strand of a dsRNA molecule are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide or a modified nucleotide. In addition, as used in this specification, an "iRNA" may include ribonucleotides with chemical modifications; an iRNA may include substantial modifications at multiple nucleotides. As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, or modified nucleobase, or any combination thereof. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.,* a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the invention include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "iRNA" or "RNAi agent" for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide if present within an RNAi agent can be considered to constitute a modified nucleotide.

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

pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

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

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not be, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs. In one embodiment of the RNAi agent, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In certain embodiments, an iRNA agent of the invention is a dsRNA, each strand of which comprises 19-23 nucleotides, that interacts with a target RNA sequence, *e.g.*, a TMPRSS6 gene, to direct cleavage of the target RNA.

In some embodiments, an iRNA of the invention is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, *e.g.*, a TMPRSS6 target mRNA sequence, to direct the cleavage of the target RNA.

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of a double stranded iRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or *vice versa*, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside

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

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

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

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotides, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, *e.g.*, 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, 10-25 nucleotides, 10-20 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the extended overhang is replaced with a nucleoside thiophosphate. In certain embodiments, the overhang includes a self-complementary portion such that the overhang is capable of forming a hairpin structure that is stable under physiological conditions.

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

The term "antisense strand" or "guide strand" refers to the strand of an iRNA, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.,* a TMPRSS6 mRNA.

As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, *e.g.,* a TMPRSS6 nucleotide sequence, as defined herein. Where the region of complementarity is not fully

complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, or 3 nucleotides of the 5'- or 3'-end of the iRNA. In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the antisense strand. In some embodiments, the antisense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the target mRNA, *e.g.*, the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the target mRNA. In some embodiments, the antisense strand double stranded RNA agent of the invention includes no more than 4 mismatches with the sense strand, *e.g.*, the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the sense strand. In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the sense strand. In some embodiments, the sense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the antisense strand, *e.g.*, the sense strand includes 4, 3, 2, 1, or 0 mismatches with the antisense strand. In some embodiments, the nucleotide mismatch is, for example, within 5, 4, 3 nucleotides from the 3'-end of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide of the iRNA agent. In some embodiments, the mismatch(s) is not in the seed region.

Thus, an RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, an RNAi agent as described herein contains no more than 3 mismatches (*i.e.,* 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments, if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of a TMPRSS6 gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of a TMPRSS6 gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a TMPRSS6 gene is important, especially if the particular region of complementarity in a TMPRSS6 gene is known to have polymorphic sequence variation within the population.

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

As used herein, "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.

As used herein, the term "cleavage region" refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately

adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

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

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

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

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

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding a TMPRSS6 gene). For example, a polynucleotide is complementary to at least a part of a TMPRSS6 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding a TMPRSS6 gene.

Accordingly, in some embodiments, the antisense polynucleotides disclosed herein are fully complementary to the target TMPRSS6 sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target TMPRSS6 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NOs:1, 3, 5, 7, or 9, or a fragment of any one of SEQ ID NOs:1, 3, 5, 7, or 9, such as about 85%, 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, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target TMPRSS6 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 187-210; 227-254;322-363; 362-390; 398-420; 404-429; 410-435; 439-461; 443-467; 448-474; 460-483; 466-488; 496-519; 519-542; 526-548; 557-593; 641-671; 652-676; 687-713; 725-762; 757-794; 886-908; 921-951; 956-987; 1051-1082; 1233-1269; 1279-1313; 1313-1341; 1327-1351; 1415-1439; 1447-1480; 1464-1486; 1486-1509; 1559-1589; 1571-1595; 1579-1609; 1707-1735; 1738-1764; 1806-1828; 1864-1886; 1934-1966; 1967-1991; 2008-2031; 2015-2043; 2042-2072; 2287-2311; 2297-2354; 2336-2361; 2360-2384; 2416-2438; 2481-2510; 2496-2527; 2526-2558; 2665-2693; 2693-2719; 2707-2729; 2799-2821; 2851-2874; 2971-2999; 2981-3006; and 3155-3195 of SEQ ID NO: 1, such as about 85%, 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, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target TMPRSS6 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 230-252, 324-346, 560-578, 560-582, 2338-2360, 3163-3185, 3169-3191, and 3172-3194 of SEQ ID NO: 1, such as about 85%, 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, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target TMPRSS6 sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 560-578, 2338-2360, and 3169-3191 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target TMPRSS6 sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of any one of Tables 2-7, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2-7, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In one embodiment, an RNAi agent of the disclosure includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is the same as a target TMPRSS6 sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 2, 4, 6, 8, or 10, or a fragment of any one of SEQ ID NOs:2, 4, 6, 8, or 10, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In some embodiments, an iRNA of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target TMPRSS6 sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in any one of any one of Tables 2-7, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2-7, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In certain embodiments, the sense and antisense strands are selected from any one of duplexes AD-1556360, AD-1571158, AD-1571033, AD-1554875, AD-1571160, AD-1555117, AD-1554911, and AD-1556915.

In certain embodiments, the sense and antisense strands are selected from any one of duplexes AD-1556360, AD-1571158, and AD-1571033.

In general, an "iRNA" includes ribonucleotides with chemical modifications. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a dsRNA molecule, are encompassed by "iRNA" for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide if present within an RNAi agent can be considered to constitute a modified nucleotide.

In an aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense oligonucleotide molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense oligonucleotide molecule is complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al.*, (2002) *Mol Cancer Ther* 1:347-

355. The single-stranded antisense oligonucleotide molecule may be about 14 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense oligonucleotide molecule may comprise a sequence that is at least about 14, 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

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

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

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

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

As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, or a mouse), or a bird that expresses the target gene, either endogenously or heterologously. In an embodiment, the subject is a human, such as a human being treated or assessed for a disease or disorder that would benefit from reduction in TMPRSS6 expression; a human at risk for a disease or disorder that would benefit from reduction in TMPRSS6 expression; a human having a disease or disorder that would benefit from reduction in TMPRSS6 expression; or human being treated for a disease or disorder that would

benefit from reduction in TMPRSS6 expression as described herein. In some embodiments, the subject is a female human. In other embodiments, the subject is a male human. In one embodiment, the subject is an adult subject. In another embodiment, the subject is a pediatric subject.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result, such as reducing at least one sign or symptom of a TMPRSS6-associated disorder in a subject. Treatment also includes a reduction of one or more sign or symptoms associated with unwanted TMPRSS6 expression; diminishing the extent of unwanted TMPRSS6 activation or stabilization; amelioration or palliation of unwanted TMPRSS6 activation or stabilization. "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 TMPRSS6 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%, 15%, 20%, 25%, 30%, %, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more. In certain embodiments, a decrease is at least 20%. In certain embodiments, the decrease is at least 50% in a disease marker, *e.g.*, protein or gene expression level. "Lower" in the context of the level of TMPRSS6 in a subject is a decrease to a level accepted as within the range of normal for an individual without such disorder. In certain embodiments, "lower" is the decrease in the difference between the level of a marker or symptom for a subject suffering from a disease and a level accepted within the range of normal for an individual, *e.g.*, the level of decrease in bodyweight between an obese individual and an individual having a weight accepted within the range of normal.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder or condition thereof, may be treated or ameliorated by a reduction in expression of a TMPRSS6 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 of unwanted or excessive TMPRSS6 expression, such as elevated iron levels or iron dyregulation. The likelihood of developing elevated iron levels or iron dyregulation is reduced, for example, when an individual having one or more risk factors for elevated iron levels or iron dyregulation either fails to develop elevated iron levels or iron dyregulation, or develops elevated iron levels or iron dyregulation 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 "Transmembrane protease, serine 6-associated disease" or "TMPRSS6-associated disease," is a disease or disorder that is caused by, or associated with TMPRSS6 gene expression or TMPRSS6 protein production. The term "TMPRSS6-associated disease" includes a disease, disorder or condition that would benefit from a decrease in TMPRSS6 gene expression, replication, or protein activity.

In some embodiments, the TMPRSS6-associated disease is a disorder associated with iron overload, a condition characterized by elevated iron levels, or iron dysregulation. Iron overload may

be caused, for example, by hereditary conditions, by elevated iron uptake from diet, or by excess iron administered parenterally that includes intravenous injection of excess iron, and transfusional iron overload.

In some embodiments, the TMPRSS6-associated disease is a disorder of ineffective erythropoiesis. Ineffective erythropoiesis is an abnormal expansion of the number of erythroid progenitor cells with unproductive synthesis of enucleated erythrocytes, leading to anemia and hypoxia. In particular, an increase in erythroid cells fails to produce a corresponding increase in red blood cells. As a consequence, iron absorption is still increased in response to stress, but the iron is deposited in the organs rather than being used to generate more erythrocytes.

In some embodiments, TMPRSS6-associated disorders include, but are not limited to, hereditary hemochromatosis, idiopathic hemochromatosis, primary hemochromatosis, secondary hemochromatosis, severe juvenile hemochromatosis, neonatal hemochromatosis, sideroblastic anemia, hemolytic anemia, dyserythropoietic anemia, sickle-cell anemia, hemoglobinopathy, thalassemia (*e.g.*, β-thalassemia and α-thalassemia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, chronic liver diseases, porphyria cutanea tarda, erythropoietic porphyria, atransferrinemia, hereditary tyrosinemia, cerebrohepatorenal syndrome, idiopathic pulmonary hemosiderosis, renal hemosiderosis.

In some embodiments, TMPRSS6 associated disorders include disorders associated with oral administration of excess iron, transfusional iron overload and intravenous injection of excess iron.

In other embodiments, TMPRSS6-associated disorders also include disorders with symptoms that are associated with or may be caused by iron overload. Such symptoms include increased risk for liver disease (cirrhosis, cancer), heart attack or heart failure, diabetes mellitus, osteoarthritis, osteoporosis, metabolic syndrome, hypothyroidism, hypogonadism, and in some cases premature death. In one embodiment, TMPRSS6-associated disorders include neurodegenerative disorders associated with iron overload and/or iron dysregulation, such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Friedreich's Ataxia, epilepsy and multiple sclerosis. Administration of an iRNA that targets TMPRSS6, *e.g.*, an iRNA described in any one of Tables 2-7 can treat one or more of these symptoms, or prevent the development or progression of a disease or disorder that is aggravated by increased iron levels.

In one embodiment, a TMPRSS6-associated disorder is a β-thalassemia. A β-thalassemia is any one of a group of hereditary disorders characterized by a genetic deficiency in the synthesis of beta-globin chains. In the homozygous state, beta thalassemia ("thalassemia major") causes severe, transfusion-dependent anemia. In the heterozygous state, the beta thalassemia trait ("thalassemia minor") causes mild to moderate microcytic anemia. "Thalassemia intermedia" is a β-thalassemia that results in subjects in whom the clinical severity of the disease is somewhere between the mild symptoms of β-thalassemia minor and the β-thalassemia major. Several laboratory tests may be used to help detect and diagnose thalassemia, for example, a complete blood count to determine the number of red blood cells and the number of hemoglobin, blood smear test, hemoglobin electrophoresis, gene sequencing, or iron tests to examine the level of iron, ferritin, unstaturated iron binding capacity, total

iron binding capacity, or the transferrin saturation level. The type and relative amounts of hemoglobin present in red blood cells are another indicator for thalassemia. β-thalassemia upsets the balance of beta and alpha hemoglobin chain formation and causes an increase in minor hemoglobin components. So individuals with the β-thalassemia major usually have larger percentages of Hb F. Those with β-thalassemia minor usually have elevated fraction of Hb A2.

In one embodiment, a β-thalassemia is thalassemia major. In another embodiment, a β-thalassemia is thalassemia intermedia.

In some embodiments, the TMPRSS6-associated disorder is polycythemia vera. Polycythemia vera is a type of blood cancer which causes the bone marrow to make excess red blood cells. These excess cells usually thinken the blood vessels, which make the patients more prone to develop blood clots, and other complications such as stroke or heart attack. Several tests may be performed to help detect and diagnose polycythemia vera, for example, a complete blood count, blood smear test, erythropoietin level test, bone marrow aspiration or biopsy, or gene sequencing.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a TMPRSS6-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 RNAi agent, 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 RNAi agent that, when administered to a subject having a TMPRSS6-associated disorder, 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 RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

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

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

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Such carriers are known in the art. Pharmaceutically acceptable carriers include carriers for administration by injection.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs, or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain 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 some embodiments, a "sample derived from a subject" refers to urine obtained from the subject. A "sample derived from a subject" can refer to blood or blood derived serum or plasma from the subject.

II. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of a TMPRSS6 gene. In certain embodiments, the iRNA includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a TMPRSS6 gene in a cell, such as a cell within a subject, *e.g.,* a mammal, such as a human susceptible to developing a TMPRSS6-associated disorder, *e.g.*, a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermiedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia. The dsRNAi agent includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of a TMPRSS6 gene. The region of complementarity is about 19-30 nucleotides in length (*e.g.,* about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, or 19 nucleotides in length).

Upon contact with a cell expressing the TMPRSS6 gene, the iRNA inhibits the expression of the TMPRSS6 gene (*e.g.,* a human, a primate, a non-primate, or a rat TMPRSS6 gene) by at least about 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting or flow cytometric techniques. In certain embodiments, inhibition of expression is determined by the qPCR method provided in the examples herein with the siRNA at, *e.g.,* a 10 nM concentration, in an appropriate organism cell line provided therein. In certain embodiments, inhibition of expression *in*

vivo is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, a mouse or an AAV-infected mouse expressing the human target gene, *e.g.*, when administered as single dose, *e.g.,* at 3 mg/kg at the nadir of RNA expression.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of a TMPRSS6 gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is 15 to 30 base pairs in length, *e.g.,* 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. In certain embodiments, the duplex structure is 18 to 25 base pairs in length, *e.g.*, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-25, 20-24,20-23, 20-22, 20-21, 21-25, 21-24, 21-23, 21-22, 22-25, 22-24, 22-23, 23-25, 23-24 or 24-25 base pairs in length, for example, 19-21 basepairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

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

In some embodiments, the duplex structure is 19 to 30 base pairs in length. Similarly, the region of complementarity to the target sequence is 19 to 30 nucleotides in length.

In some embodiments, the dsRNA is about 19 to about 23 nucleotides in length, or about 25 to about 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well-known in the art that dsRNAs longer than about 21-23 nucleotides in length may serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous

sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 19 to about 30 base pairs, *e.g.*, about 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target TMPRSS6 gene expression is not generated in the target cell by cleavage of a larger dsRNA.

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

A dsRNA can be synthesized by standard methods known in the art. Double stranded RNAi compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide strands comprising unnatural or modified nucleotides can be easily prepared. Similarly, single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In an aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences provided in any one of Tables 2-7, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 2-7. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a TMPRSS6 gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any one of Tables 2-7, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 2-7.

In certain embodiments, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In other embodiments, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

In one embodiment, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences in any one of Tables 2-7.

It will be understood that, although the sequences in, for example, Tables 3 or 5, are not described as modified or conjugated sequences, the RNA of the iRNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth in any one of Tables 2-7 that is un-modified, un-conjugated, or modified or conjugated differently than described therein. In other words, the invention encompasses dsRNA of Tables 2-7 which are un-modified, un-conjugated, modified, or conjugated, as described herein.

The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 23 base pairs, *e.g.,* 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, *EMBO* 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in any one of Tables 2-7. dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having any one of the sequences in any one of Tables 2-7 minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 19, 20, or more contiguous nucleotides derived from any one of the sequences of any one of Tables 2-7, and differing in their ability to inhibit the expression of a TMPRSS6 gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs provided in Tables 2-7 identify a site(s) in a TMPRSS6 transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of these sites. As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 19 contiguous nucleotides from any one of the sequences provided in any one of Tables 2-7 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a TMPRSS6 gene.

III. Modified iRNAs of the Invention

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

The nucleic acids featured in the invention can be synthesized or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, 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; or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral 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. In some embodiments of the invention, the dsRNA agents of the invention are in a free acid form. In other embodiments of the invention, the dsRNA agents of the invention are in a salt form. In one embodiment, the dsRNA agents of the invention are in a sodium salt form. In certain embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for substantially all of the phosphodiester and/or phosphorothiotate groups present in the agent. Agents in which substantially all of the phosphodiester and/or phosphorothioate linkages have a sodium counterion include not more than 5, 4, 3, 2, or 1 phosphodiester and/or phosphorothioate linkages without a sodium counterion. In some embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for all of the phosphodiester and/or phosphorothiotate groups present in the agent.

Representative U.S. Patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6, 239,265;

6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl 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.

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

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular $--CH_2--NH--CH_2-$, $--CH_2--N(CH_3)--O--CH_2--$[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 $--N(CH_3)--CH_2--CH_2--$ of the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506. The native phosphodiester backbone can be represented as O-P(O)(OH)-OCH2-.

Modified RNAs can also contain one or more substituted sugar moieties. The iRNAs, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_nO]_mCH_3$, $O(CH_2).{}_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.*, a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--$N(CH_3)_2$. Further exemplary modifications include : 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

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

An iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxythimidine (dT), 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil

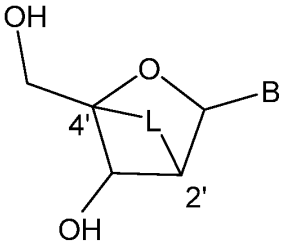
(pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and 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.

In some embodiments, an RNAi agent of the disclosure can also be modified to include one or more bicyclic sugar moieties. A "bicyclic sugar" is a furanosyl ring modified by a ring formed by the bridging of two carbons, whether adjacent or non-adjacent. A "bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a ring formed by bridging two carbons, whether adjacent or non-adjacent, 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, optionally, via the 2'-acyclic oxygen atom. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH_2-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the 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.

A locked nucleoside can be represented by the structure (omitting stereochemistry),



wherein B is a nucleobase or modified nucleobase and L is the linking group that joins the 2'-carbon to the 4'-carbon of the ribose ring. Examples of such 4′ to 2′ bridged bicyclic nucleosides, include but are not limited to 4′-(CH_2)—O-2′ (LNA); 4′-(CH_2)—S-2′; 4′-(CH_2)$_2$—O-2′ (ENA); 4′-CH(CH_3)—O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′-CH(CH_2OCH_3)—O-2′ (and analogs thereof; see, *e.g.*, U.S. Patent No. 7,399,845); 4′-C(CH_3)(CH_3)—O-2′ (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,283); 4′-CH_2—N(OCH_3)-2′ (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,425); 4′-CH_2—O—N(CH_3)-2′ (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4′-CH_2—N(R)—O-2′, wherein R is H, C1-C12 alkyl, or a nitrogen protecting group (see, *e.g.*, U.S. Patent No. 7,427,672); 4′-CH_2—C(H)(CH_3)-2′ (see, *e.g.*, Chattopadhyaya *et al., J. Org. Chem.*, 2009, 74, 118-134); and 4′-CH_2—C(=CH_2)-2′ (and analogs thereof; see, *e.g.*, U.S. Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

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

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

The RNA of an iRNA can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH_3)-O-2' bridge (*i.e.*, L in the preceding structure). In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An iRNA of the invention may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2'and C4' carbons of ribose or the C3 and -C5′ carbons of ribose. CRN lock the ribose ring into a stable conformation

and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, U.S. Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

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

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

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3'- phosphate, inverted 2'-deoxy-modified ribonucleotide, such as inverted dT(idT), inverted dA (idA), and inverted abasic 2'-deoxyribonucleotide (iAb) and others. Disclosure of this modification can be found in WO 2011/005861.

In one example, the 3' or 5' terminal end of a oligonucleotide is linked to an inverted 2'-deoxy-modified ribonucleotide, such as inverted dT(idT), inverted dA (idA), or a inverted abasic 2'-deoxyribonucleotide (iAb). In one particular example, the inverted 2'-deoxy-modified ribonucleotide is linked to the 3'end of an oligonucleotide, such as the 3'-end of a sense strand described herein, where the linking is via a 3'-3' phosphodiester linkage or a 3'-3'-phosphorothioate linkage.

In another example, the 3'-end of a sense strand is linked via a 3'-3'-phosphorothioate linkage to an inverted abasic ribonucleotide (iAb). In another example, the 3'-end of a sense strand is linked via a 3'-3'-phosphorothioate linkage to an inverted dA (idA).

In one particular example, the inverted 2'-deoxy-modified ribonucleotide is linked to the 3'end of an oligonucleotide, such as the 3'-end of a sense strand described herein, where the linking is via a 3'-3' phosphodiester linkage or a 3'-3'-phosphorothioate linkage.

In another example, the 3'-terminal nucleotides of a sense strand is an inverted dA (idA) and is linked to the preceding nucleotide via a 3'-3'- linkage (e.g., 3'-3'-phosphorothioate linkage).

Other modifications of the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, *e.g.,* a 5'-terminal phosphate or phosphate mimic on the antisense strand of an

iRNA. Suitable phosphate mimics are disclosed in, for example U.S. Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

A. Modified iRNAs Comprising Motifs of the Invention

In certain aspects of the invention, the double stranded RNA agents of the invention include agents with chemical modifications as disclosed, for example, in WO2013/075035, the entire contents of each of which are incorporated herein by reference. As shown herein and in WO2013/075035, one or more motifs of three identical modifications on three consecutive nucleotides may be introduced into a sense strand or antisense strand of a dsRNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the dsRNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The dsRNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the sense strand.

More specifically, when the sense strand and antisense strand of the double stranded RNA agent are completely modified to have one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of a dsRNAi agent, the gene silencing activity of the dsRNAi agent was observed.

Accordingly, the invention provides double stranded RNA agents capable of inhibiting the expression of a target gene (*i.e.*, TMPRSS6 gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be, for example, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

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

In certain embodiments, the dsRNAi agent may contain one or more overhang regions or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be, independently, 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. In certain embodiments, the overhang regions can include extended overhang regions as provided above. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence. The first and

second strands can also be joined, *e.g.*, by additional bases to form a hairpin, or by other non-base linkers.

In certain embodiments, the nucleotides in the overhang region of the dsRNAi agent can each independently be a modified or unmodified nucleotide including, but no limited to 2'-sugar modified, such as, 2'-F, 2'-O-methyl, thymidine (T), 2`-O-methoxyethyl-5-methyluridine (Teo), 2`-O-methoxyethyladenosine (Aeo), 2`-O-methoxyethyl-5-methylcytidine (m5Ceo), and any combinations thereof.

For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

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

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

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

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

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

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

When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In certain embodiments, every nucleotide in the sense strand and the antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In certain embodiments each residue is independently modified with a 2'-O-methyl or 3'-fluoro, *e.g.,* in an alternating motif. Optionally, the dsRNAi agent further comprises a ligand (such as, $GalNAc_3$).

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

In certain embodiments, the dsRNAi agent comprises sense and antisense strands, wherein the dsRNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end;

wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region which is at least 25 nucleotides in length, and the second strand is sufficiently complementary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein Dicer cleavage of the dsRNAi agent results in an siRNA comprising the 3'-end of the second strand, thereby reducing expression of the target gene in the mammal. Optionally, the dsRNAi agent further comprises a ligand.

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

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

For a dsRNAi agent having a duplex region of 19-23 nucleotides in length, the cleavage site of the antisense strand is typically around the 10, 11, and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, 11 positions; the 10, 11, 12 positions; the 11, 12, 13 positions; the 12, 13, 14 positions; or the 13, 14, 15 positions of the antisense strand, the count starting from the first nucleotide from the 5'-end of the antisense strand, or, the count starting from the first paired nucleotide within the duplex region from the 5'- end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the dsRNAi agent from the 5'-end.

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

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

each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

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

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

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

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

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

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

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

only occur in a terminal region, *e.g.,* at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at termini. The 5'-end or ends can be phosphorylated.

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

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

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

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

The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, *i.e.*, modifications on every other nucleotide, may be the same, but each of the sense strand or antisense strand can be selected from several possibilities of modifications within the alternating motif such as "ABABAB…", "ACACAC…" "BDBDBD…" or "CDCDCD…," *etc*.

In some embodiments, the dsRNAi agent of the invention comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and *vice versa*. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5'to 3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5' to 3' of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with

"AABBAABB" from 5' to 3' of the strand and the alternating motif in the antisense strand may start with "BBAABBAA" from 5' to 3' of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

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

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

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

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

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

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

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

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

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

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

In certain embodiments, the sense strand sequence may be represented by formula (I):

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

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

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

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

each n_p and n_q independently represent an overhang nucleotide;

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

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

In some embodiments, the N_a or N_b comprises modifications of alternating pattern.

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

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

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

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

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

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

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

When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. In one

embodiment, N_b is 0, 1, 2, 3, 4, 5, or 6 Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

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

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

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

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

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

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

wherein:

k and l are each independently 0 or 1;

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

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

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

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

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

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

In some embodiments, the N_a' or N_b' comprises modifications of alternating pattern.

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

In certain embodiments, Y'Y'Y' motif is all 2'-OMe modified nucleotides.

In certain embodiments, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

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

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

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

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

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

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

When the antisense strand is represented as formula (IId), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. In one embodiment, N_b is 0, 1, 2, 3, 4, 5, or 6.

In other embodiments, k is 0 and l is 0 and the antisense strand may be represented by the formula:

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

When the antisense strand is represented as formula (IIa), each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

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

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

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

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

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

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

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

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

(III)

wherein:

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

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

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

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

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

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

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

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

5' n_p - N_a -Y Y Y -N_a-n_q 3'

3' n_p'-N_a'-Y'Y'Y' -$N_a'$$n_q'$ 5'

(IIIa)

5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-Y'Y'Y'-N_b'-Z'Z'Z'-$N_a'$$n_q'$ 5'

(IIIb)

5' n_p-N_a- X X X -N_b -Y Y Y - N_a-n_q 3'

3' n_p'-N_a'-X'X'X'-N_b'-Y'Y'Y'-N_a'-n_q' 5'

(IIIc)

5' n_p -N_a -X X X -N_b-Y Y Y -N_b- Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-X'X'X'-N_b'-Y'Y'Y'-N_b'-Z'Z'Z'-N_a-n_q' 5'

(IIId)

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

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

When the dsRNAi agent is represented as formula (IIIc), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified

nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

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

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

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

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

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

In certain embodiments, the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, or the modification on the X nucleotide is different than the modification on the X' nucleotide.

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

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

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

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

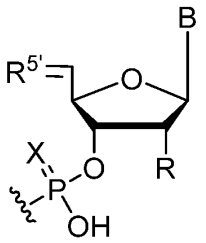
In one embodiment, two dsRNAi agents represented by at least one of formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends, and are optionally conjugated to a ligand. Each of the agents can target the same gene or two different genes;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

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

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

Various publications describe multimeric iRNAs that can be used in the methods of the invention. Such publications include WO2007/091269, U.S. Patent No. 7,858,769, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520 the entire contents of each of which are hereby incorporated herein by reference.

In certain embodiments, the compositions and methods of the disclosure include a vinyl phosphonate (VP) modification of an RNAi agent as described herein. In exemplary embodiments, a 5' vinyl phosphonate modified nucleotide of the disclosure has the structure:



wherein X is O or S;

R is hydrogen, hydroxy, fluoro, or C_{1-20}alkoxy (*e.g.*, methoxy or n-hexadecyloxy);

$R^{5'}$ is =C(H)-P(O)$(OH)_2$ and the double bond between the C5' carbon and $R^{5'}$ is in the *E* or *Z* orientation (*e.g.*, *E* orientation); and

B is a nucleobase or a modified nucleobase, optionally where B is adenine, guanine, cytosine, thymine, or uracil.

A vinyl phosphonate of the instant disclosure may be attached to either the antisense or the sense strand of a dsRNA of the disclosure. In certain embodiments, a vinyl phosphonate of the instant disclosure is attached to the antisense strand of a dsRNA, optionally at the 5' end of the antisense strand of the dsRNA.

Vinyl phosphonate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphonate structure includes the preceding structure, where R5' is =C(H)-OP(O)(OH)2 and the double bond between the C5' carbon and R5' is in the E or Z orientation (*e.g.*, E orientation).

As described in more detail below, the iRNA that contains conjugations of one or more carbohydrate moieties to an iRNA can optimize one or more properties of the iRNA. In many cases, the carbohydrate moiety will be attached to a modified subunit of the iRNA. For example, the ribose sugar of one or more ribonucleotide subunits of a iRNA can be replaced with another moiety, *e.g.*, a non-carbohydrate (such as, 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," such as, 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, or polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, *e.g.*, an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, *e.g.*, a ligand to the constituent ring.

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

i. Thermally Destabilizing Modifications

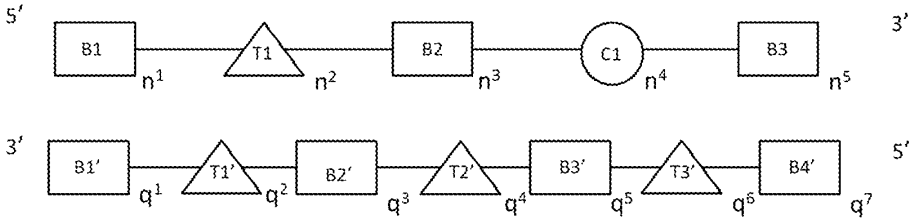
In certain embodiments, a dsRNA molecule can be optimized for RNA interference by incorporating thermally destabilizing modifications in the seed region of the antisense strand. As used herein "seed region" means at positions 2-9 of the 5'-end of the referenced strand or at positions 2-8 of the 5'-end of the refrenced strand. For example, thermally destabilizing modifications can be incorporated in the seed region of the antisense strand to reduce or inhibit off-target gene silencing.

The term "thermally destabilizing modification(s)" includes modification(s) that would result with a dsRNA with a lower overall melting temperature (T_m) than the T_m of the dsRNA without having such modification(s). For example, the thermally destabilizing modification(s) can decrease the T_m of the dsRNA by 1 – 4 °C, such as one, two, three or four degrees Celcius. And, the term "thermally destabilizing nucleotide" refers to a nucleotide containing one or more thermally destabilizing modifications.

It has been discovered that dsRNAs with an antisense strand comprising at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5' end, of the antisense strand have reduced off-target gene silencing activity. Accordingly, in some embodiments, the antisense strand comprises at least one (*e.g.*, one, two, three, four, five or more) thermally destabilizing modification of the duplex within the first 9 nucleotide positions of the 5' region of the antisense strand. In some embodiments, one or more thermally destabilizing modification(s) of the duplex is/are located in positions 2-9, such as, positions 4-8, from the 5'-end of the antisense strand. In some further embodiments, the thermally destabilizing modification(s) of the duplex is/are located at position 6, 7 or 8 from the 5'-end of the antisense strand. In still some further embodiments, the thermally destabilizing modification of the duplex is located at position 7 from the

5'-end of the antisense strand. In some embodiments, the thermally destabilizing modification of the duplex is located at position 2, 3, 4, 5 or 9 from the 5'-end of the antisense strand.

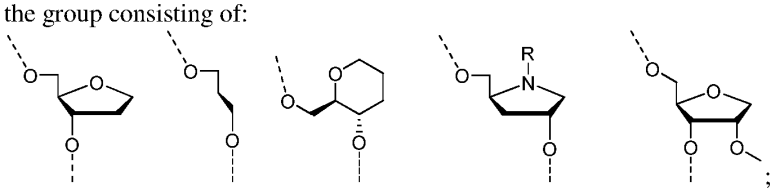
An iRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The RNAi agent may be represented by formula (L):



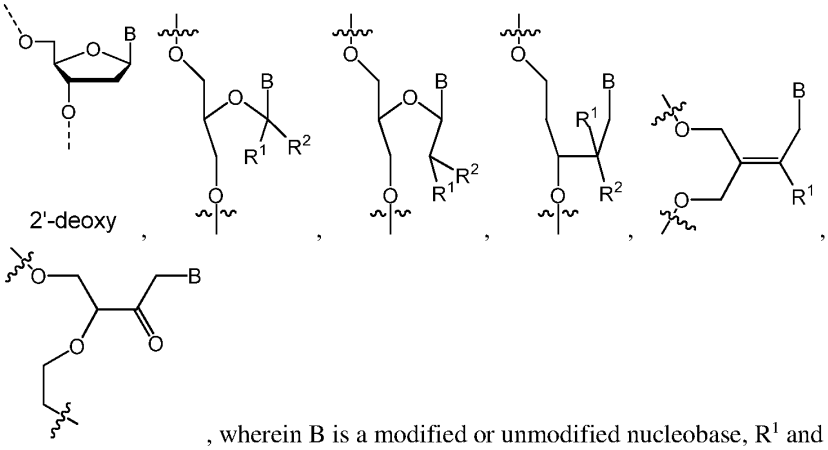
(L),

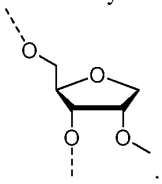
In formula (L), B1, B2, B3, B1', B2', B3', and B4' each are independently a nucleotide containing a modification selected from the group consisting of 2'-O-alkyl, 2'-substituted alkoxy, 2'-substituted alkyl, 2'-halo, ENA, and BNA/LNA. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe modifications. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe or 2'-F modifications. In one embodiment, at least one of B1, B2, B3, B1', B2', B3', and B4' contain 2'-O-N-methylacetamido (2'-O-NMA, 2'O-CH2C(O)N(Me)H) modification.

C1 is a thermally destabilizing nucleotide placed at a site opposite to the seed region of the antisense strand (*i.e.*, at positions 2-8 of the 5'-end of the antisense strand, or at positions 2-9 of the 5'-end of the antisense strand). For example, C1 is at a position of the sense strand that pairs with a nucleotide at positions 2-8 of the 5'-end of the antisense strand. In one example, C1 is at position 15 from the 5'-end of the sense strand. C1 nucleotide bears the thermally destabilizing modification which can include abasic modification; mismatch with the opposing nucleotide in the duplex; and sugar modification such as 2'-deoxy modification or acyclic nucleotide *e.g.*, unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA), or 2'-5'-linked ribonucleotides ("3'-RNA").. In one embodiment, C1 has thermally destabilizing modification selected from the group consisting of: i) mismatch with the opposing nucleotide in the antisense strand; ii) abasic modification selected from the group consisting of:



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



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

T1, T1', T2', and T3' each independently represent a nucleotide comprising a modification providing the nucleotide a steric bulk that is less or equal to the steric bulk of a 2'-OMe modification. A steric bulk refers to the sum of steric effects of a modification. Methods for determining steric effects of a modification of a nucleotide are known to one skilled in the art. The modification can be at the 2' position of a ribose sugar of the nucleotide, or a modification to a non-ribose nucleotide, acyclic nucleotide, or the backbone of the nucleotide that is similar or equivalent to the 2' position of the ribose sugar, and provides the nucleotide a steric bulk that is less than or equal to the steric bulk of a 2'-OMe modification. For example, T1, T1', T2', and T3' are each independently selected from DNA, RNA, LNA, 2'-F, and 2'-F-5'-methyl. In one embodiment, T1 is DNA. In one embodiment, T1' is DNA, RNA or LNA. In one embodiment, T2' is DNA or RNA. In one embodiment, T3' is DNA or RNA.

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

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

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

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

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

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

In one embodiment, n^4 can be 0. In one example, n^4 is 0, and q^2 and q^6 are 1. In another example, n^4 is 0, and q^2 and q^6 are 1, with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two

phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, n^4, q^2, and q^6 are each 1.

In one embodiment, n^2, n^4, q^2, q^4, and q^6 are each 1.

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

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

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

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

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

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

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

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

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

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

q^6 is equal to 1, and the modification to T3' is at the 2' position or at positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

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

In one embodiment, T2' starts at position 9 from the 5' end of the antisense strand. In one example, T2' is at position 9 from the 5' end of the antisense strand, and q^4 is 1.

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

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

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

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

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

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 6, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 7, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is

1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

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

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

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

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

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

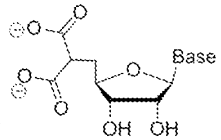
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and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

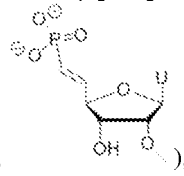
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4'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

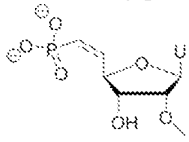
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4'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

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

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

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

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

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

 ), or mixtures thereof.

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

In one embodiment, the RNAi agent comprises a 5'-P. In one embodiment, the RNAi agent comprises a 5'-P in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS. In one embodiment, the RNAi agent comprises a 5'-PS in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-VP. In one embodiment, the RNAi agent comprises a 5'-VP in the antisense strand. In one embodiment, the RNAi agent comprises a 5'-*E*-VP in the antisense strand. In one embodiment, the RNAi agent comprises a 5'-*Z*-VP in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS_2. In one embodiment, the RNAi agent comprises a 5'-PS_2 in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS_2. In one embodiment, the RNAi agent comprises a 5'-deoxy-5'-*C*-malonyl in the antisense strand.

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

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

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

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

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

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

modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- P.

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

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

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

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

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

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

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

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

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

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

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

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two

phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

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

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

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

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide

linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

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

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

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

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

within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2 and a targeting ligand. In one embodiment, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

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

In a particular embodiment, an RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker; and

(iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14 to 16, 18, and 20 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 9, 11 to 13, 15, 17, 19, 21, and 23, and 2'F modifications at positions 2, 4, 6 to 8, 10, 14, 16, 18, 20, and 22 (counting from the 5' end); and

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

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

In another particular embodiment, an RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 15, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14, 16, 18, and 20 (counting from the 5' end); and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

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

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, 10, and 12 to 21, 2'-F modifications at positions 7, and 9, and a deoxy-nucleotide (*e.g.* dT) at position 11 (counting from the 5' end); and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 7, 9, 11, 13, 15, 17, and 19 to 23, and 2'-F modifications at positions 2, 4 to 6, 8, 10, 12, 14, 16, and 18 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

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

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, 10, 12, 14, and 16 to 21, and 2'-F modifications at positions 7, 9, 11, 13, and 15; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 5, 7, 9, 11, 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2 to 4, 6, 8, 10, 12, 14, 16, 18, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

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

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 9, and 12 to 21, and 2'-F modifications at positions 10, and 11; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

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

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

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

and

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

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

In another particular embodiment, a RNAi agent of the present invention comprises:

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

and

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

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

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:
(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 8, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 9, 14, and 16 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

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

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

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

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 19 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 4, 6, and 10 to 19, and 2'-F modifications at positions 5, and 7 to 9; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 21, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and

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

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

In certain embodiments, the iRNA for use in the methods of the invention is an agent selected from agents listed in any one of Tables 2-7. These agents may further comprise a ligand.

III. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the iRNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the iRNA *e.g.*, into a cell. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acid. Sci. USA*, 1989, 86: 6553-6556). In other embodiments, the ligand is cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J*, 1991, 10:1111-1118; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327-330; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al.*, J. *Pharmacol. Exp. Ther.*, 1996, 277:923-937).

In certain embodiments, a ligand alters the distribution, targeting, or lifetime of an iRNA agent into which it is incorporated. In some 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. In some embodiments, ligands do not take part in duplex pairing in a duplexed nucleic acid.

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

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

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-glucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

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

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins, *etc*. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin. 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 iRNAs of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

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

In the ligand-conjugated iRNAs and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then

reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites 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 certain embodiments, the ligand or conjugate is a lipid or lipid-based molecule. In one embodiment, such a lipid or lipid-based molecule binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

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 certain embodiments, the lipid based ligand binds HSA. In one embodiment, it binds HSA with a sufficient affinity such that the conjugate will be 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 other embodiments, the lipid based ligand binds HSA weakly or not at all. In one embodiment, the conjugate will be 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, such as, a helical cell-permeation agent. In one embodiment, 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. In one embodiment, the helical agent is an alpha-helical agent, which has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure

similar to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp, or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 14). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO:15) 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:16) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:17) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent *via* an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.,* glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand, *e.g.,* 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 iRNA further comprises a carbohydrate. The carbohydrate conjugated iRNA is advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri-, and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.,* C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.,* C5, C6, C7, or C8).

In certain embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide.

In certain embodiments, the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc conjugates, which comprise one or more N-acetylgalactosamine (GalNAc) derivatives, are described, for example, in US 8,106,022, the entire content of which is hereby incorporated herein by reference. In some embodiments, the GalNAc conjugate serves as a ligand that targets the iRNA to particular cells. In some embodiments, the GalNAc conjugate targets the iRNA to liver cells, *e.g.*, by serving as a ligand for the asialoglycoprotein receptor of liver cells (*e.g.*, hepatocytes).

In some embodiments, the carbohydrate conjugate comprises one or more GalNAc derivatives. The GalNAc derivatives may be attached *via* a linker, *e.g.*, a bivalent or trivalent branched linker. In some embodiments the GalNAc conjugate is conjugated to the 3' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 3' end of the sense strand) *via* a linker, *e.g.*, a linker as described herein. In some embodiments the GalNAc conjugate is conjugated to the 5' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 5' end of the sense strand) *via* a linker, *e.g.*, a linker as described herein.

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

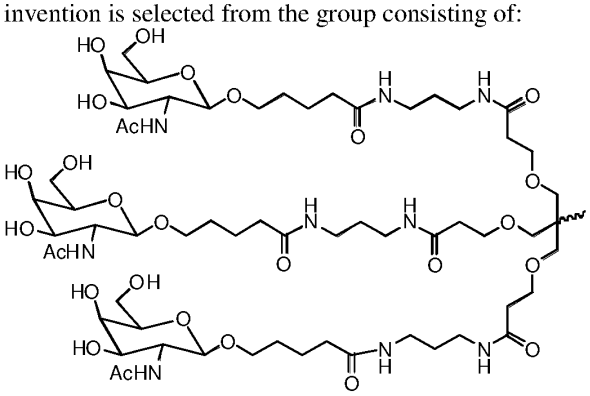
In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc

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

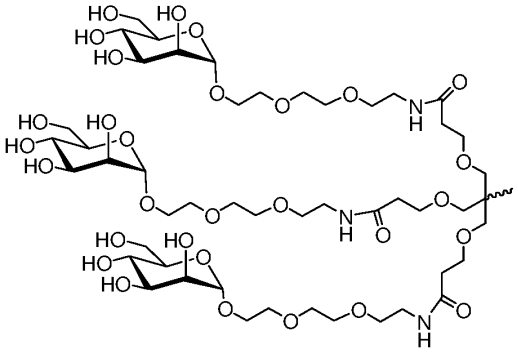
In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

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



Formula II,



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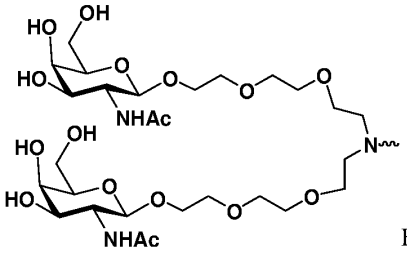

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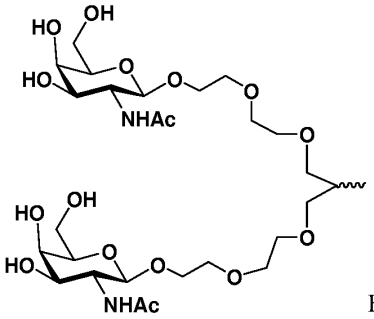

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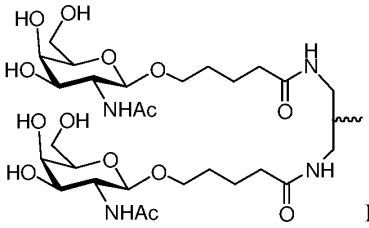

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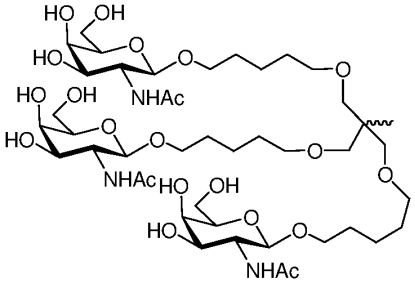
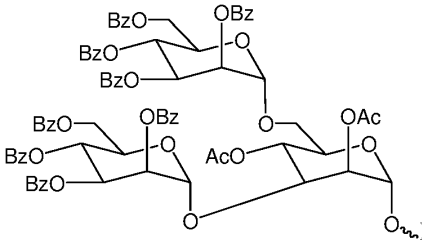
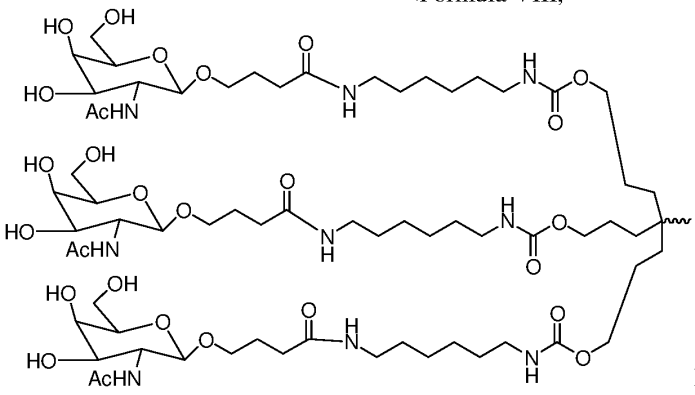


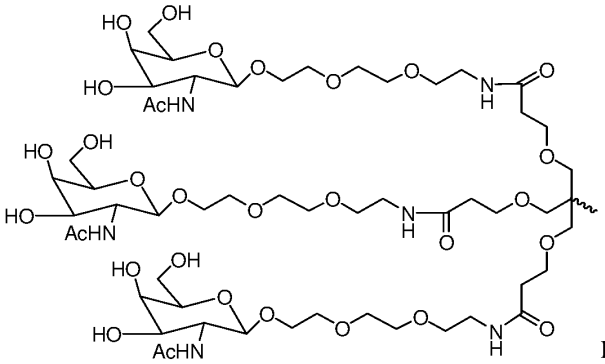
Formula VII,



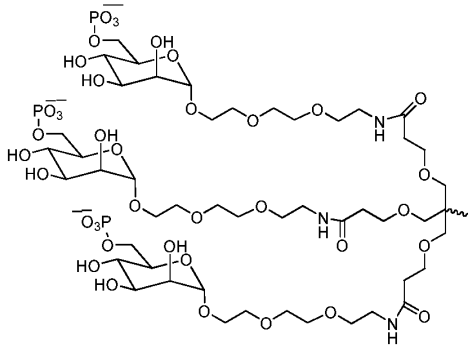
Formula VIII,



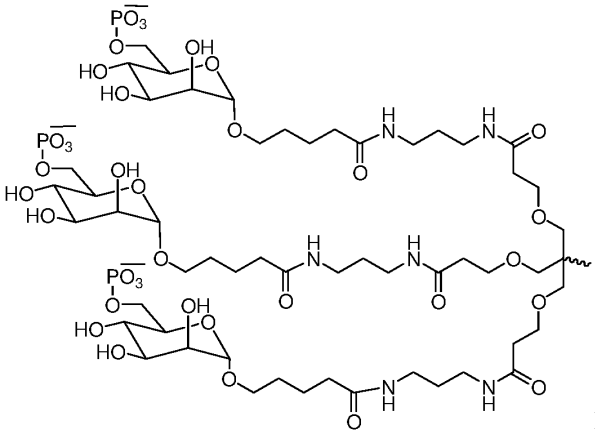
Formula IX,



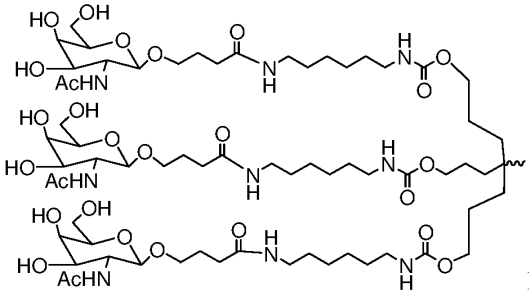
Formula X,



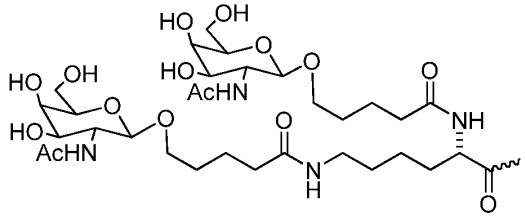
Formula XI,



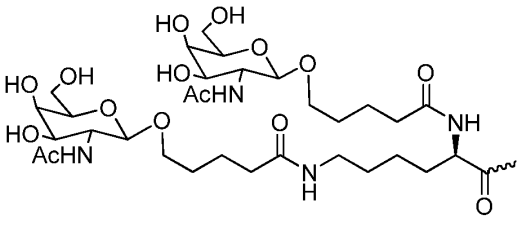
Formula XII,

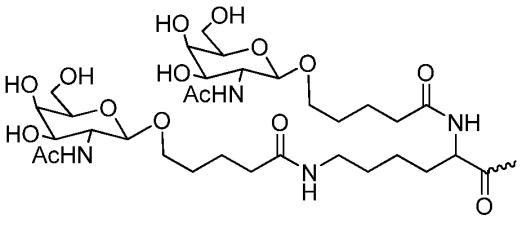


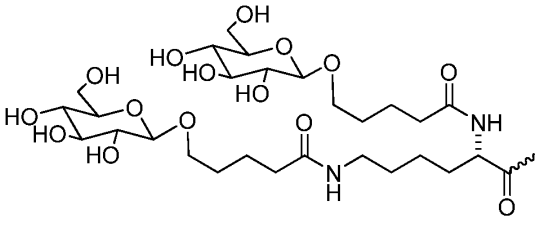
Formula XIII,

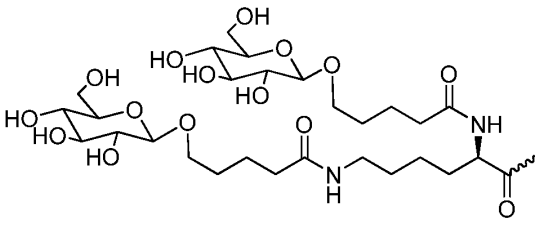


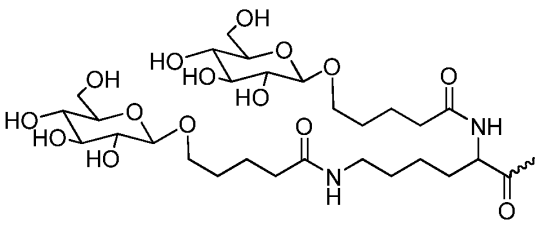
Formula XIV,

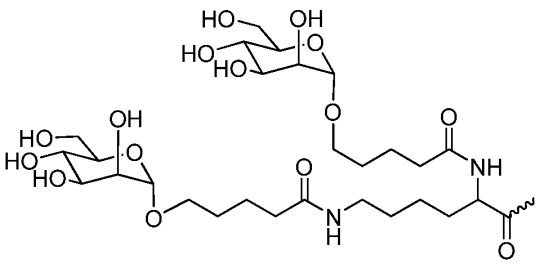

Formula XV,

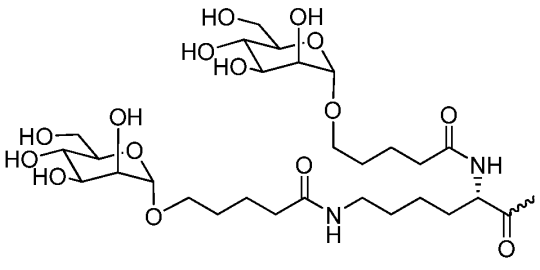

Formula XVI,

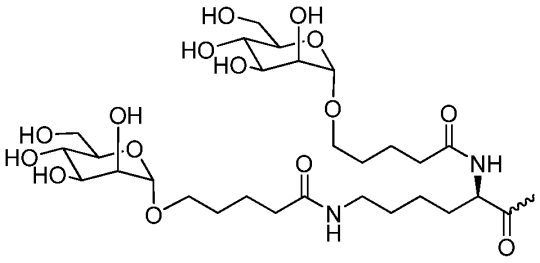

Formula XVII,


Formula XVIII,

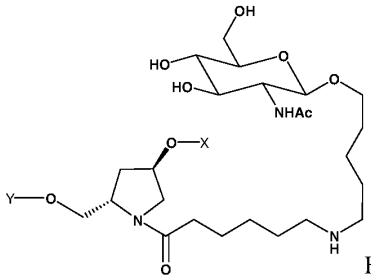

Formula XIX,


Formula XX,

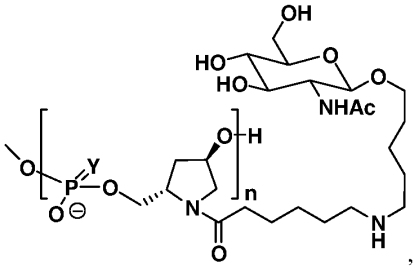

Formula XXI,



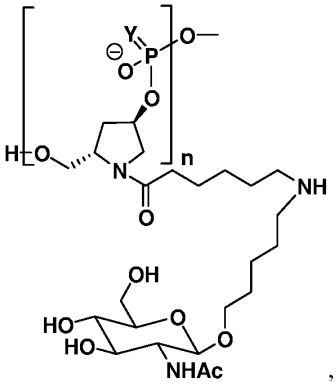
Formula XXII,



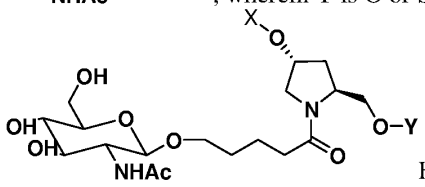
Formula XXIII;



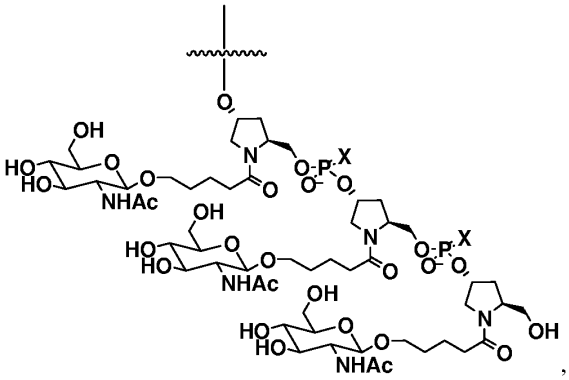
, wherein Y is O or S and n is 3 -6 (Formula XXIV);



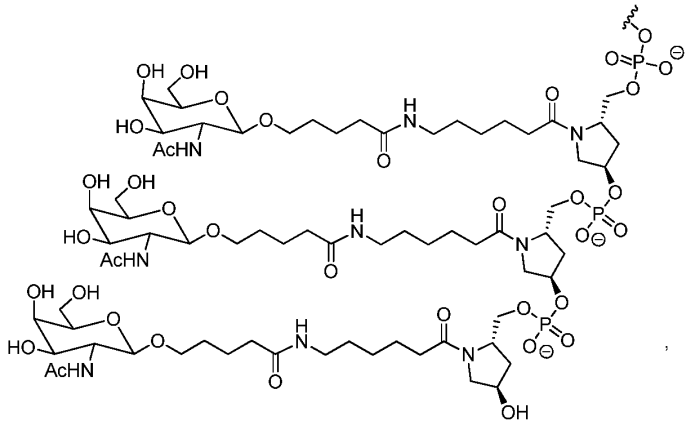
, wherein Y is O or S and n is 3-6 (Formula XXV);



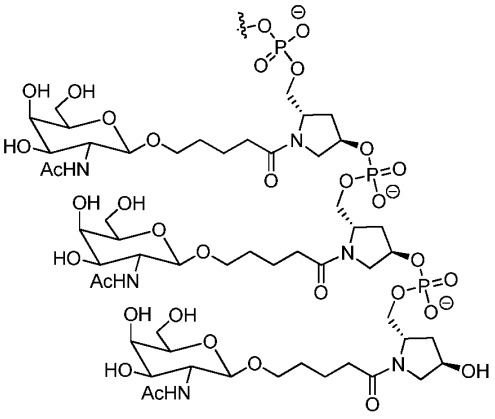
Formula XXVI;



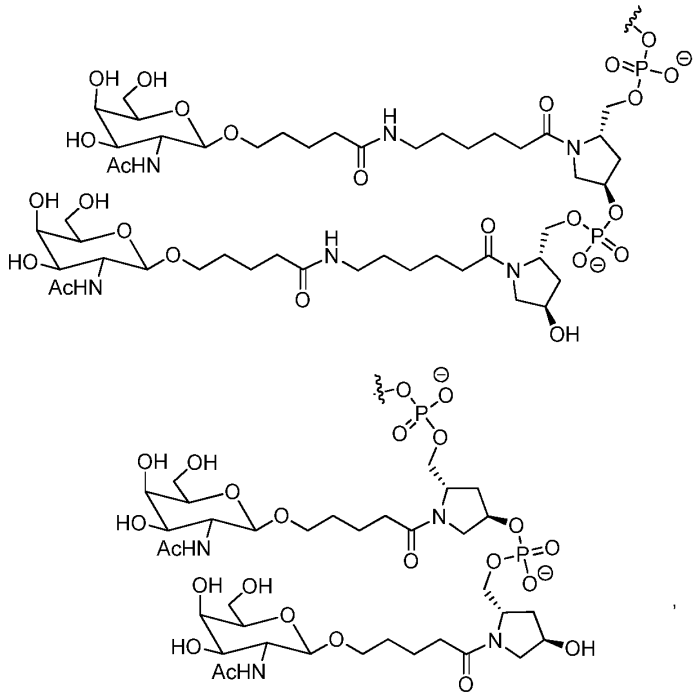
, wherein X is O or S (Formula XXVII);



,

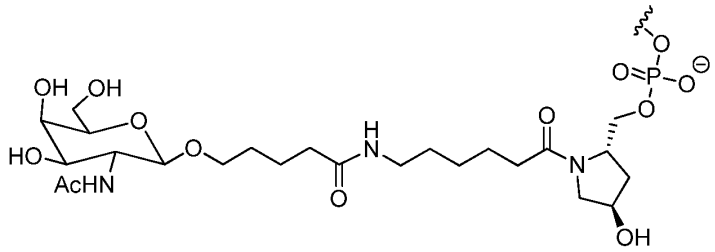


Formula XXVII; Formula XXIX;

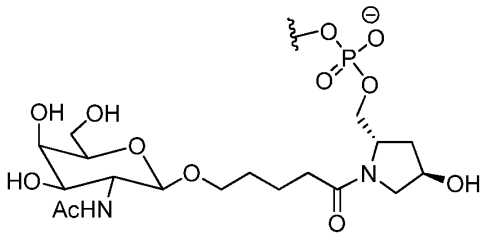


Formula XXX;

Formula XXXI;

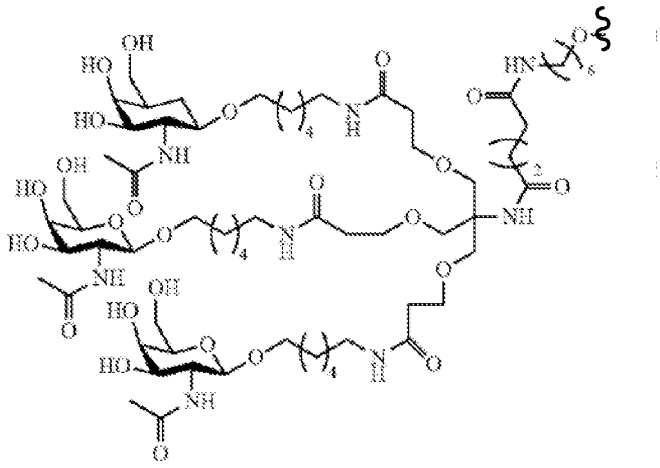


, and



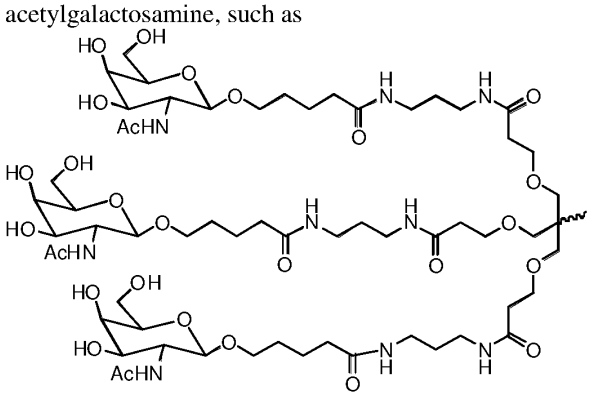
Formula XXXII;

Formula XXXIII.



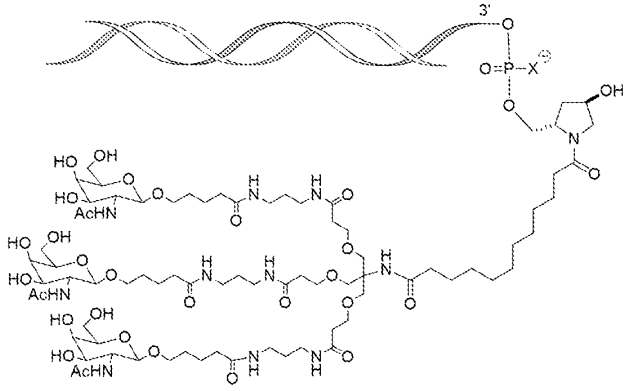
Formula XXXIV.

In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In one embodiment, the monosaccharide is an N-acetylgalactosamine, such as

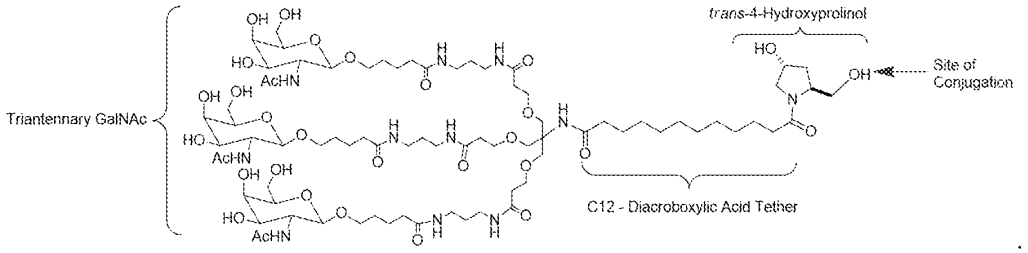


Formula II.

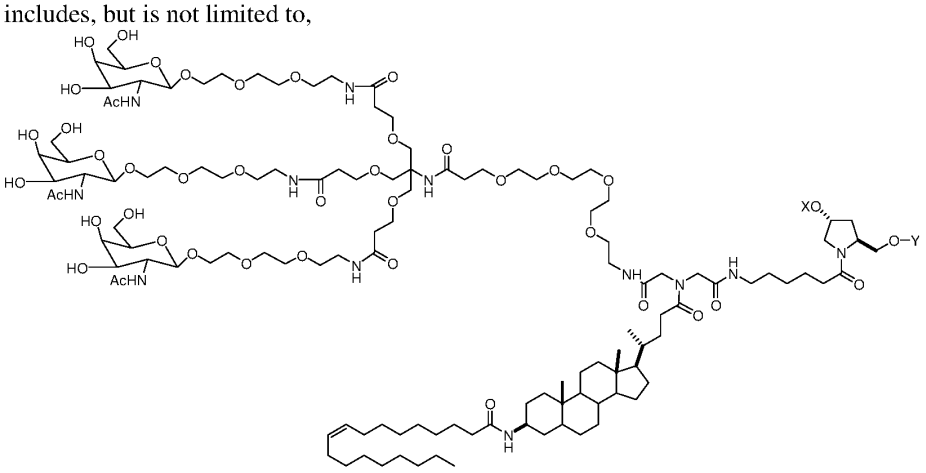
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate *via* a linker as shown in the following schematic, wherein X is O or S



In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below:

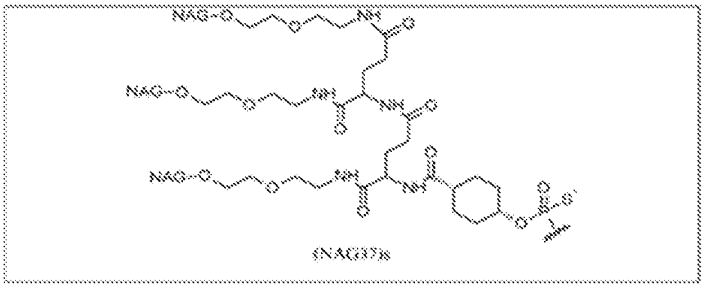


Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



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

In some embodiments, a suitable ligand is a ligand disclosed in WO 2019/055633, the entire contents of which are incorporated herein by reference. In one embodiment the ligand comprises the structure below:



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

In one embodiment, the double stranded RNAi agents of the invention comprise one or more GalNAc or GalNAc derivative attached to the iRNA agent. The GalNAc may be attached to any nucleotide *via* a linker on the sense strand or antsisense strand. The GalNac may be attached to the 5'-end of the sense strand, the 3' end of the sense strand, the 5'-end of the antisense strand, or the 3' – end of the antisense strand. In one embodiment, the GalNAc is attached to the 3' end of the sense strand, *e.g., via* a trivalent linker.

In other embodiments, the double stranded RNAi 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 double stranded RNAi agent through a plurality of linkers, *e.g.,* monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention is part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker.

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 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 iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O), substituted or unsubstituted aryl,

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

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 an exemplary 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 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 selected 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 certain embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

i. Redox cleavable linking groups

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

ii. Phosphate-based cleavable linking groups

In other embodiments, 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-, wherein Rk at each occurrence can be, independently, C1-C20 alkyl, C1-C20 haloalkyl, C6-C10 aryl, or C7-C12 aralkyl. Exemplary embodiments include -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-, and -O-P(S)(H)-S-. In certain embodiments a phosphate-based linking group is -O-

P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

In other embodiments, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In certain 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, 5.5, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). An exemplary 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.

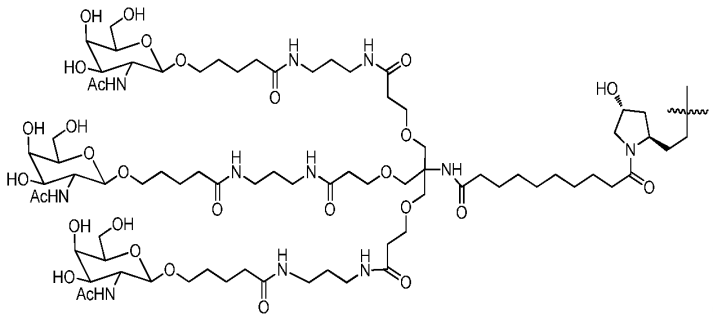
iv. Ester-based linking groups

In other embodiments, 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.

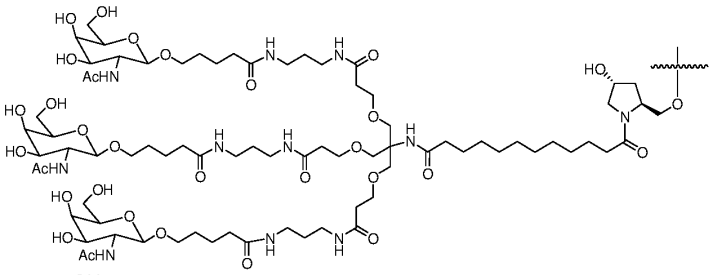
v. Peptide-based cleaving groups

In yet other embodiments, 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.

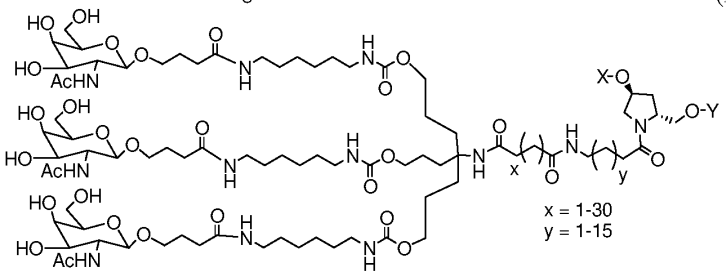
In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



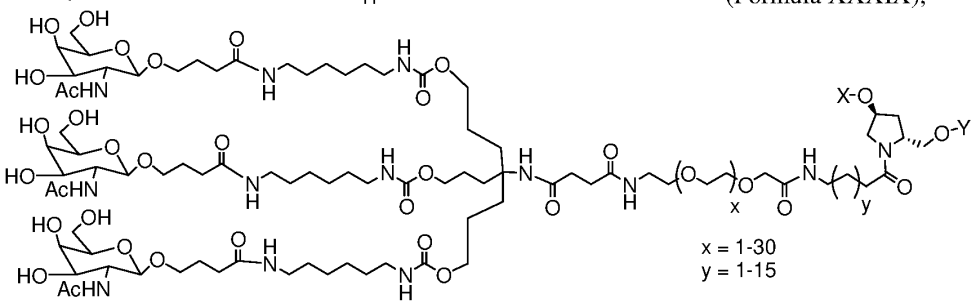
(Formula XXXVII),



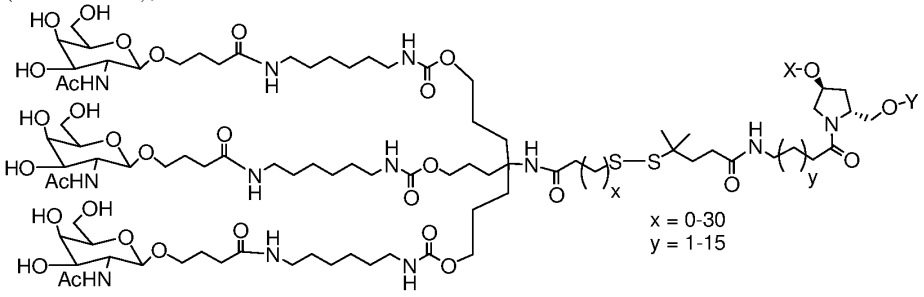
(Formula XXXVIII),



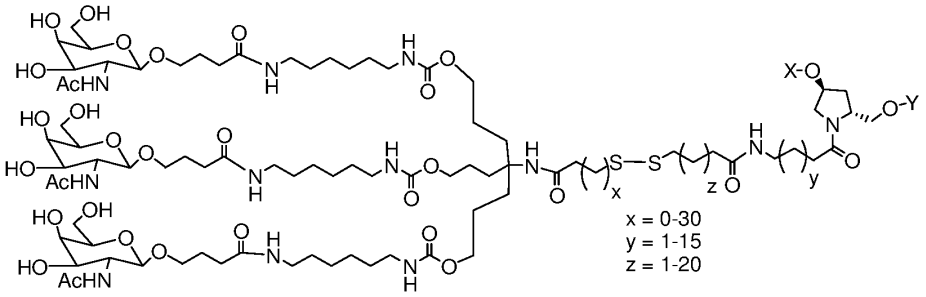
(Formula XXXIX),



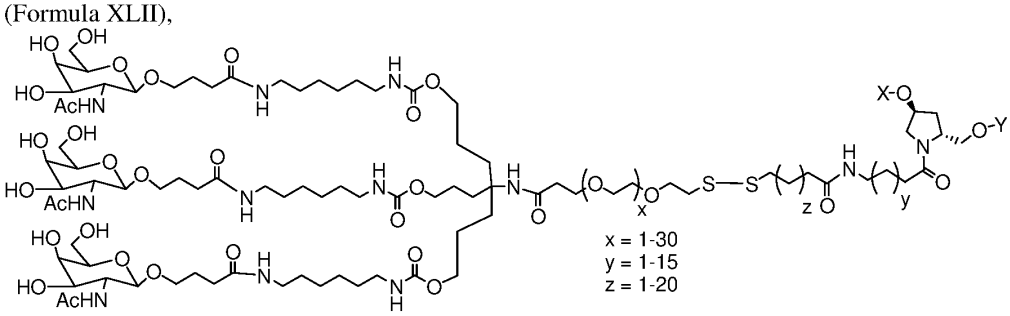
(Formula XL),



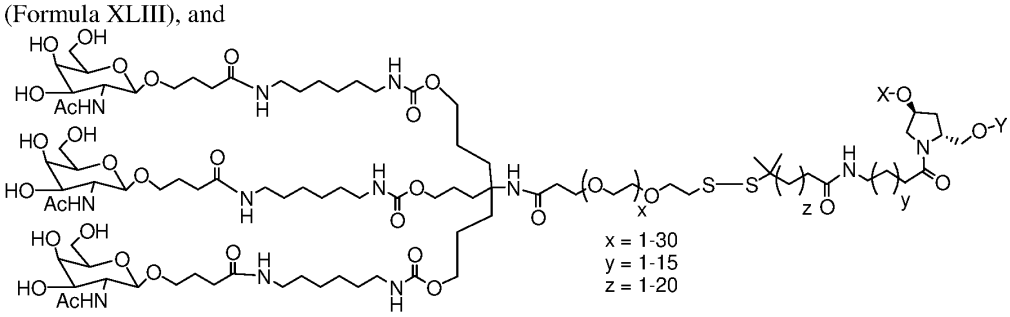
(Formula XLI),



(Formula XLII),



(Formula XLIII), and

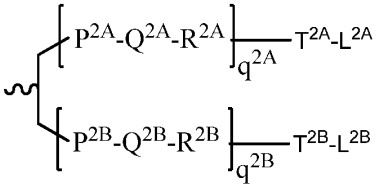


(Formula XLIV), when one of X or Y is an oligonucleotide, the other is a hydrogen.

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

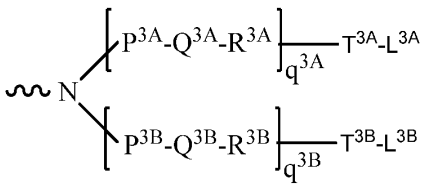
In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XLV) – (XLVI):

Formula XXXXV



,

Formula XLVI



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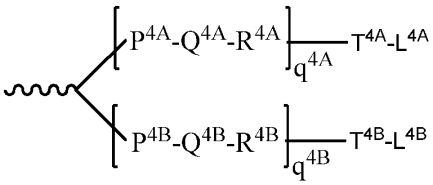
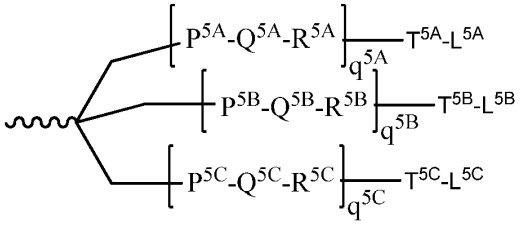


, or



;

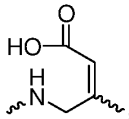
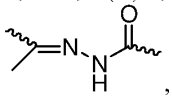
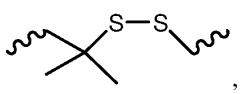
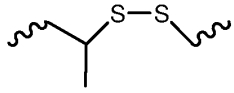
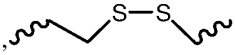
Formula XLVII Formula XLVIII

wherein:

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

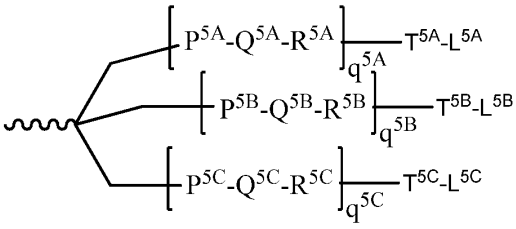
P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

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

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

L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; and R^a is H or amino acid side chain. Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (XLIX):

Formula XLIX



,

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

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. Patent Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928;5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; and 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

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

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

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular

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

IV. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention to a cell *e.g.,* a cell within a subject, such as a human subject (*e.g.,* a subject in need thereof, such as a subject susceptible to or diagnosed with a TMPRSS6-associated disorder, *e.g.*, a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an iRNA, *e.g.,* a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an iRNA of the invention (see *e.g.*, Akhtar S. and Julian RL. (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G., *et al.* (2004) *Nucleic Acids* 32:e49; Tan, PH., *et al*

(2005) *Gene Ther.* 12:59-66; Makimura, H., *et al* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya,Y., *et al* (2005) *J. Neurophysiol.* 93:594-602). Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J., *et al* (2004) *Nature* 432:173-178).

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

A. Vector encoded iRNAs of the Invention

iRNA targeting the TMPRSS6 gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; Skillern, A, *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Patent No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular

plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

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

V. Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for preventing or treating a TMPRSS6-associated disorder, *e.g.*, a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration *via* parenteral delivery, *e.g.*, by subcutaneous (SC), intramuscular (IM), or intravenous (IV) delivery. The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a TMPRSS6 gene.

In some embodiments, the pharmaceutical compositions of the invention are sterile. In another embodiment, the pharmaceutical compositions of the invention are pyrogen free.

The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a TMPRSS6 gene. In general, a suitable dose of an iRNA 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. Typically, a suitable dose of an iRNA of the invention will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, such as, about 0.3 mg/kg and about 3.0 mg/kg. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every month, once every 3-6 months, or once a year. In certain embodiments, the iRNA is administered about once per month to about once per six months.

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

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

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to mutations present in the subject, previous treatments, the general health or age of the subject, and other diseases present. Moreover, treatment of a subject with a prophylactically or therapeutically effective amount, as appropriate, of a composition can include a single treatment or a series of treatments.

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

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. Formulations include those that target the liver.

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.

A. Additional Formulations

i. Emulsions

The compositions of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed

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

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

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).

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).

The application of emulsion formulations *via* dermatological, oral, and parenteral routes, and methods for their manufacture have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

ii. Microemulsions

In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil, and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215).

iii. Microparticles

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

iv. Penetration Enhancers

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

aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

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

v. 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. Such agent are well known in the art.

vi. 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, 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, or dextran. The suspension can also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA and (b) one or more agents which function by a non-iRNA mechanism and which are useful in treating a TMPRSS63-associated disorder, *e.g.*, a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermiedia), polycythemia vera, myelodysplastic syndrome,

congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia.

Toxicity and prophylactic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose prophylactically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

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 ED50, such as, an ED80 or ED90, with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the prophylactically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) or higher levels of inhibition as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents used for the prevention or treatment of a TMPRSS6-associated disorder, *e.g.*, a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis. In any event, the administering physician can adjust the amount and timing of iRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VI. Methods For Inhibiting TMPRSS6 Expression

The present invention also provides methods of inhibiting expression of a TMPRSS6 gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNA agent, in an amount effective to inhibit expression of TMPRSS6 in the cell, thereby inhibiting expression of TMPRSS6 in the cell.

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

described herein or known in the art. In some embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a $GalNAc_3$ ligand, or any other ligand that directs the RNAi agent to a site of interest.

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

The phrase "inhibiting expression of a TMPRSS6" is intended to refer to inhibition of expression of any TMPRSS6 gene (such as, *e.g.*, a mouse TMPRSS6 3 gene, a rat TMPRSS6 gene, a monkey TMPRSS6 gene, or a human TMPRSS6 gene) as well as variants or mutants of a TMPRSS6 gene. Thus, the TMPRSS6 gene may be a wild-type TMPRSS6 gene, a mutant TMPRSS6 gene, or a transgenic TMPRSS6 gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of a TMPRSS6 gene" includes any level of inhibition of a TMPRSS6 gene, *e.g.*, at least partial suppression of the expression of a TMPRSS6 gene. The expression of the TMPRSS6 gene may be assessed based on the level, or the change in the level, of any variable associated with TMPRSS6 gene expression, *e.g.*, TMPRSS6 mRNA level or TMPRSS6 protein level. The expression of a TMPRSS6 may also be assessed indirectly based on the hepcidin mRNA level, hepcidin protein level, or iron levels in tissues or serum. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject. It is understood that TMPRSS6 is expressed predominantly in the liver.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with TMPRSS6 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 a TMPRSS6 gene is inhibited by at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In some embodiments, expression of a TMPRSS6 gene is inhibited by at least 70%. It is further understood that inhibition of TMPRSS6 expression in certain tissues, *e.g.*, in liver, without a significant inhibition of expression in other tissues, *e.g.*, brain, may be desirable. In some embodiments, expression level is determined using the assay method provided in Example 2 with a 10 nM siRNA concentration in the appropriate species matched cell line.

In certain embodiments, inhibition of expression *in vivo* is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, an AAV-infected mouse expressing the human target gene (*i.e.*, TMPRSS6), *e.g.*, when administered as a single dose, *e.g.,* at 3 mg/kg at the nadir of RNA expression. Knockdown of expression of an endogenous gene in a model animal system can also be determined, *e.g.*, after administration of a single dose at, *e.g.,* 3 mg/kg at the nadir of RNA expression. Such systems are useful when the nucleic acid sequence of the human gene and the model animal gene are sufficiently close such that the human iRNA provides effective knockdown of the model animal gene. RNA expression in liver is determined using the PCR methods provided in Example 2.

Inhibition of the expression of a TMPRSS6 gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a TMPRSS6 gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an iRNA of the invention, or by administering an iRNA of the invention to a subject in which the cells are or were present) such that the expression of a TMPRSS6 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s) not treated with an iRNA or not treated with an iRNA targeted to the gene of interest). In some embodiments, the inhibition is assessed by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line and expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

In other embodiments, inhibition of the expression of a TMPRSS6 gene may be assessed in terms of a reduction of a parameter that is functionally linked to TMPRSS6 gene expression, *e.g.*, TMPRSS6 protein level in blood or serum from a subject. TMPRSS6 gene silencing may be determined in any cell expressing TMPRSS6, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of a TMPRSS6 protein may be manifested by a reduction in the level of the TMPRSS6 protein that is expressed by a cell or group of cells or in a subject sample (*e.g.*, the level of protein in a blood sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibition of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells, or the change in the level of protein in a subject sample, *e.g.*, blood or serum derived therefrom.

A control cell, a group of cells, or subject sample that may be used to assess the inhibition of the expression of a TMPRSS6 gene includes a cell, group of cells, or subject sample that has not yet been contacted with an RNAi agent of the invention. For example, the control cell, group of cells, or subject sample may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent or an appropriately matched population control.

The level of TMPRSS6 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 TMPRSS6 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA of the TMPRSS6 gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy™ RNA preparation kits (Qiagen®) or PAXgene™ (PreAnalytix™, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, *in situ* hybridization, and microarray analysis.

In some embodiments, the level of expression of TMPRSS6 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 TMPRSS6. 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 TMPRSS6 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 TMPRSS6 mRNA.

An alternative method for determining the level of expression of TMPRSS6 in a sample involves the process of nucleic acid amplification or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.*, U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of TMPRSS6 is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System). In some embodiments, expression level is determined by the method provided in Example 2 using, *e.g.*, a 10 nM siRNA concentration, in the species matched cell line.

The expression levels of TMPRSS6 mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of TMPRSS6 expression level may also comprise using nucleic acid probes in solution.

In some 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. In some embodiments, expression level is determined by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line.

The level of TMPRSS6 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.

In some embodiments, the efficacy of the methods of the invention are assessed by a decrease in TMPRSS6 mRNA or protein level (*e.g.*, in a liver biopsy).

In some embodiments of the methods of the invention, the iRNA is administered to a subject such that the iRNA is delivered to a specific site within the subject. The inhibition of expression of TMPRSS6 may be assessed using measurements of the level or change in the level of TMPRSS6 mRNA or TMPRSS6 protein in a sample derived from fluid or tissue from the specific site within the subject (*e.g.*, liver or blood).

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

VII. Prophylactic and Treatment Methods of the Invention

The present invention also provides methods of using an iRNA of the invention or a composition containing an iRNA of the invention to inhibit expression of TMPRSS6, thereby preventing or treating a TMPRSS6-associated disorder, *e.g.*, a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis. In the methods of the invention the cell may be contacted with the siRNA *in vitro* or *in vivo, i.e.,* the cell may be within a subject.

A cell suitable for treatment using the methods of the invention may be any cell that expresses a TMPRSS6 gene, *e.g.*, a liver cell. A cell suitable for use in the methods of the invention may be a mammalian cell, *e.g.,* a primate cell (such as a human cell, including human cell in a chimeric non-human animal, or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), or a non-primate cell. In certain embodiments, the cell is a human cell, *e.g.,* a human liver cell. In the methods of the invention, TMPRSS6 expression is inhibited in the cell by at least 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95, or to a level below the level of detection of the assay.

The *in vivo* methods of the invention may include administering to a subject a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the TMPRSS6 gene of the mammal to which the RNAi agent is to be administered. The composition can be administered by any means known in the art including, but not

limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal, and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection. In certain embodiments, the compositions are administered by intramuscular injection.

In one aspect, the present invention also provides methods for inhibiting the expression of a TMPRSS6 gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a TMPRSS6 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the TMPRSS6 gene, thereby inhibiting expression of the TMPRSS6 gene in the cell. Reduction in gene expression can be assessed by any methods known in the art and by methods, *e.g.* qRT-PCR, described herein, *e.g.*, in Example 2. Reduction in protein production can be assessed by any methods known it the art, *e.g.* ELISA. In certain embodiments, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the TMPRSS6 gene or protein expression. In other embodiments, a blood sample serves as the subject sample for monitoring the reduction in the TMPRSS6 protein expression.

The present invention further provides methods of treatment in a subject in need thereof, *e.g.*, a subject diagnosed with a TMPRSS6-associated disorder, such as a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermiedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia. In one embodiment, a subject having a TMPRSS6-associated disorder has hereditary hemochromatosis. In another embodiment, a subject having a TMPRSS6-associated disorder has β-thalassemia. In another embodiment, a subject having a TMPRSS6-associated disorder has polycythemia vera.

The present invention further provides methods of prophylaxis in a subject in need thereof. The treatment methods of the invention include administering an iRNA of the invention to a subject, *e.g.*, a subject that would benefit from a reduction of TMPRSS6 expression, in a prophylactically effective amount of a dsRNA targeting a TMPRSS6 gene or a pharmaceutical composition comprising a dsRNA targeting a TMPRSS6 gene.

In one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in TMPRSS6 expression, *e.g.,* a TMPRSS6-associated disease, such as a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermiedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia. Treatment of a subject that would benefit from a reduction and/or inhibition of TMPRSS6 gene expression includes therapeutic treatment (*e.g.*, a subject is having elevated iron levels) and prophylactic treatment (*e.g.*, the subject is not having elevated iron levels or a subject may be at risk of developing elevated iron levels).

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

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

Subjects that would benefit from an inhibition of TMPRSS6 gene expression are subjects susceptible to or diagnosed with a TMPRSS6-associated disorder, such as a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermiedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia. In an embodiment, the method includes administering a composition featured herein such that expression of the target a TMPRSS6 gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 1-6, 1-3, or 3-6 months per dose. In certain embodiments, the composition is administered once every 3-6 months.

In one embodiment, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target TMPRSS6 gene. Compositions and methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

Administration of the iRNA according to the methods of the invention may result prevention or treatment of a TMPRSS6-associated disorder, *e.g.*, a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis, *e.g.*, hereditary hemochromatosis, β-thalassemia (*e.g.*, β-thalassemia major and β-thalassemia intermiedia), polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease or Friedreich's Ataxia. Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg.

In one embodiment, the iRNA is administered subcutaneously, *i.e.*, by subcutaneous injection. In another embodiment, the iRNA is administered intravenously, *i.e.,* by intravenous injection. One or more injections may be used to deliver the desired dose of iRNA to a subject. The injections may be repeated over a period of time.

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

The invention further provides methods and uses of an iRNA agent or a pharmaceutical composition thereof for treating a subject that would benefit from reduction and/or inhibition of

TMPRSS6 gene expression, *e.g.*, a subject having a TMPRSS6-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.

Accordingly, in some aspects of the invention, the methods which include either a single iRNA agent of the invention, further include administering to the subject one or more additional therapeutic agents.

For example, in certain embodiments, an iRNA targeting TMPRSS6 is administered in combination with, *e.g.*, an agent useful in treating a disorder associated with iron overload. For example, additional agents suitable for treating a subject that would benefit from reducton in TMPRSS6 expression, *e.g.*, a subject having a disorder associated with iron overload, may include iron chelators (*e.g.*, desferoxamine), folic acid, a blood transfusion, a phlebotomy, agents to manage ulcers, agents to increase fetal hemoglobin levels (*e.g.*, hydroxyurea), agents to control infection (*e.g.*, antibiotics and antivirals), agents to treat thrombotic state, or a stem cell or bone marrow transplant. A stem cell transplant can utilize stem cells from an umbilical cord, such as from a relative, *e.g.*, a sibling. Exemplary iron chelators include desferoxamine, Deferasirox (Exjade), deferiprone, vitamin E, wheat germ oil, tocophersolan, and indicaxanthin.

The iRNA agent 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.

VIII. Kits

In certain aspects, the instant disclosure provides kits that include a suitable container containing a pharmaceutical formulation of a siRNA compound, *e.g.*, a double-stranded siRNA compound, or siRNA compound, (*e.g.*, a precursor, *e.g.*, a larger siRNA compound which can be processed into a siRNA compound, or a DNA which encodes an siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, or precursor thereof).

Such kits include one or more dsRNA agent(s) and instructions for use, *e.g.*, instructions for administering a prophylactically or therapeutically effective amount of a dsRNA agent(s). The dsRNA agent may be in a vial or a pre-filled syringe. The kits may optionally further comprise means for administering the dsRNA agent (*e.g.*, an injection device, such as a pre-filled syringe), or means for measuring the inhibition of TMPRSS6 (*e.g.,* means for measuring the inhibition of TMPRSS6 mRNA, TMPRSS6 protein, and/or TMPRSS6 activity). Such means for measuring the inhibition of TMPRSS6 may comprise a means for obtaining a sample from a subject, such as, *e.g.*, a plasma sample. The kits of the invention may optionally further comprise means for determining the therapeutically effective or prophylactically effective amount.

In certain embodiments the individual components of the pharmaceutical formulation may be provided in one container, *e.g.,* a vial or a pre-filled syringe. Alternatively, it may be desirable to

provide the components of the pharmaceutical formulation separately in two or more containers, *e.g.*, one container for a siRNA compound preparation, and at least another for a carrier compound. The kit may be packaged in a number of different configurations such as one or more containers in a single box. The different components can be combined, *e.g.*, according to instructions provided with the kit. The components can be combined according to a method described herein, *e.g.*, to prepare and administer a pharmaceutical composition. The kit can also include a delivery device.

This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the informal Sequence Listing and Figures, are hereby incorporated herein by reference.

EXAMPLES

Example 1. iRNA Synthesis

Source of reagents

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

siRNA Design

siRNAs targeting the human Transmembrane protease, serine 6 (TMPRSS6) gene (human: NCBI refseqID NM_153609.4, NCBI GeneID: 164656) were designed using custom R and Python scripts. The human NM_153609.4 REFSEQ mRNA, has a length of 3197 bases.

Detailed lists of the unmodified TMPRSS6 sense and antisense strand nucleotide sequences are shown in Tables 2, 4 and 6. Detailed lists of the modified TMPRSS6 sense and antisense strand nucleotide sequences are shown in Tables 3, 5 and 7.

It is to be understood that, throughout the application, a duplex name without a decimal is equivalent to a duplex name with a decimal which merely references the batch number of the duplex. For example, AD-959917 is equivalent to AD-959917.1.

siRNA Synthesis

siRNAs were designed, synthesized, and prepared using methods known in the art.

Briefly, siRNA sequences were synthesized on a 1 µmol scale using a Mermade 192 synthesizer (BioAutomation) with phosphoramidite chemistry on solid supports. The solid support was controlled pore glass (500-1000 Å) loaded with a custom GalNAc ligand (3'-GalNAc conjugates), universal solid support (AM Chemicals), or the first nucleotide of interest. Ancillary synthesis reagents and standard 2-cyanoethyl phosphoramidite monomers (2'-deoxy-2'-fluoro, 2'-O-

methyl, RNA, DNA) were obtained from Thermo-Fisher (Milwaukee, WI), Hongene (China), or Chemgenes (Wilmington, MA, USA). Additional phosphoramidite monomers were procured from commercial suppliers, prepared in-house, or procured using custom synthesis from various CMOs. Phosphoramidites were prepared at a concentration of 100 mM in either acetonitrile or 9:1 acetonitrile:DMF and were coupled using 5-Ethylthio-1H-tetrazole (ETT, 0.25 M in acetonitrile) with a reaction time of 400 s. Phosphorothioate linkages were generated using a 100 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (9:1 v/v). Oxidation time was 5 minutes. All sequences were synthesized with final removal of the DMT group ("DMT-Off").

Upon completion of the solid phase synthesis, solid-supported oligoribonucleotides were treated with 300 µL of Methylamine (40% aqueous) at room temperature in 96 well plates for approximately 2 hours to afford cleavage from the solid support and subsequent removal of all additional base-labile protecting groups. For sequences containing any natural ribonucleotide linkages (2'-OH) protected with a tert-butyl dimethyl silyl (TBDMS) group, a second deprotection step was performed using TEA.3HF (triethylamine trihydrofluoride). To each oligonucleotide solution in aqueous methylamine was added 200 µL of dimethyl sulfoxide (DMSO) and 300 µL TEA.3HF and the solution was incubated for approximately 30 mins at 60 °C. After incubation, the plate was allowed to come to room temperature and crude oligonucleotides were precipitated by the addition of 1 mL of 9:1 acetontrile:ethanol or 1:1 ethanol:isopropanol. The plates were then centrifuged at 4 °C for 45 mins and the supernatant carefully decanted with the aid of a multichannel pipette. The oligonucleotide pellet was resuspended in 20 mM NaOAc and subsequently desalted using a HiTrap size exclusion column (5 mL, GE Healthcare) on an Agilent LC system equipped with an autosampler, UV detector, conductivity meter, and fraction collector. Desalted samples were collected in 96 well plates and then analyzed by LC-MS and UV spectrometry to confirm identity and quantify the amount of material, respectively.

Duplexing of single strands was performed on a Tecan liquid handling robot. Sense and antisense single strands were combined in an equimolar ratio to a final concentration of 10 µM in 1x PBS in 96 well plates, the plate sealed, incubated at 100 °C for 10 minutes, and subsequently allowed to return slowly to room temperature over a period of 2-3 hours. The concentration and identity of each duplex was confirmed and then subsequently utilized for in vitro screening assays.

Example 2. *In vitro* screening methods

Cell culture and 384-well transfections

For transfections, Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 7.5 µl of Opti-MEM plus 0.1 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 2.5 µl of each siRNA duplex to an individual well in a 384-well plate. The mixture was then incubated at room temperature for 15 minutes. Forty µl of complete

growth media without antibiotic containing ~1.5 x10^4 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10 nM, 1 nM, and 0.1 nM final duplex concentration.

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

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

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

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

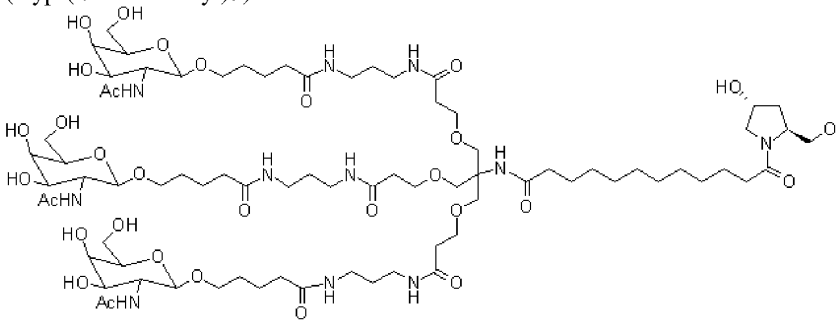
Real time PCR

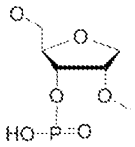
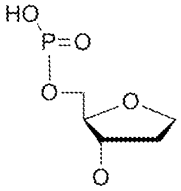
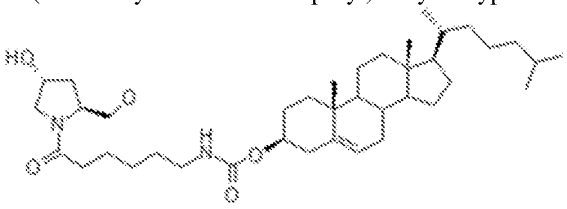
Two microlitre (µl) of cDNA were added to a master mix containing 0.5µl of human GAPDH TaqMan Probe (4326317E), 0.5µl human TMPRSS6, 2µl nuclease-free water and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche).

To calculate relative fold change, data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC_{50}s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or mock-transfected. The sense and antisense sequences of AD-1955 are: sense: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO: 18) and antisense UCGAAGuACUcAGCGuAAGdTsdT (SEQ ID NO: 19).

The results of the single dose screen of the agents in Tables 2, 3, 6 and 7 in Hep3b cells are shown in Table 8.

Table 1. 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; and it is understood that when the nucleotide contains a 2'-fluoro modification, then the fluoro replaces the hydroxy at that position in the parent nucleotide (*i.e.*, it is a 2'-deoxy-2'-fluoronucleotide).

Abbre*via*tion	Nucleotide(s)
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3`-phosphate
Abs	beta-L-adenosine-3`-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3`-phosphate
Cbs	beta-L-cytidine-3'-phosphorothioate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gb	beta-L-guanosine-3`-phosphate
Gbs	beta-L-guanosine-3`-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
Us	uridine -3'-phosphorothioate
N	any nucleotide, modified or unmodified
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'- phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'- phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'- phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage
L10	N-(cholesterylcarboxamidocaproyl)-4-hydroxyprolinol (Hyp-C6-Chol)
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol (Hyp-(GalNAc-alkyl)3) 

Abbre*via*tion	Nucleotide(s)
Y34	2-hydroxymethyl-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose) 
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofurane-5-phosphate) 
L10	N-(cholesterylcarboxamidocaproyl)-4-hydroxyprolinol (Hyp-C6-Chol) 
(Agn)	Adenosine-glycol nucleic acid (GNA) S-Isomer
(Cgn)	Cytidine-glycol nucleic acid (GNA) S-Isomer
(Ggn)	Guanosine-glycol nucleic acid (GNA) S-Isomer
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
P	Phosphate
VP	Vinyl-phosphonate
dA	2`-deoxyadenosine-3`-phosphate
dAs	2`-deoxyadenosine-3`-phosphorothioate
dC	2`-deoxycytidine-3`-phosphate
dCs	2`-deoxycytidine-3`-phosphorothioate
dG	2`-deoxyguanosine-3`-phosphate
dGs	2`-deoxyguanosine-3`-phosphorothioate
dT	2`-deoxythimidine -3`-phosphate
dTs	2`-deoxythimidine-3`-phosphorothioate
dU	2`-deoxyuridine
dUs	2`-deoxyuridine-3`-phosphorothioate
(C2p)	cytidine-2`-phosphate
(G2p)	guanosine-2`-phosphate
(U2p)	uridine-2`-phosphate
(A2p)	adenosine-2`-phosphate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Ahd)	2'-O-hexadecyl-adenosine-3'-phosphate
(Ghd)	2'-O-hexadecyl-guanosine-3'-phosphate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate

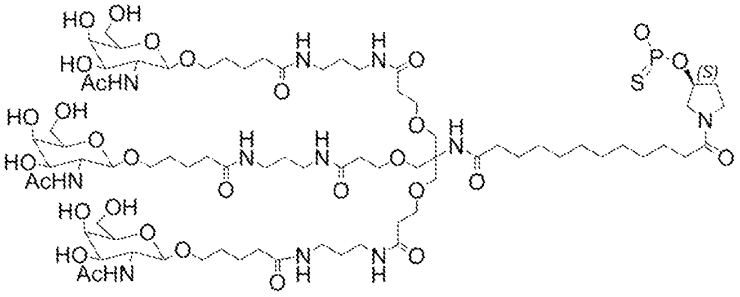
Abbre*via*tion	Nucleotide(s)
Q191s	N-[tris(GalNAc-alkyl)-amidododecanoyl]-(S)-pyrrolidin-3-ol-phosphorothioate (p-C12-(GalNAc-alkyl)3) 

Table 2. Unmodified Sense and Antisense Strand Sequences of TMPRSS6 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1554875	GCCUGUGAGGACUCCAAGAGU	20	232-252	ACUCUUGGAGUCCUCACAGGCCU	146	230-252
AD-1554909	GGUGCUACUCUGGUAUUUCCU	21	324-344	AGGAAATACCAGAGUAGCACCCC	147	322-344
AD-1554910	GUGCUACUCUGGUAUUUCCUU	22	325-345	AAGGAAAUACCAGAGUAGCACCC	148	323-345
AD-1554911	UGCUACUCUGGUAUUUCCUAU	23	326-346	ATAGGAAAUACCAGAGUAGCACC	149	324-346
AD-1554912	GCUACUCUGGUAUUUCCUAGU	24	327-347	ACUAGGAAAUACCAGAGUAGCAC	150	325-347
AD-1554913	CUACUCUGGUAUUUCCUAGGU	25	328-348	ACCUAGGAAAUACCAGAGUAGCA	151	326-348
AD-1554914	UACUCUGGUAUUUCCUAGGGU	26	329-349	ACCCTAGGAAATACCAGAGUAGC	152	327-349
AD-1554915	ACUCUGGUAUUUCCUAGGGUU	27	330-350	AACCCUAGGAAAUACCAGAGUAG	153	328-350
AD-1554916	CUCUGGUAUUUCCUAGGGUAU	28	331-351	ATACCCTAGGAAAUACCAGAGUA	154	329-351
AD-1554917	UCUGGUAUUUCCUAGGGUACU	29	332-352	AGUACCCUAGGAAAUACCAGAGU	155	330-352
AD-1554923	AUUUCCUAGGGUACAAGGCGU	30	338-358	ACGCCUTGUACCCUAGGAAAUAC	156	336-358
AD-1554951	GGUCAGCCAGGUGUACUCAGU	31	366-386	ACUGAGTACACCUGGCUGACCAU	157	364-386
AD-1554955	AGCCAGGUGUACUCAGGCAGU	32	370-390	ACUGCCTGAGUACACCUGGCUGA	158	368-390
AD-1554992	GCCACUUCUCCCAGGAUCUUU	33	407-427	AAAGAUCCUGGGAGAAGUGGGCGA	159	405-427
AD-1554997	UUCUCCCAGGAUCUUACCCGU	34	412-432	ACGGGUAAGAUCCUGGGAGAAGU	160	410-432
AD-1555000	UCCCAGGAUCUUACCCGCCGU	35	415-435	ACGGCGGGUAAGAUCCUGGGAGA	161	413-435
AD-1555030	GCCUUCCGCAGUGAAACCGCU	36	445-465	AGCGGUTUCACTGCGGAAGGCAC	162	443-465
AD-1555106	CAACUCCAGCUCCGUCUAUUU	37	522-542	AAAUAGACGGAGCUGGAGUUGUA	163	520-542
AD-1555112	CAGCUCCGUCUAUUCCUUUGU	38	528-548	ACAAAGGAAUAGACGGAGCUGGA	164	526-548
AD-1555114	CUCACCUGCUUCUUCUGGUUU	39	559-579	AAACCAGAAGAAGCAGGUGAGGG	165	557-579
AD-1555115	UCACCUGCUUCUUCUGGUUCU	40	560-580	AGAACCAGAAGAAGCAGGUGAGG	166	558-580
AD-1555117	ACCUGCUUCUUCUGGUUCAUU	41	562-582	AAUGAACCAGAAGAAGCAGGUGA	167	560-582
AD-1555118	CCUGCUUCUUCUGGUUCAUUU	42	563-583	AAAUGAACCAGAAGAAGCAGGUG	168	561-583
AD-1555120	UGCUUCUUCUGGUUCAUUCUU	43	565-585	AAGAAUGAACCAGAAGAAGCAGG	169	563-585
AD-1555121	GCUUCUUCUGGUUCAUUCUCU	44	566-586	AGAGAATGAACCAGAAGAAGCAG	170	564-586
AD-1555122	CUUCUUCUGGUUCAUUCUCCU	45	567-587	AGGAGAAUGAACCAGAAGAAGCA	171	565-587

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1555123	UUCUUCUGGUUCAUUCUCCAU	46	568-588	ATGGAGAAUGAACCAGAAGAAGC	172	566-588
AD-1555128	CUGGUUCAUUCUCCAAAUCCU	47	573-593	AGGATUTGGAGAAUGAACCAGAA	173	571-593
AD-1555184	ACAGGGCCGAGUACGAAGUGU	48	689-709	ACACTUCGUACTCGGCCCUGUAG	174	687-709
AD-1555185	CAGGGCCGAGUACGAAGUGGU	49	690-710	ACCACUTCGUACUCGGCCCUGUA	175	688-710
AD-1555212	CCAGUGUGAAAGACAUAGCUU	50	737-757	AAGCTATGUCUTUCACACUGGCU	176	735-757
AD-1555213	CAGUGUGAAAGACAUAGCUGU	51	738-758	ACAGCUAUGUCTUUCACACUGGC	177	736-758
AD-1555234	AUUGAAUUCCACGCUGGGUUU	52	759-779	AAACCCAGCGUGGAAUUCAAUGC	178	757-779
AD-1555235	UUGAAUUCCACGCUGGGUUGU	53	760-780	ACAACCCAGCGTGGAAUUCAAUG	179	758-780
AD-1555236	UGAAUUCCACGCUGGGUUGUU	54	761-781	AACAACCCAGCGUGGAAUUCAAU	180	759-781
AD-1555238	AAUUCCACGCUGGGUUGUUAU	55	763-783	ATAACAACCCAGCGUGGAAUUCA	181	761-783
AD-1555241	UCCACGCUGGGUUGUUACCGU	56	766-786	ACGGTAACAACCCAGCGUGGAAU	182	764-786
AD-1555242	CCACGCUGGGUUGUUACCGCU	57	767-787	AGCGGUAACAACCCAGCGUGGAA	183	765-787
AD-1555243	CACGCUGGGUUGUUACCGCUU	58	768-788	AAGCGGTAACAACCCAGCGUGGA	184	766-788
AD-1555247	CUGGGUUGUUACCGCUACAGU	59	772-792	ACUGTAGCGGUAACAACCCAGCG	185	770-792
AD-1555342	GGGACCGACUGGCCAUGUAUU	60	923-943	AAUACATGGCCAGUCGGUCCCGG	186	921-943
AD-1555343	GGACCGACUGGCCAUGUAUGU	61	924-944	ACAUACAUGGCCAGUCGGUCCCG	187	922-944
AD-1555345	ACCGACUGGCCAUGUAUGACU	62	926-946	AGUCAUACAUGGCCAGUCGGUCC	188	924-946
AD-1555346	CCGACUGGCCAUGUAUGACGU	63	927-947	ACGUCATACAUGGCCAGUCGGUC	189	925-947
AD-1555348	GACUGGCCAUGUAUGACGUGU	64	929-949	ACACGUCAUACAUGGCCAGUCGG	190	927-949
AD-1555349	ACUGGCCAUGUAUGACGUGGU	65	930-950	ACCACGTCAUACAUGGCCAGUCG	191	928-950
AD-1555350	CUGGCCAUGUAUGACGUGGCU	66	931-951	AGCCACGUCAUACAUGGCCAGUC	192	929-951
AD-1555366	AGGCUCAUCACCUCGGUGUAU	67	967-987	ATACACCGAGGTGAUGAGCCUCU	193	965-987
AD-1555428	GCCUGCACAGCUACUACGACU	68	1061-1081	AGUCGUAGUAGCUGUGCAGGCCC	194	1059-1081
AD-1555429	CCUGCACAGCUACUACGACCU	69	1062-1082	AGGUCGTAGUAGCUGUGCAGGCC	195	1060-1082
AD-1555535	CCUCUCUGGACUACGGCUUGU	70	1235-1255	ACAAGCCGUAGTCCAGAGAGGGC	196	1233-1255
AD-1555537	UCUCUGGACUACGGCUUGGCU	71	1237-1257	AGCCAAGCCGUAGUCCAGAGAGG	197	1235-1257
AD-1555546	UACGGCUUGGCCCUCUGGUUU	72	1246-1266	AAACCAGAGGGCCAAGCCGUAGU	198	1244-1266

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1555547	ACGGCUUGGCCCUCUGGUUUU	73	1247-1267	AAAACCAGAGGGCCAAGCCGUAG	199	1245-1267
AD-1555548	CGGCUUGGCCCUCUGGUUUGU	74	1248-1268	ACAAACCAGAGGGCCAAGCCGUA	200	1246-1268
AD-1555549	GGCUUGGCCCUCUGGUUUGAU	75	1249-1269	ATCAAACCAGAGGGCCAAGCCGU	201	1247-1269
AD-1555581	GAGGAGGCAGAAGUAUGAUUU	76	1281-1301	AAAUCATACUUCUGCCUCCUCAG	202	1279-1301
AD-1555583	GGAGGCAGAAGUAUGAUUUGU	77	1283-1303	ACAAAUCAUACTUCUGCCUCCUC	203	1281-1303
AD-1555584	GAGGCAGAAGUAUGAUUUGCU	78	1284-1304	AGCAAATCAUACUUCUGCCUCCU	204	1282-1304
AD-1555585	AGGCAGAAGUAUGAUUUGCCU	79	1285-1305	AGGCAAAUCAUACUUCUGCCUCC	205	1283-1305
AD-1555586	GGCAGAAGUAUGAUUUGCCGU	80	1286-1306	ACGGCAAAUCATACUUCUGCCUC	206	1284-1306
AD-1555587	GCAGAAGUAUGAUUUGCCGUU	81	1287-1307	AACGGCAAAUCAUACUUCUGCCU	207	1285-1307
AD-1555588	CAGAAGUAUGAUUUGCCGUGU	82	1288-1308	ACACGGCAAAUCAUACUUCUGCC	208	1286-1308
AD-1555589	AGAAGUAUGAUUUGCCGUGCU	83	1289-1309	AGCACGGCAAATCAUACUUCUGC	209	1287-1309
AD-1555590	GAAGUAUGAUUUGCCGUGCAU	84	1290-1310	ATGCACGGCAAAUCAUACUUCUG	210	1288-1310
AD-1555615	CAGUGGACGAUCCAGAACAGU	85	1318-1338	ACUGTUCUGGATCGUCCACUGGC	211	1316-1338
AD-1555616	AGUGGACGAUCCAGAACAGGU	86	1319-1339	ACCUGUTCUGGAUCGUCCACUGG	212	1317-1339
AD-1555626	CCAGAACAGGAGGCUGUGUGU	87	1329-1349	ACACACAGCCUCCUGUUCUGGAU	213	1327-1349
AD-1555628	AGAACAGGAGGCUGUGUGGCU	88	1331-1351	AGCCACACAGCCUCCUGUUCUGG	214	1329-1351
AD-1555706	UGUGCGGGUGCACUAUGGCUU	89	1449-1469	AAGCCATAGUGCACCCGCACACC	215	1447-1469
AD-1555707	GUGCGGGUGCACUAUGGCUUU	90	1450-1470	AAAGCCAUAGUGCACCCGCACAC	216	1448-1470
AD-1555709	GCGGGUGCACUAUGGCUUGUU	91	1452-1472	AACAAGCCAUAGUGCACCCGCAC	217	1450-1472
AD-1555711	GGGUGCACUAUGGCUUGUACU	92	1454-1474	AGUACAAGCCATAGUGCACCCGC	218	1452-1474
AD-1555717	ACUAUGGCUUGUACAACCAGU	93	1460-1480	ACUGGUTGUACAAGCCAUAGUGC	219	1458-1480
AD-1555723	GCUUGUACAACCAGUCGGACU	94	1466-1486	AGUCCGACUGGTUGUACAAGCCA	220	1464-1486
AD-1555725	CUGCCCUGGAGAGUUCCUCUU	95	1488-1508	AAGAGGAACUCTCCAGGGCAGGG	221	1486-1508
AD-1555768	GCCUGGAUGAGAGAAACUGCU	96	1565-1585	AGCAGUTUCUCTCAUCCAGGCCG	222	1563-1585
AD-1555771	UGGAUGAGAGAAACUGCGUUU	97	1568-1588	AAACGCAGUUUCUCUCAUCCAGG	223	1566-1588
AD-1555772	GGAUGAGAGAAACUGCGUUUU	98	1569-1589	AAAACGCAGUUTCUCUCAUCCAG	224	1567-1589
AD-1555776	GAGAGAAACUGCGUUUGCAGU	99	1573-1593	ACUGCAAACGCAGUUUCUCUCAU	225	1571-1593

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1555789	UUUGCAGAGCCACACAUUCCAGU	100	1586-1606	ACUGGAAUGUGGCUCUGCAAACG	226	1584-1606
AD-1555894	GUGGGACAUUCACCUUCCAGU	101	1709-1729	ACUGGAAGGUGAAUGUCCCACAU	227	1707-1729
AD-1555895	UGGGACAUUCACCUUCCAGUU	102	1710-1730	AACUGGAAGGUGAAUGUCCCACA	228	1708-1730
AD-1555897	GGACAUUCACCUUCCAGUGUU	103	1712-1732	AACACUGGAAGGUGAAUGUCCCA	229	1710-1732
AD-1555898	GACAUUCACCUUCCAGUGUGU	104	1713-1733	ACACACUGGAAGGUGAAUGUCCC	230	1711-1733
AD-1555899	ACAUUCACCUUCCAGUGUGAU	105	1714-1734	ATCACACUGGAAGGUGAAUGUCC	231	1712-1734
AD-1555900	CAUUCACCUUCCAGUGUGAGU	106	1715-1735	ACUCACACUGGAAGGUGAAUGUC	232	1713-1735
AD-1556052	AUCGCUGACCGCUGGGUGAUU	107	1936-1956	AAUCACCCAGCGGUCAGCGAUGA	233	1934-1956
AD-1556057	UGACCGCUGGGUGAUAACAGU	108	1941-1961	ACUGUUAUCACCCAGCGGUCAGC	234	1939-1961
AD-1556126	CGUGUUCCUGGGCAAGGUGUU	109	2010-2030	AACACCUGCCCAGGAACACGGU	235	2008-2030
AD-1556127	GUGUUCCUGGGCAAGGUGUGU	110	2011-2031	ACACACCUUGCCCAGGAACACGG	236	2009-2031
AD-1556137	GCAAGGUGUGGCAGAACUCGU	111	2021-2041	ACGAGUUCUGCCACACCUUGCCC	237	2019-2041
AD-1556139	AAGGUGUGGCAGAACUCGCGU	112	2023-2043	ACGCGAGUUCUGCCACACCUUGC	238	2021-2043
AD-1556163	CUGGAGAGGUGUCCUUCAAGU	113	2048-2068	ACUUGAAGGACACCUCUCCAGGC	239	2046-2068
AD-1556164	UGGAGAGGUGUCCUUCAAGGU	114	2049-2069	ACCUUGAAGGACACCUCUCCAGG	240	2047-2069
AD-1556166	GAGAGGUGUCCUUCAAGGUGU	115	2051-2071	ACACCUUGAAGGACACCUCUCCA	241	2049-2071
AD-1556167	AGAGGUGUCCUUCAAGGUGAU	116	2052-2072	ATCACCUUGAAGGACACCUCUCC	242	2050-2072
AD-1556319	AUCCCACAGGACCUGUGCAGU	117	2299-2319	ACUGCACAGGUCCUGUGGGAUCA	243	2297-2319
AD-1556359	UGACGCCACGCAUGCUGUGUU	118	2339-2359	AACACAGCAUGCGUGGCGUCACC	244	2337-2359
AD-1556360	GACGCCACGCAUGCUGUGUGU	119	2340-2360	ACACACAGCAUGCGUGGCGUCAC	245	2338-2360
AD-1556382	GCUACCGCAAGGGCAAGAAGU	120	2363-2383	ACUUCUUGCCCUUGCGGUAGCCG	246	2361-2383
AD-1556383	CUACCGCAAGGGCAAGAAGGU	121	2364-2384	ACCUUCUUGCCCUUGCGGUAGCC	247	2362-2384
AD-1556465	GGCCUAACUACUUCGGCGUCU	122	2483-2503	AGACGCCGAAGUAGUUAGGCCGG	248	2481-2503
AD-1556466	GCCUAACUACUUCGGCGUCUU	123	2484-2504	AAGACGCCGAAGUAGUUAGGCCG	249	2482-2504
AD-1556484	CUACACCCGCAUCACAGGUGU	124	2502-2522	ACACCUGUGAUGCGGGUGUAGAC	250	2500-2522
AD-1556510	GCUGGAUCCAGCAAGUGGUGU	125	2528-2548	ACACCACUUGCUGGAUCCAGCUG	251	2526-2548
AD-1556584	UGGCAGGAGGUGGCAUCUUGU	126	2670-2690	ACAAGATGCCACCUCCUGCCACC	252	2668-2690

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1556585	GGCAGGAGGUGGCAUCUUGUU	127	2671-2691	AACAAGAUGCCACCUCCUGCCAC	253	2669-2691
AD-1556586	GCAGGAGGUGGCAUCUUGUCU	128	2672-2692	AGACAAGAUGCCACCUCCUGCCA	254	2670-2692
AD-1556587	CAGGAGGUGGCAUCUUGUCUU	129	2673-2693	AAGACAAGAUGCCACCUCCUGCC	255	2671-2693
AD-1556613	UGAUGUCUGCUCCAGUGAUGU	130	2699-2719	ACAUCACUGGAGCAGACAUCAGG	256	2697-2719
AD-1556677	CAAUUCUCUCUCCUCCGUCCU	131	2801-2821	AGGACGGAGGAGAGAGAAUUGGG	257	2799-2821
AD-1556709	GGCUCAGCAGCAAGAAUGCUU	132	2853-2873	AAGCAUUCUUGCUGCUGAGCCAC	258	2851-2873
AD-1556710	GCUCAGCAGCAAGAAUGCUGU	133	2854-2874	ACAGCAUUCUUGCUGCUGAGCCA	259	2852-2874
AD-1556789	CUGGUCUAACUUGGGAUCUGU	134	2973-2993	ACAGAUCCCAAGUUAGACCAGGG	260	2971-2993
AD-1556790	UGGUCUAACUUGGGAUCUGGU	135	2974-2994	ACCAGATCCCAAGUUAGACCAGG	261	2972-2994
AD-1556791	GGUCUAACUUGGGAUCUGGGU	136	2975-2995	ACCCAGAUCCCAAGUUAGACCAG	262	2973-2995
AD-1556795	UAACUUGGGAUCUGGGAAUGU	137	2979-2999	ACAUTCCCAGATCCCAAGUUAGA	263	2977-2999
AD-1556799	UUGGGAUCUGGGAAUGGAAGU	138	2983-3003	ACUUCCAUUCCCAGAUCCCAAGU	264	2981-3003
AD-1556802	GGAUCUGGGAAUGGAAGGUGU	139	2986-3006	ACACCUTCCAUTCCCAGAUCCCA	265	2984-3006
AD-1556908	UGAGCUCAGCUGCCCUUUGGU	140	3158-3178	ACCAAAGGGCAGCUGAGCUCACC	266	3156-3178
AD-1556909	GAGCUCAGCUGCCCUUUGGAU	141	3159-3179	ATCCAAAGGGCAGCUGAGCUCAC	267	3157-3179
AD-1556911	GCUCAGCUGCCCUUUGGAAUU	142	3161-3181	AAUUCCAAAGGCAGCUGAGCUC	268	3159-3181
AD-1556915	AGCUGCCCUUUGGAAUAAAGU	143	3165-3185	ACUUTATUCCAAAGGGCAGCUGA	269	3163-3185
AD-1556917	CUGCCCUUUGGAAUAAAGCUU	144	3167-3187	AAGCTUTAUUCCAAAGGGCAGCU	270	3165-3187
AD-1556918	UGCCCUUUGGAAUAAAGCUGU	145	3168-3188	ACAGCUTUAUUCCAAAGGGCAGC	271	3166-3188

Table 3. Modified Sense and Antisense Strand Sequences of TMPRSS6 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO.	Antisense Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1554875	gscscugugaGfGfAfcuccaagaguL96	272	asdCsucdTudGgagudCcUfcacaggcscsu	398	AGGCCUGUGAGGACUCCAAGAGA	524
AD-1554909	gsgsugcuacUfCfUfgguauuuccuL96	273	asdGsgadAadTaccadGaGfuagcaccscsc	399	GGGGUGCUACUCUGGUAUUUCCU	525
AD-1554910	gsusgcuacuCfUfGfguauuuccuuL96	274	asdAsggdAadAuaccdAgAfguagcacscsc	400	GGGUGCUACUCUGGUAUUUCCUA	526
AD-1554911	usgscuacucUfGfGfuauuuccuauL96	275	asdTsagdGadAauacdCaGfaguagcascsc	401	GGUGCUACUCUGGUAUUUCCUAG	527

AD-1554912	gscsuacucuGfGfUfauuuccuaguL96	276	asdCsuadGgdAaauadCcAfgaguagcsasc	402	GUGCUACUCUGGUAUUUCCUAGG	528
AD-1554913	csusacucugGfUfAfuuuccuagguL96	277	asdCscudAgdGaaaudAcCfagaguagscsa	403	UGCUACUCUGGUAUUUCCUAGGG	529
AD-1554914	usascucuggUfAfUfuuccuaggguL96	278	asdCsccdTadGgaaadTaCfcagaguasgsc	404	GCUACUCUGGUAUUUCCUAGGGU	530
AD-1554915	ascsucugguAfUfUfuccuaggguuL96	279	asdAsccdCudAggaadAuAfccagagusasg	405	CUACUCUGGUAUUUCCUAGGGUA	531
AD-1554916	csuscugguaUfUfUfccuaggguauL96	280	asdTsacdCcdTaggadAaUfaccagagsusa	406	UACUCUGGUAUUUCCUAGGGUAC	532
AD-1554917	uscsugguauUfUfCfcuaggguacuL96	281	asdGsuadCcdCuaggdAaAfuaccagasgsu	407	ACUCUGGUAUUUCCUAGGGUACA	533
AD-1554923	asusuuccuaGfGfGfuacaaggcguL96	282	asdCsgcdCudTguacdCcUfaggaaausasc	408	GUAUUUCCUAGGGUACAAGGCGG	534
AD-1554951	gsgsucagccAfGfGfuguacucaguL96	283	asdCsugdAgdTacacdCuGfgcugaccsasu	409	AUGGUCAGCCAGGUGUACUCAGG	535
AD-1554955	asgsccagguGfUfUfAfcucaggcaguL96	284	asdCsugdCcdTgagudAcAfccuggcusgsa	410	UCAGCCAGGUGUACUCAGGCAGU	536
AD-1554992	gscscacuucUfCfCfcaggaucuuuL96	285	asdAsagdAudCcuggdGaGfaaguggcsgsa	411	UCGCCACUUCUCCCAGGAUCUUA	537
AD-1554997	ususcucccaGfGfAfucuuaccguL96	286	asdCsggdGudAagaudCcUfggagaasgsu	412	ACUUCUCCCAGGAUCUUACCCGC	538
AD-1555000	uscsccaggaUfCfUfuacccgccguL96	287	asdCsggdCgdGguaadGaUfccugggasgsa	413	UCUCCCAGGAUCUUACCCGCCGG	539
AD-1555030	gscscuuccgCfAfGfugaaaccgcuL96	288	asdGscgdGudTucacdTgCfggaaggcsasc	414	GUGCCUUCCGCAGUGAAACCGCC	540
AD-1555106	csasacuccaGfCfUfccgucuauuuL96	289	asdAsaudAgdAcggadGcUfggaguugsusa	415	UACAACUCCAGCUCCGUCUAUUC	541
AD-1555112	csasgcuccgUfCfUfauuccuuuguL96	290	asdCsaadAgdGaauadGaCfggagcugsgsa	416	UCCAGCUCCGUCUAUUCCUUUGG	542
AD-1555114	csuscaccugCfUfUfcuucugguuuL96	291	asdAsacdCadGaagadAgCfaggugagsgsg	417	CCCUCACCUGCUUCUUCUGGUUC	543
AD-1555115	uscsaccugcUfUfCfuucugguucuL96	292	asdGsaadCcdAgaagdAaGfcaggugasgsg	418	CCUCACCUGCUUCUUCUGGUUCA	544
AD-1555117	ascscugcuuCfUfUfcugguucauuL96	293	asdAsugdAadCcagadAgAfagcaggusgsa	419	UCACCUGCUUCUUCUGGUUCAUU	545
AD-1555118	cscsugcuucUfUfCfugguucauuuL96	294	asdAsaudGadAccagdAaGfaagcaggsusg	420	CACCUGCUUCUUCUGGUUCAUUC	546
AD-1555120	usgscuucuuCfUfGfguucauucuuL96	295	asdAsgadAudGaaccdAgAfagaagcasgsg	421	CCUGCUUCUUCUGGUUCAUUCUC	547
AD-1555121	gscsuucuucUfGfGfuucauucucuL96	296	asdGsagdAadTgaacdCaGfaagaagcsasg	422	CUGCUUCUUCUGGUUCAUUCUCC	548
AD-1555122	csusucuucuGfGfUfucauucucccuL96	297	asdGsgadGadAugaadCcAfgaagaagscsa	423	UGCUUCUUCUGGUUCAUUCUCCA	549
AD-1555123	ususcuucugGfUfUfcauucuccauL96	298	asdTsggdAgdAaugadAcCfagaagaasgsc	424	GCUUCUUCUGGUUCAUUCUCCAA	550
AD-1555128	csusgguucaUfUfCfuccaaaucccuL96	299	asdGsgadTudTggagdAaUfgaaccagsasa	425	UUCUGGUUCAUUCUCCCAAAUCCC	551
AD-1555184	ascsagggccGfAfGfuacgaaguguL96	300	asdCsacdTudCguacdTcGfgcccugusasg	426	CUACAGGGCCGAGUACGAAGUGG	552
AD-1555185	csasgggccgAfGfUfacgaaguggguL96	301	asdCscadCudTcguadCuCfggcccugsusa	427	UACAGGGCCGAGUACGAAGUGGA	553
AD-1555212	cscsagugugAfAfAfgacauagcuuL96	302	asdAsgcdTadTgucudTuCfacacuggscsu	428	AGCCAGUGUGAAAGACAUAGCUG	554

AD-1555213	csasguguga AfAfGfacuuagcuguL96	303	asdCsagdCudAugucdTuUfcacacugsgsc	429	GCCAGUGUGAAAGACAUAGCUGC	555
AD-1555234	asusugaauuCfCfAfcgcuggguuuL96	304	asdAsacdCcdAgcgudGgAfauucaausgsc	430	GCAUUGAAUUCCACGCUGGGUUG	556
AD-1555235	ususgaauucCfAfCfgcuggguuguL96	305	asdCsaadCcdCagcgdTgGfaauucaasusg	431	CAUUGAAUUCCACGCUGGGUUGU	557
AD-1555236	usgsaauuccAfCfGfcuggguuguL96	306	asdAscadAcdCcagcdGuGfgaauucasasu	432	AUUGAAUUCCACGCUGGGUUGUU	558
AD-1555238	asasuuccacGfCfUfggguuguuauL96	307	asdTsaadCadAcccadGcGfuggaauuscsa	433	UGAAUUCCACGCUGGGUUGUUAC	559
AD-1555241	uscscacgcuGfGfGfuugguuaccguL96	308	asdCsggdTadAcaacdCcAfgcuggasasu	434	AUUCCACGCUGGGUUGUUACCGC	560
AD-1555242	cscsacgcugGfGfUfuguuaccgcuL96	309	asdGscgdGudAacaadCcCfagcuggsasa	435	UUCCACGCUGGGUUGUUACCGCU	561
AD-1555243	csascgcuggGfUfUfguuaccgcuuL96	310	asdAsgcdGgdTaacadAcCfcagcgugsgsa	436	UCCACGCUGGGUUGUUACCGCUA	562
AD-1555247	csusggguugUfUfAfccgcuacaguL96	311	asdCsugdTadGcggudAaCfaacccagscsg	437	CGCUGGGUUGUUACCGCUACAGC	563
AD-1555342	gsgsgaccgaCfUfGfgccauguauuL96	312	asdAsuadCadTggccdAgUfcggucccsgsg	438	CCGGGACCGACUGGCCAUGUAUG	564
AD-1555343	gsgsaccgacUfGfGfccauguauguL96	313	asdCsaudAcdAuggcdCaGfucggucccsg	439	CGGGACCGACUGGCCAUGUAUGA	565
AD-1555345	ascscgacugGfCfCfauguaugacuL96	314	asdGsucdAudAcaugdGcCfagucgguscsc	440	GGACCGACUGGCCAUGUAUGACG	566
AD-1555346	cscsgacuggCfCfAfuguaugacguL96	315	asdCsgudCadTacaudGgCfcagucggsusc	441	GACCGACUGGCCAUGUAUGACGU	567
AD-1555348	gsascuggccAfUfGfuaugacguguL96	316	asdCsacdGudCauacdAuGfgccagucsgsg	442	CCGACUGGCCAUGUAUGACGUGG	568
AD-1555349	ascsuggccaUfGfUfaugacgugguL96	317	asdCscadCgdTcauadCaUfggccaguscsg	443	CGACUGGCCAUGUAUGACGUGGC	569
AD-1555350	csusggccauGfUfAfugacgugguL96	318	asdGsccdAcdGucaudAcAfuggccagsusc	444	GACUGGCCAUGUAUGACGUGGCC	570
AD-1555366	asgsgucauCfAfCfcucgguguauL96	319	asdTsacdAcdCgaggdTgAfugagccuscsu	445	AGAGGCUCAUCACCUCGGUGUAC	571
AD-1555428	gscscugcacAfGfCfuacuacgacuL96	320	asdGsucdGudAguagdCuGfugcaggcscsc	446	GGGCCUGCACAGCUACUACGACC	572
AD-1555429	cscsugcacaGfCfUfacuacgaccuL96	321	asdGsgudCgdTaguadGcUfgugcaggscsc	447	GGCCUGCACAGCUACUACGACCC	573
AD-1555535	cscsucucugGfAfCfuacggcuuguL96	322	asdCsaadGcdCguagdTcCfagagaggsgsc	448	GCCCUCUCUGGACUACGGCUUGG	574
AD-1555537	uscsucuggaCfUfAfcggcuuggcuL96	323	asdGsccdAadGccgudAgUfccagagasgsg	449	CCUCUCUGGACUACGGCUUGGCC	575
AD-1555546	usascggcuuGfGfCfCfccucugguuuL96	324	asdAsacdCadGaggdCcAfagccguasgsu	450	ACUACGGCUUGGCCCCUCUGGUUU	576
AD-1555547	ascsggcuugGfCfCfCfcucugguuuuL96	325	asdAsaadCcdAgaggdGcCfaagccgusasg	451	CUACGGCUUGGCCCCUCUGGUUUG	577
AD-1555548	csgsgcuuggCfCfCfCfucugguuuguL96	326	asdCsaadAcdCagagdGgCfcaagccgsusa	452	UACGGCUUGGCCCCUCUGGUUUGA	578
AD-1555549	gsgscuuggcCfCfUfCfuggguuugauL96	327	asdTscadAadCcagadGgGfccaagccsgsu	453	ACGGCUUGGCCCCUCUGGUUUGAU	579
AD-1555581	gsasggaggcAfGfAfaguaugauuuL96	328	asdAsaudCadTacuudCuGfccucccusasg	454	CUGAGGAGGCAGAAGUAUGAUUU	580
AD-1555583	gsgsaggcagAfAfGfuauguauuuguL96	329	asdCsaadAudCauacdTuCfugccucccsusc	455	GAGGAGGCAGAAGUAUGAUUUGC	581

AD-1555584	gsasggcagaAfGfUfaugauuugcuL96	330	asdGscadAadTcauadCuUfcugccucscsu	456	AGGAGGCAGAAGUAUGAUUUGCC	582
AD-1555585	asgsgcagaaGfUfAfugauuugccuL96	331	asdGsgcdAadAucaudAcUfucugccuscsc	457	GGAGGCAGAAGUAUGAUUUGCCG	583
AD-1555586	gsgscagaagUfAfUfgauuugccguL96	332	asdCsggdCadAaucadTaCfuucugccsusc	458	GAGGCAGAAGUAUGAUUUGCCGU	584
AD-1555587	gscsagaaguAfUfGfauuugccguuL96	333	asdAscgdGcdAaaucdAuAfcuucugcscsu	459	AGGCAGAAGUAUGAUUUGCCGUG	585
AD-1555588	csasgaaguaUfGfAfuuugccguguL96	334	asdCsacdGgdCaaaudCaUfacuucugscsc	460	GGCAGAAGUAUGAUUUGCCGUGC	586
AD-1555589	asgsaaguauGfAfUfuugccgugcuL96	335	asdGscadCgdGcaaadTcAfuacuucusgsc	461	GCAGAAGUAUGAUUUGCCGUGCA	587
AD-1555590	gsasaguaugAfUfUfugccgugcauL96	336	asdTsgcdAcdGgcaadAuCfauacuucsusg	462	CAGAAGUAUGAUUUGCCGUGCAC	588
AD-1555615	csasguggacGfAfUfccagaacaguL96	337	asdCsugdTudCuggadTcGfuccacugsgsc	463	GCCAGUGGACGAUCCAGAACAGG	589
AD-1555616	asgsuggacgAfUfCfcagaacagguL96	338	asdCscudGudTcuggdAuCfguccacusgsg	464	CCAGUGGACGAUCCAGAACAGGA	590
AD-1555626	cscsagaacaGfGfGfAfggcuguguguL96	339	asdCsacdAcdAgccudCcUfguucuggsasu	465	AUCCAGAACAGGAGGCUGUGUGG	591
AD-1555628	asgsaacaggAfGfGfCfugugugguL96	340	asdGsccdAcdAcagcdCuCfcuguucusgsg	466	CCAGAACAGGAGGCUGUGUGGCU	592
AD-1555706	usgsugcgggUfGfCfCfacuauggcuuL96	341	asdAsgcdCadTagugdCaCfccgcacascsc	467	GGUGUGCGGGUGCACUAUGGCUU	593
AD-1555707	gsusgcggguGfCfCfAfcuauggcuuuL96	342	asdAsagdCcdAuagudGcAfccgcacsasc	468	GUGUGCGGGUGCACUAUGGCUUG	594
AD-1555709	gscsgggugcAfCfUfauggcuuguuL96	343	asdAscadAgdCcauadGuGfcacccgcsasc	469	GUGCGGGUGCACUAUGGCUUGUA	595
AD-1555711	gsgsgugcacUfAfUfggcuuguacuL96	344	asdGsuadCadAgccadTaGfugcacccsgsc	470	GCGGGUGCACUAUGGCUUGUACA	596
AD-1555717	ascsuauggcUfUfGfuacaaccaguL96	345	asdCsugdGudTguacdAaGfccauagusgsc	471	GCACUAUGGCUUGUACAACCAGU	597
AD-1555723	gscsuuguacAfAfCfcagucggacuL96	346	asdGsucdCgdAcuggdTuGfuacaagcscsa	472	UGGCUUGUACAACCAGUCGGACC	598
AD-1555725	csusgcccugGfAfGfaguccucuuL96	347	asdAsgadGgdAacucdTcCfagggcagsgsg	473	CCCUGCCCUGGAGAGUUCCUCUG	599
AD-1555768	gscscuggauGfAfGfagaaacugcuL96	348	asdGscadGudTucucdTcAfuccaggcscsg	474	CGGCCUGGAUGAGAGAAACUGCG	600
AD-1555771	usgsgaugagAfGfAfaacugcguuuL96	349	asdAsacdGcdAguuudCuCfucauccasgsg	475	CCUGGAUGAGAGAAACUGCGUUU	601
AD-1555772	gsgsaugagaGfAfAfacugcguuuuL96	350	asdAsaadCgdCaguudTcUfcucauccsasg	476	CUGGAUGAGAGAAACUGCGUUUG	602
AD-1555776	gsasgagaaaCfUfGfcguuugcaguL96	351	asdCsugdCadAacgcdAgUfuucucucsasu	477	AUGAGAGAAACUGCGUUUGCAGA	603
AD-1555789	ususugcagaGfCfCfacauuccaguL96	352	asdCsugdGadAugugdGcUfcugcaaascsg	478	CGUUUGCAGAGCCACAUUCCAGU	604
AD-1555894	gsusgggacaUfUfCfaccuuccaguL96	353	asdCsugdGadAggugdAaUfgucccacsasu	479	AUGUGGGACAUUCACCUUCCAGU	605
AD-1555895	usgsggacauUfCfAfccuuccaguuL96	354	asdAscudGgdAaggudGaAfugucccascsa	480	UGUGGGACAUUCACCUUCCAGUG	606
AD-1555897	gsgsacauucAfCfCfuuccaguguuL96	355	asdAscadCudGgaagdGuGfaauguccscsa	481	UGGGACAUUCACCUUCCAGUGUG	607
AD-1555898	gsascauucaCfCfUfuccaguguguL96	356	asdCsacdAcdTggaadGgUfgaaugucscsc	482	GGGACAUUCACCUUCCAGUGUGA	608

AD-1555899	ascsauuucacCfUfUfccagugugauL96	357	asdTscadCadCuggadAgGfugaauguscsc	483	GGACAUUCACCUUCCAGUGUGAG	609
AD-1555900	csasuucaccUfUfCfcagugugaguL96	358	asdCsucdAcdAcugguAaGfgugaaugsusc	484	GACAUUCACCUUCCAGUGUGAGG	610
AD-1556052	asuscgcugaCfCfGfcuggguguuuL96	359	asdAsucdAcdCcagcdGgUfcagcgausgsa	485	UCAUCGCUGACCGCUGGGUGAUA	611
AD-1556057	usgsaccgcuGfGfGfugauaacaguL96	360	asdCsugdTudAucacdCcAfgcggucasgsc	486	GCUGACCGCUGGGUGAUAACAGC	612
AD-1556126	csgsuguuccUfGfGfgcaagguguuuL96	361	asdAscadCcdTugccdCaGfgaacacgsgsu	487	ACCGUGUUCCUGGGCAAGGUGUG	613
AD-1556127	gsusguuccuGfGfGfcaaggugugguL96	362	asdCsacdAcdCuugcdCcAfggaacacsgsg	488	CCGUGUUCCUGGGCAAGGUGUGG	614
AD-1556137	gscsaaggugUfGfGfcagaacucguL96	363	asdCsgadGudTcugcdCaCfaccuugcscsc	489	GGGCAAGGUGUGGCAGAACUCGC	615
AD-1556139	asasggugugGfCfAfgaacucgcguL96	364	asdCsgcdGadGuucudGcCfacaccuusgsc	490	GCAAGGUGUGGCAGAACUCGCGC	616
AD-1556163	csusggagagGfUfGfuccuucaaguL96	365	asdCsuudGadAggacdAcCfucuccagsgsc	491	GCCUGGAGAGGUGUCCUUCAAGG	617
AD-1556164	usgsgagaggUfGfUfccuucaagguL96	366	asdCscudTgdAaggadCaCfcucuccasgsg	492	CCUGGAGAGGUGUCCUUCAAGGU	618
AD-1556166	gsasgaggugUfCfCfuucaaggugcuL96	367	asdCsacdCudTgaagdGaCfaccucucscsa	493	UGGAGAGGUGUCCUUCAAGGUGA	619
AD-1556167	asgsagguguCfCfUfucaaggugauL96	368	asdTscadCcdTugaadGgAfcaccucuscsc	494	GGAGAGGUGUCCUUCAAGGUGAG	620
AD-1556319	asuscccacaGfGfAfccugugcaguL96	369	asdCsugdCadCaggudCcUfgugggauuscsa	495	UGAUCCCACAGGACCUGUGCAGC	621
AD-1556359	usgsacgccaCfGfCfaugcugguuuL96	370	asdAscadCadGcaugdCgUfggcgucascsc	496	GGUGACGCCACGCAUGCUGUGUG	622
AD-1556360	gsascgccacGfCfAfugcugugugcuL96	371	asdCsacdAcdAgcaudGcGfuggcgucsasc	497	GUGACGCCACGCAUGCUGUGUGC	623
AD-1556382	gscsuaccgcAfAfGfggcaagaaguL96	372	asdCsuudCudTgcccdTuGfcgguagcscsg	498	CGGCUACCGCAAGGGCAAGAAGG	624
AD-1556383	csusaccgcaAfGfGfgcaagaaggguL96	373	asdCscudTcdTugccdCuUfgcgguagscsc	499	GGCUACCGCAAGGGCAAGAAGGA	625
AD-1556465	gsgsccuaacUfAfCfuucggcgucuuL96	374	asdGsacdGcdCgaagdTaGfuuaggccsgsg	500	CCGGCCUAACUACUUCGGCGUCU	626
AD-1556466	gscscuaacuAfCfUfucggcgucuuuL96	375	asdAsgadCgdCcgaadGuAfguuaggccscsg	501	CGGCCUAACUACUUCGGCGUCUA	627
AD-1556484	csusacacccGfCfAfucacagggugL96	376	asdCsacdCudGugaudGcGfgguguagsasc	502	GUCUACACCCGCAUCACAGGGUGU	628
AD-1556510	gscsuggaucCfAfGfcaaguggguguL96	377	asdCsacdCadCuugcdTgGfauccagcsusg	503	CAGCUGGAUCCAGCAAGUGGGUGA	629
AD-1556584	usgsgcaggaGfGfUfggcaucuuguL96	378	asdCsaadGadTgccadCcUfccugccascsc	504	GGUGGCAGGAGGUGGCAUCUUGU	630
AD-1556585	gsgscaggagGfUfGfgcaucuuguuL96	379	asdAscadAgdAugccdAcCfuccugccsasc	505	GUGGCAGGAGGUGGCAUCUUGUC	631
AD-1556586	gscsaggaggUfGfGfcaucuugucuL96	380	asdGsacdAadGaugcdCaCfcuccugcscsa	506	UGGCAGGAGGUGGCAUCUUGUCU	632
AD-1556587	csasggagguGfGfCfaucuugucuuL96	381	asdAsgadCadAgaugdCcAfccuccugscsc	507	GGCAGGAGGUGGCAUCUUGUCUC	633
AD-1556613	usgsaugucuGfCfUfccagugauguL96	382	asdCsaudCadCuggadGcAfgacaucasgsg	508	CCUGAUGUCUGCUCCCAGUGAUGG	634
AD-1556677	csasauucucUfCfUfccuccguccuL96	383	asdGsgadCgdGaggadGaGfagaauugsgsg	509	CCCAAUUCUCUCCUCCCGUCCC	635

AD-1556709	gsgscucagcAfGfCfaagaaugcuuL96	384	asdAsgcdAudTcuugdCuGfcugagccsasc	510	GUGGCUCAGCAGCAAGAAUGCUG	636
AD-1556710	gscsucagcaGfCfAfagaaugcuguL96	385	asdCsagdCadTucuudGcUfgcugagcscsa	511	UGGCUCAGCAGCAAGAAUGCUGG	637
AD-1556789	csusggucuaAfCfUfugggaucuguL96	386	asdCsagdAudCccaadGuUfagaccagsgsg	512	CCCUGGUCUAACUUGGGAUCUGG	638
AD-1556790	usgsgucuaaCfUfUfgggaucugguL96	387	asdCscadGadTcccadAgUfuagaccasgsg	513	CCUGGUCUAACUUGGGAUCUGGG	639
AD-1556791	gsgsucuaacUfUfGfggaucuggguL96	388	asdCsccdAgdAucccdAaGfuuagaccsasg	514	CUGGUCUAACUUGGGAUCUGGGA	640
AD-1556795	usasacuuggGfAfUfcugggaauguL96	389	asdCsaudTcdCcagadTcCfcaaguuasgsa	515	UCUAACUUGGGAUCUGGGAAUGG	641
AD-1556799	ususgggaucUfGfGfgaauggaaguL96	390	asdCsuudCcdAuuccdCaGfaucccaasgsu	516	ACUUGGGAUCUGGGAAUGGAAGG	642
AD-1556802	gsgsaucuggGfAfAfuggaagguguL96	391	asdCsacdCudTccaudTcCfcagauccscsa	517	UGGGAUCUGGGAAUGGAAGGUGC	643
AD-1556908	usgsagcucaGfCfUfgcccuuugguL96	392	asdCscadAadGggcadGcUfgagcucascsc	518	GGUGAGCUCAGCUGCCCUUUGGA	644
AD-1556909	gsasgcucagCfUfGfcccuuuggauL96	393	asdTsccdAadAgggcdAgCfugagcucsasc	519	GUGAGCUCAGCUGCCCUUUGGAA	645
AD-1556911	gscsucagcuGfCfCfcuuuggaauuL96	394	asdAsuudCcdAaaggdGcAfgcugagcsusc	520	GAGCUCAGCUGCCCUUUGGAAUA	646
AD-1556915	asgscugcccUfUfUfggaauaaaguL96	395	asdCsuudTadTuccadAaGfggcagcusgsa	521	UCAGCUGCCCUUUGGAAUAAAGC	647
AD-1556917	csusgcccuuUfGfGfaauaaagcuuL96	396	asdAsgcdTudTauucdCaAfagggcagscsu	522	AGCUGCCCUUUGGAAUAAAGCUG	648
AD-1556918	usgscccuuuGfGfAfauaaagcuguL96	397	asdCsagdCudTuauudCcAfaagggcasgsc	523	GCUGCCCUUUGGAAUAAAGCUGC	649

Table 4. Unmodified Sense and Antisense Strand Sequences of TMPRSS6 dsRNA Agent

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1557376	CGGAGGUGAUGGCGAGGAAGU	650	189-209	ACUUCCTCGCCAUCACCUCCGUC	848	187-209
AD-1557377	GGAGGUGATGGCGAGGAAGCU	651	190-210	AGCUUCCUCGCCATCACCUCCGU	849	188-210
AD-1557396	AAGGCCUGTGAGGACUCCAAU	652	229-249	ATUGGAGUCCUCACAGGCCUUGA	850	227-249
AD-1557398	GGCCUGUGAGGACUCCAAGAU	653	231-251	ATCUUGGAGUCCUCACAGGCCUU	851	229-251
AD-1557399	GCCUGUGAGGACUCCAAGAGU	20	232-252	ACUCUUGGAGUCCTCACAGGCCU	852	230-252
AD-1557400	CCUGUGAGGACUCCAAGAGAU	654	233-253	ATCUCUTGGAGTCCUCACAGGCC	853	231-253
AD-1557401	CUGUGAGGACTCCAAGAGAAU	655	234-254	ATUCUCTUGGAGUCCUCACAGGC	854	232-254
AD-1557437	CUACUCUGGUAUUUCCUAGGU	25	328-348	ACCUAGGAAAUACCAGAGUAGCA	151	326-348
AD-1557440	CUCUGGUATUTCCUAGGGUAU	656	331-351	ATACCCTAGGAAATACCAGAGUA	855	329-351
AD-1557441	UCUGGUAUTUCCUAGGGUACU	657	332-352	AGUACCCUAGGAAAUACCAGAGU	155	330-352
AD-1557442	CUGGUAUUTCCUAGGGUACAU	658	333-353	ATGUACCCUAGGAAAUACCAGAG	856	331-353

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1557443	UGGUAUUUCCTAGGGUACAAU	659	334-354	ATUGUACCCUAGGAAAUACCAGA	857	332-354
AD-1557444	GGUAUUUCCUAGGGUACAAGU	660	335-355	ACUUGUACCCUAGGAAAUACCAG	858	333-355
AD-1557445	GUAUUUCCTAGGGUACAAGGU	661	336-356	ACCUUGTACCCTAGGAAAUACCA	859	334-356
AD-1557452	CUAGGGUACAAGGCGGAGGUU	662	343-363	AACCUCCGCCUTGTACCCUAGGA	860	341-363
AD-1557473	AUGGUCAGCCAGGUGUACUCU	663	364-384	AGAGUACACCUGGCUGACCAUCA	861	362-384
AD-1557475	GGUCAGCCAGGUGUACUCAGU	31	366-386	ACUGAGTACACCUGGCUGACCAU	157	364-386
AD-1557476	GUCAGCCAGGTGUACUCAGGU	664	367-387	ACCUGAGUACACCTGGCUGACCA	862	365-387
AD-1557477	UCAGCCAGGUGUACUCAGGCU	665	368-388	AGCCUGAGUACACCUGGCUGACC	863	366-388
AD-1557478	CAGCCAGGTGTACUCAGGCAU	666	369-389	ATGCCUGAGUACACCUGGCUGAC	864	367-389
AD-1557479	AGCCAGGUGUACUCAGGCAGU	32	370-390	ACUGCCTGAGUACACCUGGCUGA	158	368-390
AD-1557509	CUCAAUCGCCACUUCUCCCAU	667	400-420	ATGGGAGAAGUGGCGAUUGAGUA	865	398-420
AD-1557515	CGCCACUUCUCCCAGGAUCUU	668	406-426	AAGAUCCUGGGAGAAGUGGCGAU	866	404-426
AD-1557516	GCCACUUCTCCCAGGAUCUUU	669	407-427	AAAGAUCCUGGGAGAAGUGGCGA	159	405-427
AD-1557518	CACUUCUCCCAGGAUCUUACU	670	409-429	AGUAAGAUCCUGGGAGAAGUGGC	867	407-429
AD-1557522	UCUCCCAGGATCUUACCCGCU	671	413-433	AGCGGGTAAGATCCUGGGAGAAG	868	411-433
AD-1557523	CUCCCAGGAUCUUACCCGCCU	672	414-434	AGGCGGGUAAGAUCCUGGGAGAA	869	412-434
AD-1557524	UCCCAGGATCTUACCCGCCGU	673	415-435	ACGGCGGGUAAGATCCUGGGAGA	870	413-435
AD-1557550	UAGUGCCUTCCGCAGUGAAAU	674	441-461	ATUUCACUGCGGAAGGCACUAGA	871	439-461
AD-1557554	GCCUUCCGCAGUGAAACCGCU	36	445-465	AGCGGUTUCACTGCGGAAGGCAC	162	443-465
AD-1557555	CCUUCCGCAGTGAAACCGCCU	675	446-466	AGGCGGTUUCACUGCGGAAGGCA	872	444-466
AD-1557556	CUUCCGCAGUGAAACCGCCAU	676	447-467	ATGGCGGUUUCACTGCGGAAGGC	873	445-467
AD-1557559	CCGCAGUGAAACCGCCAAAGU	677	450-470	ACUUUGGCGGUTUCACUGCGGAA	874	448-470
AD-1557560	CGCAGUGAAACCGCCAAAGCU	678	451-471	AGCUUUGGCGGTUTCACUGCGGA	875	449-471
AD-1557561	GCAGUGAAACCGCCAAAGCCU	679	452-472	AGGCUUTGGCGGUTUCACUGCGG	876	450-472
AD-1557562	CAGUGAAACCGCCAAAGCCCU	680	453-473	AGGGCUTUGGCGGTUUCACUGCG	877	451-473
AD-1557563	AGUGAAACCGCCAAAGCCCAU	681	454-474	ATGGGCTUUGGCGGUUUCACUGC	878	452-474
AD-1557571	CGCCAAAGCCCAGAAGAUGCU	682	462-482	AGCAUCTUCUGGGCUUUGGCGGU	879	460-482
AD-1557572	GCCAAAGCCCAGAAGAUGCUU	683	463-483	AAGCAUCUUCUGGGCUUUGGCGG	880	461-483
AD-1557577	AGCCCAGAAGAUGCUCAAGGU	684	468-488	ACCUUGAGCAUCUTCUGGGCUUU	881	466-488
AD-1557606	CAGCACCCGCCUGGGAACUUU	685	498-518	AAAGUUCCCAGGCGGGUGCUGGU	882	496-518
AD-1557607	AGCACCCGCCTGGGAACUUAU	686	499-519	ATAAGUTCCCAGGCGGGUGCUGG	883	497-519
AD-1557629	ACAACUCCAGCUCCGUCUAUU	687	521-541	AAUAGACGGAGCUGGAGUUGUAG	884	519-541
AD-1557630	CAACUCCAGCTCCGUCUAUUU	688	522-542	AAAUAGACGGAGCTGGAGUUGUA	885	520-542
AD-1557639	UCACCUGCTUCUUCUGGUUCU	689	560-580	AGAACCAGAAGAAGCAGGUGAGG	166	558-580
AD-1557640	CACCUGCUTCTUCUGGUUCAU	690	561-581	ATGAACCAGAAGAAGCAGGUGAG	886	559-581

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1557642	CCUGCUUCUCUGGUUCAUUU	691	563-583	AAAUGAACCAGAAGAAGCAGGUG	168	561-583
AD-1557643	CUGCUUCUCUTCTGGUUCAUUCU	692	564-584	AGAAUGAACCAGAAGAAGCAGGU	887	562-584
AD-1557644	UGCUUCUUCUGGUUCAUUCUU	43	565-585	AAGAAUGAACCAGAAGAAGCAGG	169	563-585
AD-1557646	CUUCUUCUGGTUCAUUCUCCU	693	567-587	AGGAGAAUGAACCAGAAGAAGCA	171	565-587
AD-1557647	UUCUUCUGGUTCAUUCUCCAU	694	568-588	ATGGAGAAUGAACCAGAAGAAGC	172	566-588
AD-1557648	UCUUCUGGUTCAUUCUCCAAU	695	569-589	ATUGGAGAAUGAACCAGAAGAAG	888	567-589
AD-1557649	CUUCUGGUTCAUUCUCCAAAU	696	570-590	ATUUGGAGAAUGAACCAGAAGAA	889	568-590
AD-1557650	UUCUGGUUCATUCUCCAAAUU	697	571-591	AAUUUGGAGAATGAACCAGAAGA	890	569-591
AD-1557651	UCUGGUUCAUTCUCCAAAUCU	698	572-592	AGAUUUGGAGAAUGAACCAGAAG	891	570-592
AD-1557652	CUGGUUCATUCUCCAAAUCCU	699	573-593	AGGAUUTGGAGAATGAACCAGAA	892	571-593
AD-1557682	GUGGAGGAGCTGCUGUCCACU	700	643-663	AGUGGACAGCAGCTCCUCCACCA	893	641-663
AD-1557685	GAGGAGCUGCTGUCCACAGUU	701	646-666	AACUGUGGACAGCAGCUCCUCCA	894	644-666
AD-1557689	AGCUGCUGTCCACAGUCAACU	702	650-670	AGUUGACUGUGGACAGCAGCUCC	895	648-670
AD-1557690	GCUGCUGUCCACAGUCAACAU	703	651-671	ATGUUGACUGUGGACAGCAGCUC	896	649-671
AD-1557693	GCUGUCCACAGUCAACAGCUU	704	654-674	AAGCUGTUGACTGTGGACAGCAG	897	652-674
AD-1557694	CUGUCCACAGTCAACAGCUCU	705	655-675	AGAGCUGUUGACUGUGGACAGCA	898	653-675
AD-1557695	UGUCCACAGUCAACAGCUCGU	706	656-676	ACGAGCTGUUGACTGUGGACAGC	899	654-676
AD-1557708	ACAGGGCCGAGUACGAAGUGU	48	689-709	ACACUUCGUACTCGGCCCUGUAG	900	687-709
AD-1557711	GGGCCGAGTACGAAGUGGACU	707	692-712	AGUCCACUUCGTACUCGGCCCUG	901	690-712
AD-1557712	GGCCGAGUACGAAGUGGACCU	708	693-713	AGGUCCACUUCGUACUCGGCCCU	902	691-713
AD-1557726	AUCCUGGAAGCCAGUGUGAAU	709	727-747	ATUCACACUGGCUTCCAGGAUCA	903	725-747
AD-1557727	UCCUGGAAGCCAGUGUGAAAU	710	728-748	ATUUCACACUGGCTUCCAGGAUC	904	726-748
AD-1557728	CCUGGAAGCCAGUGUGAAAGU	711	729-749	ACUUUCACACUGGCUUCCAGGAU	905	727-749
AD-1557729	CUGGAAGCCAGUGUGAAAGAU	712	730-750	ATCUUUCACACTGGCUUCCAGGA	906	728-750
AD-1557730	UGGAAGCCAGTGUGAAAGACU	713	731-751	AGUCUUTCACACUGGCUUCCAGG	907	729-751
AD-1557731	GGAAGCCAGUGUGAAAGACAU	714	732-752	ATGUCUTUCACACTGGCUUCCAG	908	730-752
AD-1557732	GAAGCCAGTGTGAAAGACAUU	715	733-753	AAUGUCTUUCACACUGGCUUCCA	909	731-753
AD-1557733	AAGCCAGUGUGAAAGACAUAU	716	734-754	ATAUGUCUUUCACACUGGCUUCC	910	732-754
AD-1557734	AGCCAGUGTGAAAGACAUAGU	717	735-755	ACUAUGTCUUUCACACUGGCUUC	911	733-755
AD-1557735	GCCAGUGUGAAAGACAUAGCU	718	736-756	AGCUAUGUCUUTCACACUGGCUU	912	734-756
AD-1557736	CCAGUGUGAAAGACAUAGCUU	50	737-757	AAGCUATGUCUTUCACACUGGCU	913	735-757
AD-1557738	AGUGUGAAAGACAUAGCUGCU	719	739-759	AGCAGCTAUGUCUTUCACACUGG	914	737-759
AD-1557739	GUGUGAAAGACAUAGCUGCAU	720	740-760	ATGCAGCUAUGTCTUUCACACUG	915	738-760
AD-1557740	UGUGAAAGACAUAGCUGCAUU	721	741-761	AAUGCAGCUAUGUCUUUCACACU	916	739-761
AD-1557741	GUGAAAGACATAGCUGCAUUU	722	742-762	AAAUGCAGCUATGTCUUUCACAC	917	740-762

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1557758	AUUGAAUUCCACGCUGGGUUU	52	759-779	AAACCCAGCGUGGAAUUCAAUGC	178	757-779
AD-1557762	AAUUCCACGCUGGGUUGUUAU	723	763-783	ATAACAACCCAGCGUGGAAUUCA	181	761-783
AD-1557767	CACGCUGGGUUGUUACCGCUU	724	768-788	AAGCGGTAACAACCCAGCGUGGA	184	766-788
AD-1557768	ACGCUGGGUUGUUACCGCUAU	725	769-789	ATAGCGGUAACAACCCAGCGUGG	918	767-789
AD-1557769	CGCUGGGUUGUUACCGCUACU	726	770-790	AGUAGCGGUAACAACCCAGCGUG	919	768-790
AD-1557770	GCUGGGUUGUUACCGCUACAU	727	771-791	ATGUAGCGGUAACAACCCAGCGU	920	769-791
AD-1557771	CUGGGUUGUUACCGCUACAGU	728	772-792	ACUGUAGCGGUAACAACCCAGCG	921	770-792
AD-1557772	UGGGUUGUUACCGCUACAGCU	729	773-793	AGCUGUAGCGGTAACAACCCAGC	922	771-793
AD-1557773	GGGUUGUUACCGCUACAGCUU	730	774-794	AAGCUGTAGCGGUAACAACCCAG	923	772-794
AD-1557836	CAAACUCCGGCUGGAGUGGAU	731	888-908	ATCCACTCCAGCCGGAGUUUGAG	924	886-908
AD-1557866	GGGACCGACUGGCCAUGUAUU	60	923-943	AAUACATGGCCAGTCGGUCCCGG	925	921-943
AD-1557871	CGACUGGCCATGUAUGACGUU	732	928-948	AACGUCAUACATGGCCAGUCGGU	926	926-948
AD-1557881	CUGGAGAAGAGGCUCAUCACU	733	958-978	AGUGAUGAGCCTCTUCUCCAGGG	927	956-978
AD-1557882	UGGAGAAGAGGCUCAUCACCU	734	959-979	AGGUGATGAGCCUCUUCUCCAGG	928	957-979
AD-1557883	GGAGAAGAGGCUCAUCACCUU	735	960-980	AAGGUGAUGAGCCTCUUCUCCAG	929	958-980
AD-1557884	GAGAAGAGGCTCAUCACCUCU	736	961-981	AGAGGUGAUGAGCCUCUUCUCCA	930	959-981
AD-1557886	GAAGAGGCTCAUCACCUCGGU	737	963-983	ACCGAGGUGAUGAGCCUCUUCUC	931	961-983
AD-1557890	AGGCUCAUCACCUCGGUGUAU	67	967-987	ATACACCGAGGTGAUGAGCCUCU	193	965-987
AD-1557944	GAAGAAGGGCCUGCACAGCUU	738	1053-1073	AAGCUGTGCAGGCCCUUCUUCCA	932	1051-1073
AD-1557945	AAGAAGGGCCTGCACAGCUAU	739	1054-1074	ATAGCUGUGCAGGCCCUUCUUCC	933	1052-1074
AD-1557948	AAGGGCCUGCACAGCUACUAU	740	1057-1077	ATAGUAGCUGUGCAGGCCCUUCU	934	1055-1077
AD-1557949	AGGGCCUGCACAGCUACUACU	741	1058-1078	AGUAGUAGCUGTGCAGGCCCUUC	935	1056-1078
AD-1557953	CCUGCACAGCTACUACGACCU	742	1062-1082	AGGUCGTAGUAGCTGUGCAGGCC	936	1060-1082
AD-1558059	CCUCUCUGGACUACGGCUUGU	70	1235-1255	ACAAGCCGUAGTCCAGAGAGGGC	196	1233-1255
AD-1558061	UCUCUGGACUACGGCUUGGCU	71	1237-1257	AGCCAAGCCGUAGTCCAGAGAGG	937	1235-1257
AD-1558065	UGGACUACGGCUUGGCCCUCU	743	1241-1261	AGAGGGCCAAGCCGUAGUCCAGA	938	1239-1261
AD-1558066	GGACUACGGCTUGGCCCUCUU	744	1242-1262	AAGAGGGCCAAGCCGUAGUCCAG	939	1240-1262
AD-1558105	GAGGAGGCAGAAGUAUGAUUU	76	1281-1301	AAAUCATACUUCUGCCUCCUCAG	202	1279-1301
AD-1558106	AGGAGGCAGAAGUAUGAUUUU	745	1282-1302	AAAAUCAUACUTCTGCCUCCUCA	940	1280-1302
AD-1558113	AGAAGUAUGATUUGCCGUGCU	746	1289-1309	AGCACGGCAAATCAUACUUCUGC	209	1287-1309
AD-1558114	GAAGUAUGAUTUGCCGUGCAU	747	1290-1310	ATGCACGGCAAAUCAUACUUCUG	210	1288-1310
AD-1558115	AAGUAUGATUTGCCGUGCACU	748	1291-1311	AGUGCACGGCAAATCAUACUUCU	941	1289-1311
AD-1558116	AGUAUGAUTUGCCGUGCACCU	749	1292-1312	AGGUGCACGGCAAAUCAUACUUC	942	1290-1312
AD-1558117	GUAUGAUUTGCCGUGCACCCU	750	1293-1313	AGGGUGCACGGCAAAUCAUACUU	943	1291-1313
AD-1558136	GGCCAGUGGACGAUCCAGAAU	751	1315-1335	ATUCUGGAUCGTCCACUGGCCCU	944	1313-1335

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1558137	GCCAGUGGACGAUCCAGAACU	752	1316-1336	AGUUCUGGAUCGUCCACUGGCCC	945	1314-1336
AD-1558138	CCAGUGGACGAUCCAGAACAU	753	1317-1337	ATGUUCUGGAUCGTCCACUGGCC	946	1315-1337
AD-1558139	CAGUGGACGATCCAGAACAGU	754	1318-1338	ACUGUUCUGGGATCGUCCACUGGC	947	1316-1338
AD-1558142	UGGACGAUCCAGAACAGGAGU	755	1321-1341	ACUCCUGUUCUGGAUCGUCCACU	948	1319-1341
AD-1558150	CCAGAACAGGAGGCUGUGUGU	87	1329-1349	ACACACAGCCUCCTGUUCUGGAU	949	1327-1349
AD-1558152	AGAACAGGAGGCUGUGUGGCU	88	1331-1351	AGCCACACAGCCUCCUGUUCUGG	214	1329-1351
AD-1558211	ACUUCACCTCCCAGAUCUCCU	756	1415-1435	AGGAGATCUGGGAGGUGAAGUUG	950	1413-1435
AD-1558215	CACCUCCCAGAUCUCCCUCAU	757	1419-1439	ATGAGGGAGAUCUGGGAGGUGAA	951	1417-1439
AD-1558230	UGUGCGGGTGCACUAUGGCUU	758	1449-1469	AAGCCATAGUGCACCCGCACACC	215	1447-1469
AD-1558231	GUGCGGGUGCACUAUGGCUUU	90	1450-1470	AAAGCCAUAGUGCACCCGCACAC	216	1448-1470
AD-1558232	UGCGGGUGCACUAUGGCUUGU	759	1451-1471	ACAAGCCAUAGTGCACCCGCACA	952	1449-1471
AD-1558233	GCGGGUGCACTAUGGCUUGUU	760	1452-1472	AACAAGCCAUAGUGCACCCGCAC	217	1450-1472
AD-1558234	CGGGUGCACUAUGGCUUGUAU	761	1453-1473	ATACAAGCCAUAGTGCACCCGCA	953	1451-1473
AD-1558235	GGGUGCACTATGGCUUGUACU	762	1454-1474	AGUACAAGCCATAGUGCACCCGC	218	1452-1474
AD-1558236	GGUGCACUAUGGCUUGUACAU	763	1455-1475	ATGUACAAGCCAUAGUGCACCCG	954	1453-1475
AD-1558238	UGCACUAUGGCUUGUACAACU	764	1457-1477	AGUUGUACAAGCCAUAGUGCACC	955	1455-1477
AD-1558239	GCACUAUGGCTUGUACAACCU	765	1458-1478	AGGUUGTACAAGCCAUAGUGCAC	956	1456-1478
AD-1558249	CUGCCCUGGAGAGUUCCUCUU	95	1488-1508	AAGAGGAACUCTCCAGGGCAGGG	221	1486-1508
AD-1558250	UGCCCUGGAGAGUUCCUCUGU	766	1489-1509	ACAGAGGAACUCUCCAGGGCAGG	957	1487-1509
AD-1558288	AACGGCCUGGAUGAGAGAAAU	767	1561-1581	ATUUCUCUCAUCCAGGCCGUUGG	958	1559-1581
AD-1558289	ACGGCCUGGATGAGAGAAACU	768	1562-1582	AGUUUCTCUCATCCAGGCCGUUG	959	1560-1582
AD-1558290	CGGCCUGGAUGAGAGAAACUU	769	1563-1583	AAGUUUCUCUCAUCCAGGCCGUU	960	1561-1583
AD-1558292	GCCUGGAUGAGAGAAACUGCU	96	1565-1585	AGCAGUTUCUCTCAUCCAGGCCG	222	1563-1585
AD-1558293	CCUGGAUGAGAGAAACUGCGU	770	1566-1586	ACGCAGTUUCUCUCAUCCAGGCC	961	1564-1586
AD-1558301	AGAGAAACTGCGUUUUGCAGAU	771	1574-1594	ATCUGCAAACGCAGUUUUCUCUCA	962	1572-1594
AD-1558302	GAGAAACUGCGUUUUGCAGAGU	772	1575-1595	ACUCUGCAAACGCAGUUUUCUCUC	963	1573-1595
AD-1558308	CUGCGUUUUGCAGAGCCACAUU	773	1581-1601	AAUGUGGCUCUGCAAACGCAGUU	964	1579-1601
AD-1558309	UGCGUUUUGCAGAGCCACAUUU	774	1582-1602	AAAUGUGGCUCTGCAAACGCAGU	965	1580-1602
AD-1558310	GCGUUUUGCAGAGCCACAUUCU	775	1583-1603	AGAAUGTGGCUCUGCAAACGCAG	966	1581-1603
AD-1558311	CGUUUUGCAGAGCCACAUUCCU	776	1584-1604	AGGAAUGUGGCTCTGCAAACGCA	967	1582-1604
AD-1558316	GCAGAGCCACAUUCCAGUGCU	777	1589-1609	AGCACUGGAAUGUGGCUCUGCAA	968	1587-1609
AD-1558419	UGGGACAUTCACCUUCCAGUU	778	1710-1730	AACUGGAAGGUGAAUGUCCCACA	228	1708-1730
AD-1558420	GGGACAUUCACCUUCCAGUGU	779	1711-1731	ACACUGGAAGGTGAAUGUCCCAC	969	1709-1731
AD-1558421	GGACAUUCACCUUCCAGUGUU	103	1712-1732	AACACUGGAAGGUGAAUGUCCCA	229	1710-1732
AD-1558423	ACAUUCACCUTCCAGUGUGAU	780	1714-1734	ATCACACUGGAAGGUGAAUGUCC	231	1712-1734

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1558449	GAGCUGCGTGAAGAAGCCCAU	781	1740-1760	ATGGGCTUCUUCACGCAGCUCCG	970	1738-1760
AD-1558450	AGCUGCGUGAAGAAGCCCAAU	782	1741-1761	ATUGGGCUUCUTCACGCAGCUCC	971	1739-1761
AD-1558451	GCUGCGUGAAGAAGCCCAACU	783	1742-1762	AGUUGGGCUUCTUCACGCAGCUC	972	1740-1762
AD-1558452	CUGCGUGAAGAAGCCCAACCU	784	1743-1763	AGGUUGGGCUUCUTCACGCAGCU	973	1741-1763
AD-1558453	UGCGUGAAGAAGCCCAACCCU	785	1744-1764	AGGGUUGGGCUTCTUCACGCAGC	974	1742-1764
AD-1558508	AGCACUGUGACUGUGGCCUCU	786	1808-1828	AGAGGCCACAGTCACAGUGCUCC	975	1806-1828
AD-1558546	CUCCGAGGGUGAGUGGCCAUU	787	1866-1886	AAUGGCCACUCACCCUCGGAGGA	976	1864-1886
AD-1558576	AUCGCUGACCGCUGGGUGAUU	107	1936-1956	AAUCACCCAGCGGTCAGCGAUGA	977	1934-1956
AD-1558577	UCGCUGACCGCUGGGUGAUAU	788	1937-1957	ATAUCACCCAGCGGUCAGCGAUG	978	1935-1957
AD-1558578	CGCUGACCGCTGGGUGAUAAU	789	1938-1958	ATUAUCACCCAGCGGUCAGCGAU	979	1936-1958
AD-1558579	GCUGACCGCUGGGUGAUAACU	790	1939-1959	AGUUAUCACCCAGCGGUCAGCGA	980	1937-1959
AD-1558586	GCUGGGUGAUAACAGCUGCCU	791	1946-1966	AGGCAGCUGUUAUCACCCAGCGG	981	1944-1966
AD-1558609	UGCUUCCAGGAGGACAGCAUU	792	1969-1989	AAUGCUGUCCUCCTGGAAGCAGU	982	1967-1989
AD-1558610	GCUUCCAGGAGGACAGCAUGU	793	1970-1990	ACAUGCTGUCCTCCUGGAAGCAG	983	1968-1990
AD-1558611	CUUCCAGGAGGACAGCAUGGU	794	1971-1991	ACCAUGCUGUCCUCCUGGAAGCA	984	1969-1991
AD-1558650	CGUGUUCCTGGGCAAGGUGUU	795	2010-2030	AACACCTUGCCCAGGAACACGGU	235	2008-2030
AD-1558657	CUGGGCAAGGTGUGGCAGAAU	796	2017-2037	ATUCUGCCACACCTUGCCCAGGA	985	2015-2037
AD-1558658	UGGGCAAGGUGUGGCAGAACU	797	2018-2038	AGUUCUGCCACACCUUGCCCAGG	986	2016-2038
AD-1558659	GGGCAAGGTGTGGCAGAACUU	798	2019-2039	AAGUUCTGCCACACCUUGCCCAG	987	2017-2039
AD-1558660	GGCAAGGUGUGGCAGAACUCU	799	2020-2040	AGAGUUCUGCCACACCUUGCCCA	988	2018-2040
AD-1558661	GCAAGGUGTGGCAGAACUCGU	800	2021-2041	ACGAGUTCUGCCACACCUUGCCC	237	2019-2041
AD-1558662	CAAGGUGUGGCAGAACUCGCU	801	2022-2042	AGCGAGTUCUGCCACACCUUGCC	989	2020-2042
AD-1558683	UGGCCUGGAGAGGUGUCCUUU	802	2044-2064	AAAGGACACCUCUCCAGGCCAGC	990	2042-2064
AD-1558684	GGCCUGGAGAGGUGUCCUUCU	803	2045-2065	AGAAGGACACCTCTCCAGGCCAG	991	2043-2065
AD-1558685	GCCUGGAGAGGUGUCCUUCAU	804	2046-2066	ATGAAGGACACCUCUCCAGGCCA	992	2044-2066
AD-1558686	CCUGGAGAGGTGTCCUUCAAU	805	2047-2067	ATUGAAGGACACCTCTCCAGGCC	993	2045-2067
AD-1558687	CUGGAGAGGUGUCCUUCAAGU	113	2048-2068	ACUUGAAGGACACCUCUCCAGGC	239	2046-2068
AD-1558691	AGAGGUGUCCTTCAAGGUGAU	806	2052-2072	ATCACCTUGAAGGACACCUCUCC	242	2050-2072
AD-1558833	UGUGCAGUTGAUCCCACAGGU	807	2289-2309	ACCUGUGGGAUCAACUGCACAUC	994	2287-2309
AD-1558835	UGCAGUUGAUCCCACAGGACU	808	2291-2311	AGUCCUGUGGGAUCAACUGCACA	995	2289-2311
AD-1558843	AUCCCACAGGACCUGUGCAGU	117	2299-2319	ACUGCACAGGUCCTGUGGGAUCA	996	2297-2319
AD-1558845	CCCACAGGACCUGUGCAGCGU	809	2301-2321	ACGCUGCACAGGUCCUGUGGGAU	997	2299-2321
AD-1558846	CCACAGGACCTGUGCAGCGAU	810	2302-2322	ATCGCUGCACAGGTCCUGUGGGA	998	2300-2322
AD-1558878	CCAGGUGACGCCACGCAUGCU	811	2334-2354	AGCAUGCGUGGGCGTCACCUGGUA	999	2332-2354
AD-1558882	GUGACGCCACGCAUGCUGUGU	812	2338-2358	ACACAGCAUGCGUGGGCGUCACCU	1000	2336-2358

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1558883	UGACGCCACGCAUGCUGUGUU	118	2339-2359	AACACAGCAUGCGTGGCGUCACC	1001	2337-2359
AD-1558885	ACGCCACGCATGCUGUGUGCU	813	2341-2361	AGCACACAGCATGCGUGGCGUCA	1002	2339-2361
AD-1558905	GGCUACCGCAAGGGCAAGAAU	814	2362-2382	ATUCUUGCCCUTGCGGUAGCCGG	1003	2360-2382
AD-1558906	GCUACCGCAAGGGCAAGAAGU	120	2363-2383	ACUUCUTGCCCTUGCGGUAGCCG	246	2361-2383
AD-1558907	CUACCGCAAGGGCAAGAAGGU	121	2364-2384	ACCUUCTUGCCCUTGCGGUAGCC	1004	2362-2384
AD-1558961	GUGCAAGGCACUCAGUGGCCU	815	2418-2438	AGGCCACUGAGTGCCUUGCACAC	1005	2416-2438
AD-1558992	CUAACUACTUCGGCGUCUACU	816	2486-2506	AGUAGACGCCGAAGUAGUUAGGC	1006	2484-2506
AD-1558995	ACUACUUCGGCGUCUACACCU	817	2489-2509	AGGUGUAGACGCCGAAGUAGUUA	1007	2487-2509
AD-1558996	CUACUUCGGCGUCUACACCCU	818	2490-2510	AGGGUGTAGACGCCGAAGUAGUU	1008	2488-2510
AD-1559004	GCGUCUACACCCGCAUCACAU	819	2498-2518	ATGUGATGCGGGUGUAGACGCCG	1009	2496-2518
AD-1559005	CGUCUACACCCGCAUCACAGU	820	2499-2519	ACUGUGAUGCGGGTGUAGACGCC	1010	2497-2519
AD-1559008	CUACACCCGCAUCACACAGUGU	124	2502-2522	ACACCUGUGAUGCGGGUGUAGAC	250	2500-2522
AD-1559012	ACCCGCAUCACAGGUGUGAUU	821	2506-2526	AAUCACACCUGTGAUGCGGGUGU	1011	2504-2526
AD-1559013	CCCGCAUCACAGGUGUGAUCU	822	2507-2527	AGAUCACACCUGUGAUGCGGGUG	1012	2505-2527
AD-1559036	UGGAUCCAGCAAGUGGUGACU	823	2530-2550	AGUCACCACUUGCTGGAUCCAGC	1013	2528-2550
AD-1559038	GAUCCAGCAAGUGGUGACCUU	824	2532-2552	AAGGUCACCACTUGCUGGAUCCA	1014	2530-2552
AD-1559039	AUCCAGCAAGTGGUGACCUGU	825	2533-2553	ACAGGUCACCACUTGCUGGAUCC	1015	2531-2553
AD-1559041	CCAGCAAGTGGUGACCUGAGU	826	2535-2555	ACUCAGGUCACCACUUGCUGGAU	1016	2533-2555
AD-1559042	CAGCAAGUGGTGACCUGAGGU	827	2536-2556	ACCUCAGGUCACCACUUGCUGGA	1017	2534-2556
AD-1559044	GCAAGUGGTGACCUGAGGAAU	828	2538-2558	ATUCCUCAGGUCACCACUUGCUG	1018	2536-2558
AD-1559105	UGGUGGCAGGAGGUGGCAUCU	829	2667-2687	AGAUGCCACCUCCTGCCACCACA	1019	2665-2687
AD-1559106	GGUGGCAGGAGGUGGCAUCUU	830	2668-2688	AAGAUGCCACCTCCUGCCACCAC	1020	2666-2688
AD-1559107	GUGGCAGGAGGUGGCAUCUUU	831	2669-2689	AAAGAUGCCACCUCCUGCCACCA	1021	2667-2689
AD-1559109	GGCAGGAGGUGGCAUCUUGUU	127	2671-2691	AACAAGAUGCCACCUCCUGCCAC	253	2669-2691
AD-1559133	UCCCUGAUGUCUGCUCCAGUU	832	2695-2715	AACUGGAGCAGACAUCAGGGACG	1022	2693-2715
AD-1559136	CUGAUGUCTGCUCCAGUGAUU	833	2698-2718	AAUCACTGGAGCAGACAUCAGGG	1023	2696-2718
AD-1559147	UCCAGUGATGGCAGGAGGAUU	834	2709-2729	AAUCCUCCUGCCATCACUGGAGC	1024	2707-2729
AD-1559233	GGCUCAGCAGCAAGAAUGCUU	132	2853-2873	AAGCAUTCUUGCUGCUGAGCCAC	258	2851-2873
AD-1559318	CUAACUUGGGAUCUGGGAAUU	835	2978-2998	AAUUCCCAGAUCCCAAGUUAGAC	1025	2976-2998
AD-1559323	UUGGGAUCTGGGAAUGGAAGU	836	2983-3003	ACUUCCCAUUCCCCAGAUCCCAAGU	264	2981-3003
AD-1559431	GUGAGCUCAGCUGCCCUUUGU	837	3157-3177	ACAAAGGGCAGCUGAGCUCACCU	1026	3155-3177
AD-1559436	CUCAGCUGCCCUUUGGAAUAU	838	3162-3182	ATAUUCCAAAGGGCAGCUGAGCU	1027	3160-3182
AD-1559437	UCAGCUGCCCTUUGGAAUAAU	839	3163-3183	ATUAUUCCAAAGGGCAGCUGAGC	1028	3161-3183
AD-1559438	CAGCUGCCCUTUGGAAUAAAU	840	3164-3184	ATUUAUTCCAAAGGGCAGCUGAG	1029	3162-3184
AD-1559441	CUGCCCUUTGGAAUAAAGCUU	841	3167-3187	AAGCUUTAUUCCAAAGGGCAGCU	1030	3165-3187

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_153609.4
AD-1559443	GCCCUUUGGAAUAAAGCUGCU	842	3169-3189	AGCAGCTUUAUTCCAAAGGGCAG	1031	3167-3189
AD-1559444	CCCUUUGGAATAAAGCUGCCU	843	3170-3190	AGGCAGCUUUATUCCAAAGGGCA	1032	3168-3190
AD-1559445	CCUUUGGAAUAAAGCUGCCUU	844	3171-3191	AAGGCAGCUUUAUTCCAAAGGGC	1033	3169-3191
AD-1559447	UUUGGAAUAAAGCUGCCUGAU	845	3173-3193	ATCAGGCAGCUTUAUUCCAAAGG	1034	3171-3193
AD-1559448	UUGGAAUAAAGCUGCCUGAUU	846	3174-3194	AAUCAGGCAGCTUTAUUCCAAAG	1035	3172-3194
AD-1559449	UGGAAUAAAGCUGCCUGAUCU	847	3175-3195	AGAUCAGGCAGCUTUAUUCCAAA	1036	3173-3195

Table 5. Modified Sense and Antisense Strand Sequences of TMPRSS6 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Antisense Sequence 5' to 3'	SEQ ID NO	mRNA target sequence 5' to 3'	SEQ ID NO
AD-1557376	csgsgaggugdAudGgcgaggaaguL96	1037	asdCsuucc(Tgn)cgccdAudCaccuccgsusc	1264	GACGGAGGUGAUGGCGAGGAAGC	1491
AD-1557377	gsgsaggugadTgdGcgaggaagcuL96	1038	asdGscuuc(C2p)ucgcdCadTcaccuccsgsu	1265	ACGGAGGUGAUGGCGAGGAAGCG	1492
AD-1557396	asasggccugdTgdAggacuccaauL96	1039	asdTsugga(G2p)uccudCadCaggccuusgsa	1266	UCAAGGCCUGUGAGGACUCCAAG	1493
AD-1557398	gsgsccugugdAgdGacuccaagauL96	1040	asdTscuug(G2p)agucdCudCacaggccsusu	1267	AAGGCCUGUGAGGACUCCAAGAG	1494
AD-1557399	gscscugugadGgdAcuccaagaguL96	1041	asdCsucuu(G2p)gagudCcdTcacaggcscsu	1268	AGGCCUGUGAGGACUCCAAGAGA	524
AD-1557400	cscsugugagdGadCuccaagagauL96	1042	asdTscucu(Tgn)ggagdTcdCucacaggcscsc	1269	GGCCUGUGAGGACUCCAAGAGAA	1495
AD-1557401	csusgugaggdAcdTccaagagaauL96	1043	asdTsucuc(Tgn)uggadGudCcucacagsgsc	1270	GCCUGUGAGGACUCCAAGAGAAA	1496
AD-1557437	csusacucugdGudAuuuccuagguL96	1044	asdCscuag(G2p)aaaudAcdCagaguagscsa	1271	UGCUACUCUGGUAUUUCCUAGGG	529
AD-1557440	csuscugguadTudTccuaggguauL96	1045	asdTsaccc(Tgn)aggadAadTaccagagsusa	1272	UACUCUGGUAUUUCCUAGGGUAC	532
AD-1557441	uscsugguaudTudCcuaggguacuL96	1046	asdGsuacc(C2p)uaggdAadAuaccagasgsu	1273	ACUCUGGUAUUUCCUAGGGUACA	533
AD-1557442	csusgguauudTcdCuaggguacauL96	1047	asdTsguac(C2p)cuagdGadAauaccagsasg	1274	CUCUGGUAUUUCCUAGGGUACAA	1497
AD-1557443	usgsguauuudCcdTaggguacaauL96	1048	asdTsugua(C2p)ccuadGgdAaauaccasgsa	1275	UCUGGUAUUUCCUAGGGUACAAG	1498
AD-1557444	gsgsuauuucdCudAggguacaaguL96	1049	asdCsuugu(Agn)cccudAgdGaaauaccsasg	1276	CUGGUAUUUCCUAGGGUACAAGG	1499
AD-1557445	gsusauuuccdTadGgguacaaggguL96	1050	asdCscuug(Tgn)acccdTadGgaaauacscsa	1277	UGGUAUUUCCUAGGGUACAAGGC	1500
AD-1557452	csusaggguadCadAggcggagguuL96	1051	asdAsccuc(C2p)gccudTgdTacccuagsgsa	1278	UCCUAGGGUACAAGGCGGAGGUG	1501
AD-1557473	asusggucagdCcdAgguguacucuL96	1052	asdGsagua(C2p)accudGgdCugaccauscsa	1279	UGAUGGUCAGCCAGGUGUACUCA	1502
AD-1557475	gsgsucagccdAgdGuguacucaguL96	1053	asdCsugag(Tgn)acacdCudGgcugaccsasu	1280	AUGGUCAGCCAGGUGUACUCAGG	535
AD-1557476	gsuscagccadGgdTguacucagguL96	1054	asdCscuga(G2p)uacadCcdTggcugacscsa	1281	UGGUCAGCCAGGUGUACUCAGGC	1503
AD-1557477	uscsagccagdGudGuacucaggcuL96	1055	asdGsccug(Agn)guacdAcdCuggcugascsc	1282	GGUCAGCCAGGUGUACUCAGGCA	1504
AD-1557478	csasgccaggdTgdTacucaggcauL96	1056	asdTsgccu(G2p)aguadCadCcuggcugsasc	1283	GUCAGCCAGGUGUACUCAGGCAG	1505

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Antisense Sequence 5' to 3'	SEQ ID NO	mRNA target sequence 5' to 3'	SEQ ID NO
AD-1557479	asgsccaggudGudAcucaggcagcaguL96	1057	asdCsugccc(Tgn)gagudAcdAccuggcugsga	1284	UCAGCCAGGUGUACUCAGGCAGU	536
AD-1557509	csusccaaucgdCcdAcuucucccauL96	1058	asdTsggga(G2p)aagudGgdCgauugagsusa	1285	UACUCAAUCGCCACUUCUCCCAG	1506
AD-1557515	csgsccacuudCudCccaggaucuuL96	1059	asdAsgauc(C2p)ugggdAgdAaguggcgsasu	1286	AUCGCCACUUCUCCCAGGAUCUU	1507
AD-1557516	gscscacuucdTcdCcaggaucuuuL96	1060	asdAsagau(C2p)cuggdGadGaaguggcsgsa	1287	UCGCCACUUCUCCCAGGAUCUUA	537
AD-1557518	csascuucucdCcdAggaucuuacuL96	1061	asdGsuaag(Agn)uccudGgdGagaagugsgsc	1288	GCCACUUCUCCCAGGAUCUUACC	1508
AD-1557522	uscsuccccagdGadTcuuaccgcuL96	1062	asdGscggg(Tgn)aagadTcdCuggagagasag	1289	CUUCUCCCCAGGAUCUUACCCGCC	1509
AD-1557523	csusccccaggdAudCuuaccgcccuL96	1063	asdGsgcgg(G2p)uaagdAudCcuggggagsasa	1290	UUCUCCCCAGGAUCUUACCCGCCG	1510
AD-1557524	uscsccccaggadTcdTuaccgccguL96	1064	asdCsggcg(G2p)guaadGadTccuggggasgsa	1291	UCUCCCCAGGAUCUUACCCGCCGG	539
AD-1557550	usasgugccudTcdCgcagugaaauL96	1065	asdTsuuca(C2p)ugcgdGadAggcacuasgsa	1292	UCUAGUGCCUUCCGCAGUGAAAC	1511
AD-1557554	gscscuuuccgdCadGugaaaccgcuL96	1066	asdGscggu(Tgn)ucacdTgdCggaaggcsasc	1293	GUGCCUUUCCGCAGUGAAACCGCC	540
AD-1557555	cscsuuuccgcdAgdTgaaaccgccuL96	1067	asdGsgcgg(Tgn)uucadCudGcggaaaggcsa	1294	UGCCUUUCCGCAGUGAAACCGCCA	1512
AD-1557556	csusuccgcadGudGaaaccgccauL96	1068	asdTsggcg(G2p)uuucdAcdTgcggaaggsgc	1295	GCCUUUCCGCAGUGAAACCGCCAA	1513
AD-1557559	cscsgcagugdAadAccgccaaaguL96	1069	asdCsuuug(G2p)cggudTudCacugcggsasa	1296	UUCCGCAGUGAAACCGCCAAAGC	1514
AD-1557560	csgscagugadAadCcgccaaagcuL96	1070	asdGscuuu(G2p)gcggdTudTcacugcgsgsa	1297	UCCGCAGUGAAACCGCCAAAGCC	1515
AD-1557561	gscsagugaadAcdCgccaaagccuL96	1071	asdGsgcuu(Tgn)ggcgdGudTucacugcgsgg	1298	CCGCAGUGAAACCGCCAAAGCCC	1516
AD-1557562	csasgugaaadCcdGccaaagcccuL96	1072	asdGsggcu(Tgn)uggcdGgdTuucacugscsg	1299	CGCAGUGAAACCGCCAAAGCCCA	1517
AD-1557563	asgsugaaacdCgdCcaaagcccauL96	1073	asdTsgggc(Tgn)uuggdCgdGuuucacusgsc	1300	GCAGUGAAACCGCCAAAGCCCAG	1518
AD-1557571	csgsccaaagdCcdCagaagugcuL96	1074	asdGscauc(Tgn)ucugdGgdCuuugcgsgsu	1301	ACCGCCAAAGCCCAGAAGAUGCU	1519
AD-1557572	gscscaaagcdCcdAgaagaugcuuL96	1075	asdAsgcau(C2p)uucudGgdGcuuugcgsgsg	1302	CCGCCAAAGCCCAGAAGAUGCUC	1520
AD-1557577	asgscccagadAgdAugcucaagguL96	1076	asdCscuug(Agn)gcaudCudTcuggcgcususu	1303	AAAGCCCAGAAGAUGCUCAAGGA	1521
AD-1557606	csasgcacccdGcdCuggaacuuuuL96	1077	asdAsaguu(C2p)ccagdGcdGggugcugsgsu	1304	ACCAGCACCCGCCUGGGAACUUA	1522
AD-1557607	asgscacccgdCcdTgggaacuuauL96	1078	asdTsaagu(Tgn)cccadGgdCgggugcusgsg	1305	CCAGCACCCGCCUGGGAACUUAC	1523
AD-1557629	ascsaacuccdAgdCuccgcuauuuL96	1079	asdAsuaga(C2p)ggagdCudGgaguugusasg	1306	CUACAACUCCAGCUCCGUCUAUU	1524
AD-1557630	csasacuccadGcdTccgcuauuuuL96	1080	asdAsauag(Agn)cggadGcdTggaguugsusa	1307	UACAACUCCAGCUCCGUCUAUUC	541
AD-1557639	uscsaccugcdTudCuucgguucuuL96	1081	asdGsaacc(Agn)gaagdAadGcaggugasgsg	1308	CCUCACCUGCUUCUUCUGGUUCA	544
AD-1557640	csasccugcudTcdTucgguucauL96	1082	asdTsgaac(C2p)agaadGadAgccaggugsasg	1309	CUCACCUGCUUCUUCUGGUUCAU	1525
AD-1557642	cscsugcuucdTudCugguucauuuL96	1083	asdAsauga(Agn)ccagdAadGaagcaggsusg	1310	CACCUGCUUCUUCUGGUUCAUUC	546
AD-1557643	csusgcuucudTcdTgguucauucuL96	1084	asdGsaaug(Agn)accadGadAgaagcagsgsu	1311	ACCUGCUUCUUCUGGUUCAUUCU	1526
AD-1557644	usgscuucuudCudGguucauucuuL96	1085	asdAsgaau(G2p)aaccdAgdAagaagcasgsg	1312	CCUGCUUCUUCUGGUUCAUUCUC	547
AD-1557646	csusucuucudGgdTucauucucccuL96	1086	asdGsgaga(Agn)ugaadCcdAgaagaagscsa	1313	UGCUUCUUCUGGUUCAUUCUCCCA	549
AD-1557647	ususcuucugdGudTcauucuccauL96	1087	asdTsggag(Agn)augadAcdCagaagaagsgsc	1314	GCUUCUUCUGGUUCAUUCUCCCAA	550
AD-1557648	uscsuucuggdTudCauucuccaauL96	1088	asdTsugga(G2p)aaugdAadCcagaagasasg	1315	CUUCUUCUGGUUCAUUCUCCCAAA	1527
AD-1557649	csusucuggudTcdAuucuccaaauL96	1089	asdTsuugg(Agn)gaaudGadAccagaagsasa	1316	UUCUUCUGGUUCAUUCUCCCAAAU	1528
AD-1557650	ususcuggudCadTucuccaaauuL96	1090	asdAsuuug(G2p)agaadTgdAaccagaasgsa	1317	UCUUCUGGUUCAUUCUCCCAAAUC	1529

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Antisense Sequence 5' to 3'	SEQ ID NO	mRNA target sequence 5' to 3'	SEQ ID NO
AD-1557651	uscsuggguucdAudTcuccaaaucuL96	1091	asdGsauuuu(G2p)gagadAudGaaccagasasg	1318	CUUCUGGGUUCAUUCUCCCAAAUCC	1530
AD-1557652	csusgguucadTudCuccaaaucccuL96	1092	asdGsgauuu(Tgn)ggagdAadTgaaccagsasa	1319	UUCUGGGUUCAUUCUCCCAAAUCCC	551
AD-1557682	gsusggaggadGcdTgcuguccacuL96	1093	asdGsugga(C2p)agcadGcdTccuccacscsa	1320	UGGUGGAGGAGCUGCUGUCCCACA	1531
AD-1557685	gsasggagcudGcdTguccacaguuL96	1094	asdAscugu(G2p)gacadGcdAgcuccucscsa	1321	UGGAGGAGCUGCUGUCCCACAGUC	1532
AD-1557689	asgscugcugdTcdCacagucaacuL96	1095	asdGsuuga(C2p)ugugdGadCagcagcuscsc	1322	GGAGCUGCUGUCCCACAGUCAACA	1533
AD-1557690	gscsugcugudCcdAcagucaacauL96	1096	asdTsguug(Agn)cugudGgdAcagcagcsusc	1323	GAGCUGCUGUCCCACAGUCAACAG	1534
AD-1557693	gscsugcuccadCadGucaacagcuuL96	1097	asdAsgcug(Tgn)ugacdTgdTggacagcsasg	1324	CUGCUGUCCCACAGUCAACAGCUC	1535
AD-1557694	csusgcuccacdAgdTcaacagcucuL96	1098	asdGsagcu(G2p)uugadCudGuggacagscsa	1325	UGCUGUCCCACAGUCAACAGCUCG	1536
AD-1557695	usgscuccacadGudCaacagcucguL96	1099	asdCsgagc(Tgn)guugdAcdTguggacasgsc	1326	GCUGUCCCACAGUCAACAGCUCGG	1537
AD-1557708	ascsagggccdGadGuacgaaguguL96	1100	asdCsacuu(C2p)guacdTcdGgcccugusasg	1327	CUACAGGGCCGAGUACGAAGUGG	552
AD-1557711	gsgsgccgagdTadCgaaguggacuL96	1101	asdGsucca(C2p)uucgdTadCucggcccsusg	1328	CAGGGCCGAGUACGAAGUGGACC	1538
AD-1557712	gsgsccgagudAcdGaaguggaccuL96	1102	asdGsgucc(Agn)cuucdGudAcucggcccscsu	1329	AGGGCCGAGUACGAAGUGGACCC	1539
AD-1557726	asusccuggadAgdCcagugugaauL96	1103	asdTsucac(Agn)cuggdCudTccaggauscsa	1330	UGAUCCUGGAAGCCAGUGUGAAA	1540
AD-1557727	uscscuggaadGcdCagugugaaauL96	1104	asdTsuuca(C2p)acugdGcdTuccaggasusc	1331	GAUCCUGGAAGCCAGUGUGAAAG	1541
AD-1557728	cscsuggaagdCcdAgugugaaaguL96	1105	asdCsuuuc(Agn)cacudGgdCuuccaggsasu	1332	AUCCUGGAAGCCAGUGUGAAAGA	1542
AD-1557729	csusggaagcdCadGugugaaagauL96	1106	asdTscuuu(C2p)acacdTgdGcuuccaggsgsa	1333	UCCUGGAAGCCAGUGUGAAAGAC	1543
AD-1557730	usgsgaagccdAgdTgugaaagacuL96	1107	asdGsucuu(Tgn)cacadCudGgcuuccasgsg	1334	CCUGGAAGCCAGUGUGAAAGACA	1544
AD-1557731	gsgsaagccadGudGugaaagacauL96	1108	asdTsgucu(Tgn)ucacdAcdTggcuuccsasg	1335	CUGGAAGCCAGUGUGAAAGACAU	1545
AD-1557732	gsasagccagdTgdTgaaagacauuL96	1109	asdAsuguc(Tgn)uucadCadCuggcuuccsa	1336	UGGAAGCCAGUGUGAAAGACAUA	1546
AD-1557733	asasgccagudGudGaaagacauauL96	1110	asdTsaugu(C2p)uuucdAcdAcuggcuuscsc	1337	GGAAGCCAGUGUGAAAGACAUAG	1547
AD-1557734	asgsccagugdTgdAaagacauaguL96	1111	asdCsuaug(Tgn)cuuudCadCacuggcuusc	1338	GAAGCCAGUGUGAAAGACAUAGC	1548
AD-1557735	gscscagugudGadAagacauagcuL96	1112	asdGscuau(G2p)ucuudTcdAcacuggcsusu	1339	AAGCCAGUGUGAAAGACAUAGCU	1549
AD-1557736	cscsagugudAadAgacauagcuuL96	1113	asdAsgcua(Tgn)gucudTudCacacuggscsu	1340	AGCCAGUGUGAAAGACAUAGCUG	554
AD-1557738	asgsugugaadAgdAcauagcugcuL96	1114	asdGscagc(Tgn)augudCudTucacacusgsg	1341	CCAGUGUGAAAGACAUAGCUGCA	1550
AD-1557739	gsusgugaaadGadCauagcugcauL96	1115	asdTsgcag(C2p)uaugdTcdTuucacacsusg	1342	CAGUGUGAAAGACAUAGCUGCAU	1551
AD-1557740	usgsugaaagdAcdAuagcugcauuL96	1116	asdAsugca(G2p)cuaudGudCuuucacascsu	1343	AGUGUGAAAGACAUAGCUGCAUU	1552
AD-1557741	gsusgaaagadCadTagcugcauuuL96	1117	asdAsaugc(Agn)gcuadTgdTcuuucacsasc	1344	GUGUGAAAGACAUAGCUGCAUUG	1553
AD-1557758	asusugaauudCcdAcgcuggguuuL96	1118	asdAsaccc(Agn)gcgudGgdAauucaausgsc	1345	GCAUUGAAUUCCACGCUGGGUUG	556
AD-1557762	asasuuccacdGcdTggguguuauL96	1119	asdTsaaca(Agn)cccadGcdGuggaauuscsa	1346	UGAAUUCCACGCUGGGUUGUUAC	559
AD-1557767	csascgcuggdGudTguuaccgcuuL96	1120	asdAsgcgg(Tgn)aacadAcdCcagcgugsgsa	1347	UCCACGCUGGGUUGUUACCGCUA	562
AD-1557768	ascsgcugggdTudGuuaccgcuauL96	1121	asdTsagcg(G2p)uaacdAadCccagcgusgsg	1348	CCACGCUGGGUUGUUACCGCUAC	1554
AD-1557769	csgscugggudTgdTuaccgcuacuL96	1122	asdGsuagc(G2p)guaadCadAcccagcgsusg	1349	CACGCUGGGUUGUUACCGCUACA	1555
AD-1557770	gscsugggudGudTaccgcuacauL96	1123	asdTsguag(C2p)gguadAcdAacccagcgsgsu	1350	ACGCUGGGUUGUUACCGCUACAG	1556
AD-1557771	csusgggugdTudAccgcuacaguL96	1124	asdCsugua(G2p)cggudAadCaacccagscsg	1351	CGCUGGGUUGUUACCGCUACAGC	563

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Antisense Sequence 5' to 3'	SEQ ID NO	mRNA target sequence 5' to 3'	SEQ ID NO
AD-1557772	usgsgguugudTadCcgcuacagcuL96	1125	asdGscugu(Agn)gcggdTadAcaacccasgsc	1352	GCUGGGUUGUUACCGCUACAGCU	1557
AD-1557773	gsgsguugudAcdCgcuacagcuuL96	1126	asdAsgcug(Tgn)agcgdGudAacaacccsasg	1353	CUGGGUUGUUACCGCUACAGCUA	1558
AD-1557836	csasaacuccdGgdCuggaguggaaL96	1127	asdTsccac(Tgn)ccagdCcdGgaguuugsasg	1354	CUCAAACUCCGGCUGGAGUGGAC	1559
AD-1557866	gsgsgaccgadCudGgccauguauuL96	1128	asdAsuaca(Tgn)ggcccdAgdTcggucccsgsg	1355	CCGGGACCGACUGGCCAUGUAUG	564
AD-1557871	csgsacuggcdCadTguaugacguuL96	1129	asdAscguc(Agn)uacadTgdGccagucggsgsu	1356	ACCGACUGGCCAUGUAUGACGUG	1560
AD-1557881	csusggagaadGadGgcucaucacuL96	1130	asdGsugau(G2p)agccdTcdTucuccagsgsg	1357	CCCUGGGAGAAGAGGCUCAUCACC	1561
AD-1557882	usgsgagaggdAgdGcucaucaccuL96	1131	asdGsguga(Tgn)gagcdCudCuucuccasgsg	1358	CCUGGGAGAAGAGGCUCAUCACCU	1562
AD-1557883	gsgsagaagadGgdCucaucaccuuL96	1132	asdAsggug(Agn)ugagdCcdTcuucuccsasg	1359	CUGGAGAAGAGGCUCAUCACCUC	1563
AD-1557884	gsasgaagaggdGcdTcaucaccucuL96	1133	asdGsaggu(G2p)augadGcdCucuucucscsa	1360	UGGAGAAGAGGCUCAUCACCUCG	1564
AD-1557886	gsasagaggcdTcdAucaccucgguL96	1134	asdCscgag(G2p)ugaudGadGccucuucsusc	1361	GAGAAGAGGCUCAUCACCUCGGU	1565
AD-1557890	asgsgcucaudCadCcucgguguuL96	1135	asdTsacac(C2p)gaggdTgdAuggccucscsu	1362	AGAGGCUCAUCACCUCGGUGUAC	571
AD-1557944	gsasagaaggdGcdCugcacagcuuL96	1136	asdAsgcug(Tgn)gcagdGcdCcuucuucscsa	1363	UGGAAGAAGGGCCUGCACAGCUA	1566
AD-1557945	asasgaaggggdCcdTgcacagcuauL96	1137	asdTsagcu(G2p)ugcadGgdCccuucuuscsc	1364	GGAAGAAGGGCCUGCACAGCUAC	1567
AD-1557948	asasgggccudGcdAcagcuacuauL96	1138	asdTsagua(G2p)cugudGcdAggcccuuscsu	1365	AGAAGGGCCUGCACAGCUACUAC	1568
AD-1557949	asgsggccugdCadCagcuacuacuL96	1139	asdGsuagu(Agn)gcugdTgdCaggcccususc	1366	GAAGGGCCUGCACAGCUACUACG	1569
AD-1557953	cscsugccacadGcdTacuacgaccuL96	1140	asdGsgucg(Tgn)aguadGcdTgugcaggscsc	1367	GGCCUGCACAGCUACUACGACCC	573
AD-1558059	cscsucucugdGadCuacggcuuguL96	1141	asdCsaagc(C2p)guagdTcdCagagaggsgsc	1368	GCCCUCUCUGGACUACGGCUUGG	574
AD-1558061	uscsucuggadCudAcggcuuggcuL96	1142	asdGsccaa(G2p)ccgudAgdTccagagasgsg	1369	CCUCUCUGGACUACGGCUUGGCC	575
AD-1558065	usgsgacuacdGgdCuuggcccucuL96	1143	asdGsaggg(C2p)caagdCcdGuaguccasgsa	1370	UCUGGACUACGGCUUGGGCCCUCU	1570
AD-1558066	gsgsacuacgdGcdTuggcccucuuL96	1144	asdAsgagg(G2p)ccaadGcdCguaguccsasg	1371	CUGGACUACGGCUUGGGCCCUCUG	1571
AD-1558105	gsasggaggcdAgdAaguaugauuuuL96	1145	asdAsauca(Tgn)acuudCudGcccuccusasg	1372	CUGAGGAGGCAGAAGUAUGAUUU	580
AD-1558106	asgsgaggcadGadAguaugauuuuL96	1146	asdAsaauc(Agn)uacudTcdTgccucucscsa	1373	UGAGGAGGCAGAAGUAUGAUUUG	1572
AD-1558113	asgsaaguaudGadTuugccgugcuL96	1147	asdGscacg(G2p)caaadTcdAuacuucusgsc	1374	GCAGAAGUAUGAUUUGCCGUGCA	587
AD-1558114	gsasaguaugdAudTugccgugcauL96	1148	asdTsgcac(G2p)gcaadAudCauacuucsusg	1375	CAGAAGUAUGAUUUGCCGUGCAC	588
AD-1558115	asasguaugadTudTgccgugcacuL96	1149	asdGsugca(C2p)ggcadAadTcauacuuscsu	1376	AGAAGUAUGAUUUGCCGUGCACC	1573
AD-1558116	asgsuaugaudTudGccgugcaccuL96	1150	asdGsgugc(Agn)cggcdAadAucauacususc	1377	GAAGUAUGAUUUGCCGUGCACCC	1574
AD-1558117	gsusaugauudTgdCcgugcacccuL96	1151	asdGsggug(C2p)acggdCadAaucauacsusu	1378	AAGUAUGAUUUGCCGUGCACCCA	1575
AD-1558136	gsgsccagugdGadCgauccagaauL96	1152	asdTsucug(G2p)aucgdTcdCacuggccscsu	1379	AGGGCCAGUGGACGAUCCAGAAC	1576
AD-1558137	gscscaguggdAcdGauccagaacuL96	1153	asdGsuucu(G2p)gaucdGudCcacuggcscsc	1380	GGGCCAGUGGACGAUCCAGAACA	1577
AD-1558138	cscsaguggadCgdAuccagaacauL96	1154	asdTsguuc(Tgn)ggaudCgdTccacuggscsc	1381	GGCCAGUGGACGAUCCAGAACAG	1578
AD-1558139	csasguggacdGadTccagaacagL96	1155	asdCsuguu(C2p)uggadTcdGuccacugsgsc	1382	GCCAGUGGACGAUCCAGAACAGG	589
AD-1558142	usgsgacgaudCcdAgaacaggaguL96	1156	asdCsuccu(G2p)uucudGgdAucguccacscsu	1383	AGUGGACGAUCCAGAACAGGAGG	1579
AD-1558150	cscsagaacadGgdAggcugugugL96	1157	asdCsacac(Agn)gccudCcdTguucuggsasu	1384	AUCCAGAACAGGAGGCUGUGUGG	591
AD-1558152	asgsaacaggdAgdGcugugggcuL96	1158	asdGsccac(Agn)cagcdCudCcuguucusgsg	1385	CCAGAACAGGAGGCUGUGUGGGCU	592

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Antisense Sequence 5' to 3'	SEQ ID NO	mRNA target sequence 5' to 3'	SEQ ID NO
AD-1558211	ascsuucaccdTcdCcagaucuccuL96	1159	asdGsgaga(Tgn)cuggdGadGgugaagususg	1386	CAACUUCACCUCCCAGAUCUCCC	1580
AD-1558215	csascucccdAgdAucucccucauL96	1160	asdTsgagg(G2p)agaudCudGggaggugsasa	1387	UUCACCUCCCAGAUCUCCCUCAC	1581
AD-1558230	usgsugcgggdTgdCacuauggcuuL96	1161	asdAsgcca(Tgn)agugdCadCccgcacascsc	1388	GGUGUGCGGGUGCACUAUGGCUU	593
AD-1558231	gsusgcgggudGcdAcuauggcuuuL96	1162	asdAsagcc(Agn)uagudGcdAcccgcacsasc	1389	GUGUGCGGGUGCACUAUGGCUUG	594
AD-1558232	usgscgggugdCadCuauggcuuguL96	1163	asdCsaagc(C2p)auagdTgdCacccgcascsa	1390	UGUGCGGGUGCACUAUGGCUUGU	1582
AD-1558233	gscsgggugcdAcdTauggcuuguuL96	1164	asdAscaag(C2p)ccauadGudGcacccgcsasc	1391	GUGCGGGUGCACUAUGGCUUGUA	595
AD-1558234	csgsgggugcadCudAuggcuuguauL96	1165	asdTsacaa(G2p)ccaudAgdTgcacccgscsa	1392	UGCGGGUGCACUAUGGCUUGUAC	1583
AD-1558235	gsgsgugcacdTadTggcuuguacuL96	1166	asdGsuaca(Agn)gccadTadGugcacccgsgsc	1393	GCGGGUGCACUAUGGCUUGUACA	596
AD-1558236	gsgsugcacudAudGgcuuguacauL96	1167	asdTsguac(Agn)agccdAudAgugcacccsgg	1394	CGGGUGCACUAUGGCUUGUACAA	1584
AD-1558238	usgscacuaudGgdCuuguacaacuL96	1168	asdGsuugu(Agn)caagdCcdAuagugcascsc	1395	GGUGCACUAUGGCUUGUACAACC	1585
AD-1558239	gscsacuaugdGcdTuguacaaccuL96	1169	asdGsguug(Tgn)acaadGcdCauagugcsasc	1396	GUGCACUAUGGCUUGUACAACCA	1586
AD-1558249	csusgcccugdGadGaguucucuuuL96	1170	asdAsgagg(Agn)acucdTcdCagggcagsgsg	1397	CCCUGCCCUGGAGAGUUCCUCUG	599
AD-1558250	usgscccuggdAgdAguucucuguL96	1171	asdCsagag(G2p)aacudCudCcagggcasgsg	1398	CCUGCCCUGGAGAGUUCCUCUGU	1587
AD-1558288	asascggccudGgdAugagagaaauL96	1172	asdTsuucu(C2p)ucaudCcdAggccguusgsg	1399	CCAACGGCCUGGAUGAGAGAAAC	1588
AD-1558289	ascsggccugdGadTgagagaaacuL96	1173	asdGsuuuc(Tgn)cucadTcdCaggccgususg	1400	CAACGGCCUGGAUGAGAGAAACU	1589
AD-1558290	csgsgccuggdAudGagagaaacuuL96	1174	asdAsguuu(C2p)ucucdAudCcaggccgsusu	1401	AACGGCCUGGAUGAGAGAAACUG	1590
AD-1558292	gscscuggaudGadGagaaacugcuL96	1175	asdGscagu(Tgn)ucucdTcdAuccaggcscsg	1402	CGGCCUGGAUGAGAGAAACUGCG	600
AD-1558293	cscsuggaugdAgdAgaaacugcguL96	1176	asdCsgcag(Tgn)uucudCudCauccaggcscsc	1403	GGCCUGGAUGAGAGAAACUGCGU	1591
AD-1558301	asgsagaaacdTgdCguuugcagauL96	1177	asdTscugc(Agn)aacgdCadGuuucucuscsa	1404	UGAGAGAAACUGCGUUUGCAGAG	1592
AD-1558302	gsasgaaacudGcdGuuugcagaguL96	1178	asdCsucug(C2p)aaacdGcdAguuucucusuc	1405	GAGAGAAACUGCGUUUGCAGAGC	1593
AD-1558308	csusgcguuudGcdAgagccacauuL96	1179	asdAsugug(G2p)cucudGcdAaacgcagsusu	1406	AACUGCGUUUGCAGAGCCACAUU	1594
AD-1558309	usgscguuugdCadGagccacauuuL96	1180	asdAsaugu(G2p)gcucdTgdCaaacgcasgsu	1407	ACUGCGUUUGCAGAGCCACAUUC	1595
AD-1558310	gscsguuugcdAgdAgccacauucuL96	1181	asdGsaaug(Tgn)ggcudCudGcaaacgcsasg	1408	CUGCGUUUGCAGAGCCACAUUCC	1596
AD-1558311	csgsuuugcadGadGccacauuccuL96	1182	asdGsgaau(G2p)uggcdTcdTgcaaacgscsa	1409	UGCGUUUGCAGAGCCACAUUCCA	1597
AD-1558316	gscsagagccdAcdAuuccaguguL96	1183	asdGscacu(G2p)gaaudGudGgcucugcsasa	1410	UUGCAGAGCCACAUUCCAGUGCA	1598
AD-1558419	usgsggacaudTcdAccuuccaguuL96	1184	asdAscugg(Agn)aggudGadAugucccascsa	1411	UGUGGGACAUUCACCUUCCAGUG	606
AD-1558420	gsgsgacauudCadCcuuccaguguL96	1185	asdCsacug(G2p)aaggdTgdAaugucccsasc	1412	GUGGGACAUUCACCUUCCAGUGU	1599
AD-1558421	gsgsacauucdAcdCuuccaguguuL96	1186	asdAscacu(G2p)gaagdGudGaauguccscsa	1413	UGGGACAUUCACCUUCCAGUGUG	607
AD-1558423	ascsauucacdCudTccaguguuuL96	1187	asdTscaca(C2p)uggadAgdGugaauguscsc	1414	GGACAUUCACCUUCCAGUGUGAG	609
AD-1558449	gsasgcugcgdTgdAagaagcccauL96	1188	asdTsgggc(Tgn)ucuudCadCgcagcucscsg	1415	CGGAGCUGCGUGAAGAAGCCCAA	1600
AD-1558450	asgscugcgudGadAgaagcccaauL96	1189	asdTsuggg(C2p)uucudTcdAcgcagcuscsc	1416	GGAGCUGCGUGAAGAAGCCCAAC	1601
AD-1558451	gscsugcgugdAadGaagcccaacuL96	1190	asdGsuugg(G2p)cuucdTudCacgcagcsusc	1417	GAGCUGCGUGAAGAAGCCCAACC	1602
AD-1558452	csusgcgugadAgdAagcccaaccuL96	1191	asdGsguug(G2p)gcuudCudTcacgcagscsu	1418	AGCUGCGUGAAGAAGCCCAACCC	1603
AD-1558453	usgscgugaadGadAgcccaacccuL96	1192	asdGsgguu(G2p)ggcudTcdTucacgcasgsc	1419	GCUGCGUGAAGAAGCCCAACCCG	1604

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Antisense Sequence 5' to 3'	SEQ ID NO	mRNA target sequence 5' to 3'	SEQ ID NO
AD-1558508	asgscacugudGadCuguggccucuL96	1193	asdGsaggc(C2p)acagdTcdAcagugcuscsc	1420	GGAGCACUGUGACUGUGGCCUCC	1605
AD-1558546	csuscgaggdGudGaguggccauuL96	1194	asdAsuggc(C2p)acucdAcdCcucggagsgsa	1421	UCCUCCGAGGGUGAGUGGCCAUG	1606
AD-1558576	asuscgcugadCcdGcuggguguuuL96	1195	asdAsucac(C2p)cagcdGgdTcagcgauusgsa	1422	UCAUCGCUGACCGCUGGGUGAUA	611
AD-1558577	uscsgcugacdCgdCuggguguuauL96	1196	asdTsauca(C2p)ccagdCgdGucagcgasusg	1423	CAUCGCUGACCGCUGGGUGAUAA	1607
AD-1558578	csgscugaccdGcdTgggugauaaauL96	1197	asdTsuauc(Agn)cccadGcdGgucagcgsasu	1424	AUCGCUGACCGCUGGGUGAUAAC	1608
AD-1558579	gscsugaccgdCudGggugauaacuL96	1198	asdGsuuau(C2p)acccdAgdCggucagcsgsa	1425	UCGCUGACCGCUGGGUGAUAACA	1609
AD-1558586	gscsuggggugdAudAacagcugccuL96	1199	asdGsgcag(C2p)uguudAudCacccagcsgsg	1426	CCGCUGGGGUGAUAACAGCUGCCC	1610
AD-1558609	usgscuuuccadGgdAggacagcauuL96	1200	asdAsugcu(G2p)uccudCcdTggaagcasgsu	1427	ACUGCUUCCAGGAGGACAGCAUG	1611
AD-1558610	gscsuuccagdGadGgacagcauguL96	1201	asdCsaugc(Tgn)guccdTcdCuggaagcsasg	1428	CUGCUUCCAGGAGGACAGCAUGG	1612
AD-1558611	csusuccaggdAgdGacagcaugguL96	1202	asdCscaug(C2p)ugucdCudCcuggaagscsa	1429	UGCUUCCAGGAGGACAGCAUGGC	1613
AD-1558650	csgsuguuccdTgdGgcaagguguuL96	1203	asdAscacc(Tgn)ugccdCadGgaacacgsgsu	1430	ACCGUGUUCCUGGGCAAGGUGUG	613
AD-1558657	csusgggcaadGgdTguggcagaauL96	1204	asdTsucug(C2p)cacadCcdTugcccagsgsa	1431	UCCUGGGCAAGGUGUGGCAGAAC	1614
AD-1558658	usgsggcaagdGudGuggcagaacuL96	1205	asdGsuucu(G2p)ccacdAcdCuugcccasgsg	1432	CCUGGGCAAGGUGUGGCAGAACU	1615
AD-1558659	gsgsgcaaggdTgdTggcagaacuuL96	1206	asdAsguuc(Tgn)gccadCadCcuugcccsasg	1433	CUGGGCAAGGUGUGGCAGAACUC	1616
AD-1558660	gsgscaaggudGudGgcagaacucuL96	1207	asdGsaguu(C2p)ugccdAcdAccuugcccsa	1434	UGGGCAAGGUGUGGCAGAACUCG	1617
AD-1558661	gscsaaggugdTgdGcagaacucguL96	1208	asdCsgagu(Tgn)cugcdCadCaccuugcscsc	1435	GGGCAAGGUGUGGCAGAACUCGC	615
AD-1558662	csasaggugudGgdCagaacucgcuL96	1209	asdGscgag(Tgn)ucugdCcdAcaccuugscsc	1436	GGCAAGGUGUGGCAGAACUCGCG	1618
AD-1558683	usgsgccuggdAgdAgguguccuuuL96	1210	asdAsagga(C2p)accudCudCcaggccasgsc	1437	GCUGGCCUGGAGAGGUGUCCUUC	1619
AD-1558684	gsgsccuggadGadGgugcuccucuL96	1211	asdGsaagg(Agn)caccdTcdTccaggccsasg	1438	CUGGCCUGGAGAGGUGUCCUUCA	1620
AD-1558685	gscscuggagdAgdGugcccuucauL96	1212	asdTsgaag(G2p)acacdCudCuccaggcscsa	1439	UGGCCUGGAGAGGUGUCCUUCAA	1621
AD-1558686	cscsuggagadGgdTgucccuucaauL96	1213	asdTsugaa(G2p)gacadCcdTcuccaggscsc	1440	GGCCUGGAGAGGUGUCCUUCAAG	1622
AD-1558687	csusggagagdGudGucccuucaaguL96	1214	asdCsuuga(Agn)ggacdAcdCucuccagsgsc	1441	GCCUGGAGAGGUGUCCUUCAAGG	617
AD-1558691	asgsaggugudCcdTucaaggugauuL96	1215	asdTscacc(Tgn)ugaadGgdAcaccucuscsc	1442	GGAGAGGUGUCCUUCAAGGUGAG	620
AD-1558833	usgsugcagudTgdAucccacaggguL96	1216	asdCscugu(G2p)ggaudCadAcugcacasusc	1443	GAUGUGCAGUUGAUCCCACAGGA	1623
AD-1558835	usgscaguugdAudCcccacaggacuL96	1217	asdGsuccu(G2p)ugggdAudCaacugcascsa	1444	UGUGCAGUUGAUCCCACAGGACC	1624
AD-1558843	asuscccacadGgdAccugugcaguL96	1218	asdCsugca(C2p)aggudCcdTgugggauscsa	1445	UGAUCCCACAGGACCUGUGCAGC	621
AD-1558845	cscscacaggdAcdCugugcagcguL96	1219	asdCsgcug(C2p)acagdGudCcugugggasasu	1446	AUCCCACAGGACCUGUGCAGCGA	1625
AD-1558846	cscsacaggadCcdTgugcagcgauL96	1220	asdTscgcu(G2p)cacadGgdTccugugggsgsa	1447	UCCCACAGGACCUGUGCAGCGAG	1626
AD-1558878	cscsaggugadCgdCcacgcaugcuL96	1221	asdGscaug(C2p)gugggdCgdTcaccuggsusa	1448	UACCAGGUGACGCCACGCAUGCU	1627
AD-1558882	gsusgacgccdAcdGcaugcugugguL96	1222	asdCsacag(C2p)augcdGudGgcgucacscsu	1449	AGGUGACGCCACGCAUGCUGUGU	1628
AD-1558883	usgsacgccadCgdCaugcugugguL96	1223	asdAscaca(G2p)caugdCgdTggcgucascsc	1450	GGUGACGCCACGCAUGCUGUGUG	622
AD-1558885	ascsgccacgdCadTgcugugugcuL96	1224	asdGscaca(C2p)agcadTgdCgugcgcuscsa	1451	UGACGCCACGCAUGCUGUGUGCC	1629
AD-1558905	gsgscuaccgdCadAgggcaagaauL96	1225	asdTsucuu(G2p)cccudTgdCgguagccsgsg	1452	CCGGCUACCGCAAGGGCAAGAAG	1630
AD-1558906	gscsuaccgcdAadGggcaagaaguL96	1226	asdCsuucu(Tgn)gcccdTudGcgguagcscsg	1453	CGGCUACCGCAAGGGCAAGAAGG	624

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Antisense Sequence 5' to 3'	SEQ ID NO	mRNA target sequence 5' to 3'	SEQ ID NO
AD-1558907	csusaccgcadAgdGgcaagaaggguL96	1227	asdCscuuc(Tgn)ugccdCudTgcgguagscsc	1454	GGCUACCGCAAGGGCAAGAAGGA	625
AD-1558961	gsusgcaaggdCadCucaguggcccuL96	1228	asdGsgcca(C2p)ugagdTgdCcuugcacsasc	1455	GUGUGCAAGGCACUCAGUGGCCG	1631
AD-1558992	csusaacuacdTudCggcgucuacuL96	1229	asdGsuaga(C2p)gccgdAadGuaguuagsgsc	1456	GCCUAACUACUUCGGCGUCUACA	1632
AD-1558995	ascsuacuucdGgdCgucuacacccuL96	1230	asdGsgugu(Agn)gacgdCcdGaaguagususa	1457	UAACUACUUCGGCGUCUACACCC	1633
AD-1558996	csusacuucgdGcdGucuacacccuL96	1231	asdGsggug(Tgn)agacdGcdCgaaguagsusu	1458	AACUACUUCGGCGUCUACACCCG	1634
AD-1559004	gscsgucuacdAcdCcgcaucacauL96	1232	asdTsguga(Tgn)gcggdGudGuagacgcscsg	1459	CGGCGUCUACACCCGCAUCACAG	1635
AD-1559005	csgsucuacadCcdCgcaucacaguL96	1233	asdCsugug(Agn)ugcgdGgdTguagacgscsc	1460	GGCGUCUACACCCGCAUCACAGG	1636
AD-1559008	csusacacccdGcdAucacagguguL96	1234	asdCsaccu(G2p)ugaudGcdGgguagsasc	1461	GUCUACACCCGCAUCACAGGUGU	628
AD-1559012	ascscgcaudCadCaggugugauuL96	1235	asdAsucac(Agn)ccugdTgdAugcgggsusgsu	1462	ACACCCGCAUCACAGGUGUGAUC	1637
AD-1559013	cscscgcaucdAcdAggugugaucuL96	1236	asdGsauca(C2p)accudGudGaugcgggsusg	1463	CACCCGCAUCACAGGUGUGAUCA	1638
AD-1559036	usgsgauccadGcdAaguggugacuL96	1237	asdGsucac(C2p)acuudGcdTggauccasgsc	1464	GCUGGAUCCAGCAAGUGGUGACC	1639
AD-1559038	gsasuccagcdAadGuggugaccuuL96	1238	asdAsgguc(Agn)ccacdTudGcuggauscsa	1465	UGGAUCCAGCAAGUGGUGACCUG	1640
AD-1559039	asusccagcadAgdTggugaccuguL96	1239	asdCsaggu(C2p)accadCudTgcuggauscsc	1466	GGAUCCAGCAAGUGGUGACCUGA	1641
AD-1559041	cscsagcaagdTgdGugaccugaguL96	1240	asdCsucag(G2p)ucacdCadCuugcuggsasu	1467	AUCCAGCAAGUGGUGACCUGAGG	1642
AD-1559042	csasgcaagudGgdTgaccugagguL96	1241	asdCscuca(G2p)gucadCcdAcuugcugsgsa	1468	UCCAGCAAGUGGUGACCUGAGGA	1643
AD-1559044	gscsaaguggdTgdAccugaggaauL96	1242	asdTsuccu(C2p)aggudCadCcacuugcsusg	1469	CAGCAAGUGGUGACCUGAGGAAC	1644
AD-1559105	usgsguggcadGgdAgguggcaucuL96	1243	asdGsauge(C2p)accudCcdTgccaccascsa	1470	UGUGGUGGCAGGAGGUGGCAUCU	1645
AD-1559106	gsgsuggcagdGadGuggcaucuuL96	1244	asdAsgaug(C2p)caccdTcdCugccaccsasc	1471	GUGGUGGCAGGAGGUGGCAUCUU	1646
AD-1559107	gsusggcaggdAgdGuggcaucuuuL96	1245	asdAsagau(G2p)ccacdCudCcugccacscsa	1472	UGUGGUGGCAGGAGGUGGCAUCUUG	1647
AD-1559109	gsgscaggagdGudGgcaucuuguuL96	1246	asdAscaag(Agn)ugccdAcdCuccugccsasc	1473	GUGGCAGGAGGUGGCAUCUUGUC	631
AD-1559133	uscsccugaudGudCugcuccagauuL96	1247	asdAscugg(Agn)gcagdAcdAucagggascsg	1474	CGUCCCUGAUGUCUGCUCCAGUG	1648
AD-1559136	csusgaugucdTgdCuccagugauuL96	1248	asdAsucac(Tgn)ggagdCadGacaucagsgsg	1475	CCCUGAUGUCUGCUCCAGUGAUG	1649
AD-1559147	uscscagugadTgdGcaggaggauuL96	1249	asdAsuccu(C2p)cugcdCadTcacuggasgsc	1476	GCUCCAGUGAUGGCAGGAGGAUG	1650
AD-1559233	gsgscucagcdAgdCaagaaugcuuL96	1250	asdAsgcau(Tgn)cuugdCudGcugagccsasc	1477	GUGGCUCAGCAGCAAGAAUGCUG	636
AD-1559318	csusaacuugdGgdAucuggaauuL96	1251	asdAsuucc(C2p)agaudCcdCaaguuagsasc	1478	GUCUAACUUGGGAUCUGGGAAUG	1651
AD-1559323	ususggaaucdTgdGgaauggaaguL96	1252	asdCsuucc(Agn)uuccdCadGaucccaasgsu	1479	ACUUGGGAUCUGGGAAUGGAAGG	642
AD-1559431	gsusgagcucdAgdCugcccuuuguL96	1253	asdCsaaag(G2p)gcagdCudGagcucacscsu	1480	AGGUGAGCUCAGCUGCCCUUUGG	1652
AD-1559436	csuscagcugdCcdCuuuggaauauL96	1254	asdTsauuc(C2p)aaagdGgdCagcugagscsu	1481	AGCUCAGCUGCCCUUUGGAAUAA	1653
AD-1559437	uscsagcugcdCcdTuuggaauaauL96	1255	asdTsuauu(C2p)caaadGgdGcagcugagsgsc	1482	GCUCAGCUGCCCUUUGGAAUAAA	1654
AD-1559438	csasgcugccdCudTuggaauaaauL96	1256	asdTsuuau(Tgn)ccaadAgdGgcagcugsasg	1483	CUCAGCUGCCCUUUGGAAUAAAG	1655
AD-1559441	csusgcccuudTgdGaauaaagcuuL96	1257	asdAsgcuu(Tgn)auucdCadAagggcagscsu	1484	AGCUGCCCUUUGGAAUAAAGCUG	648
AD-1559443	gscscccuugdGadAuaaagcugcuL96	1258	asdGscagc(Tgn)uuaudTcdCaaagggcsasg	1485	CUGCCCUUUGGAAUAAAGCUGCC	1656
AD-1559444	cscscuuuggdAadTaaagcugccuL96	1259	asdGsgcag(C2p)uuuadTudCcaaagggcsa	1486	UGCCCUUUGGAAUAAAGCUGCCU	1657
AD-1559445	cscsuuuggadAudAaagcugccuuL96	1260	asdAsggca(G2p)cuuudAudTccaaagggsgsc	1487	GCCCUUUGGAAUAAAGCUGCCUG	1658

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Antisense Sequence 5' to 3'	SEQ ID NO	mRNA target sequence 5' to 3'	SEQ ID NO
AD-1559447	ususuggaaudAadAgcugccugauL96	1261	asdTscagg(C2p)agcudTudAuuccaaasgsg	1488	CCUUUGGAAUAAAGCUGCCUGAU	1659
AD-1559448	ususggaauadAadGcugccugauuL96	1262	asdAsucag(G2p)cagcdTudTauuccaasasg	1489	CUUUGGAAUAAAGCUGCCUGAUC	1660
AD-1559449	usgsgaauaadAgdCugccugaucuL96	1263	asdGsauca(G2p)gcagdCudTuauuccasasa	1490	UUUGGAAUAAAGCUGCCUGAUCC	1661

Table 6. Unmofidied Sense and Antisense Strand Sequences of TMPRSS6 dsRNA Agents

Duplex Name	Sense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:
AD-1570929.1	CGGAGGUGAUGGCGAGGAAGU	189-209	650	ACUUCCTCGCCAUCACCUCCGUC	187-209	848
AD-1570930.1	CCUGUGAGGACUCCAAGAGAU	233-253	654	AUCUCUTGGAGUCCUCACAGGCC	231-253	1726
AD-1570931.1	CUGUGAGGACUCCAAGAGAAU	234-254	1662	AUUCTCTUGGAGUCCUCACAGGC	232-254	1727
AD-1570932.1	CUCUGGUAUUUCCUAGGGUAU	331-351	28	AUACCCTAGGAAAUACCAGAGUA	329-351	1728
AD-1570933.1	GGUAUUUCCUAGGGUACAAGU	335-355	660	ACUUGUACCCUAGGAAAUACCAG	333-355	858
AD-1570934.1	GUAUUUCCUAGGGUACAAGGU	336-356	1663	ACCUTGTACCCUAGGAAAUACCA	334-356	1729
AD-1570935.1	GGUCAGCCAGGUGUACUCAGU	366-386	31	ACUGAGTACACCUGGCUGACCAU	364-386	157
AD-1570936.1	UCAGCCAGGUGUACUCAGGCU	368-388	665	AGCCTGAGUACACCUGGCUGACC	366-388	1730
AD-1570937.1	AGCCAGGUGUACUCAGGCAGU	370-390	32	ACUGCCTGAGUACACCUGGCUGA	368-390	158
AD-1570938.1	CACUUCUCCCAGGAUCUUACU	409-429	670	AGUAAGAUCCUGGGAGAAGUGGC	407-429	867
AD-1570939.1	UCUCCCAGGAUCUUACCCGCU	413-433	1664	AGCGGGTAAGAUCCUGGGAGAAG	411-433	1731
AD-1570940.1	GCCUUCCGCAGUGAAACCGCU	445-465	36	AGCGGUTUCACUGCGGAAGGCAC	443-465	1732
AD-1570941.1	CCUUCCGCAGUGAAACCGCCU	446-466	1665	AGGCGGTUUCACUGCGGAAGGCA	444-466	872
AD-1570942.1	GCAGUGAAACCGCCAAAGCCU	452-472	679	AGGCTUTGGCGGUUUCACUGCGG	450-472	1733
AD-1570943.1	CAGUGAAACCGCCAAAGCCCU	453-473	680	AGGGCUTUGGCGGUUUCACUGCG	451-473	1734
AD-1570944.1	AGUGAAACCGCCAAAGCCCAU	454-474	681	AUGGGCTUUGGCGGUUUCACUGC	452-474	1735
AD-1570945.1	CGCCAAAGCCCAGAAGAUGCU	462-482	682	AGCATCTUCUGGGCUUUGGCGGU	460-482	1736
AD-1570946.1	AGCCCAGAAGAUGCUCAAGGU	468-488	684	ACCUTGAGCAUCUUCUGGGCUUU	466-488	1737
AD-1570947.1	AGCACCCGCCUGGGAACUUAU	499-519	1666	AUAAGUTCCCAGGCGGGUGCUGG	497-519	1738

Duplex Name	Sense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:
AD-1570948.1	CAACUCCAGCUCCGUCUAUUU	522-542	37	AAAUAGACGGAGCUGGAGUUGUA	520-542	163
AD-1570949.1	UCACCUGCUUCUUCUGGUUCU	560-580	40	AGAACCAGAAGAAGCAGGUGAGG	558-580	166
AD-1570950.1	CCUGCUUCUUCUGGUUCAUUU	563-583	42	AAAUGAACCAGAAGAAGCAGGUG	561-583	168
AD-1570951.1	CUGCUUCUUCUGGUUCAUUCU	564-584	1667	AGAATGAACCAGAAGAAGCAGGU	562-584	1739
AD-1570952.1	CUUCUUCUGGUUCAUUCUCCU	567-587	45	AGGAGAAUGAACCAGAAGAAGCA	565-587	171
AD-1570953.1	UUCUUCUGGUUCAUUCUCCAU	568-588	46	AUGGAGAAUGAACCAGAAGAAGC	566-588	1740
AD-1570954.1	CUUCUGGUUCAUUCUCCAAAU	570-590	1668	AUUUGGAGAAUGAACCAGAAGAA	568-590	1741
AD-1570955.1	CUGGUUCAUUCUCCAAAUCCU	573-593	47	AGGATUTGGAGAAUGAACCAGAA	571-593	173
AD-1570956.1	GCUGCUGUCCACAGUCAACAU	651-671	703	AUGUTGACUGUGGACAGCAGCUC	649-671	1742
AD-1570957.1	GCUGUCCACAGUCAACAGCUU	654-674	704	AAGCTGTTUGACUGUGGACAGCAG	652-674	1743
AD-1570958.1	UGUCCACAGUCAACAGCUCGU	656-676	706	ACGAGCTGUUGACUGUGGACAGC	654-676	1744
AD-1570959.1	GGCCGAGUACGAAGUGGACCU	693-713	708	AGGUCCACUUCGUACUCGGCCCU	691-713	902
AD-1570960.1	AUCCUGGAAGCCAGUGUGAAU	727-747	709	AUUCACACUGGCUUCCAGGAUCA	725-747	1745
AD-1570961.1	CCUGGAAGCCAGUGUGAAAGU	729-749	711	ACUUTCACACUGGCUUCCAGGAU	727-749	1746
AD-1570962.1	UGGAAGCCAGUGUGAAAGACU	731-751	1669	AGUCTUTCACACUGGCUUCCAGG	729-751	1747
AD-1570963.1	GGAAGCCAGUGUGAAAGACAU	732-752	714	AUGUCUTUCACACUGGCUUCCAG	730-752	1748
AD-1570964.1	GAAGCCAGUGUGAAAGACAUU	733-753	1670	AAUGTCTUUCACACUGGCUUCCA	731-753	1749
AD-1570965.1	AGCCAGUGUGAAAGACAUAGU	735-755	1671	ACUATGTCUUUCACACUGGCUUC	733-755	1750
AD-1570966.1	CCAGUGUGAAAGACAUAGCUU	737-757	50	AAGCTATGUCUUUCACACUGGCU	735-757	1751
AD-1570967.1	AGUGUGAAAGACAUAGCUGCU	739-759	719	AGCAGCTAUGUCUUUCACACUGG	737-759	1752
AD-1570968.1	GUGAAAGACAUAGCUGCAUUU	742-762	1672	AAAUGCAGCUAUGUCUUUCACAC	740-762	1753
AD-1570969.1	AUUGAAUUCCACGCUGGGUUU	759-779	52	AAACCCAGCGUGGAAUUCAAUGC	757-779	178
AD-1570970.1	AAUUCCACGCUGGGUUGUUAU	763-783	55	AUAACAACCCAGCGUGGAAUUCA	761-783	1754
AD-1570971.1	CACGCUGGGUUGUUACCGCUU	768-788	58	AAGCGGTAACAACCCAGCGUGGA	766-788	184
AD-1570972.1	UGGGUUGUUACCGCUACAGCU	773-793	1673	AGCUGUAGCGGUAACAACCCAGC	771-793	1755
AD-1570973.1	GGGUUGUUACCGCUACAGCUU	774-794	730	AAGCTGTAGCGGUAACAACCCAG	772-794	1756

Duplex Name	Sense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:
AD-1570974.1	CAAACUCCGGCUGGAGUGGAU	888-908	731	AUCCACTCCAGCCGGAGUUUGAG	886-908	1757
AD-1570975.1	GGGACCGACUGGCCAUGUAUU	923-943	60	AAUACATGGCCAGUCGGUCCCGG	921-943	186
AD-1570976.1	CGACUGGCCAUGUAUGACGUU	928-948	1674	AACGTCAUACAUGGCCAGUCGGU	926-948	1758
AD-1570977.1	UGGAGAAGAGGCUCAUCACCU	959-979	734	AGGUGATGAGCCUCUUCUCCAGG	957-979	928
AD-1570978.1	GGAGAAGAGGCUCAUCACCUU	960-980	735	AAGGTGAUGAGCCUCUUCUCCAG	958-980	1759
AD-1570979.1	GAAGAAGGGCCUGCACAGCUU	1053-1073	738	AAGCTGTGCAGGCCCUUCUUCCA	1051-1073	1760
AD-1570980.1	AGGGCCUGCACAGCUACUACU	1058-1078	741	AGUAGUAGCUGUGCAGGCCCUUC	1056-1078	1761
AD-1570981.1	CCUGCACAGCUACUACGACCU	1062-1082	69	AGGUCGTAGUAGCUGUGCAGGCC	1060-1082	195
AD-1570982.1	GAGGAGGCAGAAGUAUGAUUU	1281-1301	76	AAAUCATACUUCUGCCUCCUCAG	1279-1301	202
AD-1570983.1	AGGAGGCAGAAGUAUGAUUUU	1282-1302	745	AAAATCAUACUUCUGCCUCCUCA	1280-1302	1762
AD-1570984.1	AGUAUGAUUUGCCGUGCACCU	1292-1312	1675	AGGUGCACGGCAAAUCAUACUUC	1290-1312	942
AD-1570985.1	CCAGUGGACGAUCCAGAACAU	1317-1337	753	AUGUTCTGGAUCGUCCACUGGCC	1315-1337	1763
AD-1570986.1	CCAGAACAGGAGGCUGUGUGU	1329-1349	87	ACACACAGCCUCCUGUUCUGGAU	1327-1349	213
AD-1570987.1	AGAACAGGAGGCUGUGUGGCU	1331-1351	88	AGCCACACAGCCUCCUGUUCUGG	1329-1351	214
AD-1570988.1	ACUUCACCUCCCAGAUCUCCU	1415-1435	1676	AGGAGATCUGGGAGGUGAAGUUG	1413-1435	950
AD-1570989.1	UGUGCGGUGCACUAUGGCUU	1449-1469	89	AAGCCATAGUGCACCCGCACACC	1447-1469	215
AD-1570990.1	GUGCGGUGCACUAUGGCUUU	1450-1470	90	AAAGCCAUAGUGCACCCGCACAC	1448-1470	216
AD-1570991.1	GGGUGCACUAUGGCUUGUACU	1454-1474	92	AGUACAAGCCAUAGUGCACCCGC	1452-1474	1764
AD-1570992.1	GGUGCACUAUGGCUUGUACAU	1455-1475	763	AUGUACAAGCCAUAGUGCACCCG	1453-1475	1765
AD-1570993.1	UGCACUAUGGCUUGUACAACU	1457-1477	764	AGUUGUACAAGCCAUAGUGCACC	1455-1477	955
AD-1570994.1	GCACUAUGGCUUGUACAACCU	1458-1478	1677	AGGUTGTACAAGCCAUAGUGCAC	1456-1478	1766
AD-1570995.1	CUGCCCUGGAGAGUUCCUCUU	1488-1508	95	AAGAGGAACUCUCCAGGGCAGGG	1486-1508	1767
AD-1570996.1	ACGGCCUGGAUGAGAGAAACU	1562-1582	1678	AGUUTCTCUCAUCCAGGCCGUUG	1560-1582	1768
AD-1570997.1	GCCUGGAUGAGAGAAACUGCU	1565-1585	96	AGCAGUTUCUCUCAUCCAGGCCG	1563-1585	1769
AD-1570998.1	CCUGGAUGAGAGAAACUGCGU	1566-1586	770	ACGCAGTUUCUCUCAUCCAGGCC	1564-1586	961
AD-1570999.1	AGAGAAACUGCGUUUGCAGAU	1574-1594	1679	AUCUGCAAACGCAGUUUCUCUCA	1572-1594	1770

Duplex Name	Sense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:
AD-1571000.1	GCGUUUGCAGAGCCACAUUCU	1583-1603	775	AGAATGTGGCUCUGCAAACGCAG	1581-1603	1771
AD-1571001.1	UGGGACAUUCACCUUCCAGUU	1710-1730	102	AACUGGAAGGUGAAUGUCCCACA	1708-1730	228
AD-1571002.1	GAGCUGCGUGAAGAAGCCCAU	1740-1760	1680	AUGGGCTUCUUCACGCAGCUCCG	1738-1760	1772
AD-1571003.1	CGCUGACCGCUGGGUGAUAAU	1938-1958	1681	AUUATCACCCAGCGGUCAGCGAU	1936-1958	1773
AD-1571004.1	GCUUCCAGGAGGACAGCAUGU	1970-1990	793	ACAUGCTGUCCUCCUGGAAGCAG	1968-1990	1774
AD-1571005.1	CGUGUUCCUGGGCAAGGUGUU	2010-2030	109	AACACCTUGCCCAGGAACACGGU	2008-2030	235
AD-1571006.1	GGGCAAGGUGUGGCAGAACUU	2019-2039	1682	AAGUTCTGCCACACCUUGCCCAG	2017-2039	1775
AD-1571007.1	GCAAGGUGUGGCAGAACUCGU	2021-2041	111	ACGAGUTCUGCCACACCUUGCCC	2019-2041	237
AD-1571008.1	CAAGGUGUGGCAGAACUCGCU	2022-2042	801	AGCGAGTUCUGCCACACCUUGCC	2020-2042	989
AD-1571009.1	GGCCUGGAGAGGUGUCCUUCU	2045-2065	803	AGAAGGACACCUCUCCAGGCCAG	2043-2065	1776
AD-1571010.1	CUGGAGAGGUGUCCUUCAAGU	2048-2068	113	ACUUGAAGGACACCUCUCCAGGC	2046-2068	239
AD-1571011.1	AGAGGUGUCCUUCAAGGUGAU	2052-2072	116	AUCACCTUGAAGGACACCUCUCC	2050-2072	1777
AD-1571012.1	GCUACCGCAAGGGCAAGAAGU	2363-2383	120	ACUUCUTGCCCUUGCGGUAGCCG	2361-2383	1778
AD-1571013.1	CUACCGCAAGGGCAAGAAGGU	2364-2384	121	ACCUTCTUGCCCUUGCGGUAGCC	2362-2384	247
AD-1571014.1	ACUACUUCGGCGUCUACACCU	2489-2509	817	AGGUGUAGACGCCGAAGUAGUUA	2487-2509	1007
AD-1571015.1	CUACUUCGGCGUCUACACCCU	2490-2510	818	AGGGTGTAGACGCCGAAGUAGUU	2488-2510	1779
AD-1571016.1	GCGUCUACACCCGCAUCACAU	2498-2518	819	AUGUGATGCGGUGUAGACGCCG	2496-2518	1780
AD-1571017.1	CGUCUACACCCGCAUCACAGU	2499-2519	820	ACUGTGAUGCGGUGUAGACGCC	2497-2519	1781
AD-1571018.1	ACCCGCAUCACAGGUGUGAUU	2506-2526	821	AAUCACACCUGUGAUGCGGGUGU	2504-2526	1782
AD-1571019.1	GAUCCAGCAAGUGGUGACCUU	2532-2552	824	AAGGTCACCACUUGCUGGAUCCA	2530-2552	1783
AD-1571020.1	GGCAGGAGGUGGCAUCUUGUU	2671-2691	127	AACAAGAUGCCACCUCCUGCCAC	2669-2691	253
AD-1571021.1	UCCCUGAUGUCUGCUCCAGUU	2695-2715	832	AACUGGAGCAGACAUCAGGGACG	2693-2715	1022
AD-1571022.1	CUGAUGUCUGCUCCAGUGAUU	2698-2718	1683	AAUCACTGGAGCAGACAUCAGGG	2696-2718	1023
AD-1571023.1	GGCUCAGCAAGAAUGCUU	2853-2873	132	AAGCAUTCUUGCUGCUGAGCCAC	2851-2873	258
AD-1571024.1	UUGGGAUCUGGGAAUGGAAGU	2983-3003	138	ACUUCCAUUCCCAGAUCCCAAGU	2981-3003	264
AD-1571025.1	CAGCUGCCCUUUGGAAUAAAU	3164-3184	1684	AUUUAUTCCAAAGGGCAGCUGAG	3162-3184	1784

Duplex Name	Sense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:
AD-1571026.1	CUGCCCUUUGGAAUAAAGCUU	3167-3187	144	AAGCUUUAUUCCAAAGGGCAGCU	3165-3187	270
AD-1571027.1	GCCCUUUGGAAUAAAGCUGCU	3169-3189	842	AGCAGCUUUAUUCCAAAGGGCAG	3167-3189	1785
AD-1571028.1	CCUCACCUGCUUCUUCUGGUU	558-578	1685	AACCAGAAGAAGCAGGUGAGGGG	556-578	1786
AD-1571029.1	CCUCACCUGCUUCUUCUGGUU	558-578	1685	AACCAGAAGAAGCAGGUGAGGCU	556-578	1787
AD-1571030.1	UCACCUGCUUCUUCUGGUU	560-578	1686	AACCAGAAGAAGCAGGUGAGG	558-578	1788
AD-1571031.1	UCACCUGCUUCUUCUGGUU	560-578	1686	AACCAGAAGAAGCAGGUGACU	558-578	1789
AD-1571032.1	ACCUGCUUCUUCUGGUU	562-578	1687	AACCAGAAGAAGCAGGUGA	560-578	1790
AD-1571033.1	UCACCUGCUUCUUCUGGUU	560-578	1686	AACCAGAAGAAGCAGGUGA	560-578	1790
AD-1571034.1	GGAGGUGAUGGCGAGGAAGCU	190-210	1688	AGCUUCCUCGCCAUCACCUCCGU	188-210	1791
AD-1571035.1	AAGGCCUGUGAGGACUCCAAU	229-249	1689	AUUGGAGUCCUCACAGGCCUUGA	227-249	1792
AD-1571036.1	GGCCUGUGAGGACUCCAAGAU	231-251	653	AUCUUGGAGUCCUCACAGGCCUU	229-251	1793
AD-1571037.1	GCCUGUGAGGACUCCAAGAGU	232-252	20	ACUCUUGGAGUCCUCACAGGCCU	230-252	146
AD-1571038.1	CUACUCUGGUAUUUCCUAGGU	328-348	25	ACCUAGGAAAUACCAGAGUAGCA	326-348	151
AD-1571039.1	UCUGGUAUUUCCUAGGGUACU	332-352	29	AGUACCCUAGGAAAUACCAGAGU	330-352	155
AD-1571040.1	CUGGUAUUUCCUAGGGUACAU	333-353	1690	AUGUACCCUAGGAAAUACCAGAG	331-353	1794
AD-1571041.1	UGGUAUUUCCUAGGGUACAAU	334-354	1691	AUUGUACCCUAGGAAAUACCAGA	332-354	1795
AD-1571042.1	CUAGGGUACAAGGCGGAGGUU	343-363	662	AACCUCCGCCUUGUACCCUAGGA	341-363	1796
AD-1571043.1	AUGGUCAGCCAGGUGUACUCU	364-384	663	AGAGUACACCUGGCUGACCAUCA	362-384	1797
AD-1571044.1	GUCAGCCAGGUGUACUCAGGU	367-387	1692	ACCUGAGUACACCUGGCUGACCA	365-387	1798
AD-1571045.1	CAGCCAGGUGUACUCAGGCAU	369-389	1693	AUGCCUGAGUACACCUGGCUGAC	367-389	1799
AD-1571046.1	CUCAAUCGCCACUUCUCCCAU	400-420	667	AUGGGAGAAGUGGCGAUUGAGUA	398-420	1800
AD-1571047.1	CGCCACUUCUCCCAGGAUCUU	406-426	668	AAGATCCUGGGAGAAGUGGCGAU	404-426	1801
AD-1571048.1	GCCACUUCUCCCAGGAUCUUU	407-427	33	AAAGAUCCUGGGAGAAGUGGCGA	405-427	159
AD-1571050.1	UCCCAGGAUCUUACCCGCCGU	415-435	35	ACGGCGGGUAAGAUCCUGGGAGA	413-435	161
AD-1571051.1	UAGUGCCUUCCGCAGUGAAAU	441-461	1694	AUUUCACUGCGGAAGGCACUAGA	439-461	1802
AD-1571052.1	CUUCCGCAGUGAAACCGCCAU	447-467	676	AUGGCGGUUUCACUGCGGAAGGC	445-467	1803

Duplex Name	Sense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:
AD-1571053.1	CCGCAGUGAAACCGCCAAAGU	450-470	677	ACUUUGGCGGUUUCACUGCGGAA	448-470	1804
AD-1571054.1	CGCAGUGAAACCGCCAAAGCU	451-471	678	AGCUUUGGCGGUUUCACUGCGGA	449-471	1805
AD-1571055.1	GCCAAAGCCCAGAAGAUGCUU	463-483	683	AAGCAUCUUCUGGGCUUUGGCGG	461-483	880
AD-1571056.1	CAGCACCCGCCUGGGAACUUU	498-518	685	AAAGUUCCCAGGCGGGUGCUGGU	496-518	1806
AD-1571057.1	ACAACUCCAGCUCCGUCUAUU	521-541	687	AAUAGACGGAGCUGGAGUUGUAG	519-541	884
AD-1571058.1	CACCUGCUUCUUCUGGUUCAU	561-581	1695	AUGAACCAGAAGAAGCAGGUGAG	559-581	1807
AD-1571059.1	UGCUUCUUCUGGUUCAUUCUU	565-585	43	AAGAAUGAACCAGAAGAAGCAGG	563-585	169
AD-1571060.1	UCUUCUGGUUCAUUCUCCAAU	569-589	1696	AUUGGAGAAUGAACCAGAAGAAG	567-589	1808
AD-1571061.1	UUCUGGUUCAUUCUCCAAAUU	571-591	1697	AAUUUGGAGAAUGAACCAGAAGA	569-591	1809
AD-1571062.1	UCUGGUUCAUUCUCCAAAUCU	572-592	1698	AGAUUUGGAGAAUGAACCAGAAG	570-592	1810
AD-1571063.1	GUGGAGGAGCUGCUGUCCACU	643-663	1699	AGUGGACAGCAGCUCCUCCACCA	641-663	1811
AD-1571064.1	GAGGAGCUGCUGUCCACAGUU	646-666	1700	AACUGUGGACAGCAGCUCCUCCA	644-666	894
AD-1571065.1	AGCUGCUGUCCACAGUCAACU	650-670	1701	AGUUGACUGUGGACAGCAGCUCC	648-670	895
AD-1571066.1	CUGUCCACAGUCAACAGCUCU	655-675	1702	AGAGCUGUUGACUGUGGACAGCA	653-675	898
AD-1571067.1	ACAGGGCCGAGUACGAAGUGU	689-709	48	ACACUCUCGUACUCGGCCCUGUAG	687-709	1812
AD-1571068.1	GGGCCGAGUACGAAGUGGACU	692-712	1703	AGUCCACUUCGUACUCGGCCCUG	690-712	1813
AD-1571069.1	UCCUGGAAGCCAGUGUGAAAU	728-748	710	AUUUCACACUGGCUUCCAGGAUC	726-748	1814
AD-1571070.1	CUGGAAGCCAGUGUGAAAGAU	730-750	712	AUCUUUCACACUGGCUUCCAGGA	728-750	1815
AD-1571071.1	AAGCCAGUGUGAAAGACAUAU	734-754	716	AUAUGUCUUUCACACUGGCUUCC	732-754	1816
AD-1571072.1	GCCAGUGUGAAAGACAUAGCU	736-756	718	AGCUAUGUCUUUCACACUGGCUU	734-756	1817
AD-1571074.1	UGUGAAAGACAUAGCUGCAUU	741-761	721	AAUGCAGCUAUGUCUUUCACACU	739-761	916
AD-1571075.1	ACGCUGGGUUGUUACCGCUAU	769-789	1704	AUAGCGGUAACAACCCAGCGUGG	767-789	1818
AD-1571076.1	CGCUGGGUUGUUACCGCUACU	770-790	1705	AGUAGCGGUAACAACCCAGCGUG	768-790	919
AD-1571077.1	GCUGGGUUGUUACCGCUACAU	771-791	1706	AUGUAGCGGUAACAACCCAGCGU	769-791	1819
AD-1571078.1	CUGGGUUGUUACCGCUACAGU	772-792	59	ACUGUAGCGGUAACAACCCAGCG	770-792	185
AD-1571079.1	CUGGAGAAGAGGCUCAUCACU	958-978	733	AGUGAUGAGCCUCUUCUCCAGGG	956-978	1820

Duplex Name	Sense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:
AD-1571080.1	GAGAAGAGGCUCAUCACCUCU	961-981	1707	AGAGGUGAUGAGCCUCUUCUCCA	959-981	930
AD-1571081.1	GAAGAGGCUCAUCACCUCGGU	963-983	1708	ACCGAGGUGAUGAGCCUCUUCUC	961-983	931
AD-1571082.1	AGGCUCAUCACCUCGGUGUAU	967-987	67	AUACACCGAGGUGAUGAGCCUCU	965-987	1821
AD-1571083.1	AAGAAGGCCCUGCACAGCUAU	1054-1074	1709	AUAGCUGUGCAGGCCCUUCUUCC	1052-1074	1822
AD-1571084.1	AAGGGCCUGCACAGCUACUAU	1057-1077	740	AUAGTAGCUGUGCAGGCCCUUCU	1055-1077	1823
AD-1571085.1	CCUCUCUGGACUACGGCUUGU	1235-1255	70	ACAAGCCGUAGUCCAGAGAGGGC	1233-1255	1824
AD-1571086.1	UCUCUGGACUACGGCUUGGCU	1237-1257	71	AGCCAAGCCGUAGUCCAGAGAGG	1235-1257	197
AD-1571087.1	UGGACUACGGCUUGGCCCUCU	1241-1261	743	AGAGGGCCAAGCCGUAGUCCAGA	1239-1261	938
AD-1571088.1	GGACUACGGCUUGGCCCUCUU	1242-1262	1710	AAGAGGGCCAAGCCGUAGUCCAG	1240-1262	939
AD-1571089.1	AGAAGUAUGAUUUGCCGUGCU	1289-1309	83	AGCACGGCAAAUCAUACUUCUGC	1287-1309	1825
AD-1571090.1	GAAGUAUGAUUUGCCGUGCAU	1290-1310	84	AUGCACGGCAAAUCAUACUUCUG	1288-1310	1826
AD-1571091.1	AAGUAUGAUUUGCCGUGCACU	1291-1311	1711	AGUGCACGGCAAAUCAUACUUCU	1289-1311	1827
AD-1571092.1	GUAUGAUUUGCCGUGCACCCU	1293-1313	1712	AGGGTGCACGGCAAAUCAUACUU	1291-1313	1828
AD-1571093.1	GGCCAGUGGACGAUCCAGAAU	1315-1335	751	AUUCTGGAUCGUCCACUGGCCCU	1313-1335	1829
AD-1571094.1	GCCAGUGGACGAUCCAGAACU	1316-1336	752	AGUUCUGGAUCGUCCACUGGCCC	1314-1336	945
AD-1571096.1	UGGACGAUCCAGAACAGGAGU	1321-1341	755	ACUCCUGUUCUGGAUCGUCCACU	1319-1341	948
AD-1571097.1	CACCUCCCAGAUCUCCCUCAU	1419-1439	757	AUGAGGGAGAUCUGGGAGGUGAA	1417-1439	1830
AD-1571098.1	UGCGGGUGCACUAUGGCUUGU	1451-1471	759	ACAAGCCAUAGUGCACCCGCACA	1449-1471	1831
AD-1571099.1	GCGGGUGCACUAUGGCUUGUU	1452-1472	91	AACAAGCCAUAGUGCACCCGCAC	1450-1472	217
AD-1571100.1	CGGGUGCACUAUGGCUUGUAU	1453-1473	761	AUACAAGCCAUAGUGCACCCGCA	1451-1473	1832
AD-1571102.1	AACGGCCUGGAUGAGAGAAAU	1561-1581	767	AUUUCUCUCCAUCCAGGCCGUUGG	1559-1581	1833
AD-1571103.1	CGGCCUGGAUGAGAGAAACUU	1563-1583	769	AAGUTUCUCUCAUCCAGGCCGUU	1561-1583	1834
AD-1571104.1	GAGAAACUGCGUUUGCAGAGU	1575-1595	772	ACUCTGCAAACGCAGUUUCUCUC	1573-1595	1835
AD-1571105.1	CUGCGUUUGCAGAGCCACAUU	1581-1601	773	AAUGTGGCUCUGCAAACGCAGUU	1579-1601	1836
AD-1571106.1	UGCGUUUGCAGAGCCACAUUU	1582-1602	774	AAAUGUGGGCUCUGCAAACGCAGU	1580-1602	1837
AD-1571107.1	CGUUUGCAGAGCCACAUUCCU	1584-1604	776	AGGAAUGUGGGCUCUGCAAACGCA	1582-1604	1838

Duplex Name	Sense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:
AD-1571108.1	GCAGAGCCACAUUCCAGUGCU	1589-1609	777	AGCACUGGAAUGUGGCUCUGCAA	1587-1609	968
AD-1571109.1	GGGACAUUCACCUUCCAGUGU	1711-1731	779	ACACTGGAAGGUGAAUGUCCCAC	1709-1731	1839
AD-1571110.1	GGACAUUCACCUUCCAGUGUU	1712-1732	103	AACACUGGAAGGUGAAUGUCCCA	1710-1732	229
AD-1571111.1	ACAUUCACCUUCCAGUGUGAU	1714-1734	105	AUCACACUGGAAGGUGAAUGUCC	1712-1734	1840
AD-1571112.1	AGCUGCGUGAAGAAGCCCAAU	1741-1761	782	AUUGGGCUUCUUCACGCAGCUCC	1739-1761	1841
AD-1571113.1	GCUGCGUGAAGAAGCCCAACU	1742-1762	783	AGUUGGGCUUCUUCACGCAGCUC	1740-1762	1842
AD-1571114.1	CUGCGUGAAGAAGCCCAACCU	1743-1763	784	AGGUTGGGCUUCUUCACGCAGCU	1741-1763	1843
AD-1571115.1	UGCGUGAAGAAGCCCAACCCU	1744-1764	785	AGGGTUGGGCUUCUUCACGCAGC	1742-1764	1844
AD-1571116.1	AGCACUGUGACUGUGGCCUCU	1808-1828	786	AGAGGCCACAGUCACAGUGCUCC	1806-1828	1845
AD-1571117.1	CUCCGAGGGUGAGUGGCCAUU	1866-1886	787	AAUGGCCACUCACCCUCGGAGGA	1864-1886	976
AD-1571118.1	AUCGCUGACCGCUGGGUGAUU	1936-1956	107	AAUCACCCAGCGGUCAGCGAUGA	1934-1956	233
AD-1571119.1	UCGCUGACCGCUGGGUGAUAU	1937-1957	788	AUAUCACCCAGCGGUCAGCGAUG	1935-1957	1846
AD-1571120.1	GCUGACCGCUGGGUGAUAACU	1939-1959	790	AGUUAUCACCCAGCGGUCAGCGA	1937-1959	980
AD-1571121.1	GCUGGGUGAUAACAGCUGCCU	1946-1966	791	AGGCAGCUGUUAUCACCCAGCGG	1944-1966	981
AD-1571122.1	UGCUUCCAGGAGGACAGCAUU	1969-1989	792	AAUGCUGUCCUCCUGGAAGCAGU	1967-1989	1847
AD-1571123.1	CUUCCAGGAGGACAGCAUGGU	1971-1991	794	ACCATGCUGUCCUCCUGGAAGCA	1969-1991	1848
AD-1571124.1	CUGGGCAAGGUGUGGCAGAAU	2017-2037	1713	AUUCTGCCACACCUUGCCCAGGA	2015-2037	1849
AD-1571125.1	UGGGCAAGGUGUGGCAGAACU	2018-2038	797	AGUUCUGCCACACCUUGCCCAGG	2016-2038	986
AD-1571126.1	GGCAAGGUGUGGCAGAACUCU	2020-2040	799	AGAGTUCUGCCACACCUUGCCCA	2018-2040	1850
AD-1571127.1	UGGCCUGGAGAGGUGUCCUUU	2044-2064	802	AAAGGACACCUCUCCAGGCCAGC	2042-2064	990
AD-1571128.1	GCCUGGAGAGGUGUCCUUCAU	2046-2066	804	AUGAAGGACACCUCUCCAGGCCA	2044-2066	1851
AD-1571129.1	CCUGGAGAGGUGUCCUUCAAU	2047-2067	1714	AUUGAAGGACACCUCUCCAGGCC	2045-2067	1852
AD-1571130.1	UGUGCAGUUGAUCCCACAGGU	2289-2309	1715	ACCUGUGGGAUCAACUGCACAUC	2287-2309	994
AD-1571131.1	UGCAGUUGAUCCCACAGGACU	2291-2311	808	AGUCCUGUGGGAUCAACUGCACA	2289-2311	995
AD-1571132.1	AUCCCACAGGACCUGUGCAGU	2299-2319	117	ACUGCACAGGUCCUGUGGGAUCA	2297-2319	243
AD-1571133.1	CCCACAGGACCUGUGCAGCGU	2301-2321	809	ACGCTGCACAGGUCCUGUGGGAU	2299-2321	1853

Duplex Name	Sense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:
AD-1571134.1	CCACAGGACCUGUGCAGCGAU	2302-2322	1716	AUCGCUGCACAGGUCCUGUGGGA	2300-2322	1854
AD-1571135.1	CCAGGUGACGCCACGCAUGCU	2334-2354	811	AGCATGCGUGGCGUCACCUGGUA	2332-2354	1855
AD-1571136.1	GUGACGCCACGCAUGCUGUGU	2338-2358	812	ACACAGCAUGCGUGGCGUCACCU	2336-2358	1000
AD-1571137.1	UGACGCCACGCAUGCUGUGUU	2339-2359	118	AACACAGCAUGCGUGGCGUCACC	2337-2359	244
AD-1571138.1	ACGCCACGCAUGCUGUGUGCU	2341-2361	1717	AGCACACAGCAUGCGUGGCGUCA	2339-2361	1856
AD-1571139.1	GCUACCGCAAGGCAAGAAU	2362-2382	814	AUUCUUGCCCUUGCGGUAGCCGG	2360-2382	1857
AD-1571140.1	GUGCAAGGCACUCAGUGGCCU	2418-2438	815	AGGCCACUGAGUGCCUUGCACAC	2416-2438	1858
AD-1571141.1	CUAACUACUUCGGCGUCUACU	2486-2506	1718	AGUAGACGCCGAAGUAGUUAGGC	2484-2506	1006
AD-1571142.1	CUACACCCGCAUCACAGGUGU	2502-2522	124	ACACCUGUGAUGCGGGUGUAGAC	2500-2522	250
AD-1571143.1	CCCGCAUCACAGGUGUGAUCU	2507-2527	822	AGAUCACACCUGUGAUGCGGGUG	2505-2527	1012
AD-1571144.1	UGGAUCCAGCAAGUGGUGACU	2530-2550	823	AGUCACCACUUGCUGGAUCCAGC	2528-2550	1859
AD-1571145.1	AUCCAGCAAGUGGUGACCUGU	2533-2553	1719	ACAGGUCACCACUUGCUGGAUCC	2531-2553	1860
AD-1571146.1	CCAGCAAGUGGUGACCUGAGU	2535-2555	1720	ACUCAGGUCACCACUUGCUGGAU	2533-2555	1016
AD-1571147.1	CAGCAAGUGGUGACCUGAGGU	2536-2556	1721	ACCUCAGGUCACCACUUGCUGGA	2534-2556	1017
AD-1571148.1	GCAAGUGGUGACCUGAGGAAU	2538-2558	1722	AUUCCUCAGGUCACCACUUGCUG	2536-2558	1861
AD-1571149.1	UGGUGGCAGGAGGUGGCAUCU	2667-2687	829	AGAUGCCACCUCCUGCCACCACA	2665-2687	1862
AD-1571150.1	GGUGGCAGGAGGUGGCAUCUU	2668-2688	830	AAGATGCCACCUCCUGCCACCAC	2666-2688	1863
AD-1571151.1	GUGGCAGGAGGUGGCAUCUUU	2669-2689	831	AAAGAUGCCACCUCCUGCCACCA	2667-2689	1021
AD-1571152.1	UCCAGUGAUGGCAGGAGGAUU	2709-2729	1723	AAUCCUCCUGCCAUCACUGGAGC	2707-2729	1864
AD-1571153.1	CUAACUUGGGAUCUGGGAAUU	2978-2998	835	AAUUCCCAGAUCCCAAGUUAGAC	2976-2998	1025
AD-1571154.1	GUGAGCUCAGCUGCCCUUUGU	3157-3177	837	ACAAAGGGCAGCUGAGCUCACCU	3155-3177	1026
AD-1571155.1	CUCAGCUGCCCUUUGGAAUAU	3162-3182	838	AUAUTCCAAAGGGCAGCUGAGCU	3160-3182	1865
AD-1571156.1	UCAGCUGCCCUUUGGAAUAAU	3163-3183	1724	AUUATUCCAAAGGGCAGCUGAGC	3161-3183	1866
AD-1571157.1	CCCUUUGGAAUAAAGCUGCCU	3170-3190	1725	AGGCAGCUUUAUUCCAAAGGGCA	3168-3190	1867
AD-1571158.1	CCUUUGGAAUAAAGCUGCCUU	3171-3191	844	AAGGCAGCUUUAUUCCAAAGGGC	3169-3191	1868
AD-1571159.1	UUUGGAAUAAAGCUGCCUGAU	3173-3193	845	AUCAGGCAGCUUUAUUCCAAAGG	3171-3193	1869

Duplex Name	Sense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	Range in NM_153609.4	SEQ ID NO:
AD-1571160.1	UUGGAAUAAAGCUGCCUGAUU	3174-3194	846	AAUCAGGCAGCUUUAUUCCAAAG	3172-3194	1870
AD-1571161.1	UGGAAUAAAGCUGCCUGAUCU	3175-3195	847	AGAUCAGGCAGCUUUAUUCCAAA	3173-3195	1871

Table 7. Modified Sense and Antisense Strand Sequences of TMPRSS6 dsRNA Agents

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1570929.1	csgsgaggUfgAfUfGfgcgaggaaguL96	1872	asCfsuudCc(Tgn)cgccauCfaCfcuccgsusc	2099	GACGGAGGUGAUGGCGAGGAAGC	1491
AD-1570930.1	cscsugugAfgGfAfCfuccaagagauL96	1873	asUfscudCu(Tgn)ggagucCfuCfacaggscsc	2100	GGCCUGUGAGGACUCCAAGAGAA	1495
AD-1570931.1	csusgugaGfgAfCfUfccaagagaauL96	1874	asUfsucdTc(Tgn)uggaguCfcUfcacagsgsc	2101	GCCUGUGAGGACUCCAAGAGAAA	1496
AD-1570932.1	csuscuggUfaUfUfUfccuaggguauL96	1875	asUfsacdCc(Tgn)aggaaaUfaCfcagagsusa	2102	UACUCUGGUAUUUCCUAGGGUAC	532
AD-1570933.1	gsgsuauuUfcCfUfAfggguacaaguL96	1876	asCfsuudGu(Agn)cccuagGfaAfauaccsasg	2103	CUGGUAUUUCCUAGGGUACAAGG	1499
AD-1570934.1	gsusauuuCfcUfAfGfgguacaagguL96	1877	asCfscudTg(Tgn)acccuaGfgAfaauacscsa	2104	UGGUAUUUCCUAGGGUACAAGGC	1500
AD-1570935.1	gsgsucagCfcAfGfGfuguacucaguL96	1878	asCfsugdAg(Tgn)acaccuGfgCfugaccsasu	2105	AUGGUCAGCCAGGUGUACUCAGG	535
AD-1570936.1	uscsagccAfgGfUfGfuacucaggcuL96	1879	asGfsccdTg(Agn)guacacCfuGfgcugascsc	2106	GGUCAGCCAGGUGUACUCAGGCA	1504
AD-1570937.1	asgsccagGfuGfUfAfcucaggcaguL96	1880	asCfsugdCc(Tgn)gaguacAfcCfuggcusgsa	2107	UCAGCCAGGUGUACUCAGGCAGU	536
AD-1570938.1	csascuucUfcCfCfAfggaucuuacuL96	1881	asGfsuadAg(Agn)uccuggGfaGfaagugsgsc	2108	GCCACUUCUCCCAGGAUCUUACC	1508
AD-1570939.1	uscsucccAfgGfAfUfcuuacccgcuL96	1882	asGfscgdGg(Tgn)aagaucCfuGfggagasasg	2109	CUUCUCCCAGGAUCUUACCCGCC	1509
AD-1570940.1	gscscuucCfgCfAfGfugaaaccgcuL96	1883	asGfscgdGu(Tgn)ucacugCfgGfaaggcsasc	2110	GUGCCUUCCGCAGUGAAACCGCC	540

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1570941.1	cscsuuccGfcAfGfUfgaaaccgccuL96	1884	asGfsgcdGg(Tgn)uucacuGfcGfgaaggscsa	2111	UGCCUUCCGCAGUGAAACCGC CA	1512
AD-1570942.1	gscsagugAfaAfCfCfgccaaagccuL96	1885	asGfsgcdTu(Tgn)ggcggguUfuCfacugcsgsg	2112	CCGCAGUGAAACCGCCAAAGC CC	1516
AD-1570943.1	csasgugaAfaCfCfGfccaaagccccuL96	1886	asGfsggdCu(Tgn)uggcggUfuUfcacugscsg	2113	CGCAGUGAAACCGCCAAAGCC CA	1517
AD-1570944.1	asgsugaaAfcCfGfCfcaaagcccauL96	1887	asUfsggdGc(Tgn)uuggcgGfuUfucacusgsc	2114	GCAGUGAAACCGCCAAAGCCC AG	1518
AD-1570945.1	csgsccaaAfgCfCfCfagaagaugcuL96	1888	asGfscadTc(Tgn)ucugggCfuUfuggcgsgsu	2115	ACCGCCAAAGCCCAGAAGAUG CU	1519
AD-1570946.1	asgscccaGfaAfGfAfugcucaaggulL96	1889	asCfscudTg(Agn)gcaucuUfcUfgggcususu	2116	AAAGCCCAGAAGAUGCUCAAG GA	1521
AD-1570947.1	asgscaccCfgCfCfUfgggaacuuauL96	1890	asUfsaadGu(Tgn)cccaggCfgGfgugcusgsg	2117	CCAGCACCCGCCUGGGAACUU AC	1523
AD-1570948.1	csasacucCfaGfCfUfccgucuauuuL96	1891	asAfsaudAg(Agn)cggagcUfgGfaguugsus a	2118	UACAACUCCAGCUCCGUCUAU UC	541
AD-1570949.1	uscsaccuGfcUfUfCfuucugguucuL96	1892	asGfsaadCc(Agn)gaagaaGfcAfggugasgsg	2119	CCUCACCUGCUUCUUCUGGUUU CA	544
AD-1570950.1	cscsugcuUfcUfUfCfugguucauuuL96	1893	asAfsaudGa(Agn)ccagaaGfaAfgcaggsusg	2120	CACCUGCUUCUUCUGGUUCAU UC	546
AD-1570951.1	csusgcuuCfuUfCfUfgguucauucuL96	1894	asGfsaadTg(Agn)accagaAfgAfagcagsgsu	2121	ACCUGCUUCUUCUGGUUCAUU CU	1526
AD-1570952.1	csusucuuCfuGfGfUfucauucuccuL96	1895	asGfsgadGa(Agn)ugaaccAfgAfagaagscsa	2122	UGCUUCUUCUGGUUCAUUCUC CA	549
AD-1570953.1	ususcuucUfgGfUfUfcauucuccauL96	1896	asUfsggdAg(Agn)augaacCfaGfaagaasgsc	2123	GCUUCUUCUGGUUCAUUCUCC AA	550
AD-1570954.1	csusucugGfuUfCfAfuucuccaaauL96	1897	asUfsuudGg(Agn)gaaugaAfcCfagaagsasa	2124	UUCUUCUGGUUCAUUCUCCAA AU	1528
AD-1570955.1	csusgguuCfaUfUfCfuccaaauccuL96	1898	asGfsgadTu(Tgn)ggagaaUfgAfaccagsasa	2125	UUCUGGUUCAUUCUCCAAAUC CC	551
AD-1570956.1	gscsugcuGfuCfCfAfcagucaacauL96	1899	asUfsgudTg(Agn)cuguggAfcAfgcagcsusc	2126	GAGCUGCUGUCCACAGUCAAC AG	1534
AD-1570957.1	gscsugucCfaCfAfGfucaacagcuuL96	1900	asAfsgcdTg(Tgn)ugacugUfgGfacagcsasg	2127	CUGCUGUCCACAGUCAACAGC UC	1535

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1570958.1	usgsuccaCfaGfUfCfaacagcucguL96	1901	asCfsgadGc(Tgn)guugacUfgUfggacasgsc	2128	GCUGUCCACAGUCAACAGCUCGG	1537
AD-1570959.1	gsgsccgaGfuAfCfGfaaguggaccuL96	1902	asGfsgudCc(Agn)cuucguAfcUfcggccscsu	2129	AGGGCCGAGUACGAAGUGGACCC	1539
AD-1570960.1	asusccugGfaAfGfCfcagugugaauL96	1903	asUfsucdAc(Agn)cuggcuUfcCfaggauscsa	2130	UGAUCCUGGAAGCCAGUGUGAAA	1540
AD-1570961.1	cscsuggaAfgCfCfAfgugugaaaguL96	1904	asCfsuudTc(Agn)cacuggCfuUfccaggsasu	2131	AUCCUGGAAGCCAGUGUGAAAGA	1542
AD-1570962.1	usgsgaagCfcAfGfUfgugaaagacuL96	1905	asGfsucdTu(Tgn)cacacuGfgCfuuccasgsg	2132	CCUGGAAGCCAGUGUGAAAGACA	1544
AD-1570963.1	gsgsaagcCfaGfUfGfugaaagacauL96	1906	asUfsgudCu(Tgn)ucacacUfgGfcuuccsasg	2133	CUGGAAGCCAGUGUGAAAGACAU	1545
AD-1570964.1	gsasagccAfgUfGfUfgaaagacauuL96	1907	asAfsugdTc(Tgn)uucacaCfuGfgcuucscsa	2134	UGGAAGCCAGUGUGAAAGACAUA	1546
AD-1570965.1	asgsccagUfgUfGfAfaagacauaguL96	1908	asCfsuadTg(Tgn)cuuucaCfaCfuggcususc	2135	GAAGCCAGUGUGAAAGACAUAGC	1548
AD-1570966.1	cscsagugUfgAfAfAfgacauagcuuL96	1909	asAfsgcdTa(Tgn)gucuuuCfaCfacuggscsu	2136	AGCCAGUGUGAAAGACAUAGCUG	554
AD-1570967.1	asgsugugAfaAfGfAfcauagcugcuL96	1910	asGfscadGc(Tgn)augucuUfuCfacacusgsg	2137	CCAGUGUGAAAGACAUAGCUGCA	1550
AD-1570968.1	gsusgaaaGfaCfAfUfagcugcauuuL96	1911	asAfsaudGc(Agn)gcuaugUfcUfuucacsasc	2138	GUGUGAAAGACAUAGCUGCAUUG	1553
AD-1570969.1	asusugaaUfuCfCfAfcgcuggguuuL96	1912	asAfsacdCc(Agn)gcguggAfaUfucaausgsc	2139	GCAUUGAAUUCCACGCUGGGGUUG	556
AD-1570970.1	asasuuccAfcGfCfUfggguuguuauL96	1913	asUfsaadCa(Agn)cccagcGfuGfgaauuscsa	2140	UGAAUUCCACGCUGGGGUUGUAC	559
AD-1570971.1	csascgcuGfgGfUfUfguuaccgcuuL96	1914	asAfsgcdGg(Tgn)aacaacCfcAfgcgugsgsa	2141	UCCACGCUGGGGUUGUUACCGCUA	562
AD-1570972.1	usgsgguuGfuUfAfCfcgcuacagcuL96	1915	asGfscudGu(Agn)gcgguaAfcAfacccasgsc	2142	GCUGGGUUGUUACCGCUACAGCU	1557
AD-1570973.1	ggsgsguugUfuAfCfCfgcuacagcuuL96	1916	asAfsgcdTg(Tgn)agcgguAfaCfaacccsasg	2143	CUGGGUUGUUACCGCUACAGCUA	1558
AD-1570974.1	csasaacuCfcGfGfCfuggaguggauL96	1917	asUfsccdAc(Tgn)ccagccGfgAfguuugsasg	2144	CUCAAACUCCGGCUGGAGUGGAC	1559

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1570975.1	gsgsgaccGfaCfUfGfgccauguauuL96	1918	asAfsuadCa(Tgn)ggccagUfcGfgucccsgsg	2145	CCGGGACCGACUGGCCAUGUA UG	564
AD-1570976.1	csgsacugGfcCfAfUfguaugacguuL96	1919	asAfscgdTc(Agn)uacaugGfcCfagucgsgsu	2146	ACCGACUGGCCAUGUAUGACG UG	1560
AD-1570977.1	usgsgagaAfgAfGfGfcucaucaccuL96	1920	asGfsgudGa(Tgn)gaggccuCfuUfcuccasgsg	2147	CCUGGAGAAGAGGCUCAUCAC CU	1562
AD-1570978.1	gsgsagaaGfaGfGfCfucaucaccuuL96	1921	asAfsggdTg(Agn)ugaggccUfcUfucuccsasg	2148	CUGGAGAAGAGGCUCAUCACC UC	1563
AD-1570979.1	gsasagaaGfgGfCfCfugcacagcuuL96	1922	asAfsgcdTg(Tgn)gcaggcCfcUfucuucscsa	2149	UGGAAGAAGGGCCUGCACAGC UA	1566
AD-1570980.1	asgsggccUfgCfAfCfagcuacuacuL96	1923	asGfsuadGu(Agn)gcugugCfaGfgcccususcc	2150	GAAGGGCCUGCACAGCUACUA CG	1569
AD-1570981.1	cscsugcaCfaGfCfUfuacuacgaccuL96	1924	asGfsgudCg(Tgn)aguagcUfgUfgcaggscsc	2151	GGCCUGCACAGCUACUACGAC CC	573
AD-1570982.1	gsasggagGfcAfGfAfaguaugauuuL96	1925	asAfsaudCa(Tgn)acuucuGfcCfuccucsasg	2152	CUGAGGAGGCAGAAGUAUGA UUU	580
AD-1570983.1	asgsgaggCfaGfAfAfguauguauuuL96	1926	asAfsaadTc(Agn)uacuucUfgCfcuccuscsa	2153	UGAGGAGGCAGAAGUAUGAU UUG	1572
AD-1570984.1	asgsuaugAfuUfUfGfccgugcaccuL96	1927	asGfsgudGc(Agn)cggcaaAfuCfauacususc	2154	GAAGUAUGAUUUGCCGUGCA CCC	1574
AD-1570985.1	cscsagugGfaCfGfAfuccagaacauL96	1928	asUfsgudTc(Tgn)ggaucgUfcCfacuggscsc	2155	GGCCAGUGGACGAUCCAGAAC AG	1578
AD-1570986.1	cscsagaaCfaGfGfAfggcugugugguL96	1929	asCfsacdAc(Agn)gccuccUfgUfucuggsasu	2156	AUCCAGAACAGGAGGCUGUG UGG	591
AD-1570987.1	asgsaacaGfgAfGfGfcugugugggcuL96	1930	asGfscdAc(Agn)cagccuCfcUfguucusgsg	2157	CCAGAACAGGAGGCUGUGUG GCU	592
AD-1570988.1	ascsuucaCfcUfCfCfcagaucucccuL96	1931	asGfsgadGa(Tgn)cugggaGfgUfgaagususg	2158	CAACUUCACCUCCCAGAUCUC CC	1580
AD-1570989.1	usgsuggcGfgUfGfCfacuauggcuuuL96	1932	asAfsgcdCa(Tgn)agugcaCfcCfgcacascsc	2159	GGUGUGCGGGUGCACUAUGG CUU	593
AD-1570990.1	gsusgcggGfuGfCfAfcuauggcuuuL96	1933	asAfsagdCc(Agn)uagugcAfcCfcgcacsasc	2160	GUGUGCGGGUGCACUAUGGC UUG	594
AD-1570991.1	gsgsgugcAfcUfAfUfggcuuguacuL96	1934	asGfsuadCa(Agn)gccauaGfuGfcacccsgsc	2161	GCGGGUGCACUAUGGCUUGU ACA	596

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1570992.1	gsgsugcaCfuAfUfGfgcuuguacauL96	1935	asUfsgudAc(Agn)agccauAfgUfgcaccscsg	2162	CGGGUGCACUAUGGCUUGUAC AA	1584
AD-1570993.1	usgscacuAfuGfGfCfuuguacaacuL96	1936	asGfsuudGu(Agn)caagccAfuAfgugcascsc	2163	GGUGCACUAUGGCUUGUACA ACC	1585
AD-1570994.1	gscsacuaUfgGfCfUfuguacaaccuL96	1937	asGfsgudTg(Tgn)acaagcCfaUfagugcsasc	2164	GUGCACUAUGGCUUGUACAAC CA	1586
AD-1570995.1	csusgcccUfgGfAfGfaguuccucuuL96	1938	asAfsgadGg(Agn)acucucCfaGfggcagsgsg	2165	CCCUGCCCUGGAGAGUUCCUC UG	599
AD-1570996.1	ascsggccUfgGfAfUfgagagaaacuL96	1939	asGfsuudTc(Tgn)cucaucCfaGfgccgususg	2166	CAACGGCCUGGAUGAGAGAA ACU	1589
AD-1570997.1	gscscuggAfuGfAfGfagaaacugcuL96	1940	asGfscadGu(Tgn)ucucucAfuCfcaggcscsg	2167	CGGCCUGGAUGAGAGAAACU GCG	600
AD-1570998.1	cscsuggaUfgAfGfAfgaaacugcguL96	1941	asCfsgcdAg(Tgn)uucucuCfaUfccaggscsc	2168	GGCCUGGAUGAGAGAAACUG CGU	1591
AD-1570999.1	asgsagaaAfcUfGfCfguuugcagauL96	1942	asUfscudGc(Agn)aacgcaGfuUfucucucsca	2169	UGAGAGAAACUGCGUUUGCA GAG	1592
AD-1571000.1	gscsguuuGfcAfGfAfgccacauucuL96	1943	asGfsaadTg(Tgn)ggcucuGfcAfaacgcsasg	2170	CUGCGUUUGCAGAGCCACAUU CC	1596
AD-1571001.1	usgsggacAfuUfCfAfccuuccaguuL96	1944	asAfscudGg(Agn)agggugaAfuGfucccascsa	2171	UGUGGGACAUUCACCUUCCAG UG	606
AD-1571002.1	gsasgcugCfgUfGfAfagaagcccauL96	1945	asUfsggdGc(Tgn)ucuucaCfgCfagcucscsg	2172	CGGAGCUGCGUGAAGAAGCCC AA	1600
AD-1571003.1	csgscugaCfcGfCfUfgggugauaauL96	1946	asUfsuadTc(Agn)cccagcGfgUfcagcgsasu	2173	AUCGCUGACCGCUGGGUGAUA AC	1608
AD-1571004.1	gscsuuccAfgGfAfGfgacagcauguL96	1947	asCfsaudGc(Tgn)guccucCfuGfgaagcsasg	2174	CUGCUUCCAGGAGGACAGCAU GG	1612
AD-1571005.1	csgsuguuCfcUfGfGfgcaagguguuL96	1948	asAfscadCc(Tgn)ugcccaGfgAfacacgsgsu	2175	ACCGUGUUCCUGGGCAAGGUG UG	613
AD-1571006.1	gsgsgcaaGfgUfGfUfggcagaacuuL96	1949	asAfsgudTc(Tgn)gccacaCfcUfugcccsasg	2176	CUGGGCAAGGUGUGGCAGAA CUC	1616
AD-1571007.1	gscsaaggUfgUfGfGfcagaacucguL96	1950	asCfsgadGu(Tgn)cugccaCfaCfcuugcscsc	2177	GGGCAAGGUGUGGCAGAACU CGC	615
AD-1571008.1	csasagguGfuGfGfCfagaacucguL96	1951	asGfscgdAg(Tgn)ucugccAfcAfccuugscsc	2178	GGCAAGGUGUGGCAGAACUC GCG	1618

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1571009.1	gsgsccugGfaGfAfGfguguccuucuL96	1952	asGfsaadGg(Agn)caccucUfcCfaggccsasg	2179	CUGGCCUGGAGAGGUGUCCUU CA	1620
AD-1571010.1	csusggagAfgGfUfGfuccuucaaguL96	1953	asCfsuudGa(Agn)ggacacCfuCfuccaggsgsc	2180	GCCUGGAGAGGUGUCCUUCAA GG	617
AD-1571011.1	asgsagguGfuCfCfUfucaaggugauL96	1954	asUfscadCc(Tgn)ugaaggAfcAfccucuscsc	2181	GGAGAGGUGUCCUUCAAGGU GAG	620
AD-1571012.1	gscsuaccGfcAfAfGfggcaagaaguL96	1955	asCfsuudCu(Tgn)gcccuuGfcGfguagcscsg	2182	CGGCUACCGCAAGGGCAAGAA GG	624
AD-1571013.1	csusaccgCfaAfGfGfgcaagaaggUL96	1956	asCfscudTc(Tgn)ugcccuUfgCfgguagscsc	2183	GGCUACCGCAAGGGCAAGAAG GA	625
AD-1571014.1	ascsuacuUfcGfGfCfgucuacaccuL96	1957	asGfsgudGu(Agn)gacgccGfaAfguagusus a	2184	UAACUACUUCGGCGCUCUACAC CC	1633
AD-1571015.1	csusacuuCfgGfCfGfucuacacccuL96	1958	asGfsggdTg(Tgn)agacgcCfgAfaguagsusu	2185	AACUACUUCGGCGUCUACACC CG	1634
AD-1571016.1	gscsgucuAfcAfCfCfcgcaucacauL96	1959	asUfsgudGa(Tgn)gcggguGfuAfgacgcscsg	2186	CGGCGUCUACACCCGCAUCAC AG	1635
AD-1571017.1	csgsucuaCfaCfCfCfgcaucacagUL96	1960	asCfsugdTg(Agn)ugcgggUfgUfagacgscsc	2187	GGCGUCUACACCCGCAUCACA GG	1636
AD-1571018.1	ascscgcAfuCfAfCfaggugugauuL96	1961	asAfsucdAc(Agn)ccugugAfuGfcgggusgs u	2188	ACACCCGCAUCACAGGUGUGA UC	1637
AD-1571019.1	gsasuccaGfcAfAfGfuggugaccuuL96	1962	asAfsggdTc(Agn)ccacuuGfcUfggaucscsa	2189	UGGAUCCAGCAAGUGGUGACC UG	1640
AD-1571020.1	gsgscaggAfgGfUfGfgcaucuuguuL96	1963	asAfscadAg(Agn)ugccacCfuCfcugccsasc	2190	GUGGCAGGAGGUGGCAUCUU GUC	631
AD-1571021.1	uscsccugAfuGfUfCfugcuccaguuL96	1964	asAfscudGg(Agn)gcagacAfuCfagggascsg	2191	CGUCCCUGAUGUCUGCUCCAG UG	1648
AD-1571022.1	csusgaugUfcUfGfCfuccagugauuL96	1965	asAfsucdAc(Tgn)gggcaGfaCfaucagsgsg	2192	CCCUGAUGUCUGCUCCAGUGA UG	1649
AD-1571023.1	gsgscucaGfcAfGfCfaagaaugcuuL96	1966	asAfsgcdAu(Tgn)cuugcuGfcUfgagccsasc	2193	GUGGCUCAGCAGCAAGAAUGC UG	636
AD-1571024.1	ususgggaUfcUfGfGfgaauggaaguL96	1967	asCfsuudCc(Agn)uucccaGfaUfcccaasgsu	2194	ACUUGGGAUCUGGGAAUGGA AGG	642
AD-1571025.1	csasgcugCfcCfUfUfuggaauaaauL96	1968	asUfsuudAu(Tgn)ccaaagGfgCfagcugsasg	2195	CUCAGCUGCCCUUUGGAAUAA AG	1655

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1571026.1	csusgcccUfuUfGfGfaauaaagcuuL96	1969	asAfsgcdTu(Tgn)auuccaAfaGfggcagscsu	2196	AGCUGCCCUUUGGAAUAAAGC UG	648
AD-1571027.1	gscsccuuUfgGfAfAfuaaagcugcuL96	1970	asGfscadGc(Tgn)uuaauucCfaAfagggcsasg	2197	CUGCCCUUUGGAAUAAAGCUG CC	1656
AD-1571028.1	cscsucacCfuGfCfCfUfucuucugguuL96	1971	asAfsccaGfaAfGfaagcAfgGfugaggsgsg	2198	CCCCUCACCUGCUUCUUCUGG UU	2328
AD-1571029.1	cscsucacCfuGfCfCfUfucuucugguuL96	1971	asAfsccaGfaAfGfaagcAfgGfugaggscsu	2199	CCCCUCACCUGCUUCUUCUGG UU	2328
AD-1571030.1	uscsacCfuGfCfCfUfucuucugguuL96	1972	asAfsccaGfaAfGfaagcAfgGfugasgsg	2200	CCUCACCUGCUUCUUCUGGUU	2329
AD-1571031.1	uscsacCfuGfCfCfUfucuucugguuL96	1972	asAfsccaGfaAfGfaagcAfgGfugascsu	2201	CCUCACCUGCUUCUUCUGGUU	2329
AD-1571032.1	ascsCfuGfCfUfucuucugguuL96	1973	asAfsccaGfaAfGfaagcAfgGfusgsa	2202	UCACCUGCUUCUUCUGGUU	2330
AD-1571033.1	Q191sUfcAfcCfuGfcUfuCfuUfcUfg GfsusUf	1974	asAfscCfaGfaAfgAfaGfcAfgGfusGfsa	2203	UCACCUGCUUCUUCUGGUU	2330
AD-1571034.1	ggsgsaggUfgAfUfGfCfgaggaagcuL96	1975	asGfscudTc(C2p)ucgccaUfcAfccuccsgsu	2204	ACGGAGGUGAUGGCGAGGAA GCG	1492
AD-1571035.1	asasggccUfgUfGfAfggacuccaauL96	1976	asUfsugdGa(G2p)uccucaCfaGfgccuusgsa	2205	UCAAGGCCUGUGAGGACUCCA AG	1493
AD-1571036.1	gsgsccugUfgAfGfGfacuccaagauL96	1977	asUfscudTg(G2p)aguccuCfaCfaggccsusu	2206	AAGGCCUGUGAGGACUCCAAG AG	1494
AD-1571037.1	gscscuguGfaGfGfAfcuccaagaguL96	1978	asCfsucdTu(G2p)gaguccUfcAfcaggcscsu	2207	AGGCCUGUGAGGACUCCAAGA GA	524
AD-1571038.1	csusacucUfgGfUfAfuuuccuaggguL96	1979	asCfscudAg(G2p)aaauacCfaGfaguagscsa	2208	UGCUACUCUGGUAUUUCCUAG GG	529
AD-1571039.1	uscsugguAfuUfUfCfcuaggguacuL96	1980	asGfsuadCc(C2p)uaggaaAfuAfccagasgsu	2209	ACUCUGGUAUUUCCUAGGGU ACA	533
AD-1571040.1	csusgguaUfuUfCfCfuaggguacauL96	1981	asUfsgudAc(C2p)cuaggaAfaUfaccagsasg	2210	CUCUGGUAUUUCCUAGGGUAC AA	1497
AD-1571041.1	usgsguauUfuCfCfUfaggguacaauL96	1982	asUfsugdTa(C2p)ccuaggAfaAfuaccasgsa	2211	UCUGGUAUUUCCUAGGGUAC AAG	1498
AD-1571042.1	csusagggUfaCfAfAfggcggaggguuL96	1983	asAfscdTc(C2p)gccuugUfaCfccuaggsgsa	2212	UCCUAGGGUACAAGGCGGAG GUG	1501
AD-1571043.1	asusggucAfgCfCfAfgguguacucuL96	1984	asGfsagdTa(C2p)accuggCfuGfaccauscsa	2213	UGAUGGUCAGCCAGGUGUAC UCA	1502
AD-1571044.1	gsuscagcCfaGfGfUfguacucagguL96	1985	asCfscudGa(G2p)uacaccUfgGfcugacscsa	2214	UGGUCAGCCAGGUGUACUCAG GC	1503

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1571045.1	csasgccaGfgUfGfUfacucaggcauL96	1986	asUfsgcdCu(G2p)aguacaCfcUfggcugsasc	2215	GUCAGCCAGGUGUACUCAGGC AG	1505
AD-1571046.1	csuscaauCfgCfCfAfcuucucccauL96	1987	asUfsggdGa(G2p)aaguggCfgAfuugagsus a	2216	UACUCAAUCGCCACUUCUCCC AG	1506
AD-1571047.1	csgsccacUfuCfUfCfccaggaucuuL96	1988	asAfsgadTc(C2p)ugggagAfaGfuggcgsasu	2217	AUCGCCACUUCUCCCAGGAUC UU	1507
AD-1571048.1	gscscacuUfcUfCfCfcaggaucuuuL96	1989	asAfsagdAu(C2p)cugggaGfaAfguggcsgs a	2218	UCGCCACUUCUCCCAGGAUCU UA	537
AD-1571050.1	uscsccagGfaUfCfUfuaccgccguL96	1990	asCfsggdCg(G2p)guaagaUfcCfugggasgsa	2219	UCUCCCAGGAUCUUACCCGCC GG	539
AD-1571051.1	usasgugcCfuUfCfCfgcagugaaauL96	1991	asUfsuudCa(C2p)ugcggaAfgGfcacuasgsa	2220	UCUAGUGCCUUCCGCAGUGAA AC	1511
AD-1571052.1	csusuccgCfaGfUfGfaaaccgccauL96	1992	asUfsggdCg(G2p)uuucacUfgCfggaagsgsc	2221	GCCUUCCGCAGUGAAACCGCC AA	1513
AD-1571053.1	cscsgcagUfgAfAfAfcgccaaagcuL96	1993	asCfsuudTg(G2p)cgguuuCfaCfugcggsasa	2222	UUCCGCAGUGAAACCGCCAAA GC	1514
AD-1571054.1	csgscaguGfaAfAfCfcgccaaagcuL96	1994	asGfscudTu(G2p)gcgguuUfcAfcugcgsgsa	2223	UCCGCAGUGAAACCGCCAAAG CC	1515
AD-1571055.1	gscscaaaGfcCfCfAfgaagaugcuuL96	1995	asAfsgcdAu(C2p)uucuggGfcUfuuggcsgs g	2224	CCGCCAAAGCCCAGAAGAUGC UC	1520
AD-1571056.1	csasgcacCfcGfCfCfugggaacuuuL96	1996	asAfsagdTu(C2p)ccaggcGfgGfugcugsgsu	2225	ACCAGCACCCGCCUGGGAACU UA	1522
AD-1571057.1	ascsaacuCfcAfGfCfuccgucuauuL96	1997	asAfsuadGa(C2p)ggagcuGfgAfgugusas g	2226	CUACAACUCCAGCUCCGUCUA UU	1524
AD-1571058.1	csasccugCfuUfCfUfucgguucauL96	1998	asUfsgadAc(C2p)agaagaAfgCfaggugsasg	2227	CUCACCUGCUUCUUCUGGUUC AU	1525
AD-1571059.1	usgscuucUfuCfUfGfguucauucuuL96	1999	asAfsgadAu(G2p)aaccagAfaGfaagcasgsg	2228	CCUGCUUCUUCUGGUUCAUUC UC	547
AD-1571060.1	uscsuucuGfgUfUfCfauucuccaauL96	2000	asUfsugdGa(G2p)aauguaaCfcAfgaagasasg	2229	CUUCUUCUGGUUCAUUCUCCA AA	1527
AD-1571061.1	ususcuggUfuCfAfUfucuccaaauuL96	2001	asAfsuudTg(G2p)agaaugAfaCfcagaasgsa	2230	UCUUCUGGUUCAUUCUCCAAA UC	1529
AD-1571062.1	uscsugguUfcAfUfUfcuccaaaucuL96	2002	asGfsaudTu(G2p)gagaauGfaAfccagasasg	2231	CUUCUGGUUCAUUCUCCAAAU CC	1530

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1571063.1	gsusggagGfaGfCfUfgcuguccacuL96	2003	asGfsugdGa(C2p)agcagcUfcCfuccacscsa	2232	UGGUGGAGGAGCUGCUGUCC ACA	1531
AD-1571064.1	gsasggagCfuGfCfUfguccacaguuL96	2004	asAfscudGu(G2p)gacagcAfgCfuccucscsa	2233	UGGAGGAGCUGCUGUCCACAG UC	1532
AD-1571065.1	asgscugcUfgUfCfCfacagucaacuL96	2005	asGfsuudGa(C2p)uguggaCfaGfcagcuscsc	2234	GGAGCUGCUGUCCACAGUCAA CA	1533
AD-1571066.1	csusguccAfcAfGfUfcaacagcucuL96	2006	asGfsagdCu(G2p)uugacuGfuGfgacagscsa	2235	UGCUGUCCACAGUCAACAGCU CG	1536
AD-1571067.1	ascsagggCfcGfAfGfuacgaaguguL96	2007	asCfsacdTu(C2p)guacucGfgCfccugusasg	2236	CUACAGGGCCGAGUACGAAGU GG	552
AD-1571068.1	gsgsgccgAfgUfAfCfgaaguggacuL96	2008	asGfsucdCa(C2p)uucguaCfuCfggcccsusg	2237	CAGGGCCGAGUACGAAGUGG ACC	1538
AD-1571069.1	uscscuggAfaGfCfCfcfagugugaaauL96	2009	asUfsuudCa(C2p)acuggcUfuCfcaggasusc	2238	GAUCCUGGAAGCCAGUGUGA AAG	1541
AD-1571070.1	csusggaaGfcCfAfGfugugaaagauL96	2010	asUfscudTu(C2p)acacugGfcUfuccagsgsa	2239	UCCUGGAAGCCAGUGUGAAA GAC	1543
AD-1571071.1	asasgccaGfuGfUfGfaaagacauauL96	2011	asUfsaudGu(C2p)uuucacAfcUfggcuuscsc	2240	GGAAGCCAGUGUGAAAGACA UAG	1547
AD-1571072.1	gscscaguGfuGfAfAfagacauagcuL96	2012	asGfscudAu(G2p)ucuuucAfcAfcuggcsus u	2241	AAGCCAGUGUGAAAGACAUA GCU	1549
AD-1571074.1	usgsugaaAfgAfCfAfuagcugcauuL96	2013	asAfsugdCa(G2p)cuauguCfuUfucacascsu	2242	AGUGUGAAAGACAUAGCUGC AUU	1552
AD-1571075.1	ascsgcugGfgUfUfGfuuaccgcuauL96	2014	asUfsagdCg(G2p)uaacaaCfcCfagcgusgsg	2243	CCACGCUGGGUUGUUACCGCU AC	1554
AD-1571076.1	csgscuggGfuUfGfUfuaccgcuacuL96	2015	asGfsuadGc(G2p)guaacaAfcCfcagcgsusg	2244	CACGCUGGGUUGUUACCGCUA CA	1555
AD-1571077.1	gscsugggUfuGfUfUfaccgcuacauL96	2016	asUfsgudAg(C2p)gguaacAfaCfccagcsgsu	2245	ACGCUGGGUUGUUACCGCUAC AG	1556
AD-1571078.1	csusggguUfgUfUfAfccgcuacaguL96	2017	asCfsugdTa(G2p)cgguaaCfaAfcccagscsg	2246	CGCUGGGUUGUUACCGCUACA GC	563
AD-1571079.1	csusggagAfaGfAfGfgcucaucacuL96	2018	asGfsugdAu(G2p)agccucUfuCfuccagsgsg	2247	CCCUGGGAGAAGAGGGCUCAUCA CC	1561
AD-1571080.1	gsasgaagAfgGfCfUfcaucaccucuL96	2019	asGfsagdGu(G2p)augagcCfuCfuucucscsa	2248	UGGAGAAGAGGGCUCAUCACCU CG	1564

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1571081.1	gsasagagGfcUfCfAfucaccucggguL96	2020	asCfscgdAg(G2p)ugaugaGfcCfucuucsusc	2249	GAGAAGAGGCUCAUCACCUCG GU	1565
AD-1571082.1	asgsgcucAfuCfAfCfcucgguguauL96	2021	asUfsacdAc(C2p)gaggugAfuGfagccuscsu	2250	AGAGGCUCAUCACCUCGGUGU AC	571
AD-1571083.1	asasgaagGfgCfCfUfgcacagcuauL96	2022	asUfsagdCu(G2p)ugcaggCfcCfuucuuscsc	2251	GGAAGAAGGGCCUGCACAGCU AC	1567
AD-1571084.1	asasgggcCfuGfCfAfcagcuacuauL96	2023	asUfsagdTa(G2p)cugugcAfgGfcccuuscsu	2252	AGAAGGGCCUGCACAGCUACU AC	1568
AD-1571085.1	cscsucucUfgGfAfCfuacggcuuguL96	2024	asCfsaadGc(C2p)guagucCfaGfagaggsgsc	2253	GCCCUCUCUGGACUACGGCUU GG	574
AD-1571086.1	uscsucugGfaCfUfAfcggcuuggcuL96	2025	asGfsccdAa(G2p)ccguagUfcCfagagasgsg	2254	CCUCUCUGGACUACGGCUUGG CC	575
AD-1571087.1	usgsgacuAfcGfGfCfuuggcccucuL96	2026	asGfsagdGg(C2p)caagccGfuAfguccasgsa	2255	UCUGGACUACGGCUUGGCCCU CU	1570
AD-1571088.1	gsgsacuaCfgGfCfUfuggcccucuuL96	2027	asAfsgadGg(G2p)ccaagcCfgUfaguccsasg	2256	CUGGACUACGGCUUGGCCCUC UG	1571
AD-1571089.1	asgsaaguAfuGfAfUfuugccgugcuL96	2028	asGfscadCg(G2p)caaaucAfuAfcuucusgsc	2257	GCAGAAGUAUGAUUUGCCGU GCA	587
AD-1571090.1	gsasaguaUfgAfUfUfugccgugcauL96	2029	asUfsgcdAc(G2p)gcaaauCfaUfacuucsusg	2258	CAGAAGUAUGAUUUGCCGUG CAC	588
AD-1571091.1	asasguauGfaUfUfUfgccgugcacuL96	2030	asGfsugdCa(C2p)ggcaaaUfcAfuacuuscsu	2259	AGAAGUAUGAUUUGCCGUGC ACC	1573
AD-1571092.1	gsusaugaUfuUfGfCfcgugcacccuL96	2031	asGfsggdTg(C2p)acggcaAfaUfcauacsusu	2260	AAGUAUGAUUUGCCGUGCACC CA	1575
AD-1571093.1	gsgsccagUfgGfAfCfgauccagaauL96	2032	asUfsucdTg(G2p)aucgucCfaCfuggccscsu	2261	AGGGCCAGUGGACGAUCCAGA AC	1576
AD-1571094.1	gscscaguGfgAfCfGfauccagaacuL96	2033	asGfsuudCu(G2p)gaucguCfcAfcuggcscsc	2262	GGGCCAGUGGACGAUCCAGAA CA	1577
AD-1571096.1	usgsgacgAfuCfCfAfgaacaggaguL96	2034	asCfsucdCu(G2p)uucuggAfuCfguccascsu	2263	AGUGGACGAUCCAGAACAGG AGG	1579
AD-1571097.1	csascccuCfcAfGfAfucucccucauL96	2035	asUfsgadGg(G2p)agaucuGfgGfaggugsas a	2264	UUCACCUCCCAGAUCUCCCUC AC	1581
AD-1571098.1	usgscgggUfgCfAfCfuauggcuuguL96	2036	asCfsaadGc(C2p)auagugCfaCfcgcascsa	2265	UGUGCGGGUGCACUAUGGCU UGU	1582

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1571099.1	gscsggguGfcAfCfUfauggcuuguuL96	2037	asAfscadAg(C2p)cauaguGfcAfccgcsasc	2266	GUGCGGGUGCACUAUGGCUU GUA	595
AD-1571100.1	csgsggugCfaCfUfAfuggcuuguauL96	2038	asUfsacdAa(G2p)ccauagUfgCfacccgscsa	2267	UGCGGGUGCACUAUGGCUUG UAC	1583
AD-1571102.1	asascggcCfuGfGfAfugagagaaauL96	2039	asUfsuudCu(C2p)ucauccAfgGfccguusggg	2268	CCAACGGCCUGGAUGAGAGAA AC	1588
AD-1571103.1	csgsgccuGfgAfUfGfagagaaacuuL96	2040	asAfsgudTu(C2p)ucucauCfcAfggccgsusu	2269	AACGGCCUGGAUGAGAGAAA CUG	1590
AD-1571104.1	gsasgaaaCfuGfCfGfuuugcagaguL96	2041	asCfsucdTg(C2p)aaacgcAfgUfuucucsusc	2270	GAGAGAAACUGCGUUUGCAG AGC	1593
AD-1571105.1	csusgcguUfuGfCfAfgagccacauuL96	2042	asAfsugdTg(G2p)cucugcAfaAfcgcagsusu	2271	AACUGCGUUUGCAGAGCCACA UU	1594
AD-1571106.1	usgscguuUfgCfAfGfagccacauuuL96	2043	asAfsaudGu(G2p)gcucugCfaAfacgcasgsu	2272	ACUGCGUUUGCAGAGCCACAU UC	1595
AD-1571107.1	csgsuuugCfaGfAfGfccacauuccuL96	2044	asGfsgadAu(G2p)uggcucUfgCfaaacgscsa	2273	UGCGUUUGCAGAGCCACAUUC CA	1597
AD-1571108.1	gscsagagCfcAfCfAfuuccagugcuL96	2045	asGfscadCu(G2p)gaauguGfgCfucugcsasa	2274	UUGCAGAGCCACAUUCCAGUG CA	1598
AD-1571109.1	gsgsgacaUfuCfAfCfcuuccaguguL96	2046	asCfsacdTg(G2p)aaggugAfaUfgucccsasc	2275	GUGGGACAUUCACCUUCCAGU GU	1599
AD-1571110.1	gsgsacauUfcAfCfCfuuccaguguuL96	2047	asAfscadCu(G2p)gaagguGfaAfugucccsca	2276	UGGGACAUUCACCUUCCAGUG UG	607
AD-1571111.1	ascsauucAfcCfUfUfccagugugauL96	2048	asUfscadCa(C2p)uggaagGfuGfaauguscsc	2277	GGACAUUCACCUUCCAGUGUG AG	609
AD-1571112.1	asgscugcGfuGfAfAfgaagcccaauL96	2049	asUfsugdGg(C2p)uucuucAfcGfcagcuscsc	2278	GGAGCUGCGUGAAGAAGCCCA AC	1601
AD-1571113.1	gscsugcgUfgAfAfGfaagcccaacuL96	2050	asGfsuudGg(G2p)cuucuuCfaCfgcagcsusc	2279	GAGCUGCGUGAAGAAGCCCAA CC	1602
AD-1571114.1	csusgcguGfaAfGfAfagcccaaccuL96	2051	asGfsgudTg(G2p)gcuucuUfcAfcgcagscsu	2280	AGCUGCGUGAAGAAGCCCAAC CC	1603
AD-1571115.1	usgscgugAfaGfAfAfgcccaacccuL96	2052	asGfsggdTu(G2p)ggcuucUfuCfacgcasgsc	2281	GCUGCGUGAAGAAGCCCAACC CG	1604
AD-1571116.1	asgscacuGfuGfAfCfuguggccucuL96	2053	asGfsagdGc(C2p)acagucAfcAfgugcuscsc	2282	GGAGCACUGUGACUGUGGCCU CC	1605

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1571117.1	csuscgaGfgGfUfGfagugggccauuL96	2054	asAfsugdGc(C2p)acucacCfcUfcggagsgsa	2283	UCCUCCGAGGGUGAGUGGCCA UG	1606
AD-1571118.1	asuscgccuGfaCfCfGfcugggugauuL96	2055	asAfsucdAc(C2p)cagcggUfcAfgcgausgsa	2284	UCAUCGCUGACCGCUGGGUGA UA	611
AD-1571119.1	uscsgcugAfcCfGfCfugggugauauuL96	2056	asUfsaudCa(C2p)ccagcgGfuCfagcgasusg	2285	CAUCGCUGACCGCUGGGUGAU AA	1607
AD-1571120.1	gscsugacCfgCfUfGfgguguaaacuL96	2057	asGfsuudAu(C2p)acccagCfgGfucagcsgsa	2286	UCGCUGACCGCUGGGUGAUAA CA	1609
AD-1571121.1	gscsugggUfgAfUfAfacagcugccuL96	2058	asGfsgcdAg(C2p)uguuauuCfaCfccagcsgsg	2287	CCGCUGGGUGAUAACAGCUGC CC	1610
AD-1571122.1	usgscuucCfaGfGfAfggacagcauuL96	2059	asAfsugdCu(G2p)uccuccUfgGfaagcasgsu	2288	ACUGCUUCCAGGAGGACAGCA UG	1611
AD-1571123.1	csusuccaGfgAfGfGfacagcaugguL96	2060	asCfscadTg(C2p)uguccuCfcUfggaagscsa	2289	UGCUUCCAGGAGGACAGCAUG GC	1613
AD-1571124.1	csusgggcAfaGfGfUfguggcagaauL96	2061	asUfsucdTg(C2p)cacaccUfuGfcccagsgsa	2290	UCCUGGGCAAGGUGUGGCAG AAC	1614
AD-1571125.1	usgsggcaAfgGfUfGfuggcagaacuL96	2062	asGfsuudCu(G2p)ccacacCfuUfgcccasgsg	2291	CCUGGGCAAGGUGUGGCAGAA ACU	1615
AD-1571126.1	gsgscaagGfuGfUfGfgcagaacucuL96	2063	asGfsagdTu(C2p)ugccacAfcCfuugccscsa	2292	UGGGCAAGGUGUGGCAGAAC UCG	1617
AD-1571127.1	usgsgccuGfgAfGfAfgguguccuuuL96	2064	asAfsagdGa(C2p)accucuCfcAfggccasgsc	2293	GCUGGCCUGGAGAGGUGUCCU UC	1619
AD-1571128.1	gscscuggAfgAfGfGfuguccuucauL96	2065	asUfsgadAg(G2p)acaccuCfuCfcaggcscsa	2294	UGGCCUGGAGAGGUGUCCUUC AA	1621
AD-1571129.1	cscsuggaGfaGfGfUfguccuucaauL96	2066	asUfsugdAa(G2p)gacaccUfcUfccaggscsc	2295	GGCCUGGAGAGGUGUCCUUCA AG	1622
AD-1571130.1	usgsugcaGfuUfGfAfucccacagguL96	2067	asCfscudGu(G2p)ggaucaAfcUfgcacasusc	2296	GAUGUGCAGUUGAUCCCACAG GA	1623
AD-1571131.1	usgscaguUfgAfUfCfccacaggacuL96	2068	asGfsucdCu(G2p)uggguuCfaAfcugcascsa	2297	UGUGCAGUUGAUCCCACAGGA CC	1624
AD-1571132.1	asuscccaCfaGfGfAfccugugcaguL96	2069	asCfsugdCa(C2p)agguccUfgUfgggauscsa	2298	UGAUCCCACAGGACCUGUGCA GC	621
AD-1571133.1	cscscacaGfgAfCfCfugugcagcguL96	2070	asCfsgcdTg(C2p)acagguCfcUfgugggsasu	2299	AUCCCACAGGACCUGUGCAGC GA	1625

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1571134.1	cscsacagGfaCfCfUfgugcagcgauL96	2071	asUfscgdCu(G2p)cacaggUfcCfuguggsgsa	2300	UCCCACAGGACCUGUGCAGCG AG	1626
AD-1571135.1	cscsagguGfaCfGfCfcacgcaugcuL96	2072	asGfscadTg(C2p)guggcgUfcAfccuggsusa	2301	UACCAGGUGACGCCACGCAUG CU	1627
AD-1571136.1	gsusgacgCfcAfCfGfcaugcuguguL96	2073	asCfsacdAg(C2p)augcguGfgCfgucacscsu	2302	AGGUGACGCCACGCAUGCUGU GU	1628
AD-1571137.1	usgsacgcCfaCfGfCfaugcuguguuL96	2074	asAfscadCa(G2p)caugcgUfgGfcgucascsc	2303	GGUGACGCCACGCAUGCUGUG UG	622
AD-1571138.1	ascsgccaCfgCfAfUfgcugugugcuL96	2075	asGfscadCa(C2p)agcaugCfgUfggcguscsa	2304	UGACGCCACGCAUGCUGUGUG CC	1629
AD-1571139.1	gsgscuacCfgCfAfAfgggcaagaauL96	2076	asUfsucdTu(G2p)cccuugCfgGfuagccsgsg	2305	CCGGCUACCGCAAGGGCAAGA AG	1630
AD-1571140.1	gsusgcaaGfgCfAfCfucaguggccuL96	2077	asGfsgcdCa(C2p)ugagugCfcUfugcacsasc	2306	GUGUGCAAGGCACUCAGUGGC CG	1631
AD-1571141.1	csusaacuAfcUfUfCfggcgucuacuL96	2078	asGfsuadGa(C2p)gccgaaGfuAfguuagsgsc	2307	GCCUAACUACUUCGGCGUCUA CA	1632
AD-1571142.1	csusacacCfcGfCfAfucagguguL96	2079	asCfsacdCu(G2p)ugaugcGfgGfuguagsasc	2308	GUCUACACCCGCAUCACAGGU GU	628
AD-1571143.1	cscscgcaUfcAfCfAfggugugaucuL96	2080	asGfsaudCa(C2p)accuguGfaUfgcgggsusg	2309	CACCCGCAUCACAGGUGUGAU CA	1638
AD-1571144.1	usgsgaucCfaGfCfAfaguggugacuL96	2081	asGfsucdAc(C2p)acuugcUfgGfauccasgsc	2310	GCUGGAUCCAGCAAGUGGUG ACC	1639
AD-1571145.1	asusccagCfaAfGfUfggugaccuguL96	2082	asCfsagdGu(C2p)accacuUfgCfuggauscsc	2311	GGAUCCAGCAAGUGGUGACCU GA	1641
AD-1571146.1	cscsagcaAfgUfGfGfugaccugaguL96	2083	asCfsucdAg(G2p)ucaccaCfuUfgcuggsasu	2312	AUCCAGCAAGUGGUGACCUGA GG	1642
AD-1571147.1	csasgcaaGfuGfGfUfgaccugaggguL96	2084	asCfscudCa(G2p)gucaccAfcUfugcugsgsa	2313	UCCAGCAAGUGGUGACCUGAG GA	1643
AD-1571148.1	gscsaaguGfgUfGfAfccugaggaauL96	2085	asUfsucdCu(C2p)aggucaCfcAfcuugcsusg	2314	CAGCAAGUGGUGACCUGAGG AAC	1644
AD-1571149.1	usgsguggCfaGfGfAfgguggcaucuL96	2086	asGfsaudGc(C2p)accuccUfgCfcaccascsa	2315	UGUGGUGGCAGGAGGUGGGCA UCU	1645
AD-1571150.1	ggsgsuggcAfgGfAfGfguggcaucuuL96	2087	asAfsgadTg(C2p)caccucCfuGfccaccsasc	2316	GUGGUGGCAGGAGGUGGGCAU CUU	1646

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1571151.1	gsusggcaGfgAfGfGfuggcaucuuuL96	2088	asAfsagdAu(G2p)ccaccuCfcUfgccacscsa	2317	UGGUGGCAGGAGGUGGCAUC UUG	1647
AD-1571152.1	uscscaguGfaUfGfGfcaggaggauuL96	2089	asAfsucdCu(C2p)cugccaUfcAfcuggasgsc	2318	GCUCCAGUGAUGGCAGGAGG AUG	1650
AD-1571153.1	csusaacuUfgGfGfAfucugggaauuL96	2090	asAfsuudCc(C2p)agauccCfaAfguuagsasc	2319	GUCUAACUUGGGAUCUGGGA AUG	1651
AD-1571154.1	gsusgagcUfcAfGfCfugcccuuuguL96	2091	asCfsaadAg(G2p)gcagcuGfaGfcucacscsu	2320	AGGUGAGCUCAGCUGCCCUUU GG	1652
AD-1571155.1	csuscagcUfgCfCfCfuuuggaauauL96	2092	asUfsaudTc(C2p)aaagggCfaGfcugagscsu	2321	AGCUCAGCUGCCCUUUGGAAU AA	1653
AD-1571156.1	uscsagcuGfcCfCfUfuuggaauaauL96	2093	asUfsuadTu(C2p)caaaggGfcAfgcugasgsc	2322	GCUCAGCUGCCCUUUGGAAUA AA	1654
AD-1571157.1	cscscuuuGfgAfAfUfaaagcugccuL96	2094	asGfsgcdAg(C2p)uuuauuCfcAfaagggscsa	2323	UGCCCUUUGGAAUAAAGCUGC CU	1657
AD-1571158.1	cscsuuugGfaAfUfAfaagcugccuuL96	2095	asAfsggdCa(G2p)cuuuauUfcCfaaaggsgsc	2324	GCCCUUUGGAAUAAAGCUGCC UG	1658
AD-1571159.1	ususuggaAfuAfAfAfGfcugccugauL96	2096	asUfscadGg(C2p)agcuuuAfuUfccaaasgsg	2325	CCUUUGGAAUAAAGCUGCCUG AU	1659
AD-1571160.1	ususggaaUfaAfAfGfcugccugauuL96	2097	asAfsucdAg(G2p)cagcuuUfaUfuccaasasg	2326	CUUUGGAAUAAAGCUGCCUG AUC	1660
AD-1571161.1	usgsgaauAfaAfGfCfugccugaucuL96	2098	asGfsaudCa(G2p)gcagcuUfuAfuuccasasa	2327	UUUGGAAUAAAGCUGCCUGA UCC	1661

Table 8. Single Dose Screen in Hep3b Cells

	10 nM		1 nM		0.1 nM	
Duplex	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev
AD-1570929.1	55	8	73	7	55	8
AD-1571034.1	76	5	94	7	129	8
AD-1571035.1	62	15	73	11	82	8
AD-1571036.1	53	8	75	11	92	4
AD-1554875.1	14	3	21	4	30	5
AD-1571037.1	29	7	44	12	98	9
AD-1570930.1	15	3	22	3	30	2
AD-1570931.1	11	2	14	1	21	8
AD-1554909.1	22	6	39	1	44	8
AD-1554910.1	21	4	30	3	39	7
AD-1554911.1	21	3	32	10	36	5
AD-1554912.1	21	3	46	3	51	5
AD-1554913.1	50	6	71	15	66	13
AD-1571038.1	95	21	96	9	121	9
AD-1554914.1	47	8	74	9	70	9
AD-1554915.1	28	3	51	9	50	7
AD-1554916.1	34	5	54	8	64	8
AD-1570932.1	17	3	34	5	55	8
AD-1554917.1	25	5	47	4	52	8
AD-1571039.1	31	3	55	13	89	10
AD-1571040.1	37	8	43	11	86	11
AD-1571041.1	36	9	61	16	97	32
AD-1570933.1	92	14	109	22	97	3
AD-1570934.1	80	11	103	9	71	9
AD-1554923.1	41	6	79	16	72	7
AD-1571042.1	69	19	70	4	93	5
AD-1571043.1	56	11	81	16	107	8
AD-1554951.1	32	5	59	2	56	7
AD-1570935.1	60	12	79	12	73	7
AD-1571044.1	78	9	64	14	122	21
AD-1570936.1	103	24	105	13	102	22
AD-1571045.1	76	15	99	15	122	22
AD-1554955.1	31	6	48	4	51	6
AD-1570937.1	27	5	54	3	61	3
AD-1571046.1	37	9	60	16	87	16
AD-1571047.1	23	3	28	7	45	9
AD-1554992.1	85	6	99	9	75	2
AD-1571048.1	74	14	98	10	111	20
AD-1570938.1	36	4	71	12	70	11
AD-1554997.1	24	6	43	3	50	4
AD-1570939.1	111	16	117	11	84	8

	10 nM		1 nM		0.1 nM	
Duplex	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev
AD-1555000.1	30	5	51	4	64	12
AD-1571050.1	51	10	87	6	88	8
AD-1571051.1	44	7	68	18	77	15
AD-1555030.1	30	6	61	7	57	9
AD-1570940.1	27	4	62	6	70	6
AD-1570941.1	103	16	113	11	79	10
AD-1571052.1	23	4	38	1	40	7
AD-1571053.1	31	2	58	14	76	5
AD-1571054.1	28	5	46	5	56	6
AD-1570942.1	47	4	70	5	76	8
AD-1570943.1	27	7	42	3	68	4
AD-1570944.1	38	6	36	4	62	6
AD-1570945.1	52	8	87	7	67	4
AD-1571055.1	43	6	68	12	83	10
AD-1570946.1	80	11	89	11	82	4
AD-1571056.1	44	3	70	13	87	13
AD-1570947.1	54	9	80	14	84	9
AD-1571057.1	43	3	62	6	67	14
AD-1555106.1	16	5	17	2	35	8
AD-1570948.1	26	7	34	6	53	7
AD-1555112.1	33	5	61	4	64	8
AD-1571028.1	65	8	87	6	105	10
AD-1571029.1	69	12	83	4	112	19
AD-1555114.1	25	6	36	3	43	12
AD-1555115.1	26	5	38	4	40	6
AD-1570949.1	29	5	45	3	56	8
AD-1571030.1	37	2	61	12	74	12
AD-1571031.1	46	11	64	14	79	10
AD-1571058.1	34	4	44	3	53	5
AD-1555117.1	23	6	27	5	38	2
AD-1571032.1	54	1	80	14	86	7
AD-1571033.1	44	5	80	11	101	23
AD-1555118.1	30	7	33	3	47	5
AD-1570950.1	31	6	44	5	63	5
AD-1570951.1	28	6	33	7	46	3
AD-1555120.1	24	6	33	4	53	11
AD-1571059.1	28	5	44	6	55	5
AD-1555121.1	36	5	55	4	69	7
AD-1555122.1	22	4	32	5	49	6
AD-1570952.1	25	4	45	6	52	8
AD-1555123.1	35	7	43	1	70	5
AD-1570953.1	93	6	102	14	101	12

	10 nM		1 nM		0.1 nM	
Duplex	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev
AD-1571060.1	25	4	42	9	53	11
AD-1570954.1	23	6	32	3	72	17
AD-1571061.1	22	3	35	3	43	3
AD-1571062.1	44	7	68	5	87	15
AD-1555128.1	36	7	41	6	63	15
AD-1570955.1	31	8	35	2	48	9
AD-1571063.1	80	10	88	14	89	6
AD-1571064.1	87	8	94	6	123	6
AD-1571065.1	68	4	80	9	93	8
AD-1570956.1	48	9	76	7	93	19
AD-1570957.1	50	11	66	3	82	14
AD-1571066.1	35	5	43	9	82	26
AD-1570958.1	69	12	102	7	92	3
AD-1555184.1	87	13	100	12	99	5
AD-1571067.1	80	18	77	11	93	12
AD-1555185.1	63	15	88	15	100	13
AD-1571068.1	71	10	55	6	73	15
AD-1570959.1	104	13	106	9	85	6
AD-1570960.1	48	9	62	18	79	16
AD-1571069.1	57	5	41	10	81	9
AD-1570961.1	73	12	101	2	94	13
AD-1571070.1	48	5	44	11	78	8
AD-1570962.1	57	11	88	6	82	13
AD-1570963.1	33	6	52	4	50	8
AD-1570964.1	52	10	83	7	92	19
AD-1571071.1	59	4	65	6	85	16
AD-1570965.1	86	17	109	12	100	16
AD-1571072.1	72	6	75	4	120	8
AD-1555212.1	42	11	56	7	71	13
AD-1570966.1	32	5	39	9	58	6
AD-1555213.1	33	6	36	5	47	7
AD-1570967.1	35	8	58	10	52	4
AD-1571074.1	19	3	31	6	33	4
AD-1570968.1	30	6	41	4	44	7
AD-1555234.1	30	6	41	6	56	5
AD-1570969.1	42	8	62	10	61	8
AD-1555235.1	51	9	77	12	72	5
AD-1555236.1	59	7	67	15	68	3
AD-1555238.1	45	8	55	9	58	1
AD-1570970.1	77	10	88	32	74	6
AD-1555241.1	41	6	57	10	39	9
AD-1555242.1	47	6	83	6	71	3

	10 nM		1 nM		0.1 nM	
Duplex	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev
AD-1555243.1	41	8	65	7	67	4
AD-1570971.1	93	11	108	8	92	14
AD-1571075.1	25	5	37	5	38	3
AD-1571076.1	15	4	33	9	41	8
AD-1571077.1	39	9	43	13	46	11
AD-1555247.1	42	4	51	4	78	8
AD-1571078.1	16	3	40	14	49	4
AD-1570972.1	53	15	67	39	40	18
AD-1570973.1	45	5	35	8	55	8
AD-1570974.1	76	12	81	16	81	9
AD-1555342.1	73	16	69	3	78	18
AD-1570975.1	108	21	84	15	103	13
AD-1555343.1	80	12	92	5	91	9
AD-1555345.1	84	10	97	6	103	13
AD-1555346.1	54	12	71	7	86	3
AD-1570976.1	71	11	70	9	93	5
AD-1555348.1	57	22	64	6	84	12
AD-1555349.1	36	8	50	2	66	7
AD-1555350.1	57	9	58	10	77	10
AD-1571079.1	71	12	77	10	65	11
AD-1570977.1	34	8	68	10	92	8
AD-1570978.1	30	13	53	4	86	5
AD-1571080.1	63	11	70	3	71	14
AD-1571081.1	76	12	79	3	94	16
AD-1555366.1	42	4	48	2	78	2
AD-1571082.1	37	3	54	5	56	11
AD-1570979.1	31	8	54	12	72	10
AD-1571083.1	45	4	54	6	56	8
AD-1571084.1	34	1	53	11	58	11
AD-1570980.1	82	13	81	14	92	9
AD-1555428.1	48	12	75	8	96	4
AD-1555429.1	47	7	66	8	90	4
AD-1570981.1	34	14	66	3	92	6
AD-1555535.1	41	2	65	5	71	6
AD-1571085.1	48	6	77	6	69	8
AD-1555537.1	52	1	63	4	115	12
AD-1571086.1	40	2	54	6	61	3
AD-1571087.1	69	15	76	4	97	13
AD-1571088.1	39	7	63	8	60	7
AD-1555546.1	20	4	30	4	56	7
AD-1555547.1	24	3	47	4	73	11
AD-1555548.1	41	5	55	5	79	7

	10 nM		1 nM		0.1 nM	
Duplex	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev
AD-1555549.1	61	10	89	7	84	11
AD-1555581.1	35	5	60	9	95	12
AD-1570982.1	55	1	80	11	95	11
AD-1570983.1	61	5	84	10	100	13
AD-1555583.1	40	4	65	3	89	9
AD-1555584.1	50	5	78	11	102	8
AD-1555585.1	49	4	74	13	86	9
AD-1555586.1	48	11	70	5	86	18
AD-1555587.1	34	9	60	6	89	11
AD-1555588.1	40	7	56	7	91	10
AD-1555589.1	34	3	52	11	83	13
AD-1571089.1	32	3	42	6	60	3
AD-1555590.1	46	6	68	16	87	5
AD-1571090.1	40	8	54	10	69	12
AD-1571091.1	39	8	52	7	56	7
AD-1570984.1	77	11	100	10	110	8
AD-1571092.1	39	9	76	6	86	16
AD-1571093.1	71	9	76	7	86	10
AD-1571094.1	66	7	73	18	104	13
AD-1570985.1	25	5	43	10	60	4
AD-1555615.1	43	2	60	7	82	12
AD-1555616.1	60	10	84	22	91	9
AD-1571096.1	90	10	95	12	96	14
AD-1555626.1	69	15	67	11	99	11
AD-1570986.1	71	6	90	10	93	5
AD-1555628.1	81	7	85	11	102	15
AD-1570987.1	119	16	99	14	126	8
AD-1570988.1	82	7	96	8	116	10
AD-1571097.1	43	3	65	11	61	6
AD-1555706.1	60	10	78	16	101	18
AD-1570989.1	59	17	83	12	96	12
AD-1555707.1	34	8	57	5	81	9
AD-1570990.1	63	9	67	8	93	9
AD-1571098.1	48	3	73	3	82	10
AD-1555709.1	44	3	72	12	89	14
AD-1571099.1	50	11	79	12	92	7
AD-1571100.1	24	5	44	3	64	10
AD-1555711.1	49	5	78	4	97	15
AD-1570991.1	77	8	122	9	114	11
AD-1570992.1	78	6	127	24	97	12
AD-1570993.1	28	4	51	1	77	6
AD-1570994.1	67	1	85	18	102	11

	10 nM		1 nM		0.1 nM	
Duplex	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev
AD-1555717.1	42	2	57	1	73	8
AD-1555723.1	48	5	70	11	100	13
AD-1555725.1	42	3	71	11	98	17
AD-1570995.1	90	13	110	20	129	15
AD-1571102.1	24	4	37	4	58	5
AD-1570996.1	47	8	87	24	112	13
AD-1571103.1	43	6	68	8	92	14
AD-1555768.1	37	8	66	14	92	18
AD-1570997.1	43	6	85	17	89	20
AD-1570998.1	61	7	91	16	90	23
AD-1555771.1	17	3	34	6	44	5
AD-1555772.1	23	3	43	10	66	17
AD-1555776.1	52	12	82	12	117	23
AD-1570999.1	120	18	120	23	154	33
AD-1571104.1	70	9	56	7	91	16
AD-1571105.1	20	1	40	5	40	5
AD-1571106.1	31	2	47	7	74	14
AD-1571000.1	38	5	94	12	112	16
AD-1571107.1	25	1	52	5	70	6
AD-1555789.1	27	2	55	7	72	9
AD-1571108.1	65	9	87	6	92	21
AD-1555894.1	52	13	65	9	115	5
AD-1555895.1	37	7	58	8	78	17
AD-1571001.1	59	11	96	15	96	16
AD-1571109.1	62	4	83	7	93	10
AD-1555897.1	57	15	88	21	125	13
AD-1571110.1	79	11	109	10	118	10
AD-1555898.1	47	7	87	24	114	24
AD-1555899.1	78	6	109	14	104	4
AD-1571111.1	88	5	95	8	107	17
AD-1555900.1	45	4	99	12	86	5
AD-1571002.1	19	8	61	5	69	6
AD-1571112.1	27	3	50	6	65	11
AD-1571113.1	41	2	64	8	82	16
AD-1571114.1	39	5	62	5	77	15
AD-1571115.1	54	7	70	7	74	13
AD-1571116.1	41	4	70	8	75	13
AD-1571117.1	110	3	108	23	102	14
AD-1556052.1	19	3	42	5	73	17
AD-1571118.1	24	5	60	6	79	3
AD-1571119.1	30	3	55	10	83	13
AD-1571003.1	42	4	87	7	94	11

	10 nM		1 nM		0.1 nM	
Duplex	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev
AD-1571120.1	44	7	58	11	77	15
AD-1556057.1	33	5	69	12	71	7
AD-1571121.1	69	11	77	6	87	8
AD-1571122.1	46	4	62	13	81	19
AD-1571004.1	106	5	115	12	111	10
AD-1571123.1	90	10	103	6	102	9
AD-1556126.1	43	2	103	18	100	16
AD-1571005.1	40	16	99	10	88	10
AD-1556127.1	38	3	75	14	77	7
AD-1571124.1	44	6	84	11	102	15
AD-1571125.1	54	6	95	16	107	19
AD-1571006.1	35	0	76	9	80	2
AD-1571126.1	49	11	70	10	72	12
AD-1556137.1	40	1	85	17	86	3
AD-1571007.1	66	12	117	20	104	14
AD-1571008.1	55	5	101	25	107	8
AD-1556139.1	48	7	84	15	101	21
AD-1571127.1	60	5	76	6	79	8
AD-1571009.1	23	6	76	19	66	8
AD-1571128.1	42	5	71	11	95	15
AD-1571129.1	47	9	71	11	87	18
AD-1556163.1	27	6	81	14	85	11
AD-1571010.1	61	5	94	11	73	5
AD-1556164.1	52	5	41	7	77	2
AD-1556166.1	55	10	88	7	89	14
AD-1556167.1	43	7	93	13	114	8
AD-1571011.1	44	12	99	12	101	14
AD-1571130.1	48	3	82	12	83	15
AD-1571131.1	54	7	78	10	99	21
AD-1556319.1	34	5	47	15	62	9
AD-1571132.1	75	15	100	25	114	14
AD-1571133.1	96	24	110	24	126	31
AD-1571134.1	52	14	87	14	108	10
AD-1571135.1	47	12	65	4	138	37
AD-1571136.1	93	7	105	14	112	13
AD-1556359.1	31	6	36	1	81	0
AD-1571137.1	59	9	81	10	100	13
AD-1556360.1	26	2	49	15	48	12
AD-1571138.1	85	18	93	18	91	16
AD-1571139.1	51	10	92	15	100	24
AD-1556382.1	38	8	63	6	40	8
AD-1571012.1	58	6	71	10	54	15

	10 nM		1 nM		0.1 nM	
Duplex	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev
AD-1556383.1	44	7	81	10	70	22
AD-1571013.1	58	12	90	6	86	10
AD-1571140.1	117	32	120	16	131	16
AD-1556465.1	36	2	70	8	68	2
AD-1556466.1	8	2	24	4	41	3
AD-1571141.1	52	11	88	15	97	12
AD-1571014.1	63	13	45	10	91	24
AD-1571015.1	49	6	83	9	80	19
AD-1571016.1	47	4	67	1	59	7
AD-1571017.1	55	5	90	13	90	14
AD-1556484.1	49	13	87	2	79	16
AD-1571142.1	84	11	94	20	97	11
AD-1571018.1	48	9	83	10	94	16
AD-1571143.1	49	5	73	12	95	5
AD-1556510.1	34	6	57	3	68	10
AD-1571144.1	28	7	53	10	74	9
AD-1571019.1	28	2	54	2	75	9
AD-1571145.1	38	7	51	4	77	8
AD-1571146.1	39	3	63	3	81	11
AD-1571147.1	38	6	48	9	77	5
AD-1571148.1	25	1	46	6	68	4
AD-1571149.1	59	7	68	8	72	5
AD-1571150.1	41	11	65	8	88	5
AD-1571151.1	59	1	74	13	94	13
AD-1556584.1	67	5	102	17	89	17
AD-1556585.1	54	3	92	17	91	21
AD-1571020.1	86	13	118	16	114	9
AD-1556586.1	57	7	93	3	103	13
AD-1556587.1	47	8	75	9	94	7
AD-1571021.1	72	1	95	4	117	8
AD-1571022.1	47	3	84	8	97	8
AD-1556613.1	48	9	62	7	88	12
AD-1571152.1	52	4	67	8	92	13
AD-1556677.1	40	5	80	18	94	12
AD-1556709.1	66	16	92	5	91	9
AD-1571023.1	56	8	94	13	85	10
AD-1556710.1	51	6	69	9	91	12
AD-1556789.1	57	4	97	5	93	12
AD-1556790.1	75	5	113	21	107	13
AD-1556791.1	77	13	101	22	99	19
AD-1571153.1	53	9	65	9	95	11
AD-1556795.1	43	4	82	5	99	17

	10 nM		1 nM		0.1 nM	
Duplex	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev	Avg % message remaining	St. Dev
AD-1556799.1	64	3	87	11	104	7
AD-1571024.1	85	11	113	13	115	7
AD-1556802.1	62	10	95	14	92	14
AD-1571154.1	47	6	67	7	96	7
AD-1556908.1	37	5	82	10	93	11
AD-1556909.1	70	16	101	11	114	24
AD-1556911.1	20	1	39	3	42	8
AD-1571155.1	11	3	24	3	45	6
AD-1571156.1	8	1		0		
AD-1571025.1	40	8	49	4	56	8
AD-1556915.1	29	8	37	8	58	12
AD-1556917.1	22	4	39	6	55	9
AD-1571026.1	30	6	52	6	57	12
AD-1556918.1	18	4	33	9	46	5
AD-1571027.1	45	4	66	6	86	3
AD-1571157.1	18	7	37	8	57	10
AD-1571158.1	10	2	17	3	20	5
AD-1571159.1	18	0	22	5	42	4
AD-1571160.1	16	1	26	4	35	5
AD-1571161.1	27	3	31	10	55	12

Example 3. *In vivo Efficacy* of dsRNA Duplexes in Non-Human Primates (NHP)

Selected duplexes of interest, identified from the above *in vitro* studies, were evaluated *in vivo* in non-human primates. Figure 1 provides a depiction of the study design.

In particular, 15 male Cynomolgus monkeys were divided into 5 groups of 3 each and were subcutaneously administered a single 3 mg/kg dose of AD-1556360, a single 10 mg/kg dose of AD-1556360, a single 3 mg/kg dose of AD-1571158, or a single 3 mg/kg dose of AD-1571033, or PBS as a control (see Table 9). For each animal, two liver biopsy samples (one per lobe) of about 100 mg each were collected following 12 hours of fasting on Day 22, Day 57, and/or Day 85. Liver biopsy and serum samples were also collected from the animals 21 days prior to dosing. One mL of blood was collected into tubes without anticoagulant weekly from Day 1 for hepcidin level, iron level, transferrin saturation level, and red blood cell (RBC) count determinations. Following clotting, serum was aliquoted and stored at -80°C.

Tissue mRNA was extracted and alayzed by the RT-QPCR method. TMPRSS6 mRNA levels were compared to the levels of the housekeeping gene, GAPDH. The values were then normalized to the average of PBS vehicle control group. The data were expressed as percent of baseline value, and presented as mean plus standard deviation.

Iron and transferrin saturation levels were determined using commercially available kits from Roche.

The results, shown in Figures 2-4, demonstrate that all three exemplary duplexes, AD-1556360, AD-1571158, and AD-1571033, potently and durably inhibit the expression ofTMPRSS6 messenger RNA *in vivo* (Figure 2), potently and durably lower plasma iron levels (Figure 3), and potently and durably lower transferrin saturation levels (Figure 4). Transferrin saturation is a measure of the amount of iron bound to serum transferrin, and corresponds to the ratio of serum iron and total iron-binding capacity.

Table 9. Treatment Groups

Group No.	Duplex	Dose Level (mg/kg)	No. of males
1	PBS (control)	0	3
2	AD-1556360	3	3
3	AD-1556360	10	3
4	AD-1571158	3	3
5	AD-1571033 (benchmark comparator duplex)	3	3

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Transmembrane protease, serine 6 (TMPRSS6) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding TMPRSS6, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-7.

2. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2-7 and an antisense strand comprising at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-7.

3. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising at least 15 contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2-7 and an antisense strand comprising at least 15 contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-7.

4. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising at least 15 contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the sense strands in any one of Tables 2-7 and an antisense strand comprising at least 15 contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-7.

5. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the sense strands in any one of Tables 2-7 and an antisense strand comprising a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the antisense strands in any one of Tables 2-7.

6. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Transmembrane protease, serine 6 (TMPRSS6) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the

nucleotide sequence of nucleotides 187-210; 227-254;322-363; 362-390; 398-420; 404-429; 410-435; 439-461; 443-467; 448-474; 460-483; 466-488; 496-519; 519-542; 526-548; 557-593; 641-671; 652-676; 687-713; 725-762; 757-794; 886-908; 921-951; 956-987; 1051-1082; 1233-1269; 1279-1313; 1313-1341; 1327-1351; 1415-1439; 1447-1480; 1464-1486; 1486-1509; 1559-1589; 1571-1595; 1579-1609; 1707-1735; 1738-1764; 1806-1828; 1864-1886; 1934-1966; 1967-1991; 2008-2031; 2015-2043; 2042-2072; 2287-2311; 2297-2354; 2336-2361; 2360-2384; 2416-2438; 2481-2510; 2496-2527; 2526-2558; 2665-2693; 2693-2719; 2707-2729; 2799-2821; 2851-2874; 2971-2999; 2981-3006; and 3155-3195 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

7. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Transmembrane protease, serine 6 (TMPRSS6) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 230-252, 324-346, 560-578, 560-582, 2338-2360, 3163-3185, 3169-3191, and 3172-3194 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

8. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Transmembrane protease, serine 6 (TMPRSS6) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 560-578, 2338-2360, and 3169-3191 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

9. The dsRNA agent of any one of claims 1-8, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1556360, AD-1571158, AD-1571033, AD-1554875, AD-1571160, AD-1555117, AD-1554911, and AD-1556915.

10. The dsRNA agent of any one of claims 1-9, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1556360, AD-1571158, and AD-1571033.

11. The dsRNA agent of any one of claims 1-10, wherein the dsRNA agent comprises at least one modified nucleotide.

12. The dsRNA agent of any one of claims 1-11, wherein substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand comprise a modification; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

13. The dsRNA agent of any one of claims 1-12, wherein all of the nucleotides of the sense strand comprise a modification; all of the nucleotides of the antisense strand comprise a modification; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

14. The dsRNA agent of any one of claims 11-13, wherein at least one of the modified nucleotides is selected from the group a deoxy-nucleotide, a 3'-terminal deoxythimidine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a 2'-5'-linked ribonucleotide (3'-RNA), a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide comprising a 5' phosphate or 5' phosphate mimic, a nucleotide comprising vinyl phosphonate, a glycol nucleic acid (GNA), a glycol nucleic acid S-Isomer (S-GNA), a nucleotide comprising 2-hydroxymethyl-tetrahydrofuran-5-phosphate, a nucleotide comprising 2'-deoxythymidine-3'phosphate, a nucleotide comprising 2'-deoxyguanosine-3'-phosphate, and a terminal nucleotide linked to a cholesteryl derivative and a dodecanoic acid bisdecylamide group; and combinations thereof.

15. The dsRNA agent of any one of claims 11-13, wherein the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

16. The dsRNA agent of any one of claims 11-13, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified

nucleotide (GNA), a nucleotide comprising a 2' phosphate, and, a vinyl-phosphonate nucleotide; and combinations thereof.

17. The dsRNA agent of any one of claims 1-16, wherein the double stranded region is 19-30 nucleotide pairs in length.

18. The dsRNA agent of claim 17, wherein the double stranded region is 19-25 nucleotide pairs in length.

19. The dsRNA agent of claim 17, wherein the double stranded region is 19-23 nucleotide pairs in length.

20. The dsRNA agent of claim 17, wherein the double stranded region is 23-27 nucleotide pairs in length.

21. The dsRNA agent of claim 17, wherein the double stranded region is 21-23 nucleotide pairs in length.

22. The dsRNA agent of any one of claims 1-21, wherein each strand is independently no more than 30 nucleotides in length.

23. The dsRNA agent of any one of claims 1-22, wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

24. The dsRNA agent of any one of claims 1-23, wherein the region of complementarity is at least 17 nucleotides in length.

25. The dsRNA agent of any one of claims 1-24, wherein the region of complementarity is between 19 and 23 nucleotides in length.

26. The dsRNA agent of any one of claims 1-25, wherein the region of complementarity is 19 nucleotides in length.

27. The dsRNA agent of any one of claims 1-26, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

28. The dsRNA agent of any one of claims 1-26, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

29. The dsRNA agent of any one of claims 1-28, further comprising a ligand.

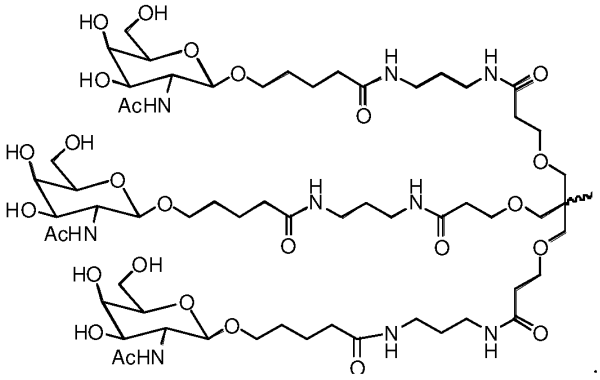
30. The dsRNA agent of claim 29, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

31. The dsRNA agent of claim 29, wherein the ligand is conjugated to the 5' end of the sense strand of the dsRNA agent.

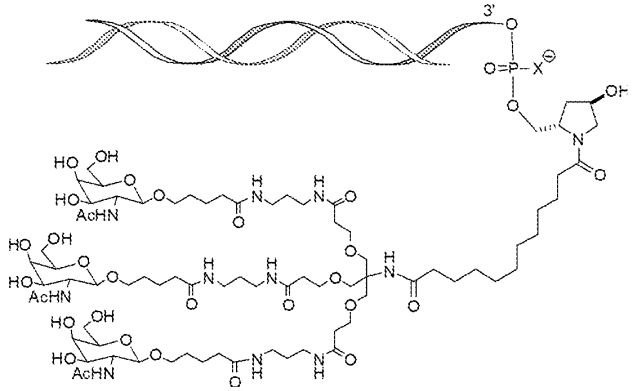
32 The dsRNA agent of any one of claims 29-31, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

33. The dsRNA agent of any one of claims 29-32, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

34. The dsRNA agent of claim 32 or 33, wherein the ligand is



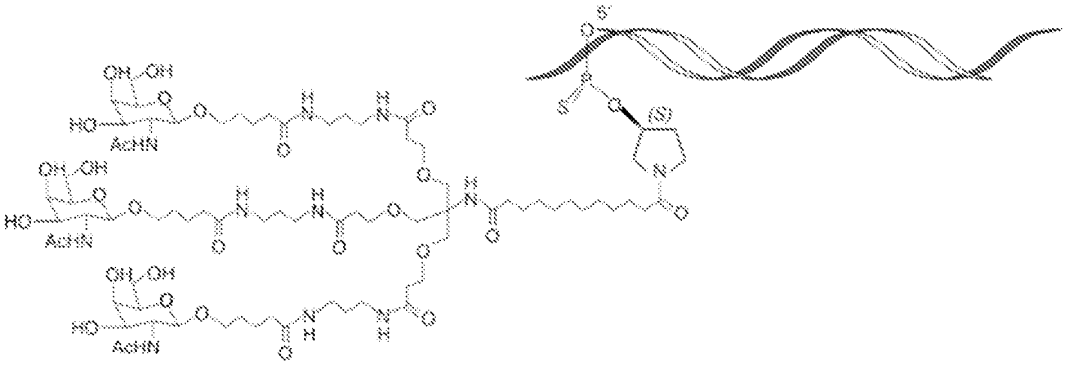
35. The dsRNA agent of claim 34, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

36. The dsRNA agent of claim 35, wherein the X is O.

37. The dsRNA agent of claim 34, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



38. The dsRNA agent of any one of claims 1-37, wherein the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

39. The dsRNA agent of claim 38, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

40. The dsRNA agent of claim 39, wherein the strand is the antisense strand.

41. The dsRNA agent of claim 39, wherein the strand is the sense strand.

42. The dsRNA agent of claim 39, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

43. The dsRNA agent of claim 42, wherein the strand is the antisense strand.

44. The dsRNA agent of claim 42, wherein the strand is the sense strand.

45. The dsRNA agent of claim 38, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at both the 5'- and 3'-terminus of one strand.

46. The dsRNA agent of claim 45, wherein the strand is the antisense strand.

47. The dsRNA agent of any one of claims 1-46, comprising 6-8 phosphorothioate or methylphosphonate internucleotide linkages.

48. The dsRNA agent of any one of claims 1-47, wherein the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

49. The dsRNA agent of any one of claims 1-48, wherein the sense strand comprises at least 17 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'-GACGCCACGCAUGCUGUGUGU -3'(SEQ ID NO: 119).

50. The dsRNA agent of any one of claims 1-49, wherein the sense strand comprises at least 19 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'-GACGCCACGCAUGCUGUGUGU -3'(SEQ ID NO: 119).

51. The dsRNA agent of any one of claims 1-50, wherein the sense strand comprises the nucleotide sequence of 5'- GACGCCACGCAUGCUGUGUGU -3'(SEQ ID NO: 119).

52. The dsRNA agent of any one of claims 1-51, wherein the sense strand consists of the nucleotide sequence of 5'- GACGCCACGCAUGCUGUGUGU -3'(SEQ ID NO: 119).

53. The dsRNA agent of any one of claims 1-52, wherein the antisense strand comprises at least 17 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'-ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

54. The dsRNA agent of any one of claims 1-53, wherein the antisense strand comprises at least 19 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'-ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

55. The dsRNA agent of any one of claims 1-54, wherein the antisense strand comprises at least 21 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'- ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

56. The dsRNA agent of any one of claims 1-55, wherein the antisense strand comprises the nucleotide sequence of 5'- ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

57. The dsRNA agent of any one of claims 1-56, wherein the antisense strand consists of the nucleotide sequence of 5'- ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

58. The dsRNA agent of any one of claims 1-57, wherein the sense strand comprises the nucleotide sequence of 5'- GACGCCACGCAUGCUGUGUGU -3'(SEQ ID NO: 119) and the antisense strand comprises the nucleotide sequence of 5'- ACACACAGCAUGCGUGGCGUCAC -3' (SEQ ID NO: 245).

59. The dsRNA agent of any one of claims 1-58, wherein the sense strand differs by no more than 3 modified nucleotides from the nucleotide sequence of 5'- gsascgccacGfCfAfugcugugugu-3' (SEQ ID NO:371) wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

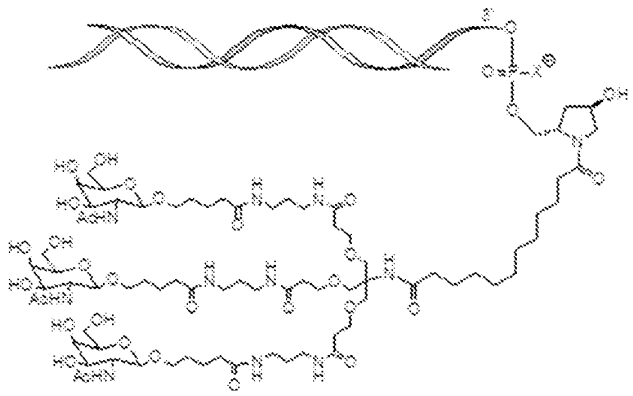
60. The dsRNA agent of any one of claims 1-59, wherein the antisense strand differs by no more than 3 modified nucleotides from the nucleotide sequence of 5'- asdCsacdAcdAgcaudGcGfuggcgucsasc -3' (SEQ ID NO: 497), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein dA, dG, and dC are 2`-deoxyadenosine-3`-phosphate, 2`-deoxyguanosine-3`-phosphate, and 2`-deoxycytidine-3`-phosphate respectively; and s is a phosphorothioate linkage.

61. The dsRNA agent of any one of claims 1-60, wherein the sense strand comprises the nucleotide sequence of 5'- gsascgccacGfCfAfugcugugugu-3' (SEQ ID NO: 371) and the antisense strand comprises the nucleotide sequence of 5'- asdCsacdAcdAgcaudGcGfuggcgucsasc -3' (SEQ ID NO: 497), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein dA, dG, and dC are 2`-deoxyadenosine-3`-phosphate, 2`-deoxyguanosine-3`-phosphate, and 2`-deoxycytidine-3`-phosphate respectively; and s is a phosphorothioate linkage.

62. The dsRNA agent of any one of claims 1-61, wherein the sense strand comprises the nucleotide sequence of 5'- gsascgccacGfCfAfugcugugugL96 -3' (SEQ ID NO: 371) and the

antisense strand comprises the nucleotide sequence of 5'- asdCsacdAcdAgcaudGcGfuggcgucsasc -3' (SEQ ID NO: 497), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein dA, dG, and dC are 2`-deoxyadenosine-3`-phosphate, 2`-deoxyguanosine-3`-phosphate, and 2`-deoxycytidine-3`-phosphate respectively; s is a phosphorothioate linkage, and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

63. The dsRNA agent of any one of claims 1-61, wherein the sense strand comprises the nucleotide sequence of 5'- gsascgccacGfCfAfugcugugugu -3' (SEQ ID NO: 371) and the antisense strand comprises the nucleotide sequence of 5'- asdCsacdAcdAgcaudGcGfuggcgucsasc -3' (SEQ ID NO: 497), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein dA, dG, and dC are 2`-deoxyadenosine-3`-phosphate, 2`-deoxyguanosine-3`-phosphate, and 2`-deoxycytidine-3`-phosphate respectively; and s is a phosphorothioate linkage, wherein the 3'-end of the sense strand is conjugated to the ligand as shown in the following schematic:



and, wherein X is O.

64. The dsRNA agent of any one of claims 1-48, wherein sense strand comprises at least 17 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'-CCUUUGGAAUAAAGCUGCCUU -3'(SEQ ID NO: 844).

65. The dsRNA agent of any one of claims 1-48 and 64, wherein the sense strand comprises at least 19 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'- CCUUUGGAAUAAAGCUGCCUU -3'(SEQ ID NO: 844).

66. The dsRNA agent of any one of claims 1-48, 64 and 65, wherein the sense strand comprises the nucleotide sequence of 5'- CCUUUGGAAUAAAGCUGCCUU -3'(SEQ ID NO: 844).

67. The dsRNA agent of any one of claims 1-48 and 64-66, wherein the sense strand consists of the nucleotide sequence of 5'- CCUUUGGAAUAAAGCUGCCUU -3'(SEQ ID NO: 844).

68. The dsRNA agent of any one of claims 1-48 and 64-67, wherein the antisense strand comprises at least 17 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'- AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

69. The dsRNA agent of any one of claims 1-48 and 64-68, wherein the antisense strand comprises at least 19 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'- AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

70. The dsRNA agent of any one of claims 1-48 and 64-69, wherein the antisense strand comprises at least 21 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'- AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

71. The dsRNA agent of any one of claims 1-48 and 64-70, wherein the antisense strand comprises the nucleotide sequence of 5'- AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

72. The dsRNA agent of any one of claims 1-48 and 64-71, wherein the antisense strand consists of the nucleotide sequence of 5'- AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

73. The dsRNA agent of any one of claims 1-48 and 64-72, wherein the sense strand comprises the nucleotide sequence of 5'- CCUUUGGAAUAAAGCUGCCUU -3'(SEQ ID NO: 844) and the antisense strand comprises the nucleotide sequence of 5'- AAGGCAGCUUUAUUCCAAAGGGC -3' (SEQ ID NO: 1868).

74. The dsRNA agent of any one of claims 1-48 and 64-73, wherein the sense strand differs by no more than 3 modified nucleotides from the nucleotide sequence of 5'- cscsuuugGfaAfUfAfaagcugccuu -3' (SEQ ID NO: 2095) wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

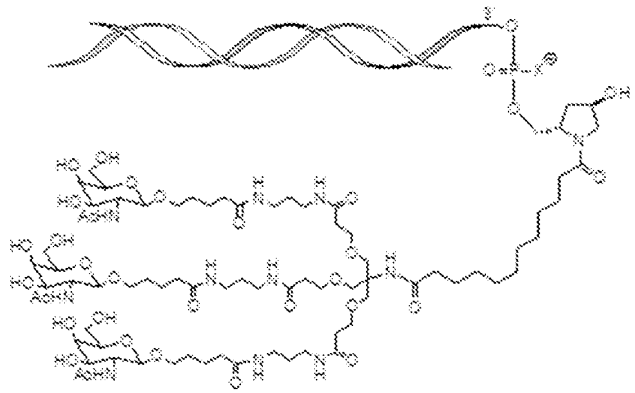
75. The dsRNA agent of any one of claims 1-48 and 64-74, wherein the antisense strand differs by no more than 3 modified nucleotides from the nucleotide sequence of 5'- asAfsggdCa(G2p)cuuuauUfcCfaaaggsgsc -3' (SEQ ID NO: 2324), wherein a, g, c and u are 2′-O-

methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein G2p is guanosine-2`-phosphate; and s is a phosphorothioate linkage.

76. The dsRNA agent of any one of claims 1-48 and 64-75, wherein the sense strand comprises the nucleotide sequence of 5’- cscsuuugGfaAfUfAfaagcugccuu -3’ (SEQ ID NO: 2095) and the antisense strand comprises the nucleotide sequence of 5’- asAfsggdCa(G2p)cuuuauUfcCfaaaggsgsc -3’ (SEQ ID NO: 2324), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein G2p is guanosine-2`-phosphate; and s is a phosphorothioate linkage.

77. The dsRNA agent of any one of claims 1-48 and 64-76, wherein the sense strand comprises the nucleotide sequence of 5’- cscsuuugGfaAfUfAfaagcugccuuL96 -3’ (SEQ ID NO: 2095) and the antisense strand comprises the nucleotide sequence of 5’- asAfsggdCa(G2p)cuuuauUfcCfaaaggsgsc -3’ (SEQ ID NO: 2324), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein G2p is guanosine-2`-phosphate; s is a phosphorothioate linkage, and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

78. The dsRNA agent of any one of claims 1-48 and 64-76, wherein the sense strand comprises the nucleotide sequence of 5’- cscsuuugGfaAfUfAfaagcugccuu -3’ (SEQ ID NO: 2095) and the antisense strand comprises the nucleotide sequence of 5’- asAfsggdCa(G2p)cuuuauUfcCfaaaggsgsc -3’ (SEQ ID NO: 2324), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; wherein G2p is guanosine-2`-phosphate, s is a phosphorothioate linkage, and wherein the 3’-end of the sense strand is conjugated to the ligand as shown in the following schematic:



and, wherein X is O.

79. The dsRNA agent of any one of claims 1-48, wherein the sense strand comprises at least 17 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'-UCACCUGCUUCUUCUGGUU-3'(SEQ ID NO: 1686).

80. The dsRNA agent of any one of claims 1-48 and 79, wherein the sense strand comprises the nucleotide sequence of 5'- UCACCUGCUUCUUCUGGUU-3'(SEQ ID NO: 1686).

81. The dsRNA agent of any one of claims 1-48, 79 and 80, wherein the sense strand consists of the nucleotide sequence of 5'- UCACCUGCUUCUUCUGGUU-3'(SEQ ID NO: 1686).

82. The dsRNA agent of any one of claims 1-48 and 79-81, wherein the antisense strand comprises at least 17 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 5'- AACCAGAAGAAGCAGGUGA-3' (SEQ ID NO: 1790).

83. The dsRNA agent of any one of claims 1-48 and 79-82, wherein the antisense strand comprises the nucleotide sequence of 5'- AACCAGAAGAAGCAGGUGA-3' (SEQ ID NO: 1790).

84. The dsRNA agent of any one of claims 1-48 and 79-83, wherein the antisense strand consists of the nucleotide sequence of 5'- AACCAGAAGAAGCAGGUGA-3' (SEQ ID NO: 1790).

85. The dsRNA agent of any one of claims 1-48 and 79-84, wherein the sense strand comprises the nucleotide sequence of 5'- UCACCUGCUUCUUCUGGUU-3'(SEQ ID NO: 1686) and the antisense strand comprises the nucleotide sequence of 5'- AACCAGAAGAAGCAGGUGA -3' (SEQ ID NO:1790).

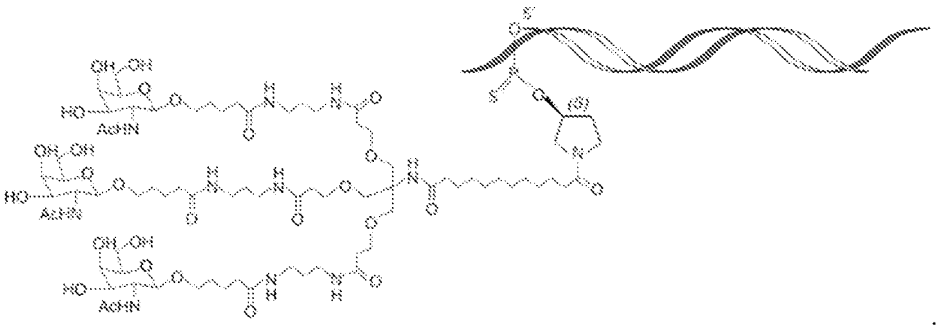
86. The dsRNA agent of any one of claims 1-48 and 79-85, wherein the sense strand differs by no more than 3 modified nucleotides from the nucleotide sequence of 5'-UfcAfcCfuGfcUfuCfuUfcUfgGfsusUf -3' (SEQ ID NO: 1974), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

87. The dsRNA agent of any one of claims 1-48 and 79-86, wherein the antisense strand differs by no more than 3 modified nucleotides from the nucleotide sequence of 5'-asAfscCfaGfaAfgAfaGfcAfgGfusGfsa -3' (SEQ ID NO: 2203), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

88. The dsRNA agent of any one of claims 1-48 and 79-87, wherein the sense strand comprises the nucleotide sequence of 5'- UfcAfcCfuGfcUfuCfuUfcUfgGfsusUf-3' (SEQ ID NO: 1974) and the antisense strand comprises the nucleotide sequence of 5'- asAfscCfaGfaAfgAfaGfcAfgGfusGfsa -3' (SEQ ID NO: 2203), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage.

89. The dsRNA agent of any one of claims 1-48 and 79-88, wherein the sense strand comprises the nucleotide sequence of 5'- Q191sUfcAfcCfuGfcUfuCfuUfcUfgGfsusUf -3' (SEQ ID NO: 1974) and the antisense strand comprises the nucleotide sequence of 5'- asAfscCfaGfaAfgAfaGfcAfgGfusGfsa -3' (SEQ ID NO: 2203), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; s is a phosphorothioate linkage, and Q191 is N-[tris(GalNAc-alkyl)-amidododecanoyl]-(S)-pyrrolidin-3-ol-phosphorothioate (p-C12-(GalNAc-alkyl)3).

90. The dsRNA agent of any one of claims 1-48 and 79-88, wherein the sense strand comprises the nucleotide sequence of 5'- UfcAfcCfuGfcUfuCfuUfcUfgGfsusUf -3' (SEQ ID NO: 1974) and the antisense strand comprises the nucleotide sequence of 5'- asAfscCfaGfaAfgAfaGfcAfgGfusGfsa -3' (SEQ ID NO: 2203), wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; and s is a phosphorothioate linkage, wherein the 5'-end of the sense strand is conjugated to the ligand as shown in the following schematic:



91. A cell containing the dsRNA agent of any one of claims 1-90.

92. A pharmaceutical composition for inhibiting expression of a gene encoding Transmembrane protease, serine 6 (TMPRSS6) comprising the dsRNA agent of any one of claims 1-90.

93. The pharmaceutical composition of claim 92, wherein dsRNA agent is in an unbuffered solution.

94. The pharmaceutical composition of claim 93, wherein the unbuffered solution is saline or water.

95. The pharmaceutical composition of claim 92, wherein said dsRNA agent is in a buffer solution.

96. The pharmaceutical composition of claim 95, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

97. The pharmaceutical composition of claim 96, wherein the buffer solution is phosphate buffered saline (PBS).

98. A method of inhibiting expression of a Transmembrane protease, serine 6 (TMPRSS6) gene in a cell, the method comprising contacting the cell with the dsRNA agent of any one of claims 1-90, or the pharmaceutical composition of any one of claims 92-97, thereby inhibiting expression of the TMPRSS6 gene in the cell.

99. The method of claim 98, wherein the cell is within a subject.

100. The method of claim 98, wherein the subject is a human.

101. The method of claim 98 or 100, wherein the subject has a TMPRSS6-associated disorder.

102. The method of claim 101, wherein the TMPRSS6-associated disorder is a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis.

103. The method of claim 101, wherein the TMPRSS6-associated disorder is selected from the group consisting of hereditary hemochromatosis, β-thalassemia, polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease and Friedreich's Ataxia.

104. The method of claim 101, wherein the TMPRSS6-associated disorder is β-thalassemia.

105. The method of claim 104, wherein the β-thalassemia is thalassemia major.

106. The method of claim 104, wherein the β-thalassemia is thalassemia intermedia.

107. The method of claim 101, wherein the TMPRSS6-associated disorder is polycythemia vera.

108. The method of any one of claims 98-107, wherein contacting the cell with the dsRNA agent inhibits the expression of TMPRSS6 by at least 50%, 60%, 70%, 80%, 90%, or 95%.

109. The method of any one of claims 98-108, wherein inhibiting expression of TMPRSS6 decreases TMPRSS6 protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

110. The method of any one of claims 98-109, wherein contacting the cell with the dsRNA agent increases the expression of hepcidin by at least 50%, 60%, 70%, 80%, 90%, or 95%.

111. The method of any one of claims 98-110, wherein increasing expression of hepicidin increases hepicidin protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

112. A method of treating a subject having a disorder that would benefit from reduction in Transmembrane protease, serine 6 (TMPRSS6) expression, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-58, or the pharmaceutical composition of any one of claims 50-55, thereby treating the subject having the disorder that would benefit from reduction in TMPRSS6 expression.

113. A method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in Transmembrane protease, serine 6 (TMPRSS6) expression, comprising administering to the subject a prophylactically effective amount of the dsRNA agent of any one of claims 1-48, or the pharmaceutical composition of any one of claims 50-55, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in TMPRSS6 expression.

114. The method of claim 112 or 113, wherein the disorder is a TMPRSS6-associated disorder.

115. The method of claim 114, wherein the TMPRSS6-associated disorder is a disorder associated with iron overload and/or a disorder of ineffective erythropoiesis.

116. The method of claim 115, wherein the TMPRSS6-associated disorder is selected from the group consisting of hereditary hemochromatosis, β-thalassemia, polycythemia vera, myelodysplastic syndrome, congenital dyserythropoietic anemias, pyruvate kinase deficiency, erythropoietic porphyria, Parkinson's Disease, Alzheimer's Disease and Friedreich's Ataxia.

117. The method of claim 114, wherein the TMPRSS6-associated disorder is β-thalassemia.

118. The method of claim 117, wherein the β-thalassemia is thalassemia major.

119. The method of claim 117, wherein the β-thalassemia is thalassemia intermedia.

120. The method of claim 114, wherein the TMPRSS6-associated disorder is polycythemia vera.

121. The method of any one of claims 112-120, wherein the subject is a human.

122. The method of any one of claims 112-121, wherein the administration of the agent to the subject causes a decrease in iron level, a decrease in ferritin level, a decrease in a transferrin saturation level, an increase in hemoglobin level, an increase in hematocrit level, and/or a decrease in TMPRSS6 protein accumulation.

123. The method of any one of claims 112-122, wherein the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

124. The method of any one of claims 112-123, wherein the dsRNA agent is administered to the subject subcutaneously.

125. The method of any one of claims 112-123, wherein the dsRNA agent is administered to the subject intravenously.

126. The method of any one of claims 112-125, further comprising determining the level of TMPRSS6 in a sample(s) from the subject.

127. The method of claim 126, wherein the level of TMPRSS6 in the subject sample(s) is a TMPRSS6 protein level in a blood, serum or liver sample(s).

128. The method of any one of claims 112-127, further comprising determining the level of iron and/or hepcidin in a sample(s) from the subject.

129. The method of any one of claims 112-128, further comprising administering to the subject an additional therapeutic agent for treatment of a TMPRSS6-associated disorder.

130. The method of claim 129, wherein the additional therapeutic agent is an iron chelator.

131 The method of claim 130, wherein the iron chelator is selected from the group consisting of deferiprone, deferoxamine, and deferasirox.

132. A kit comprising the dsRNA agent of any one of claims 1-90 or the pharmaceutical composition of any one of claims 92-97.

133. A vial comprising the dsRNA agent of any one of claims 1-90 or the pharmaceutical composition of any one of claims 92-97.

134. A syringe comprising the dsRNA agent of any one of claims 1-90 or the pharmaceutical composition of any one of claims 92-97.

135. An RNA-induced silencing complex (RISC) comprising an antisense strand of the dsRNA agent of any one of claims 1-90.