

COMPLEMENT FACTOR B (CFB) IRNA COMPOSITIONS AND METHODS OF USE THEREOF

Related Applications

5 This application claims the benefit of priority to U.S. Provisional Application No. 63/017,725, filed on April 30, 2020, U.S. Provisional Application No. 63/119,009, filed on November 30, 2020, and U.S. Provisional Application No. 63/157,899, filed on March 8, 2021. The entire contents of each of the foregoing applications are incorporated herein by reference.

10 **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 23, 2021, is named 121301_11420_SL.txt and is 436,429 bytes in size.

15 **Background of the Invention**

Complement was first discovered in the 1890s when it was found to aid or “complement” the killing of bacteria by heat-stable antibodies present in normal serum (Walport, M.J. (2001) *N Engl J Med.* 344:1058). The complement system consists of more than 30 proteins that are either present as soluble proteins in the blood or are present as membrane-associated proteins. Activation of complement leads to a sequential cascade of enzymatic reactions, known as complement activation pathways resulting in the formation of the potent anaphylatoxins C3a and C5a that elicit a plethora of physiological responses that range from chemoattraction to apoptosis. Initially, complement was thought to play a major role in innate immunity where a robust and rapid response is mounted against invading pathogens. However, recently it is becoming increasingly evident that complement also plays an important role in adaptive immunity involving T and B cells that help in elimination of pathogens (Dunkelberger JR and Song WC. (2010) *Cell Res.* 20:34; Molina H, *et al.* (1996) *Proc Natl Acad Sci U S A.* 93:3357), in maintaining immunologic memory preventing pathogenic re-invasion, and is involved in numerous human pathological states (Qu, H, *et al.* (2009) *Mol Immunol.* 47:185; Wagner, E. and Frank MM. (2010) *Nat Rev Drug Discov.* 9:43).

30 Complement activation is known to occur through three different pathways: alternate, classical and lectin (Figure 1) involving proteins that mostly exist as inactive zymogens that are then sequentially cleaved and activated.

The classical pathway is often activated by antibody-antigen complexes or by the C-reactive protein (CRP), both of which interact with complement component C1q. In addition, the classical pathway can be activated by phosphatidyl serine present in apoptotic bodies in the absence of immune complexes.

The lectin pathway is initiated by the mannose-binding lectins (MBL) that bind to complex carbohydrate residues on the surface of pathogens. The activation of the classical pathway or the lectin pathway leads to activation of the (C4b2b) C3 convertase.

The alternate pathway is activated by the binding of C3b, which is spontaneously generated by the hydrolysis of C3, on targeted surfaces. This surface-bound C3b is then recognized by factor B, forming the complex C3bB. The C3bB complex, in turn, is cleaved by factor D to yield the active form of the C3 convertase of the AP (C3bBb). Both types of C3 convertases will cleave C3, forming C3b. C3b then either binds to more factor B, enhancing the complement activation through the AP (the so-called alternative or amplification loop), or leads to the formation of the active C5 convertase (C3bBbC3b or C4bC2bC3b), which cleaves C5 and triggers the late events that result in the formation of the membrane attack complex (MAC) (C5b-9).

Inappropriate activation of the complement system is responsible for propagating or initiating pathology in many different diseases, including, for example, C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, polycystic kidney disease, membranous nephropathy, age-related macular degeneration, atypical hemolytic uremic syndrome, thrombotic microangiopathy, myasthenia gravis, ischemia and reperfusion injury, paroxysmal nocturnal hemoglobinuria, and rheumatoid arthritis.

To date, only one therapeutic that targets the alternate pathway, *e.g.*, the C5-C5a axis, is available for the treatment of complement component-associated diseases, the anti-C5 antibody, eculizumab (Soliris®). Although eculizumab has been shown to be effective for the treatment of paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and Myasthenia Gravis, and is currently being evaluated in clinical trials for additional complement component-associated diseases, eculizumab therapy requires weekly high dose infusions followed by biweekly maintenance infusions at a high cost. Furthermore, approximately 50% of eculizumab-treated PNH subjects have low level of hemolysis and require residual transfusions (Hill A, *et al.* (2010) *Haematologica* 95(4):567-73).

Accordingly, there is a need in the art for compositions and methods for treating diseases, disorders, and conditions associated with complement activation by, for example, activation of complement factor B activity.

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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 complement factor B (CFB). The complement factor B (CFB) may be within a cell, *e.g.*, a cell within a subject, such as a human subject.

Accordingly, in one aspect the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of complement factor B (CFB) 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 0, 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:8. In certain embodiments, the sense strand comprises at least 15 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:8. In certain embodiments, the sense strand comprises at least 17 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 17 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:8. In certain embodiments, the sense strand comprises at least 19 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 19 contiguous nucleotides of the nucleotide sequence of SEQ ID NO:8.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of complement factor B (CFB) 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 complement factor B (CFB), and wherein the region of complementarity comprises at least 15 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, 13, 16, 19 and 20. In certain embodiments, the region of complementarity comprises at least 15 contiguous nucleotides of any one of the antisense nucleotide sequences in any one of Tables 2-7, 13, 16, 19 and 20. In certain embodiments, the region of complementarity comprises at least 17 contiguous nucleotides of any one of the antisense nucleotide sequences in any one of Tables 2-7, 13, 16, 19 and 20. In certain embodiments, the region of complementarity comprises at least 19 contiguous nucleotides of any one of the antisense nucleotide sequences in any one of Tables 2-7, 13, 16, 19 and 20. In certain embodiments, the region of complementarity comprises at least 20 contiguous nucleotides of any one of the antisense nucleotide sequences in any one of Tables 2-7, 13, 16, 19 and 20. In certain embodiments, the region of complementarity comprises at least 21 contiguous nucleotides of any one of the antisense nucleotide sequences in any one of Tables 2-7, 13, 16, 19 and 20.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of complement factor B (CFB) 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 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 633-665, 1133-1185, 1133-1173, 1133-1167, 1143-1173, 1540-1563, 1976-2002, 2386-2438, 2386-2418, 2386-2413, and 2389-1418 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO:8, where a substitution of a T with a U in either SEQ ID NO: 1 or SEQ ID NO: 8 does not count as a difference.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of complement factor B (CFB) 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 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides *e.g.*, at least 15 nucleotides, at least 17 nucleotides, at least 19 nucleotides, or at least 20 nucleotides, from any one of the nucleotide sequence of nucleotides 633-655, 643-665, 928-950, 1133-1155, 1140-1162, 1141-1163, 1143-1165, 1145-1167, 1148-1170, 1150-1172, 1151-1173, 1185-1207, 1306-1328, 1534-1556, 1540-1562, 1541-1563, 1976-1998, 1979-2001, 1980-2002, 2078-2100, 2386-2408, 2388-2410, 2389-2411, 2391-2413, 2393-2415, 2395-2417, 2396-2418, 2438-2460, 2602-2624 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides, *e.g.*, at least 15 nucleotides, at least 17 nucleotides, at least 19 nucleotides, or at least 20 nucleotides, from the corresponding nucleotide sequence of SEQ ID NO:8, where a substitution of a T with a U in either SEQ ID NO: 1 or SEQ ID NO: 8 does not count as a difference.

In one embodiment, 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-560018, AD-559375, AD-559160, AD-559374, AD-559060, AD-559721, AD-559026, AD-558225, AD-557069, AD-558068, AD-557422, AD-558063, AD-558066, AD-556701, AD-558657, AD-559020, AD-559023, AD-558860, AD-560019, AD-560016, AD-559008, AD-559717, AD-557072, AD-558097, AD-557774, AD-557070, AD-558065, AD-557853, and AD-557079. In certain embodiments, the antisense strand comprises at least 15 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the antisense strand comprises at least 17 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the antisense strand comprises at least 19 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the antisense strand comprises at least 20 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the antisense strand comprises at least 21 contiguous nucleotides of any one of the selected duplexes.

In one embodiment, the sense 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-560018, AD-559375, AD-559160, AD-559374, AD-559060, AD-559721, AD-559026, AD-558225, AD-557069, AD-558068, AD-557422, AD-558063, AD-558066, AD-556701, AD-558657, AD-559020, AD-559023, AD-558860, AD-560019, AD-560016, AD-559008, AD-559717, AD-557072, AD-558097, AD-557774, AD-557070, AD-558065, AD-557853, and AD-557079. In certain embodiments, the sense strand comprises at least 15 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the sense strand comprises at least 17 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the sense strand comprises at least 19 contiguous nucleotides of any one of the

selected duplexes. In certain embodiments, the sense strand comprises at least 20 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the sense strand comprises at least 21 contiguous nucleotides of any one of the selected duplexes.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of complement factor B (CFB) 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 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides *e.g.*, at least 15 nucleotides, at least 17 nucleotides, at least 19 nucleotides, or at least 20 nucleotides, from any one of the nucleotide sequence of nucleotides 153-175; 202-224; 219-241; 254-276; 304-326; 321-343; 347-369; 402-424; 418-440; 447-469; 491-513; 528-550; 549-571; 566-588; 591-613; 792-814; 819-841; 967-989; 1042-1064; 1234-1256; 1250-1272; 1269-1291; 1335-1357; 1354-1376; 1372-1394; 1422-1444; 1496-1518; 1670-1692; 1716-1738; 1757-1779; 1774-1796; 1793-1815; 1844-1866; 1871-1893; 1909-1931; 1924-1947; 1947-1969; 2161-2183; 2310-2332; 2330-2352; 2355-2377; 2494-2516; and 2527-2549 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides, *e.g.*, at least 15 nucleotides, at least 17 nucleotides, at least 19 nucleotides, or at least 20 nucleotides, from the corresponding nucleotide sequence of SEQ ID NO:8, where a substitution of a T with a U in either SEQ ID NO: 1 or SEQ ID NO: 8 does not count as a difference.

In one embodiment, the antisense strand comprises at least 15 contiguous nucleotides differing by nor more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-560132.1; AD-560099.1; AD-559998.1; AD-559993.1; AD-559973.1; AD-559882.1; AD-559706.1; AD-559704.1; AD-559688.1; AD-559668.1; AD-559641.1; AD-559609.1; AD-559590.1; AD-559573.1; AD-559532.1; AD-559486.1; AD-559330.1; AD-559274.1; AD-559226.1; AD-559208.1; AD-559189.1; AD-559124.1; AD-559105.1; AD-559089.1; AD-558935.1; AD-558879.1; AD-558777.1; AD-558750.1; AD-558637.1; AD-558612.1; AD-558595.1; AD-558574.1; AD-558555.1; AD-558511.1; AD-558482.1; AD-558466.1; AD-558450.1; AD-558424.1; AD-558407.1; AD-558393.1; AD-558378.1; AD-558361.1; AD-558312.1. In certain embodiments, the antisense strand comprises at least 15 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the antisense strand comprises at least 17 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the antisense strand comprises at least 19 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the antisense strand comprises at least 20 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the antisense strand comprises at least 21 contiguous nucleotides of any one of the selected duplexes.

In one embodiment, the sense strand comprises at least 15 contiguous nucleotides differing by nor more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-560132.1; AD-560099.1; AD-559998.1; AD-559993.1; AD-559973.1; AD-559882.1; AD-559706.1; AD-559704.1; AD-559688.1; AD-559668.1; AD-

559641.1; AD-559609.1; AD-559590.1; AD-559573.1; AD-559532.1; AD-559486.1; AD-559330.1; AD-559274.1; AD-559226.1; AD-559208.1; AD-559189.1; AD-559124.1; AD-559105.1; AD-559089.1; AD-558935.1; AD-558879.1; AD-558777.1; AD-558750.1; AD-558637.1; AD-558612.1; AD-558595.1; AD-558574.1; AD-558555.1; AD-558511.1; AD-558482.1; AD-558466.1; AD-558450.1; AD-558424.1; AD-558407.1; AD-558393.1; AD-558378.1; AD-558361.1; AD-558312.1.

In certain embodiments, the sense strand comprises at least 15 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the sense strand comprises at least 17 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the sense strand comprises at least 19 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the sense strand comprises at least 20 contiguous nucleotides of any one of the selected duplexes. In certain embodiments, the sense strand comprises at least 21 contiguous nucleotides of any one of the selected duplexes.

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 deoxythymidine (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, 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 nucleotide comprising a 2'-phosphate group, *e.g.*, cytidine-2'-phosphate (C2p); guanosine-2'-phosphate (G2p); uridine-2'-phosphate (U2p); adenosine-2'-phosphate (A2p); a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), 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 comprising a 2'-phosphate group, and, a vinyl-phosphonate nucleotide; and combinations thereof.

In another embodiment, at least one of the modifications on the nucleotides is a thermally destabilizing nucleotide modification.

In one embodiment, the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acid (UNA), and a glycerol nucleic acid (GNA).

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; 19-23 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.

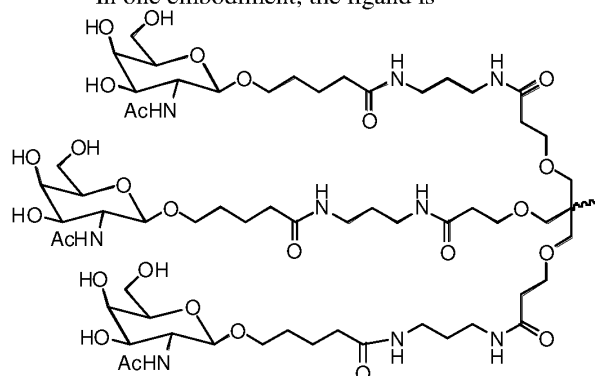
In one embodiment, the dsRNA agent further comprises a ligand.

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

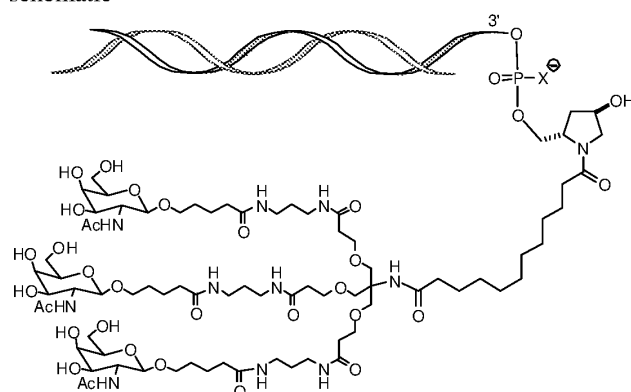
In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

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.

5 In one embodiment, the X is O.

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.

10 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.

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, 20 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 complement factor B (CFB) 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 25 inhibiting expression of the CFB gene in the cell.

In one embodiment, the cell is within a subject, *e.g.*, a human subject, *e.g.*, a subject having a complement factor B-associated disorder. Such disorders are typically associated with inflammation or immune system activation, *e.g.*, membrane attack complex-mediated lysis, anaphylaxis, or hemolysis. Non-limiting examples of complement factor B-associated disorders include paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), asthma, rheumatoid 30

arthritis (RA); antiphospholipid antibody syndrome; lupus nephritis; ischemia-reperfusion injury; typical or infectious hemolytic uremic syndrome (tHUS); dense deposit disease (DDD); neuromyelitis optica (NMO); multifocal motor neuropathy (MMN); multiple sclerosis (MS); macular degeneration (e.g., age-related macular degeneration (AMD)); hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; thrombotic thrombocytopenic purpura (TTP); spontaneous fetal loss; Pauci-immune vasculitis; epidermolysis bullosa; recurrent fetal loss; pre-eclampsia, traumatic brain injury, myasthenia gravis, cold agglutinin disease, dermatomyositis bullous pemphigoid, Shiga toxin E. coli-related hemolytic uremic syndrome, C3 neuropathy, anti-neutrophil cytoplasmic antibody-associated vasculitis (e.g., granulomatosis with polyangiitis (previously known as Wegener granulomatosis), Churg-Strauss syndrome, and microscopic polyangiitis), humoral and vascular transplant rejection, graft dysfunction, myocardial infarction (e.g., tissue damage and ischemia in myocardial infarction), an allogenic transplant, sepsis (e.g., poor outcome in sepsis), Coronary artery disease, dermatomyositis, Graves' disease, atherosclerosis, Alzheimer's disease, systemic inflammatory response sepsis, septic shock, spinal cord injury, glomerulonephritis, Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, autoimmune hemolytic anemia (AIHA), ITP, Goodpasture syndrome, Degos disease, antiphospholipid syndrome (APS), catastrophic APS (CAPS), a cardiovascular disorder, myocarditis, a cerebrovascular disorder, a peripheral (e.g., musculoskeletal) vascular disorder, a renovascular disorder, a mesenteric/enteric vascular disorder, vasculitis, Henoch-Schönlein purpura nephritis, systemic lupus erythematosus-associated vasculitis, vasculitis associated with rheumatoid arthritis, immune complex vasculitis, Takayasu's disease, dilated cardiomyopathy, diabetic angiopathy, Kawasaki's disease (arteritis), venous gas embolus (VGE), and restenosis following stent placement, rotational atherectomy, and percutaneous transluminal coronary angioplasty (PTCA) (see, e.g., Holers (2008) *Immunological Reviews* 223:300-316; Holers and Thurman (2004) *Molecular Immunology* 41:147-152; U.S. Patent Publication No. 20070172483).

In one embodiment, the complement factor B-associate disease is selected from the group consisting of C3 glomerulopathy, systemic lupus erythematosus (SLE), e.g., Lupus Nephritis, IgA nephropathy, diabetic nephropathy, polycystic kidney disease, membranous nephropathy, age-related macular degeneration, atypical hemolytic uremic syndrome, thrombotic microangiopathy, myasthenia gravis, ischemia and reperfusion injury, paroxysmal nocturnal hemoglobinuria, and rheumatoid arthritis

In another embodiment, the complement factor B-associate disease is selected from the group consisting of C3 glomerulopathy, systemic lupus erythematosus (SLE), e.g., Lupus Nephritis, IgA nephropathy, diabetic nephropathy, and polycystic kidney disease.

In one embodiment, contacting the cell with the dsRNA agent inhibits the expression of CFB by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one embodiment, inhibiting expression of CFB decreases CFB 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 complement factor B (CFB) 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 CFB expression.

In another aspect, the present invention provides a method of preventing development of a disorder that would benefit from reduction in complement factor B (CFB) expression in a subject having at least one sign or symptom of a disorder who does not yet meet the diagnostic criteria for that disorder. 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 the subject progressing to meet the diagnostic criteria of the disorder that would benefit from reduction in CFB expression.

In one embodiment, the disorder is a complement factor B- (CFB)-associated disorder.

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.

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

In one embodiment, the administration of the agent to the subject causes a decrease in hemolysis or a decrease in CFB protein accumulation.

In certain embodiments, the methods of the invention further comprise administering to the subject an additional therapeutic agent.

In some aspects, the additional therapeutic agent is an iRNA agent targeting a C5 gene, such as those described in U.S. Patent No.: 9,249,415, the entire contents of which are hereby incorporated herein by reference.

In other aspects, the additional therapeutic agent is an iRNA agent targeting a complement factor B (CFB) gene, such as those described in U.S. Patent No.: 10,465,194, the entire contents of which are hereby incorporated herein by reference.

In other aspects, the additional therapeutic agent is an inhibitor of C5, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.*, eculizumab, ravulizumab-cwvz, or pozelimab (REGN3918)) or a C5 peptide inhibitor (*e.g.*, zilucoplan). Eculizumab is a humanized monoclonal IgG2/4, kappa light chain antibody that specifically binds complement component C5 with high affinity and inhibits cleavage of C5 to C5a and C5b, thereby inhibiting the generation of the terminal complement complex C5b-9. Eculizumab is described in U.S. Patent No. 6,355,245, the entire contents of which are incorporated herein by reference. Ravulizumab-cwvz is a humanized IgG2/4 monoclonal antibody that specifically binds complement component C5 with high affinity and inhibits cleavage of C5 to C5a and C5b, thereby inhibiting the

generation of the terminal complement complex C5b-9. Ravulizumab-cwvz is described in WO2015134894, the entire contents of which are incorporated herein by reference. Pozelimab (also known as H4H12166P, described in US20170355757, the entire contents of which are incorporated herein by reference) is a fully-human IgG4 monoclonal antibody designed to block complement factor C5. Zilucoplan is a synthetic, macrocyclic peptide that binds complement component 5 (C5) with sub-nanomolar affinity and allosterically inhibits its cleavage into C5a and C5b upon activation of the classical, alternative, or lectin pathways (see, *e.g.*, WO2017105939, the entire contents of which are incorporated herein by reference).

In yet other aspects, the additional therapeutic is a C3 peptide inhibitor, or analog thereof. In one embodiment, the C3 peptide inhibitor is compstatin. Compstatin is a cyclic tridecapeptide with potent and selective C3 inhibitory activity. Compstatin, and its analogs, are described in U.S. Patent Nos. 7,888,323, 7,989,589, and 8,442,776, in U.S. Patent Publication No. 2012/0178694 and 2013/0053302, and in PCT Publication Nos. WO 2012/174055, WO 2012/2178083, WO 2013/036778, the entire contents of each of which are incorporated herein by reference.

In certain embodiments, treatments known in the art for the various CFB-associated diseases are used in combination with the RNAi agents of the invention.

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.

Brief Description of the Drawings

Figure 1 depicts the three complement pathways: alternative, classical and lectin.

Figure 2A is a heatmap depicting the effect of dual targeting of C3 and CFB on alternative hemolytic activity in human sera *in vitro*.

Figure 2B is a heatmap depicting the effect of dual targeting of C3 and C5 on alternative hemolytic activity in human sera *in vitro*.

Figure 2C is a heatmap depicting the effect of dual targeting of C3 and C5 on classical hemolytic activity in human sera *in vitro*.

Figure 2D is a heatmap depicting the effect of dual targeting of C3 and C5 on classical hemolytic activity as determined by the Wieslab® Complement Classical Pathway (CCP) assay in human sera *in vitro*.

Figure 3A is a graph depicting the effect of administration of a single 6 mg/kg dose of a dsRNA agent targeting C3; or a dsRNA agent targeting CFB; or a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and a single 6 mg/kg dose of a dsRNA agent targeting CFB; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and a single 6 mg/kg dose of a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting CFB and a single 6 mg/kg dose of a dsRNA agent targeting C5 on C3 protein levels, CFB protein levels or C5 protein levels in the sera of non-human primates.

Figure 3B is a graph depicting the effect of administration of a single 6 mg/kg dose of a dsRNA agent targeting C3; or a dsRNA agent targeting CFB; or a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and a single 6 mg/kg dose of a dsRNA agent targeting CFB; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and a single 6 mg/kg dose of a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting CFB and a single 6 mg/kg dose of a dsRNA agent targeting C5 on alternative hemolytic activity in the sera of non-human primates.

Figure 3C is a graph depicting the effect of administration of a single 6 mg/kg dose of a dsRNA agent targeting C3; or a dsRNA agent targeting CFB; or a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and a single 6 mg/kg dose of a dsRNA agent targeting CFB; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and a single 6 mg/kg dose of a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting CFB and a single 6 mg/kg dose of a dsRNA agent targeting C5 on classical hemolytic activity in the sera of non-human primates.

Figure 3D is a graph depicting the effect of administration of a single 6 mg/kg dose of a dsRNA agent targeting C3; or a dsRNA agent targeting CFB; or a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and a single 6 mg/kg dose of a dsRNA agent targeting CFB; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and a single 6 mg/kg dose of a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting CFB and a single 6 mg/kg dose of a dsRNA agent targeting C5 on alternative hemolytic activity as determined by the Wieslab® Complement Alternative Pathway (CAP) assay in the sera of non-human primates.

Figure 3E is a graph depicting the effect of administration of a single 6 mg/kg dose of a dsRNA agent targeting C3; or a dsRNA agent targeting CFB; or a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and a single 6 mg/kg dose of a dsRNA agent targeting CFB; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and a single 6 mg/kg dose of a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting CFB and a single 6 mg/kg dose of a dsRNA agent targeting C5 on alternative hemolytic activity as determined by the Wieslab® Complement Classical Pathway (CCP) assay in the sera of non-human primates.

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 complement factor B (CFB) 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 (complement factor B gene) in mammals.

The iRNAs of the invention have been designed to target the human complement factor B gene, including portions of the gene that are conserved in the complement factor B 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
 5 complement factor B-associated disorder, disease, or condition, *e.g.*, a disorder, disease, or condition with inflammation or immune system activation, *e.g.*, membrane attack complex-mediated lysis, anaphylaxis, or hemolysis, *e.g.*, C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, and polycystic kidney disease, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA
 10 transcripts of a complement factor B 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
 15 region is substantially complementary to at least part of an mRNA transcript of a complement factor B gene. In certain embodiments, the RNAi agents of the disclosure include an RNA strand (the antisense strand) having a region which is about 21-23 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a complement factor B gene.

In certain embodiments, one or both of the strands of the double stranded RNAi agents of the
 20 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 complement factor B gene. In some embodiments, such iRNA agents having longer length antisense strands may include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30
 25 contiguous nucleotides.

The use of iRNAs of the invention enables the targeted degradation of mRNAs of the corresponding gene (complement factor B gene) in mammals. Using *in vitro* and *in vivo* assays, the present inventors have demonstrated that iRNAs targeting a complement factor B gene can potently mediate RNAi, resulting in significant inhibition of expression of a complement factor B gene. Thus,
 30 methods and compositions including these iRNAs are useful for treating a subject having a complement factor B -associated disorder, *e.g.*, C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, and polycystic kidney disease.

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
 35 complement factor B gene, *e.g.*, a complement factor B-associated disease, such as C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, and polycystic kidney disease, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a CFB 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 complement factor B gene, *e.g.*, C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, and polycystic kidney disease.

5 In certain embodiments, the administration of the dsRNA to the subject causes a decrease in CFB mRNA level, CFB protein level, CH₅₀ activity (a measure of total hemolytic complement), AH₅₀ (a measure the hemolytic activity of the alternate pathway of complement), lactate dehydrogenase (LDH) (a measure of intravascular hemolysis), hemoglobin levels; the level of any one or more of C3, C9, C5, C5a, C5b, and soluble C5b-9 complex.

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

25 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.”

30 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 $\pm 10\%$. In certain embodiments, about means $\pm 5\%$. 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.

35 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, the term “Complement Factor B,” used interchangeably with the term “CFB,” refers to the well-known gene and polypeptide, also known in the art as AHUS, BF, CFAB, BFD, FB, GBG, FBI12, B-Factor, Properdin, H2-Bf, Glycine-Rich Beta Glycoprotein, C3 Proaccelerator, Properdin Factor 2B, C3 Proactivator, PBF2, Glycine-Rich Beta-Glycoprotein, C3/C5 Convertase, EC 3.4.21, and EC 3.4.21.473.

The term “CFB” includes human CFB, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:189181756; mouse CFB, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession Nos. GI:218156288 and GI:218156290; rat CFB, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:218156284; and chimpanzee CFB, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:57114201. The term “CFB” also includes *Macaca fascicularis* CFB, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:544428919 and in the entry for the gene, ENSMMUP00000000985 (locus=scaffold3881:47830:53620), in the *Macaca* genome project web site (macaque.genomics.org.cn/page/species/index.jsp). Additional examples of CFB mRNA sequences are readily available using, e.g., GenBank, UniProt, OMIM, and the *Macaca* genome project web site.

Exemplary CFB nucleotide sequences may also be found in SEQ ID NOs:1-7. SEQ ID NOs:8-14 are the antisense sequences of SEQ ID NOs: 1-7, respectively.

The term “CFB,” as used herein, also refers to naturally occurring DNA sequence variations of the CFB gene. Non-limiting examples of sequence variations within the CFB gene include 1598A>G in exon 12, which results in a lysine being changed to an arginine at amino acid residue 533; 858C>G in exon 6, which results in a phenylalanine being changed to a leucine at amino acid residue 286; and 967A>G in exon 7, which results in a lysine being changed to an alanine at amino

acid residue 323 (Tawadrous H. *et al.* (2010) *Pediatr Nephrol.* 25:947; Goicoechea de Jorge E *et al.* (2007) *Proc Natl Acad Sci. USA* 104:240). The term “CFB,” as used herein, also refers to single nucleotide polymorphisms in the CFB gene. Numerous sequence variations within the CFB gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*,

5 ncbi.nlm.nih.gov/snp).

Further information on CFB can be found, for example, at www.ncbi.nlm.nih.gov/gene/629.

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

The entire contents of each of the foregoing GenBank Accession numbers and the Gene
10 database numbers are 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 complement factor B gene, including mRNA that is a product of RNA processing of a primary transcription product. The target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that
15 portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a CFB gene. In one embodiment, the target sequence is within the protein coding region of CFB.

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-
20 25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. In some embodiments, the target sequence is about 19 to about 30 nucleotides in length. In other embodiments, the target sequence is about 19 to about 25 nucleotides in length. In still other embodiments, the target sequence is about 19 to about 23 nucleotides in length. In some embodiments, the target sequence is about 21 to about 23 nucleotides in length. Ranges and lengths
25 intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

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

“G,” “C,” “A,” “T,” and “U” each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that
30 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
35 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 complement factor B 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 complement factor B 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 complement factor B (CFB) 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 complement factor B (CFB) 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.

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
5 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
10 claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide – which is acknowledged as a naturally occurring form of nucleotide – if present within a 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
15 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
20 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
25 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 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.
30 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.

In certain embodiment, the two strands of double-stranded oligomeric compound can be linked together. The two strands can be linked to each other at both ends, or at one end only. By
35 linking at one end is meant that 5'-end of first strand is linked to the 3'-end of the second strand or 3'-end of first strand is linked to 5'-end of the second strand. When the two strands are linked to each other at both ends, 5'-end of first strand is linked to 3'-end of second strand and 3'-end of first strand is linked to 5'-end of second strand. The two strands can be linked together by an oligonucleotide linker

including, but not limited to, (N)_n; wherein N is independently a modified or unmodified nucleotide and n is 3-23. In some embodiments, n is 3-10, e.g., 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the oligonucleotide linker is selected from the group consisting of GNRA, (G)₄, (U)₄, and (dT)₄, wherein N is a modified or unmodified nucleotide and R is a modified or unmodified purine nucleotide. Some of the nucleotides in the linker can be involved in base-pair interactions with other nucleotides in the linker. The two strands can also be linked together by a non-nucleosidic linker, e.g. a linker described herein. It will be appreciated by one of skill in the art that any oligonucleotide chemical modifications or variations describe herein can be used in the oligonucleotide linker.

Hairpin and dumbbell type oligomeric compounds will have a duplex region equal to or at least 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotide pairs. The duplex region can be equal to or less than 200, 100, or 50, in length. In some embodiments, ranges for the duplex region are 15-30, 17 to 23, 19 to 23, and 19 to 21 nucleotides pairs in length.

The hairpin oligomeric compounds can have a single strand overhang or terminal unpaired region, in some embodiments at the 3', and in some embodiments on the antisense side of the hairpin. In some embodiments, the overhangs are 1-4, more generally 2-3 nucleotides in length. The hairpin oligomeric compounds that can induce RNA interference are also referred to as "shRNA" herein.

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 complement factor B (CFB) 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 CFB 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 of the dsRNA, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 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 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 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 CFB 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 complement factor B 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, a 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 CFB 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 CFB gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a CFB gene is important, especially if the

particular region of complementarity in a CFB 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 be, for example, “stringent conditions”, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50 oC or 70 oC 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 oligonucleotides 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 complement factor B gene). For example, a polynucleotide is complementary to at least a part of a complement factor B mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding a complement factor B gene.

Accordingly, in some embodiments, the antisense polynucleotides disclosed herein are fully complementary to the target CFB sequence.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target CFB 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, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 or 35, or a fragment of any one of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 or 35, 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 CFB 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 943-965; 788-810; 734-756; 1016-1038; 1013-1035; 1207-1229; 1149-1171; 574-596; 1207-1229 or 828-850 of SEQ ID NO: 1, such as about 85%, about 90%, about 95%, or fully complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target CFB 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 153-175; 202-224; 219-241; 254-276; 304-326; 321-343; 347-369; 402-424; 418-440; 447-469; 491-513; 528-550; 549-571; 566-588; 591-613; 792-814; 819-841; 967-989; 1042-1064; 1234-1256; 1250-1272; 1269-1291; 1335-1357; 1354-1376; 1372-1394; 1422-

1444; 1496-1518; 1670-1692; 1716-1738; 1757-1779; 1774-1796; 1793-1815; 1844-1866; 1871-1893; 1909-1931; 1924-1947; 1947-1969; 2161-2183; 2310-2332; 2330-2352; 2355-2377; 2494-2516; and 2527-2549 of SEQ ID NO: 1, such as about 85%, about 90%, about 95%, or fully complementary.

5 In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target CFB 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, 13, 16, 19 and 20, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2-7, 13, 16, 19 and 20, such as about 85%,
10 about 90%, about 95%, or fully 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 CFB 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
15 the nucleotide sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 or 36, or a fragment of any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 or 36, such as about 85%, about 90%, about 95%, or fully 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
20 complement factor B 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, 13, 16, 19 and 20, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2-7, 13, 16, 19 and 20, such as about 85%, about 90%, about 95%, or fully complementary

25 In certain embodiments, the sense and antisense strands are selected from any one of the chemically modified duplexes AD-560018, AD-559375, AD-559160, AD-559374, AD-559060, AD-559721, AD-559026, AD-558225, AD-557069, AD-558068, AD-557422, AD-558063, AD-558066, AD-556701, AD-558657, AD-559020, AD-559023, AD-558860, AD-560019, AD-560016, AD-559008, AD-559717, AD-557072, AD-558097, AD-557774, AD-557070, AD-558065, AD-557853,
30 AD-557079.

In certain embodiments, the sense and antisense strands are selected from any one of the chemically modified duplexes AD-560132.1; AD-560099.1; AD-559998.1; AD-559993.1; AD-559973.1; AD-559882.1; AD-559706.1; AD-559704.1; AD-559688.1; AD-559668.1; AD-559641.1; AD-559609.1; AD-559590.1; AD-559573.1; AD-559532.1; AD-559486.1; AD-559330.1; AD-
35 559274.1; AD-559226.1; AD-559208.1; AD-559189.1; AD-559124.1; AD-559105.1; AD-559089.1; AD-558935.1; AD-558879.1; AD-558777.1; AD-558750.1; AD-558637.1; AD-558612.1; AD-558595.1; AD-558574.1; AD-558555.1; AD-558511.1; AD-558482.1; AD-558466.1; AD-558450.1; AD-558424.1; AD-558407.1; AD-558393.1; AD-558378.1; AD-558361.1; AD-558312.1

In some embodiments, the double-stranded region of a double-stranded iRNA agent is equal to or at least, 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, 30 or more nucleotide pairs in length.

In some embodiments, the antisense strand of a double-stranded iRNA agent is equal to or at least 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

In some embodiments, the sense strand of a double-stranded iRNA agent is equal to or at least 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each 18 to 30 nucleotides in length.

In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each 19 to 25 nucleotides in length.

In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each 21 to 23 nucleotides in length.

In one embodiment, the sense strand of the iRNA agent is 21- nucleotides in length, and the antisense strand is 23-nucleotides in length, wherein the strands form a double-stranded region of 21 consecutive base pairs having a 2-nucleotide long single stranded overhangs at the 3'-end.

In some embodiments, the majority of nucleotides of each strand are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, e.g., a deoxyribonucleotide or a modified nucleotide. In addition, an "iRNA" may include ribonucleotides with chemical modifications. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modifications, as used in an iRNA molecule, are encompassed by "iRNA" for the purposes of this specification and claims.

In 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 one embodiment, at least partial suppression of the expression of a CFB gene, is assessed by a reduction of the amount of CFB mRNA which can be isolated from or detected in a first cell or group of cells in which a CFB gene is transcribed and which has or have been treated such that the expression of a CFB 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 or have not been so treated (control cells). The degree of inhibition may be expressed in terms of:

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

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
5 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
10 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.
15 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
20 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 rabbit, a sheep, a hamster, a guinea pig, 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
25 treated or assessed for a disease or disorder that would benefit from reduction in CFB expression; a human at risk for a disease or disorder that would benefit from reduction in CFB expression; a human having a disease or disorder that would benefit from reduction in CFB expression; or human being treated for a disease or disorder that would benefit from reduction in CFB expression as described herein. In some embodiments, the subject is a female human. In other embodiments, the subject is a
30 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 CFB-associated disorder in a subject. Treatment also includes a reduction of one or more sign or symptoms associated with unwanted CFB expression;
35 diminishing the extent of unwanted CFB activation or stabilization; amelioration or palliation of unwanted CFB 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 CFB 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%, 35%, 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 CFB 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, the expression of the target is normalized, *i.e.*, decreased towards or to a level accepted as within the range of normal for an individual without such disorder, *e.g.*, normalization of body weight, blood pressure, or a serum lipid level. As used here, “lower” in a subject can refer to lowering of gene expression or protein production in a cell in a subject does not require lowering of expression in all cells or tissues of a subject. For example, as used herein, lowering in a subject can include lowering of gene expression or protein production in the liver of a subject.

The term “lower” can also be used in association with normalizing a symptom of a disease or condition, *i.e.* decreasing the difference between a level in a subject suffering from a CFB -associated disease towards or to a level in a normal subject not suffering from a CFB-associated disease. For example, if a subject with a normal weight of 70 kg weighs 90 kg prior to treatment (20 kg overweight) and 80 kg after treatment (10 kg overweight), the subject’s weight is lowered towards a normal weight by 50% ($(10/20 \times 100\%)$). Similarly, if the HDL level of a woman is increased from 50 mg/dL (poor) to 57 mg/dL, with a normal level being 60 mg/dL, the difference between the prior level of the subject and the normal level is decreased by 70% (difference of 10 mg/dL between subject level and normal is decreased by 7 mg/dL, $7/10 \times 100\%$). As used herein, if a disease is associated with an elevated value for a symptom, “normal” is considered to be the upper limit of normal. If a disease is associated with a decreased value for a symptom, “normal” is considered to be the lower limit of normal.

As used herein, “prevention” or “preventing,” when used in reference to a disease, disorder or condition thereof, that would benefit from a reduction in expression of a CFB gene or production of CFB protein, refers to preventing a subject who has at least one sign or symptom of a disease from developing further signs and symptoms thereby meeting the diagnostic criteria for that disease. In certain embodiments, prevention includes delayed progression to meeting the diagnostic criteria of the disease by days, weeks, months or years as compared to what would be predicted by natural history studies or the typical progression of the disease.

As used herein, the term “complement factor B disease” or “CFB-associated disease,” is a disease or disorder that is caused by, or associated with, complement activation. The term “CFB-associated disease” includes a disease, disorder or condition that would benefit from a decrease in CFB gene expression, replication, or protein activity. Non-limiting examples of CFB-associated diseases include, for example, paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), asthma, rheumatoid arthritis (RA); antiphospholipid antibody syndrome;

lupus nephritis; ischemia-reperfusion injury; typical or infectious hemolytic uremic syndrome (tHUS); dense deposit disease (DDD); neuromyelitis optica (NMO); multifocal motor neuropathy (MMN); multiple sclerosis (MS); macular degeneration (e.g., age-related macular degeneration (AMD)); hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; thrombotic

5 thrombocytopenic purpura (TTP); spontaneous fetal loss; Pauci-immune vasculitis; epidermolysis bullosa; recurrent fetal loss; pre-eclampsia, traumatic brain injury, myasthenia gravis, cold agglutinin disease, dermatomyositis bullous pemphigoid, Shiga toxin E. coli-related hemolytic uremic syndrome, C3 neuropathy, anti-neutrophil cytoplasmic antibody-associated vasculitis (e.g., granulomatosis with polyangiitis (previously known as Wegener granulomatosis), Churg-Strauss

10 syndrome, and microscopic polyangiitis), humoral and vascular transplant rejection, graft dysfunction, myocardial infarction (e.g., tissue damage and ischemia in myocardial infarction), an allogeneic transplant, sepsis (e.g., poor outcome in sepsis), Coronary artery disease, dermatomyositis, Graves' disease, atherosclerosis, Alzheimer's disease, systemic inflammatory response sepsis, septic shock, spinal cord injury, glomerulonephritis, Hashimoto's thyroiditis, type I diabetes, psoriasis,

15 pemphigus, autoimmune hemolytic anemia (AIHA), ITP, Goodpasture syndrome, Degos disease, antiphospholipid syndrome (APS), catastrophic APS (CAPS), a cardiovascular disorder, myocarditis, a cerebrovascular disorder, a peripheral (e.g., musculoskeletal) vascular disorder, a renovascular disorder, a mesenteric/enteric vascular disorder, vasculitis, Henoch-Schönlein purpura nephritis, systemic lupus erythematosus-associated vasculitis, vasculitis associated with rheumatoid arthritis,

20 immune complex vasculitis, Takayasu's disease, dilated cardiomyopathy, diabetic angiopathy, Kawasaki's disease (arteritis), venous gas embolus (VGE), and restenosis following stent placement, rotational atherectomy, and percutaneous transluminal coronary angioplasty (PTCA) (see, e.g., Holers (2008) *Immunological Reviews* 223:300-316; Holers and Thurman (2004) *Molecular Immunology* 41:147-152; U.S. Patent Publication No. 20070172483).

25 In one embodiment, the complement factor B-associate disease is selected from the group consisting of C3 glomerulopathy, systemic lupus erythematosus (SLE), e.g., Lupus Nephritis, IgA nephropathy, diabetic nephropathy, polycystic kidney disease, membranous nephropathy, age-related macular degeneration, atypical hemolytic uremic syndrome, thrombotic microangiopathy, myasthenia gravis, ischemia and reperfusion injury, paroxysmal nocturnal hemoglobinuria, and

30 rheumatoid arthritis

In another embodiment, the complement factor B-associate disease is selected from the group consisting of C3 glomerulopathy, systemic lupus erythematosus (SLE), e.g., Lupus Nephritis, IgA nephropathy, diabetic nephropathy, and polycystic kidney disease.

35 Further details regarding signs and symptoms of the various diseases or conditions are provided herein and are well known in the art.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a CFB-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.

5 "Prophylactically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having at least one sign or symptom of a CFB-associated disorder, is sufficient to prevent or delay the subject's progression to meeting the full diagnostic criteria of the disease. Prevention of the disease includes slowing the course of progression to full blown disease. The "prophylactically effective amount" may vary depending on the RNAi
10 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
15 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, compositions, or dosage forms which are, within the scope of sound medical judgment,
20 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,
25 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
30 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
35 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

5 The present invention provides iRNAs which inhibit the expression of a complement factor B gene. In certain embodiments, the iRNA includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a CFB gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human susceptible to developing a complement factor B-associated disorder. The dsRNAi agent includes an antisense strand having a region of complementarity which is
 10 complementary to at least a part of an mRNA formed in the expression of a CFB 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 CFB gene, the iRNA inhibits the expression of the CFB gene (*e.g.*, a human, a primate, a non-primate, or a rat CFB gene) by at least about 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or
 15 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
 20 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
 25 complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of a CFB 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-
 30 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-
 35 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 complement factor B 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.

Regardless of the method of synthesis, the siRNA preparation can be prepared in a solution (e.g., an aqueous or organic solution) that is appropriate for formulation. For example, the siRNA preparation can be precipitated and redissolved in pure double-distilled water, and lyophilized. The dried siRNA can then be resuspended in a solution appropriate for the intended formulation process.

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, 13, 16, 19 and 20, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 2-7, 13, 16, 19 and 20. 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 complement factor B 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, 13, 16, 19 and 20, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 2-7, 13, 16, 19 and 20.

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 certain embodiments, the sense or antisense strand is selected from the sense or antisense strand of any one of duplexes AD-560018, AD-559375, AD-559160, AD-559374, AD-559060, AD-559721, AD-559026, AD-558225, AD-557069, AD-558068, AD-557422, AD-558063, AD-558066, AD-556701, AD-558657, AD-559020, AD-559023, AD-558860, AD-560019, AD-560016, AD-559008, AD-559717, AD-557072, AD-558097, AD-557774, AD-557070, AD-558065, AD-557853, or AD-557079.

It will be understood that, although the sequences in Tables 2, 4, 6 and 19 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, 13, 16, 19 and 20 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, 13, 16, 19 and 20 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)

5 *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, 13, 16, 19 and 20, 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, 13, 16, 19 and 20 minus only a few nucleotides on one or both ends can be similarly effective as compared to the
10 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, 13, 16, 19 and 20, and differing in their ability to inhibit the expression of a complement factor B 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.

15 In addition, the RNAs provided in Tables 2-7, 13, 16, 19 and 20 identify a site(s) in a complement factor B 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
20 contiguous nucleotides from any one of the sequences provided in any one of Tables 2-7, 13, 16, 19 and 20 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a complement factor B gene.

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
25 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
30 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 CFB 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 CFB gene. Consideration of the efficacy
35 of RNAi agents with mismatches in inhibiting expression of a CFB gene is important, especially if the particular region of complementarity in a CFB gene is known to have polymorphic sequence variation within the population.

III. Modified iRNAs of the Invention

In certain embodiments, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is unmodified, 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 1 unmodified 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 or phosphorothiotate groups present in the agent. Agents in

which substantially all of the phosphodiester or phosphorothioate linkages have a sodium counterion include not more than 5, 4, 3, 2, or 1 phosphodiester 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 or

5 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; 10 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 15 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; 20 alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S, and CH₂ 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; 25 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 30 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 35 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 --CH₂--NH--CH₂--, --CH₂--

5 N(CH₃)--O--CH₂--[known as a methylene (methylimino) or MMI backbone], --CH₂--O--N(CH₃)--CH₂--, --CH₂--N(CH₃)--N(CH₃)--CH₂-- and --N(CH₃)--CH₂--CH₂-- 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-
10 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 C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Exemplary
15 suitable modifications include O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl,
20 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--CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*,
25 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminoethoxy, *i.e.*, a O(CH₂)₂ON(CH₃)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--CH₂--O--CH₂--N(CH₂)₂. Further exemplary modifications include : 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-
30 deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other modifications include 2'-methoxy (2'-OCH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) 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
35 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.

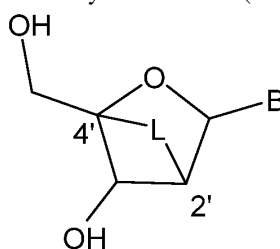
An iRNA can also include nucleobase (often referred to in the art simply as “base”) modifications or substitutions. As used herein, “unmodified” or “natural” nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxythymidine (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 and 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, those 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 iRNA 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 the bridging of two atoms. A “bicyclic sugar” is a furanosyl ring modified by a ring formed by the bridging of two

carbons, whether adjacent or non-adjacent atoms. A “bicyclic nucleoside” (“BNA”) is a nucleoside having a sugar moiety comprising a bridge a ring formed by bridging connecting two carbons, whether adjacent or non-adjacent, atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring, optionally, via the 2'-acyclic oxygen atoms. 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'-CH₂-O-2' bridge. This structure effectively “locks” the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4' to 2' bridge.

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'-(CH₂)—O-2' (LNA); 4'-(CH₂)—S-2'; 4'-(CH₂)₂—O-2' (ENA); 4'-CH(CH₃)—O-2' (also referred to as “constrained ethyl” or “cEt”) and 4'-CH(CH₂OCH₃)—O-2' (and analogs thereof; see, *e.g.*, U.S. Patent No. 7,399,845); 4'-C(CH₃)(CH₃)—O-2' (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,283); 4'-CH₂—N(OCH₃)-2' (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,425); 4'-CH₂—O—N(CH₃)-2' (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4'-CH₂—N(R)—O-2', wherein R is H, C1-C12 alkyl, or a nitrogen protecting group (see, *e.g.*, U.S. Patent No. 7,427,672); 4'-CH₂—C(H)(CH₃)-2' (see, *e.g.*, Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂—C(=CH₂)-2' (and analogs thereof; see, *e.g.*, 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).

An iRNA agent of the disclosure can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-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 CFB 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, US2013/0190383; and WO2013/036868, 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 Fluiters *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, US8,314,227; and US2013/0096289; US2013/0011922; and US2011/0313020, 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 base dT(idT) and others. Disclosure of this modification can be found in WO2011/005861.

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 US2012/0157511, the entire contents of which are incorporated herein by reference.

A. Modified iRNAs Comprising Motifs of the Invention

5 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.

15 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.*, CFB 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.

25 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.

30 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 not 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 RNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang may be located at the 3'-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, and 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 two nucleotide overhang. In one embodiment, the two nucleotide overhang is at the 3'-end of the antisense strand.

When the two 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 2'-fluoro, *e.g.*, in an alternating motif. Optionally, the dsRNAi agent further comprises a ligand (such as, GalNAc).

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, and 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, and 11 positions; the 10, 11, and 12 positions; the 11, 12, and 13 positions; the 12, 13, and 14 positions; or the 13, 14, and 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 then the chemistries can be the same or different. Two
5 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 motifs of three identical modifications on three consecutive nucleotides, with at least one of the motifs
10 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'-
15 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
20 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
25 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
30 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
35 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_aYYN_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 deoxythymidine (dT) or the nucleotide at the 3'-end of the antisense strand is deoxythymidine(dT). For example, there is a short sequence of deoxythymidine 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):



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 N_b and Y do not have the same modification; and

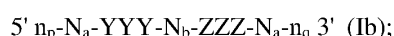
XXX, YYY, and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. In one embodiment, YYY is all 2'-F modified nucleotides.

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-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.

5 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.

10 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
15 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
20 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;

25 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;

30 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,
35 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.

5 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).

10 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.

15 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.

20 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).

25 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.

30 Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, CRN, UNA, cEt, glycol nucleic acid (GNA), hexitol nucleic acid (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.

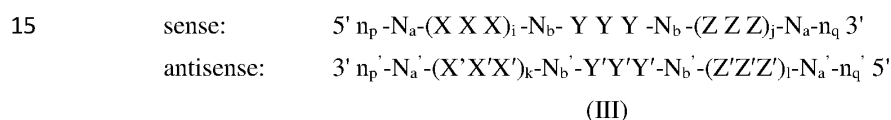
35 In some embodiments, the sense strand of the dsRNAi agent may contain YYY motif occurring at 9, 10, and 11 positions of the strand when the duplex region is 21 nt, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first paired nucleotide within 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):



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;

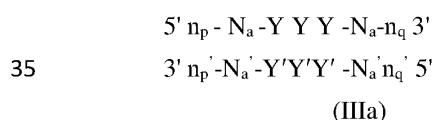
each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

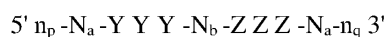
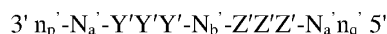
wherein each n_p , n_p' , n_q , and n_q' , each of which may or may not be present, independently represents an overhang nucleotide; and

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.

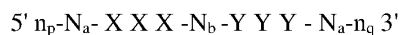
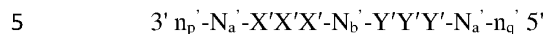
In one embodiment, i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. In another embodiment, k is 0 and l is 0; or k is 1 and l is 0; k is 0 and l is 1; or both k and l are 0; or both k and l are 1.

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





(IIIb)



(IIIc)



(IIId)

10 When the dsRNAi agent is represented by formula (IIIa), each N_a independently represents an oligonucleotide sequence comprising 0-20, 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
15 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.

20 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.

25 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
30 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.

35 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.

5 In certain embodiments, when the dsRNAi agent is represented by formula (IIIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications. In other embodiments, when the RNAi agent is represented by formula (IIIId), 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 *via* phosphorothioate linkage. In yet other embodiments, when the RNAi agent is represented by formula (IIIId), 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
10 embodiments, when the RNAi agent is represented by formula (IIIId), 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
15 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.
20

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.
25

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.
30

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.
35

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

5 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

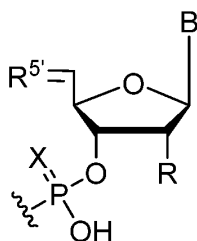
10 modification. For example, the RNAi agent may contain 2, 1 or 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent may contain 2 nucleotides with a 2'-fluoro modification, *e.g.*, 0 nucleotides with a 2'-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

Various publications describe multimeric iRNAs that can be used in the methods of the

15 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

20 5'-vinyl phosphonate modified nucleotide of the disclosure has the structure:



wherein X is O or S;

R is hydrogen, hydroxy, fluoro, or C₁₋₂₀alkoxy (*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*

25 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

30 disclosure is attached to the antisense strand of a dsRNA, optionally at the 5' end of the antisense strand of the dsRNA.

Vinyl phosphate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphate 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).

5 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 an iRNA can be replaced with another moiety, e.g., a non-carbohydrate (such as, cyclic) carrier to which is attached a carbohydrate ligand. A
 10 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
 15 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
 20 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,
 25 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
 30 or acyclic group. In some embodiments, 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 some embodiments, the acyclic group is a serinol backbone or diethanolamine backbone.

35

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

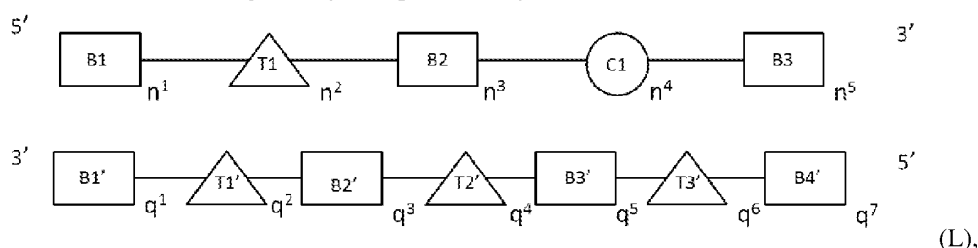
herein “seed region” means at positions 2-9 of the 5'-end of the referenced strand. For example, thermally destabilizing modifications can be incorporated in the seed region of the antisense strand to reduce or inhibit off-target gene silencing.

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.

The thermally destabilizing modifications can include, but are not limited to, abasic modification; mismatch with the opposing nucleotide in the opposing strand; and sugar modification such as 2'-deoxy modification or acyclic nucleotide, *e.g.*, unlocked nucleic acids (UNA) or glycol nucleic acid (GNA).

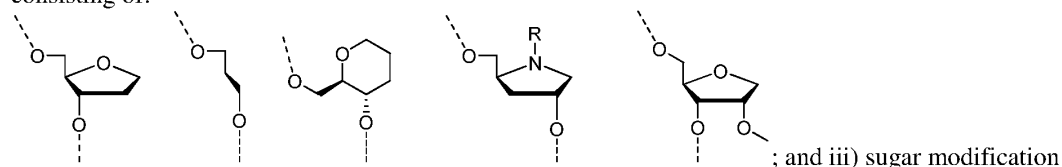
An iRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The RNAi agent may be represented by formula (L):



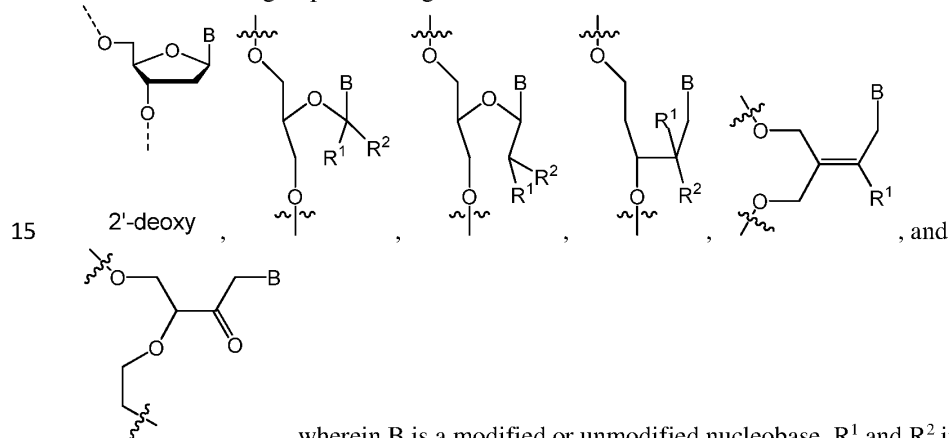
In formula (L), B1, B2, B3, B1', B2', B3', and B4' each are independently a nucleotide containing a modification selected from the group consisting of 2'-O-alkyl, 2'-substituted alkoxy, 2'-substituted alkyl, 2'-halo, ENA, and BNA/LNA. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 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) modification.

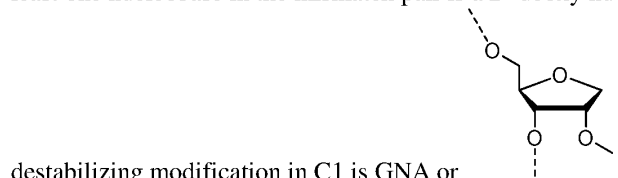
C1 is a thermally destabilizing nucleotide placed at a site opposite to the seed region of the antisense strand (*i.e.*, at positions 2-8 of the 5'-end of the antisense strand). For example, C1 is at a position of the sense strand that pairs with a nucleotide at positions 2-8 of the 5'-end of the antisense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



selected from the group consisting of:



, wherein B is a modified or unmodified nucleobase, R¹ and R² independently are H, halogen, OR₃, or alkyl; and R₃ 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
5 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
10 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
15 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
20 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
30 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, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.

- 5 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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
10 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, n¹ is 6, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 7, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.
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- In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 6, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 7, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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
20 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, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 6, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.
25

- In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 6, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with
30 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, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 5, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand.
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In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 5, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand; with two

- 5 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, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.

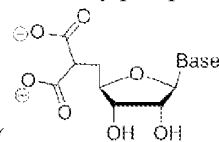
- In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two
15 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, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1.
In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with
25 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).

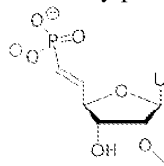
- In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1.

- In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; with two
35 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).

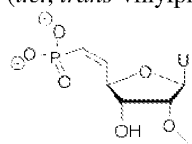
The RNAi agent can comprise a phosphorus-containing group at the 5'-end of the sense strand or antisense strand. The 5'-end phosphorus-containing group can be 5'-end phosphate (5'-P), 5'-end phosphorothioate (5'-PS), 5'-end phosphorodithioate (5'-PS₂), 5'-end vinylphosphonate (5'-



VP), 5'-end methylphosphonate (MePhos), or 5'-deoxy-5'-C-malonyl (). When the 5'-end phosphorus-containing group is 5'-end vinylphosphonate (5'-VP), the 5'-VP can be either



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



vinylphosphate,), or mixtures thereof.

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'-PS₂. In one embodiment, the RNAi agent comprises a 5'-PS₂ in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS₂. 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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'-PS₂.

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, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand

- 5 (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'-PS₂.

- 10 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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.
- 15

- In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-P.
- 20

- In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The dsRNA agent also comprises a 5'-PS.
- 25

- In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.
- 30

- In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS₂.
- 35

- In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F,

q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4,

T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The dsRNAi RNA agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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'-PS₂.

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'-PS₂.

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.

5 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
10 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,
15 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
20 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
25 (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,
30 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
35 RNAi agent also comprises a 5'-PS₂.

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,

q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.

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'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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, n¹ is 8, T1 is 2'-F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ 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'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ 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'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ 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'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ 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:

- 5 (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
 - 10 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
 - 15 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
 - 25 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
 - 30 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
 - 35 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,
 5 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:

10 (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
 15 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:
 20 (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,
 25 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:

30 (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
 35 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 desoxy-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:

- 5 (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
- 10 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:

- 15 (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
- 20 (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;
- 25 (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);

30 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;
- 35 (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:

- 5 (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
- 10 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, 13, 16, 19 and 20. These agents may further comprise a

15 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,

20 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. Acad. Sci. USA*, 1989, 86: 6553-6556). In other embodiments, the ligand is cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Lett.*, 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. Lett.*, 1993, 3:2765-2770), a thiocholesterol

25 (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,

30 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).

35 In certain embodiments, a ligand alters the distribution, targeting, or lifetime of an iRNA agent into which it is incorporated. In certain 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 as 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-glycolide) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic 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- κ B.

5 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.

10 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,
15 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
20 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
25 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
30 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
35 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. Such a lipid or lipid-based molecule may bind 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, such that 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 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 antennopodia. 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, for example, having 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:15). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO:16) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a “delivery” peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO:17) and the *Drosophila* Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:18) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent *via* an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

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 peptidomimetics 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, such as, 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

5 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, 10 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), 15 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.

20 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 25 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' 30 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 35 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

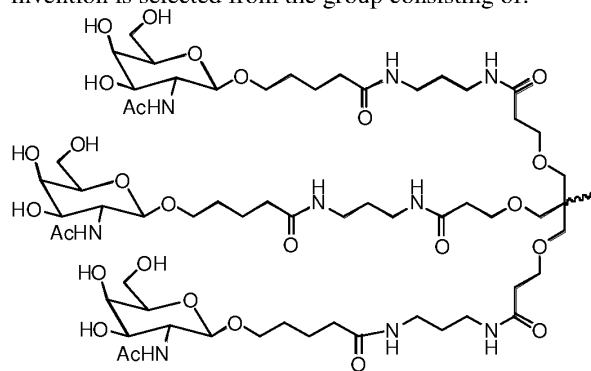
invention *via* a trivalent linker. In other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a tetravalent linker.

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.

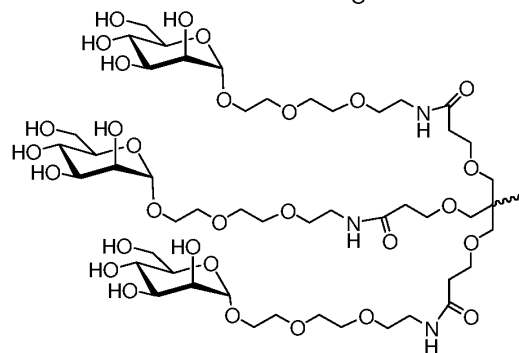
In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of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

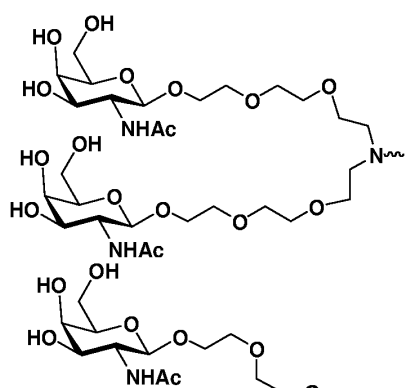
In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



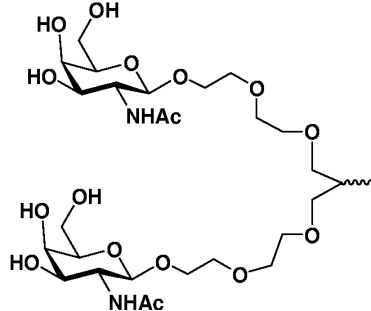
Formula II,



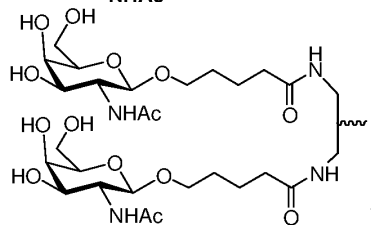
Formula III,



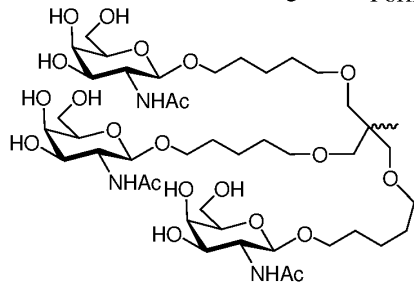
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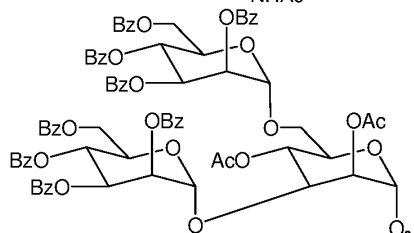
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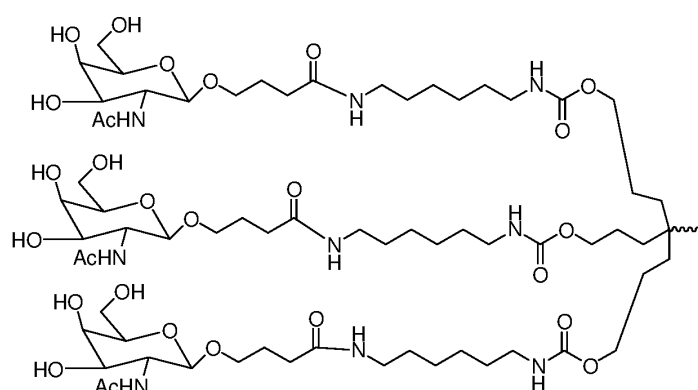
Formula VI,



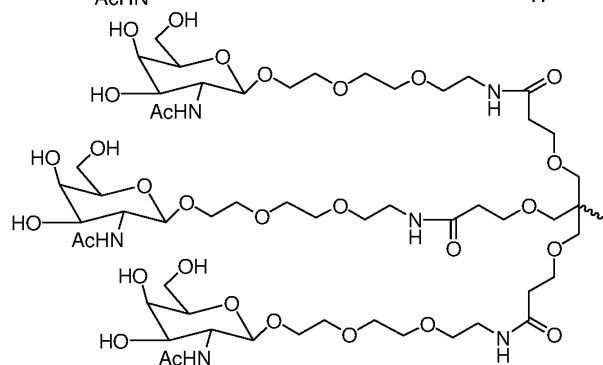
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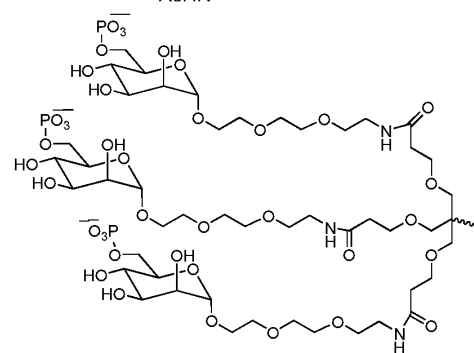
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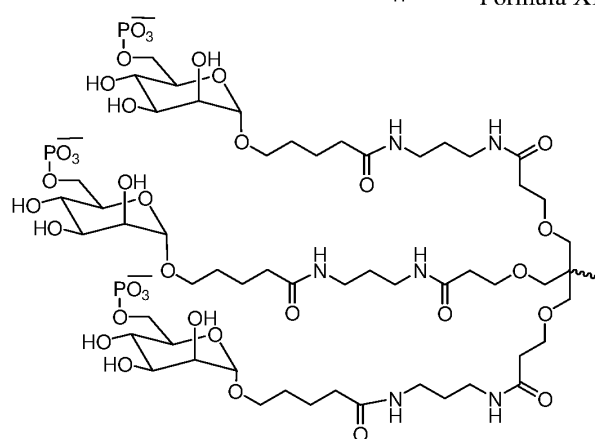
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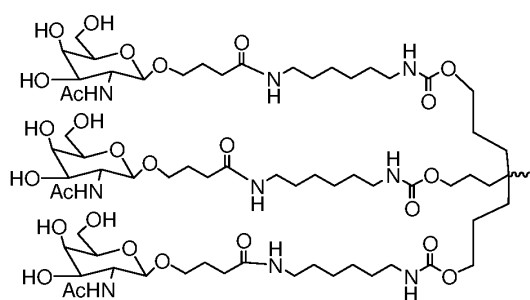
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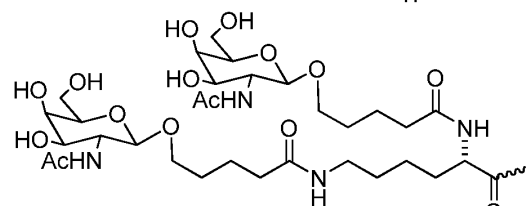
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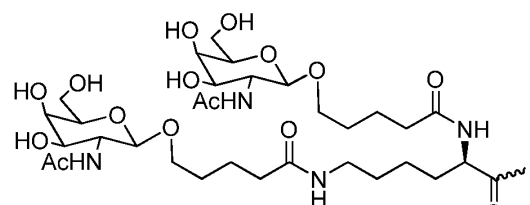
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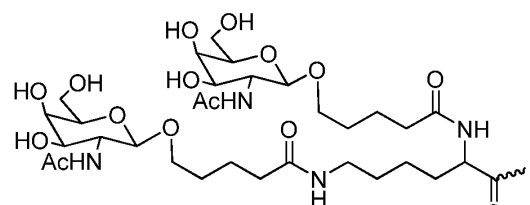
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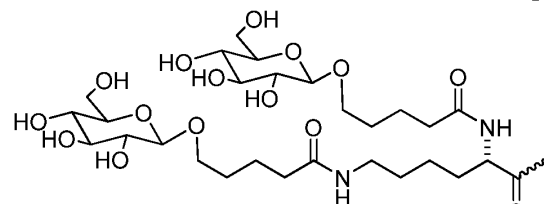
Formula XIV,



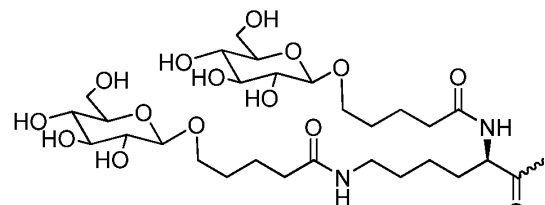
Formula XV,



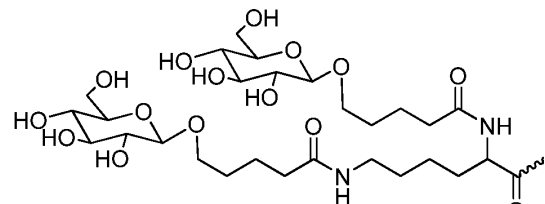
Formula XVI,



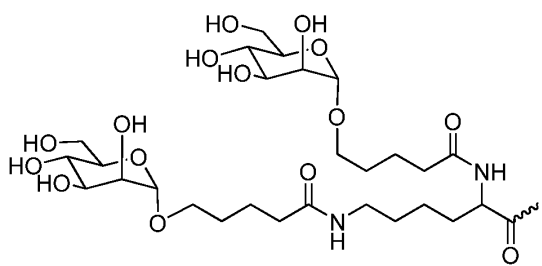
Formula XVII,



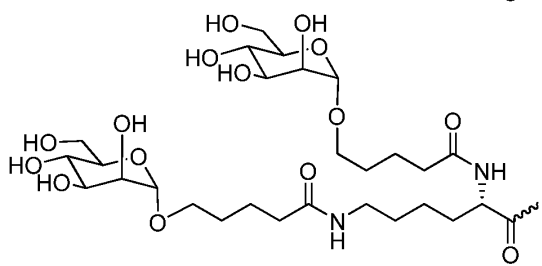
Formula XVIII,



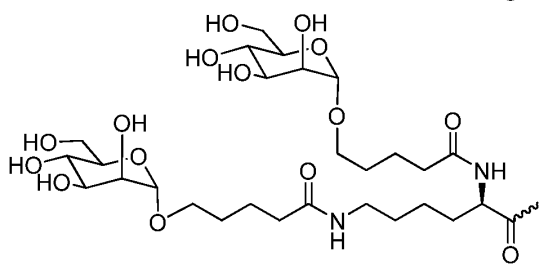
Formula XIX,



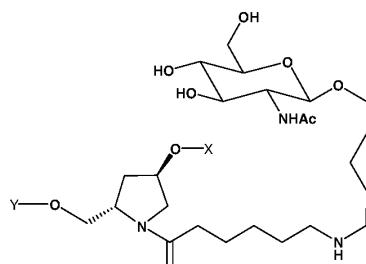
Formula XX,



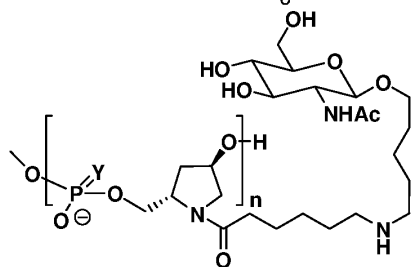
Formula XXI,



Formula XXII,

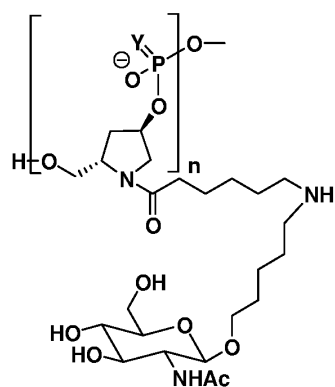


Formula XXIII;

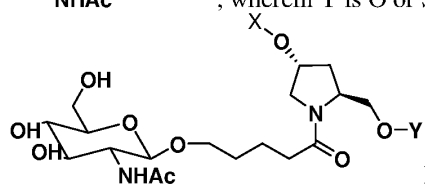


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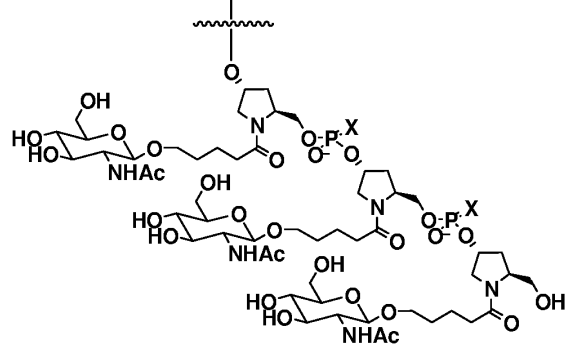
, wherein Y is O or S and n is 3 -6 (Formula XXIV);



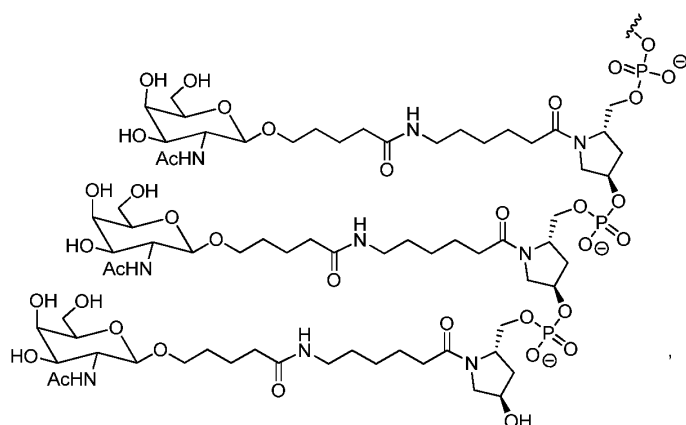
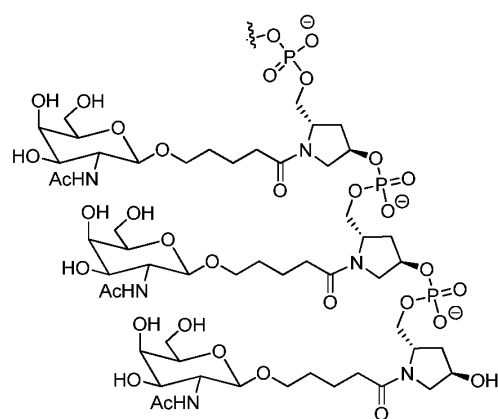
, wherein Y is O or S and n is 3-6 (Formula XXV);



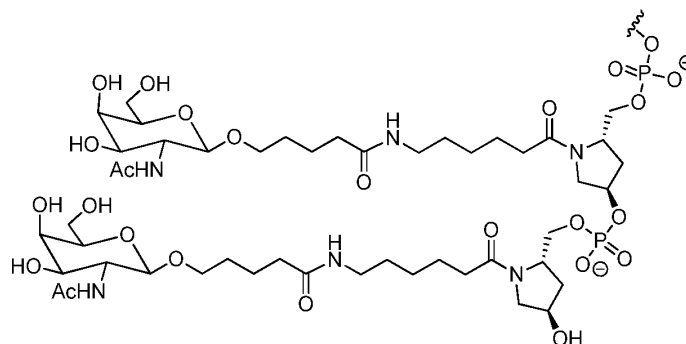
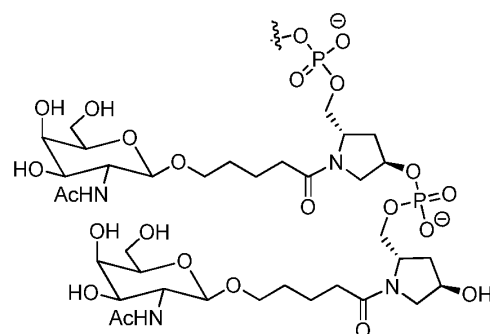
Formula XXVI;



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

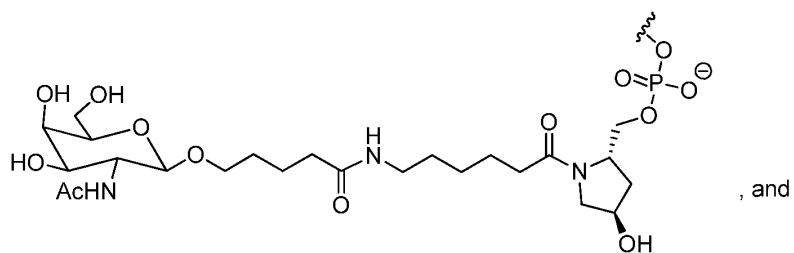
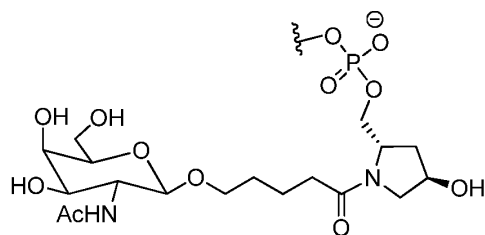


Formula XXVII; Formula XXIX;



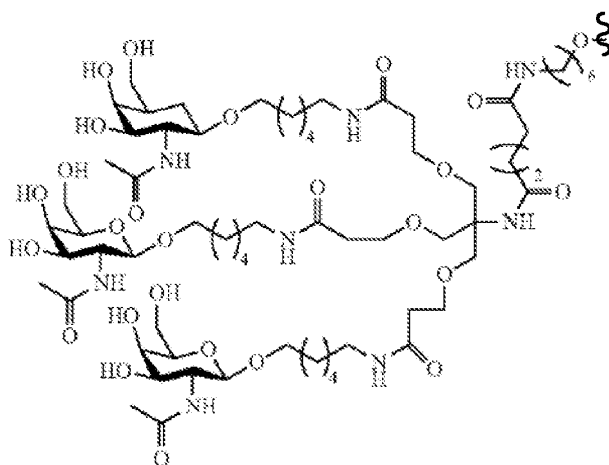
Formula XXX;

Formula XXXI;



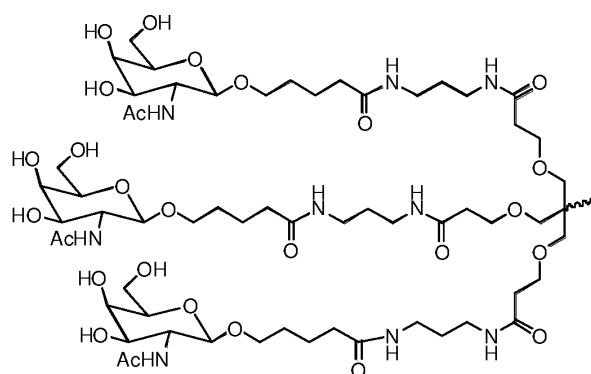
Formula XXXII;

Formula XXXIII.



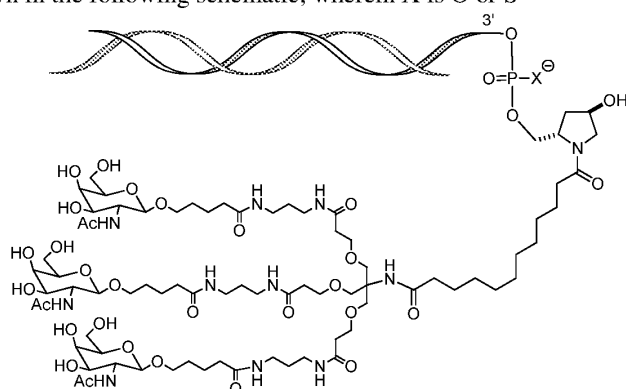
5 Formula XXXIV.

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

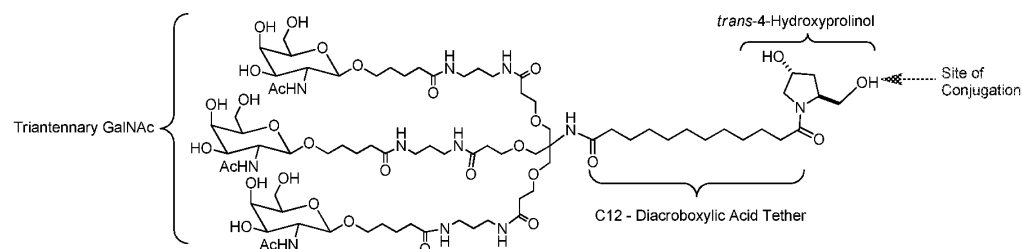


Formula II.

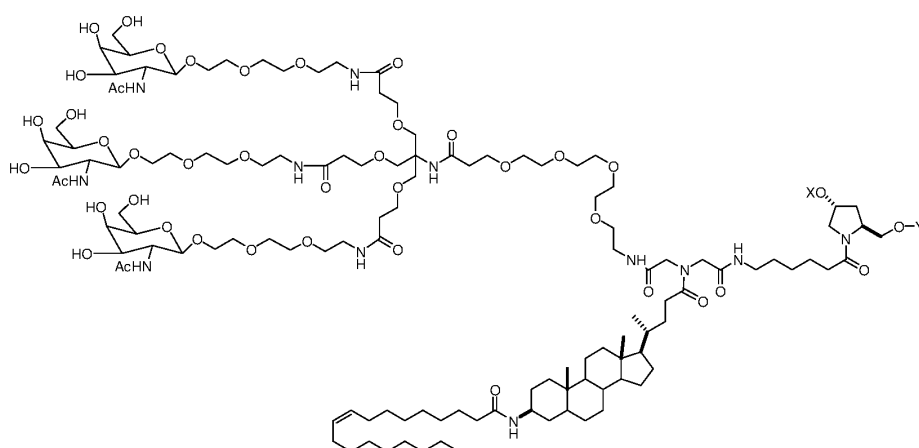
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate *via* a linker as shown in the following schematic, wherein X is O or S



- 5 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,



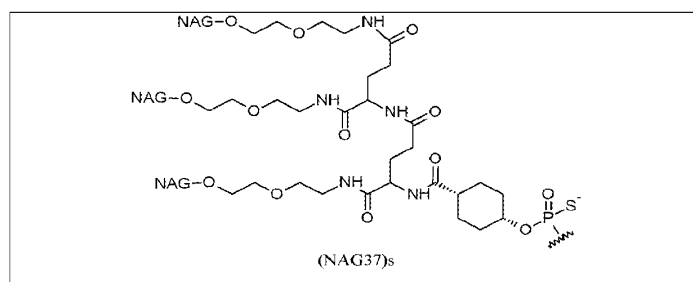
(Formula XXXVI), when one of X or Y is an oligonucleotide, the other is a hydrogen.

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:

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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 antisense 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.

25

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.

15 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, SO₂, SO₂NH 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, heterocyclalkyl, heterocyclalkenyl, heterocyclalkynyl, aryl, heteroaryl, heterocycl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclalkyl, alkylheterocyclalkenyl, alkylheterocyclalkynyl, alkenylheterocyclalkyl, alkenylheterocyclalkenyl, alkenylheterocyclalkynyl, alkynylheterocyclalkyl, alkynylheterocyclalkenyl, alkynylheterocyclalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylheteroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO₂, 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 one

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).

5 *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 known 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.

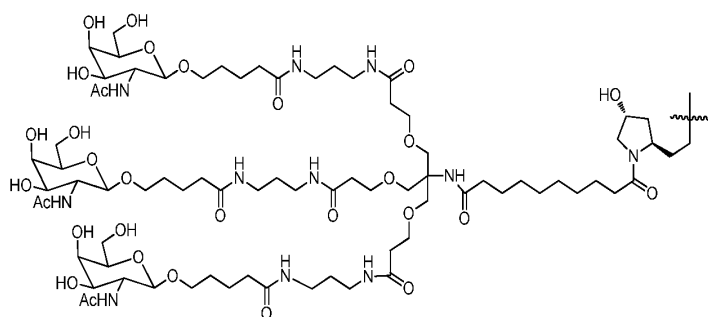
iv. Ester-based linking groups

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.

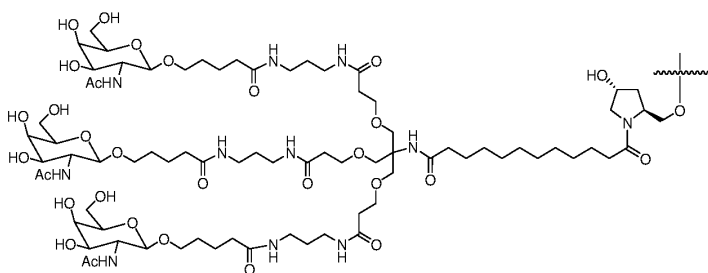
v. Peptide-based cleaving groups

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 alkynylene. 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.

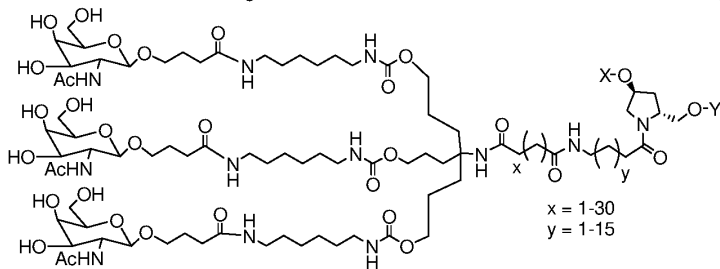
In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



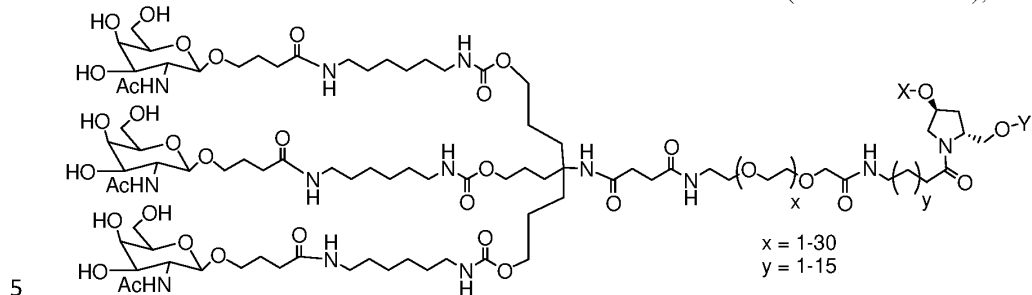
(Formula XXXVII),



(Formula XXXVIII),

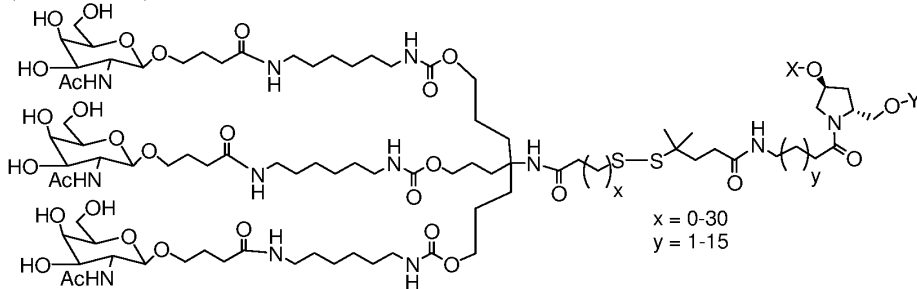


(Formula XXXIX),

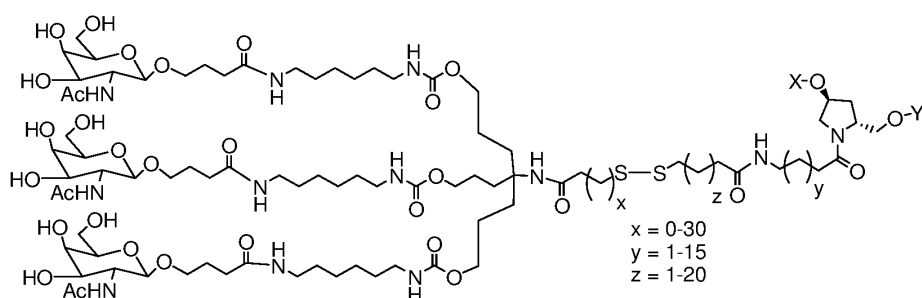


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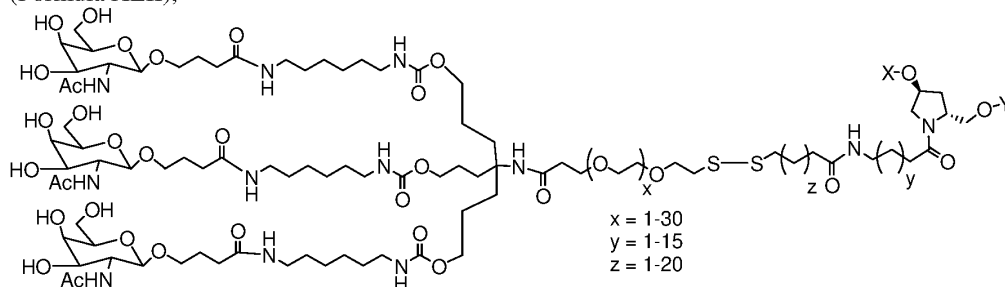
(Formula XL),



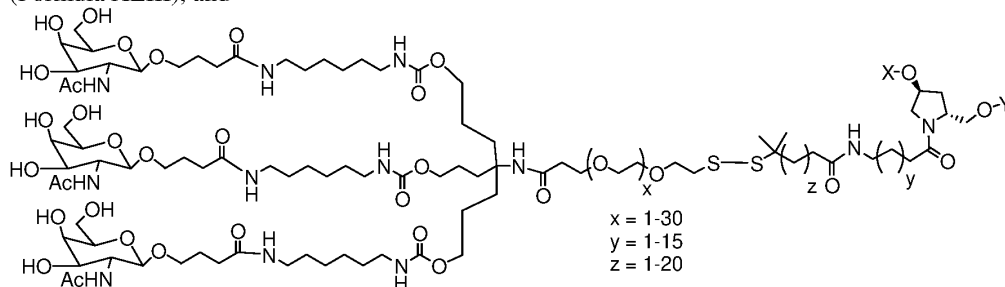
(Formula XLI),



(Formula XLII),



(Formula XLIII), and



5

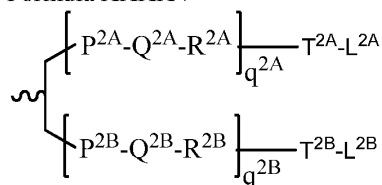
(Formula XLIV), when one of X or Y is an oligonucleotide, the other is a hydrogen.

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.

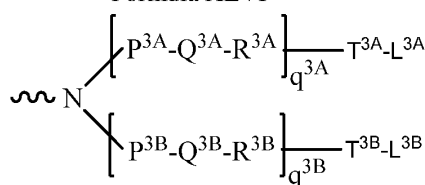
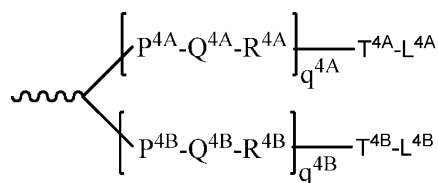
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In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XLV) – (XLVI):

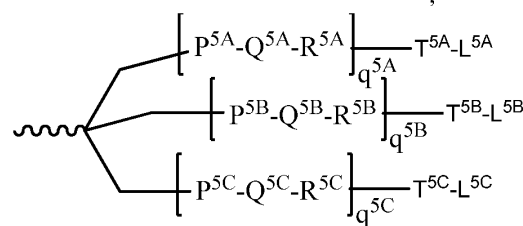
Formula XXXXV



Formula XLVI



Formula (VI)



Formula (VII)

Formula XLVII

Formula XLVIII

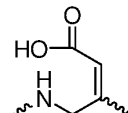
5 wherein:

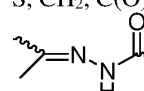
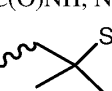
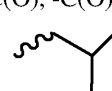
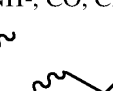
q^{2A}, q^{2B}, q^{3A}, q^{3B}, q^{4A}, q^{4B}, q^{5A}, q^{5B} and q^{5C} represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

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^{5A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH₂, CH₂NH or CH₂O;

10 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), SO₂, N(R^N), C(R')=C(R''), C≡C or C(O);

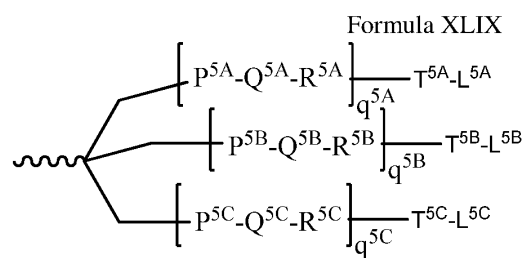
R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O,

S, CH₂, C(O)O, C(O)NH, NHCH(R^a)C(O), -C(O)-CH(R^a)-NH-, CO, CH=N-O, ,

15 , , , , 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

20 target gene, such as those of 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. Lett.*, 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 complement factor B-associated disorder) 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.*

5 101:17270-17275; 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
10 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
15 enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim SH, *et al.* (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA
20 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
25 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
30 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.

35 A. Vector encoded iRNAs of the Invention

iRNA targeting the complement factor B 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.*,
 5 *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;
 10 (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.

15 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.

20 **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
 25 complement factor B-associated disorder. 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 complement factor B gene.

30 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 complement factor B 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
 35 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.

5 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
10 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,
15 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
20 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
25 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

30 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 &
35 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).

5 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).

10 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 complement factor B-associated disorder.

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 complement factor B-associated disorder. 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 Complement Factor B Expression

The present invention also provides methods of inhibiting expression of a CFB 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 CFB in the cell, thereby inhibiting expression of CFB 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 certain embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a GalNAc 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 complement factor B gene” is intended to refer to inhibition of expression of any complement factor B gene (such as, *e.g.*, a mouse complement factor B gene, a rat complement factor B gene, a monkey complement factor B gene, or a human complement factor B gene) as well as variants or mutants of a complement factor B gene. Thus, the complement factor B gene may be a wild-type complement factor B gene, a mutant complement factor B gene, or a transgenic complement factor B gene in the context of a genetically manipulated cell, group of cells, or organism.

“Inhibiting expression of a complement factor B gene” includes any level of inhibition of a complement factor B gene, *e.g.*, at least partial suppression of the expression of a complement factor B gene, such as a clinically relevant level of suppression. The expression of the complement factor B gene may be assessed based on the level, or the change in the level, of any variable associated with complement factor B gene expression, *e.g.*, complement factor B mRNA level or complement factor B protein level, or, for example, CH₅₀ activity as a measure of total hemolytic complement, AH₅₀ to measure the hemolytic activity of the alternate pathway of complement, or lactate dehydrogenase (LDH) levels as a measure of intravascular hemolysis, or hemoglobin levels. Levels of C3, C9, C5, C5a, C5b, and soluble C5b-9 complex may also be measured to assess CFB expression. Inhibition may be assessed by a decrease in an absolute or relative level of one or more of these variables compared with a control level. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject. It is understood that complement factor B is expressed predominantly in the liver, and is present in circulation.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with complement factor B 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 complement factor B 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 certain embodiments, expression of a complement factor B gene is inhibited by at least 70%. It is further understood that inhibition of complement factor B expression in certain tissues, *e.g.*, in gall bladder, without a significant inhibition of expression in other tissues, *e.g.*, brain, may be desirable. In certain 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.*, complement factor B), *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 complement factor B 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 complement factor B 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 complement factor B 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 certain embodiments, the inhibition is assessed by the method provided in Example 2 using a 10 nM 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 complement factor B gene may be assessed in terms of a reduction of a parameter that is functionally linked to complement factor B gene expression, *e.g.*, complement factor B protein level in blood or serum from a subject. Complement factor B gene silencing may be determined in any cell expressing complement factor B, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of a complement factor B protein may be manifested by a reduction in the level of the complement factor B 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 complement factor B 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 complement factor B 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 complement factor B in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA of the complement factor B gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasyTM RNA preparation kits (Qiagen®) or PAXgeneTM (PreAnalytixTM, 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 complement factor B 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 complement factor B. 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 complement factor B 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 complement factor B mRNA.

An alternative method for determining the level of expression of complement factor B 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 CFB is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System).

- 5 In ceratin embodiments, expression level is determined by the method provided in Example 2 using, *e.g.*, a 10nM siRNA concentration, in the species matched cell line.

The expression levels of complement factor B 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).

- 10 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 complement factor B expression level may also comprise using nucleic acid probes in solution.

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

- The level of CFB 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.

- 25 In some embodiments, the efficacy of the methods of the invention are assessed by a decrease in CFB 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 complement factor B may be assessed using measurements of the level or change in the level of complement factor B mRNA or complement factor B 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.

35

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 complement factor B, thereby

preventing or treating a complement factor B-associated disorder, *e.g.*, paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), asthma, rheumatoid arthritis (RA); antiphospholipid antibody syndrome; lupus nephritis; ischemia-reperfusion injury; typical or infectious hemolytic uremic syndrome (tHUS); dense deposit disease (DDD); neuromyelitis optica (NMO); multifocal motor neuropathy (MMN); multiple sclerosis (MS); macular degeneration (*e.g.*, age-related macular degeneration (AMD)); hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; thrombotic thrombocytopenic purpura (TTP); spontaneous fetal loss; Pauci-immune vasculitis; epidermolysis bullosa; recurrent fetal loss; pre-eclampsia, traumatic brain injury, myasthenia gravis, cold agglutinin disease, dermatomyositis bullous pemphigoid, Shiga toxin *E. coli*-related hemolytic uremic syndrome, C3 neuropathy, anti-neutrophil cytoplasmic antibody-associated vasculitis (*e.g.*, granulomatosis with polyangiitis (previously known as Wegener granulomatosis), Churg-Strauss syndrome, and microscopic polyangiitis), humoral and vascular transplant rejection, graft dysfunction, myocardial infarction (*e.g.*, tissue damage and ischemia in myocardial infarction), an allogenic transplant, sepsis (*e.g.*, poor outcome in sepsis), Coronary artery disease, dermatomyositis, Graves' disease, atherosclerosis, Alzheimer's disease, systemic inflammatory response sepsis, septic shock, spinal cord injury, glomerulonephritis, Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, autoimmune hemolytic anemia (AIHA), ITP, Goodpasture syndrome, Degos disease, antiphospholipid syndrome (APS), catastrophic APS (CAPS), a cardiovascular disorder, myocarditis, a cerebrovascular disorder, a peripheral (*e.g.*, musculoskeletal) vascular disorder, a renovascular disorder, a mesenteric/enteric vascular disorder, vasculitis, Henoch-Schönlein purpura nephritis, systemic lupus erythematosus-associated vasculitis, vasculitis associated with rheumatoid arthritis, immune complex vasculitis, Takayasu's disease, dilated cardiomyopathy, diabetic angiopathy, Kawasaki's disease (arteritis), venous gas embolus (VGE), and restenosis following stent placement, rotational atherectomy, and percutaneous transluminal coronary angioplasty (PTCA) (see, *e.g.*, Holers (2008) *Immunological Reviews* 223:300-316; Holers and Thurman (2004) *Molecular Immunology* 41:147-152; U.S. Patent Publication No. 20070172483).

In one embodiment, the complement factor B-associate disease is selected from the group consisting of C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, polycystic kidney disease, membranous nephropathy, age-related macular degeneration, atypical hemolytic uremic syndrome, thrombotic microangiopathy, myasthenia gravis, ischemia and reperfusion injury, paroxysmal nocturnal hemoglobinuria, and rheumatoid arthritis

In another embodiment, the complement factor B-associate disease is selected from the group consisting of C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, and polycystic kidney disease.

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 complement factor B gene, *e.g.*, a liver cell, a brain cell, a gall bladder cell, a heart cell, or a kidney cell. In one embodiment, the cell is 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
5 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, complement factor B 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
10 containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the complement factor B 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,
15 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 some embodiments, the administration is via a depot injection. A depot injection may
20 release the iRNA in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, *e.g.*, a desired inhibition of CFB, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In certain embodiments, the depot injection is a subcutaneous injection.

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

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

In one aspect, the present invention also provides methods for inhibiting the expression of a
35 complement factor B gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a complement factor B gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the complement factor B gene, thereby inhibiting expression of the complement factor B 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 in the art, *e.g.* ELISA. In certain embodiments, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the complement factor B gene or protein expression. In other embodiments, a blood sample serves as the subject sample for monitoring the reduction in the complement factor B protein expression.

The present invention further provides methods of treatment in a subject in need thereof, *e.g.*, a subject diagnosed with a complement factor B-associated disorder, such as, C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, and polycystic kidney disease.

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 complement factor B expression, in a prophylactically effective amount of an iRNA targeting a complement factor B gene or a pharmaceutical composition comprising an iRNA targeting a complement factor B gene.

In one embodiment, a complement factor B-associated disease is selected from the group consisting of paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), asthma, rheumatoid arthritis (RA); antiphospholipid antibody syndrome; lupus nephritis; ischemia-reperfusion injury; typical or infectious hemolytic uremic syndrome (tHUS); dense deposit disease (DDD); neuromyelitis optica (NMO); multifocal motor neuropathy (MMN); multiple sclerosis (MS); macular degeneration (*e.g.*, age-related macular degeneration (AMD)); hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; thrombotic thrombocytopenic purpura (TTP); spontaneous fetal loss; Pauci-immune vasculitis; epidermolysis bullosa; recurrent fetal loss; pre-eclampsia, traumatic brain injury, myasthenia gravis, cold agglutinin disease, dermatomyositis, bullous pemphigoid, Shiga toxin E. coli-related hemolytic uremic syndrome, C3 neuropathy, anti-neutrophil cytoplasmic antibody-associated vasculitis (*e.g.*, granulomatosis with polyangiitis (previously known as Wegener granulomatosis), Churg-Strauss syndrome, and microscopic polyangiitis), humoral and vascular transplant rejection, graft dysfunction, myocardial infarction (*e.g.*, tissue damage and ischemia in myocardial infarction), an allogeneic transplant, sepsis (*e.g.*, poor outcome in sepsis), Coronary artery disease, dermatomyositis, Graves' disease, atherosclerosis, Alzheimer's disease, systemic inflammatory response sepsis, septic shock, spinal cord injury, glomerulonephritis, Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, autoimmune hemolytic anemia (AIHA), ITP, Goodpasture syndrome, Degos disease, antiphospholipid syndrome (APS), catastrophic APS (CAPS), a cardiovascular disorder, myocarditis, a cerebrovascular disorder, a peripheral (*e.g.*, musculoskeletal) vascular disorder, a renovascular disorder, a mesenteric/enteric vascular disorder, vasculitis, Henoch-Schönlein purpura nephritis, systemic lupus erythematosus-associated vasculitis, vasculitis associated with rheumatoid arthritis, immune complex vasculitis, Takayasu's disease, dilated cardiomyopathy, diabetic angiopathy, Kawasaki's disease (arteritis),

venous gas embolus (VGE), and restenosis following stent placement, rotational atherectomy, and percutaneous transluminal coronary angioplasty (PTCA) (see, *e.g.*, Holers (2008) *Immunological Reviews* 223:300-316; Holers and Thurman (2004) *Molecular Immunology* 41:147-152; US20070172483).

5 In one embodiment, the complement factor B-associate disease is selected from the group consisting of C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, polycystic kidney disease, membranous nephropathy, age-related macular degeneration, atypical hemolytic uremic syndrome, thrombotic microangiopathy, myasthenia gravis, ischemia and reperfusion injury, paroxysmal nocturnal hemoglobinuria, and
10 rheumatoid arthritis

 In another embodiment, the complement factor B-associate disease is selected from the group consisting of C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, and polycystic kidney disease.

 An iRNA of the invention may be administered as a “free iRNA.” A free iRNA is
15 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.

20 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 complement factor B gene expression are subjects susceptible to or diagnosed with a CFB-associated disorder, *e.g.*, C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy,
25 and polycystic kidney disease.

 In an embodiment, the method includes administering a composition featured herein such that expression of the target complement component B 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.

30 In one embodiment, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target complement factor B 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
35 or treatment of a complement factor B-associated disorder, *e.g.*, C3 glomerulopathy, systemic lupus erythematosus (SLE), *e.g.*, Lupus Nephritis, IgA nephropathy, diabetic nephropathy, and polycystic kidney disease.

Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg. Subjects can be administered a therapeutic amount of iRNA, such as about 5 mg to about 1000 mg as a fixed dose, regardless of body weight.

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

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 or inhibition of CFB gene expression, *e.g.*, a subject having a CFB-associated disease, in combination with other pharmaceuticals or other therapeutic methods, *e.g.*, with known pharmaceuticals 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. The iRNA agent and an additional therapeutic agent or treatment may be administered at the same time 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 or by another method known in the art or described herein.

In one embodiment, an iRNA agent of the invention is administered in combination with an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.*, eculizumab or ravulizumab-cwvz), an iRNA agent targeting complement component C5, an iRNA agent targeting complement component C3, or a C3 peptide inhibitor (*e.g.*, compstatin). In one embodiment, the iRNA agent of the invention is administered to the patient, and then the additional therapeutic agent is administered to the patient (or vice versa). In another embodiment, the iRNA agent of the invention and the additional therapeutic agent are administered at the same time.

The iRNA agent of the invention and an additional therapeutic agent or treatment may be administered at the same time 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 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 ssiRNA compound, (*e.g.*, a precursor, *e.g.*, a larger siRNA compound which can be

processed into a ssiRNA 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 CFB (*e.g.*, means for measuring the inhibition of CFB mRNA, CFB protein, or CFB activity). Such means for measuring the inhibition of CFB 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.

20

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

25

EXAMPLES

Example 1. iRNA Synthesis

30 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.

35 siRNA Design

siRNAs targeting the human complement factor B (CFB) gene, (human: NCBI refseqID NM_001710.5; NCBI GeneID: 629) was designed using custom R and Python scripts. The human NM_001710 REFSEQ mRNA, version 5, has a length of 2646 bases. Detailed lists of the unmodified

CFB sense and antisense strand nucleotide sequences are shown in Tables 2, 4, and 6. Detailed lists of the modified CFB 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.

- 5 For example, AD-959917 is equivalent to AD-959917.1.

siRNA Synthesis

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

10 **Example 2. *In vitro* screening methods**

Cell culture and 384-well transfections

- Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO₂ in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Primary mouse hepatocytes (PMH) were
 15 freshly isolated less than 1 hour prior to transfections and grown in primary hepatocyte media. For both Hep3B and PMH, transfection was carried out by adding 5 µl of Opti-MEM plus 0.1 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 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 Eagle's Minimum Essential Medium (ATCC Cat#30-2003) containing ~5
 20 x10³ Hep3B cells or PMH 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.

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

- RNA was isolated using an automated protocol on a BioTek-EL406 platform using
 25 DYNABEADS (Invitrogen, cat#61012). Briefly, 70 µl of Lysis/Binding Buffer and 10 µl of lysis buffer containing 3 µl of magnetic beads were added to the plate with cells. Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured and the supernatant was removed. Bead-bound RNA was then washed 2 times with 150 µl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150 µl Elution Buffer, re-
 30 captured and supernatant removed.

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

- A master mix of 1.2 µl 10X Buffer, 0.48 µl 25X dNTPs, 1.2 µl Random primers, 0.6 µl Reverse Transcriptase, 0.6 µl RNase inhibitor and 7.92 µl of H₂O per reaction were added per well.
 35 Plates were sealed, mixed, and then incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by incubation at 37°C for 2 hours.

Real time PCR

Two μ l of cDNA were added to a master mix containing 0.5 μ l of human GAPDH TaqMan Probe (4326317E), and 0.5 μ l CFB human probe (Hs01071998_m1) and 5 μ l Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001).

- 5 Real time PCR was done in a LightCycler480 Real Time PCR system (Roche). Each duplex was tested at least two times and data were normalized to cells transfected with a non-targeting control siRNA. To calculate relative fold change, real time data were analyzed using the $\Delta\Delta C_t$ method and normalized to assays performed with cells transfected with a non-targeting control siRNA.

- 10 The results of the single dose screen of the dsRNA agents in Tables 2 are 3 in Hep3B cells are shown in Table 8. The results of the single dose screen of the dsRNA agents Tables 4 and 5 in Hep3B cells are shown in Table 9. The results of the single dose screen of the dsRNA agents Tables 4 and 5 in PMH cells are shown in Table 10. The results of the single dose screen of the dsRNA agents Tables 6 and 7 in PMH cells are shown in Table 11. The results of the single dose screen of the dsRNA agents Tables 6 and 7 in Hep3B cells are shown in Table 12.

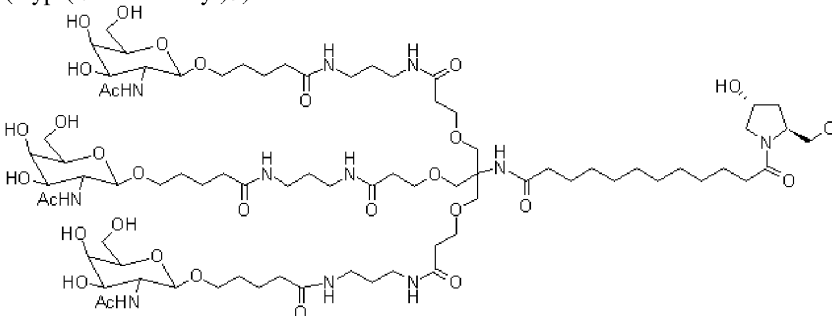
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ELISA Assay

- Human CFB protein levels were determined using a quantitative sandwich enzyme immunoassay (Human Complement Factor B AssayMax ELISA Kit – AssayPro). Briefly, samples were diluted 1:1000 and 50 μ l of sample was added to a well of a microtiter plate. Samples were
20 incubated for two hours and subsequently washed. Fifty μ l of biotinylated anti-CFB antibody was added to each well and incubated 1 hour. Samples were then washed and 50 μ l of streptavidin-peroxidase conjugate was added to each well and incubated for 30 minutes. Following another wash, 50 μ l of peroxidase enzyme substrate per well was added and samples were incubated for 15 minutes after which 50 μ l of Stop Solution per well was added. Samples were read at 450 nm immediately
25 and the amount of human CFB protein was determined by comparing the reading to a standard curve (0-280 ng of human CFB protein).

- 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'-
30 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).

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

Abbreviation	Nucleotide(s)
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)
	
Y34	2-hydroxymethyl-tetrahydrofuran-4-methoxy-3-phosphate (abasic 2'-OMe furanose)
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofuran-5-phosphate)
(Agn)	Adenosine-glycol nucleic acid (GNA)
(Cgn)	Cytidine-glycol nucleic acid (GNA)
(Ggn)	Guanosine-glycol nucleic acid (GNA)

Abbreviation	Nucleotide(s)
(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'-deoxythymidine-3'-phosphate
dTs	2'-deoxythymidine-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

Table 2. Unmodified Sense and Antisense Strand Sequences of Complement Factor B dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)	Antisense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)
AD-557072.1	CAAAAAGUGUCUAGUCAACUU	19	1145-1165	AAGUUGACUAGACACUUUUUGGC	105	1143-1165
AD-558097.1	UAGUGG AUGUCUGCAAAAACU	20	2440-2460	AGUUUUTGCAGACAUCCACUACU	106	2438-2460
AD-557774.1	GAAUCCUGAAUUUAUGACU	21	1981-2001	AGUCAUAAAUAUCAGGAUUUCCU	107	1979-2001
AD-557070.1	GCCAAAAGUGUCUAGUCAAU	22	1143-1163	AUUGACTAGACACUUUUUGGCUC	108	1141-1163
AD-558068.1	UCACAAAGAGAGUCGUUCAU	23	2393-2413	AUGAAACGACUUCUUGUGAAC	109	2391-2413
AD-557068.1	GAGCCAAAAAGUGUCUAGUCU	24	1141-1161	AGACUAGACACUUUUUGGCUCU	110	1139-1161
AD-556701.1	CUACUACAAUGUGAGUGAU	25	635-655	ACAUACUCACAUUUGUAGUAGGG	111	633-655
AD-558076.1	GAAGUCGUUUCAUUCAAGUUU	26	2401-2421	AAACUUGAAUUGAAAGACUUCUC	112	2399-2421
AD-558065.1	AGUUCACAAAGAGAGUCGUUU	27	2390-2410	AAACGACUUCUUCUUGAGACUUA	113	2388-2410
AD-557859.1	CAACUCGAGCUUUGAGGCUUU	28	2086-2106	AAAGCCTCAAAGCUCGAGUUGUU	114	2084-2106
AD-558096.1	GUAGUGG AUGUCUGCAAAAACU	29	2439-2459	AUUUUUGCAGACAUCCACUACUC	115	2437-2459
AD-557422.1	GAGGAUUUAUCUGGAUGUCUUA	30	1536-1556	AUAGACAUCACAGAUAAUCCUCCC	116	1534-1556
AD-556919.1	AGACUCCUUAUGUACGACAU	31	935-955	AUGUCGTACAUAGAGGAGUCUUG	117	933-955
AD-558069.1	CACAAAGAGAGUCGUUCAUU	32	2394-2414	AAUGAAACGACUUCUUGUGAA	118	2392-2414
AD-558063.1	AUAGUUCACAAAGAGAGUCGU	33	2388-2408	ACGACUUCUUCUUGAGAACUAUA	119	2386-2408
AD-557069.1	AGCCAAAAGUGUCUAGUCAU	34	1142-1162	AUGACUAGACACUUUUUGGCUCU	120	1140-1162
AD-558225.1	UGAAUUAAAACAGCUGCGACU	35	2604-2624	AGUCGCAGCUUUUUAAUUAUAU	121	2602-2624
AD-557853.1	AGGGAACAAACUCGAGCUUUGU	36	2080-2100	ACAAAGCUCGAGUUGUUCUCCUCG	122	2078-2100
AD-558012.1	GACAAAGUCAAGGACUUCUCU	37	2277-2297	AGAGAUGUCCUUGACUUCUUGCAU	123	2275-2297
AD-558061.1	UGAUAGUUCACAAAGAGAAAGUU	38	2386-2406	AACUUCTCUUGUGAACUAUCAAAG	124	2384-2406
AD-557079.1	UGUCUAGUCAACUUAUUUGAU	39	1152-1172	AUCAAUTAAGUUGACUAGACACU	125	1150-1172
AD-558066.1	GUUCACAAAGAGAGUCGUUUU	40	2391-2411	AAACGACUUCUUCUUGUGAACUA	126	2389-2411
AD-557353.1	GACCCAAUUAACUGUCAUUUGAU	41	1467-1487	AUCAAUGACAGUAAUUGGGUCCC	127	1465-1487
AD-557782.1	GAAUUUUUAUGACUUAUGACGUU	42	1989-2009	AACGUCAUAGUCAUAAAUAUUCAG	128	1987-2009
AD-556390.1	CUGGAGUUUUCAGCUUGGACAU	43	179-199	AUGUCCAAGCUGAAACUCCAGAC	129	177-199
AD-557078.1	GUGUCUAGUCAACUUAUUUGU	44	1151-1171	ACAAUUAAGUUGACUAGACACU	130	1149-1171
AD-557475.1	UUCCAAGAAAGACAAUAGACU	45	1607-1627	AGCUCATUGUCUUCUUGGAAGC	131	1605-1627
AD-556734.1	CUGCUAUGACGGUUAACACUCU	46	668-688	AGAGUGTAACCGUCUAUAGCAGUG	132	666-688
AD-557084.1	AGUCAACUUAUUAGAGAAAGGU	47	1157-1177	ACUUCTCAAUUAAGUUGACUAG	133	1155-1177
AD-557498.1	AUGUGUUCAAAGUCAAGGAUU	48	1630-1650	AUCCUTGACUUUGAACACACU	134	1628-1650
AD-556788.1	GACAGCGAUCUGUGACAAACGU	49	740-760	ACGUUGTCACAGAUCCGUCUCUG	135	738-760
AD-557067.1	GGAGCCAAAAGUGUCUAGUU	50	1140-1160	AACUAGACACUUCUUGGCUCUCG	136	1138-1160
AD-557852.1	GAGGGAACAAACUCGAGCUUUU	51	2079-2099	AAAAGCTCGAGUUGUUCUCCUCG	137	2077-2099

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)	Antisense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)
AD-556733.1	ACUGCUAUGACGGUUAACACUU	52	667-687	AAUGUUAACCCGUCUAAGCAGUGG	138	665-687
AD-556725.1	CUCUUCCACUCGUAUGACGU	53	659-679	ACGUCATAGCAGUGGAAAGAGAU	139	657-679
AD-557860.1	AACUCGAGCUUUGAGGCUUCU	54	2087-2107	AGAAAGCCUCAAAGCUCGAGUUGU	140	2085-2107
AD-556786.1	CAGACAGGUAUCUGUGACAAU	55	738-758	AUUGACACGUAUCGUCGUCGCC	141	736-758
AD-556581.1	CCUUCUGGCUUCUACCCGUAU	56	465-485	AUACGGGUAAGAGCCAGAGGAC	142	463-485
AD-556963.1	ACCAUAGAAAGGAGUCGAGUCU	57	999-1019	AGCAUCGACUCCUUCUAUGGUCU	143	997-1019
AD-557450.1	ACCAAGUGAAACAUCAAUGCUU	58	1582-1602	AAGCAUTGAUGUUCACUUGGUUC	144	1580-1602
AD-557204.1	UGAAAUCAAUUAUGAAGACCU	59	1298-1318	AGGUCUTCAUAAUUAUUAUUAU	145	1296-1318
AD-558062.1	GAUAGUUCACAAAGAGAGUCU	60	2387-2407	AGACUUCUUCUGAGAAUUAUAA	146	2385-2407
AD-557602.1	AAGGUACCGAUUACCAAAU	61	1734-1754	AUUGUGGUAUUCGGUACCCUCC	147	1732-1754
AD-556917.1	CAAGACUCCUUAUGUACGAU	62	933-953	AUCGUACAUGAAGGAGUUCUUGC	148	931-953
AD-557969.1	CUGACUCGGAAGGAGGUCUAU	63	2196-2216	AUAGACCUCCUCCGAGUCAGCU	149	2194-2216
AD-558064.1	UAGUUCACAAAGAGAGUUGUU	64	2389-2409	AACGACTUCUUCUUGUGAACUAC	150	2387-2409
AD-558105.1	GUCUGCAAAACCAAGAGCGU	65	2448-2468	ACGCUUCUGGUUUUUGCAGACU	151	2446-2468
AD-556791.1	AGCGAUCUGUAGACAACGGAGU	66	743-763	ACUCCGTUGUCACAGAUCCGUGU	152	741-763
AD-557972.1	ACUCGGAAGGAGGUCUACAUU	67	2199-2219	AAUGUAGACCUCCUCCGAGUCA	153	2197-2219
AD-556920.1	GACUCCUUAUGUACGACACU	68	936-956	AGUUCGUACAUGAAGGAGUCUU	154	934-956
AD-558078.1	AGUCGUUUAUUAAGUUGGU	69	2403-2423	ACCAACTUGAAUGAAACGACUUC	155	2401-2423
AD-557861.1	ACUCGAGCUUUGAGGCUUCCU	70	2088-2108	AGGAAGCCUCAAAGCUCGAGUUG	156	2086-2108
AD-557836.1	GAACAACUCGAGCUUUGAGGU	71	2083-2103	ACCUCAAAGCUCGAGUUGUCCU	157	2081-2103
AD-557041.1	UGGUGCUAUGGUAUCAGACU	72	1096-1116	AGUCUGAUCCAUCUAGCACCAGG	158	1094-1116
AD-556874.1	GUGUCAGGAAGGUGGCUUUU	73	890-910	AAAGAGCCACCUUCCUGACACGU	159	888-910
AD-557345.1	CUGAUGGAUUGCACACAUGU	74	1441-1461	ACAUGUTGUGCAUCCAUACAGUC	160	1439-1461
AD-557839.1	CAGACUAUCAGGCCCAUUUGU	75	2046-2066	ACAAUUGGGCCUGAUAGUCUGGC	161	2044-2066
AD-556962.1	GACCAUAGAAAGGAGUCGAGU	76	998-1018	ACAUCGACUCCUUCUAUGGUCUC	162	996-1018
AD-557066.1	AGGAGCCAAAGUGUCUAGU	77	1139-1159	ACUAGACACUUUUUGGCUCCUGU	163	1137-1159
AD-556961.1	AGACCAUAGAGGAGUCGUAU	78	997-1017	AUUCGACUCCUUCUAUGGUCUCU	164	995-1017
AD-557226.1	AAGUUGAAGUCAGGACUAAU	79	1320-1340	AUUAUCCUCCUGACUUAACUUGU	165	1318-1340
AD-557865.1	GAGCUUUGAGGCUUCCUCCAU	80	2092-2112	AUGGAGGAAGCCUCAAAGCUCGA	166	2090-2112
AD-557209.1	UCAAUUAUGAAGACCACAAGU	81	1303-1323	ACUUGUGGUCUUAUUAUUAUUAU	167	1301-1323
AD-556918.1	AAGACUCCUUAUGUACGACU	82	934-954	AGUCUACAUGAAGGAGUUCUGG	168	932-954
AD-557868.1	CUUUGAGGCUUCCUCCAAUUCU	83	2095-2115	AAGUUGGAGGAAGCCUCAAAGCU	169	2093-2115
AD-557873.1	AGGCUUCCUCCAAUUAUCCACU	84	2100-2120	AGUUGUAGUUGGAGGAAGCCUCA	170	2098-2120
AD-556787.1	AGACAGCGAUCUGUGACAACU	85	739-759	AGUUGUCACAGAUCCGUCUGUCG	171	737-759

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)	Antisense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)
AD-558004.1	CAGGCUAUGACAAAAAGUCAAGU	86	2269-2289	ACUUGACUUUGUCAUAGCCUUGG	172	2267-2289
AD-557867.1	GUUUGAGGCUUCCUCCAAACU	87	2094-2114	AGUUGAGGAGAACCCUACAAAGCUC	173	2092-2114
AD-557788.1	UUGACUAUGACGUUGGCCU	88	1995-2015	AAGGGCAACGUAUAGUCAUAAA	174	1993-2015
AD-558106.1	UCUGCAAAACCAGAAAGCGU	89	2449-2469	ACGCGUTCUGGUUUUGCAGACA	175	2447-2469
AD-556724.1	UCUCUUUCCACUGCUAUGACU	90	658-678	AGUCAUAGCAGUGGAAAAGAGAUC	176	656-678
AD-556739.1	AUGACGGUUACACUCUCCGGU	91	673-693	ACCGGAGAGUGUAACCGUCAUAG	177	671-693
AD-557417.1	CAAGGGAGGAUUAUCUGGAU	92	1531-1551	AAUCCAGUAUUCUCCCUUGGG	178	1529-1551
AD-556790.1	CAGCGAUCUCUGGACAAACGGAU	93	742-762	AUCCGUTGUCACAGUCGCUGUC	179	740-762
AD-557872.1	GAGGCUUCUCCCAACUACCAU	94	2099-2119	AUGGUAUGGAGGAAAGCCUCAA	180	2097-2119
AD-556738.1	UAUGACGGUUACACUCUCCGU	95	672-692	ACGGAGAGUGUAACCGUCAAGC	181	670-692
AD-557857.1	AACAACUCGAGCUUUGAGGCU	96	2084-2104	AGCCUCAAGCUCGAGUUGUUC	182	2082-2104
AD-556726.1	UCUUUCCACUGCUAUGACGGU	97	660-680	ACCGUCAUAGCAGUGGAAAAGAGA	183	658-680
AD-557495.1	AACAUGUGUUCAAAAGUCAAGU	98	1627-1647	ACUUGACUUUGAACACAUUUGC	184	1625-1647
AD-557016.1	CCUUCAGGCUCCAUGAACA	99	1071-1091	AAUGUUCAGUGGAGCCUGAAGGU	185	1069-1091
AD-558074.1	GAGAAGUCGUUUCAUUAAGU	100	2399-2419	ACUUGAAUGAAACGACUUCUCU	186	2397-2419
AD-557604.1	GGUACCGGAUUACCAACAAGU	101	1736-1756	AGCUUGTGGUAAUCGGUACCCU	187	1734-1756
AD-557346.1	UGAUGGAUUGCACAACAAGU	102	1442-1462	ACCAUGTUGGAAUCCAUACAGU	188	1440-1462
AD-557851.1	CGAGGGAACAACUCGAGCUU	103	2078-2098	AAAGCUCGAGUUGUUCUCCCGU	189	2076-2098
AD-557786.1	UUUAUGACUAUGACGUUGCCU	104	1993-2013	AGGCAACGCUCAUAGUCAUAAA	190	1991-2013

Table 3. Modified Sense and Antisense Strand Sequences of Complement Factor B dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-557072.1	csasaaaaGfuGfUfcfuagucacauL96	191	asAfsaguu(Agn)cuagacAfcUfuuuugsgsc	277	GCCAAAAAGUGUCAGUCAACUU	363
AD-558097.1	usagsgaGfuGfUfcfugcaaacauL96	192	asGfsuuu(Tgn)gcagacAfcCfcaatascsc	278	AGUAGUGGAGUGUCUGCAAAAACC	364
AD-557774.1	gsasauucCfuGfAFAfuuuuaagacuL96	193	asGfsuacu(Agn)aaauucAfcGfcaatascsc	279	AGGAAUUCUGAAUUUUU/AUGACU	365
AD-557070.1	gscscaaaAfaGfUfGfucuaiguacauL96	194	asUfsuagac(Tgn)agacacUfuUfuuuggcusuc	280	GAGCCAAAAGUGUCUAGUCAAC	366
AD-558068.1	uscsacaaGfaGfAfaGfuguuuacauL96	195	asUfsgaaa(Cgn)gacuuUfcUfugugasasc	281	GUUCACAAAGAAAGUCGUUUCAU	367
AD-557068.1	gsasgccaAfaAfARGfugucuaiguL96	196	asGfsacuac(Ggn)acacuUfuUfggcucscsc	282	AGGAGCCAAAAGUGUCUAGUCA	368
AD-556701.1	csusacuacCfaAFUfGfugagugauL96	197	asCfsauec(Cgn)ucaacuUfgUfaguagsgsg	283	CCCACUACA AUGUGAGUGAUGU	369
AD-558076.1	gsasagucGfuUfUfcauucaguuL96	198	asAfsacuac(Cgn)aaugaaAfcGfcaucuscsc	284	GAGAAGUCGUUCAUUCAGUAUG	370
AD-558065.1	asasuucaCfaAfGfAfaaagucuuuL96	199	asAfsagaa(Cgn)uuucuUfUfUfgaucuscsc	285	AUAGUUCACAGAGAAAGUCGUU	371

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-557859.1	csasacucGfaGfCfUfugagagcuuL96	200	asAfsagcc(Tgn)caagcUfcGfaguugsusu	286	AACAACUCGAGCUUUGAGGCUUC	372
AD-558096.1	gsusagugGfaUfgUfUfcgcaaaaL96	201	asUfsuuuu(Ggn)cgagcaUfcCfaucausc	287	GAGUAGUGGAGUGUCGCAAAAC	373
AD-557422.1	gsasggauUfaUfCfUfUfggaugcuauL96	202	asUfsagac(Agn)uccagaUfaAfuccuscsc	288	GGGAGGAUUUCUGGAUGUCUAU	374
AD-556919.1	asgsacucCfuUfCfAfuguaagacuL96	203	asUfsagac(Tgn)acagaUfgGfaguucusc	289	CAAGACUCCUACUGUACGACAC	375
AD-558069.1	csascaagAfgAfAfGfuguucauuL96	204	asAfsuag(Agn)cgacuuCfuCfugugsasa	290	UUCACAAAGAAAGUCGUUUAUU	376
AD-558063.1	asusaguuCfaCfAfAfagagagcuL96	205	asCfsgacu(Tgn)cucuuUfgAfauuasca	291	UGAUAGUUCACAAAGAGAGUCGU	377
AD-557069.1	asgsccaaAfaAfGfUfuguaugacuL96	206	asUfsagac(Agn)gacuuUfuUfugucusc	292	GGAGCCAAAAGUGUCUAGUCA	378
AD-558225.1	usgsaaauAfaAfAfCfagcugcacuL96	207	asGfsuegc(Agn)geuunUfuAfauucasasu	293	AUUGAAUUAAAACAGCUGCGACA	379
AD-557853.1	asgsagaaCfaAfCfUfugagcuuuL96	208	asCfsaaag(Cgn)ucaguuUfgUfuccuscsc	294	CGAGGGAACAACUCGAGCUUUGA	380
AD-558012.1	gsascaaaGfuCfAfAfagacucuuL96	209	asGfsagau(Ggn)uccuuUfgUfugucscsc	295	AUGACAAAGUCAAGGACAUCUCA	381
AD-558061.1	usgsauagUfuCfAfCfagagagauL96	210	asAfsuuc(Tgn)cuuugAfaCfaucausag	296	CUUGAUAGUUCACAAAGAGAGUC	382
AD-557079.1	usgsucuaGfuCfAfAfauuaauuL96	211	asUfseuu(Tgn)aaguuUfgUfagacuscsc	297	AGUGUCUAGUCAACUUAAUUGAG	383
AD-558066.1	gsusucacAfaGfAfGfagugcuuuL96	212	asAfsaacg(Agn)cuuucUfuGfugacuscsc	298	UAGUUCACAAAGAGAGAGUCUUUC	384
AD-557353.1	gsascccaAfuUfAfCfuguaugauL96	213	asUfscuu(Ggn)acaguuAfuUfugucscsc	299	GGGACCCAAUACUGUCAUUGAU	385
AD-557782.1	gsasaauUfaUfGfAfauagagcuL96	214	asAfsuuc(Agn)uaguuUfaAfauucscsc	300	CUGAAUUUAUGACUUAUGACGUU	386
AD-556390.1	csusggagUfuUfCfAfgeuugacuL96	215	asUfsguuc(Agn)agcuuAfaCfuccagasc	301	GUCUGGAGUUUCAGCUUGGACAC	387
AD-557078.1	gsusguuAfgUfCfAfacuuaauuL96	216	asCfsaaui(Agn)aguuuGfuAfgacuscsc	302	AAGUGUCUAGUCAACUUAAUUGA	388
AD-557475.1	usuccaaGfaAfAfGfacaugacuL96	217	asGfseuca(Tgn)uucuuUfcUfugaagasc	303	GCUUCCAAGAAAGACAAUAGACA	389
AD-556734.1	csusgcuUfgAfCfGfuguaacuuL96	218	asGfsagug(Tgn)aacuuUfaUfagcagusc	304	CACUGCUAUGACGGUUAACACUCU	390
AD-557084.1	asgsucuaCfuUfAfAfugagagauL96	219	asCfsuuc(Tgn)cauuuAfgUfugacuscsc	305	CUAGUCAACUUAAUUGAGAGAGU	391
AD-557498.1	asusguguUfcAfAfAfgucaagauL96	220	asAfsuuc(Tgn)gacuuGfaAfcaucscsc	306	ACAUGUGUUCAAAAGUCAAGGAUA	392
AD-556788.1	gsasagcGfaUfCfUfugacacacuL96	221	asCfsguug(Tgn)cacuuUfcGfugucuscsc	307	CAGACAGCGAUUCUGUGACAACGG	393
AD-557067.1	gsagsagcAfaAfAfAfugucuaauL96	222	asAfsuag(Agn)acuuuUfuGfuccuscsc	308	CAGGAGCCAAAAGUGUCUAGUC	394
AD-557852.1	gsasgggaAfcAfAfCfugagcuuuL96	223	asAfsaagc(Tgn)cgauuGfuUfuccuscsc	309	CCGAGGGAACAACUCGAGCUUUG	395
AD-556733.1	ascstuguAfuGfAfCfuguaacuuL96	224	asAfsuguu(Agn)accuuAfuAfgcaguscsc	310	CCACUGCUAUGACGGUUAACACUC	396
AD-556725.1	csuscuuuCfcAfCfUfgeuaugacuL96	225	asCfsguica(Tgn)agcuuGfuAfaagascsc	311	AUCUCUUUCCACUGCUAUGACGG	397
AD-557860.1	asascuagAfgCfUfUfugagcuuuL96	226	asGfsaagc(Cgn)ucauuCfuCfaguuscsc	312	ACAACUCGAGUUUUGAGGUUCC	398
AD-556786.1	csasgacaGfcGfAfUfugugacuauL96	227	asUfsuuc(Agn)cgauuGfcUfugucscsc	313	GGCAGACAGCGAUCUGUGACAAC	399
AD-556581.1	csusuucGfgCfUfUfuaacccguauL96	228	asUfsacgg(Ggn)uaguuCfcAfgaagascsc	314	GUCUCUUGGCUUUAACCCGUAC	400
AD-556963.1	ascscuuAfaAfGfGfagucagcuL96	229	asGfseuuc(Ggn)acuuuUfcUfauuguscsc	315	AGACCAUAGAAGGAGUCGAUGCU	401
AD-557450.1	ascscagUfgAfAfCfaucauauL96	230	asAfsuuc(Tgn)gauuuCfaCfugucuscsc	316	GAACCAAGUAGAACUUAUGUCU	402
AD-557204.1	usgsaaauCfaAfUfUfauagagacuL96	231	asGfsuuc(Tgn)gauuuUfgAfauucscsc	317	AAUGAAAUCAAUAUGAAGACCA	403
AD-558062.1	gsasuaugUfcAfCfAfagagagcuL96	232	asGfsuuc(Cgn)ucuuuGfaAfauucscsc	318	UUUGAUAGUUCACAAGAGAGAGUCG	404
AD-557602.1	asasggguAfcCfGfAfuaaccacuL96	233	asUfsugug(Ggn)uaauuGfuAfcuccuscsc	319	GGAAGGGUACCGAUUAACCCACAAG	405

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-556917.1	csasagacUfcCfUfUfcauguaacgauL96	234	asUfscgna(Cgn)auagaagGfaGfucuuagsgc	320	GCCAAGACUCCUUAUGUACGAC	406
AD-557969.1	csusgacuCfGfAfAfagagagcuauL96	235	asUfsgaac(Cgn)uccuuCfGfAfugacgscsu	321	AGCUGACUCGGAAGGAGGUCUAC	407
AD-558064.1	usasguucAfcAfAfGfagaagucguuL96	236	asAfscgac(Tgn)uccuuGfuGfaacuasusc	322	GAUAGUUCACAAGAGAAAGUCGUU	408
AD-558105.1	gsuscucaAfaAfAfAfcagaagcguL96	237	asCfsguc(Cgn)ugguuuUfuGfagaacsasu	323	AUGUCUGCAAAAACACAGAACGGG	409
AD-556791.1	asgscgauCfuGfUfGfacaacaggauL96	238	asCfsuccg(Tgn)ugucacAfgAfucgusgsu	324	ACAGCGAUCUGAGACCAACGAGGC	410
AD-557972.1	ascsucggAfaGfGfAfagucuaauL96	239	asAfsuua(Cgn)uccuccUfuCfagaguscua	325	UGACUCGGAAGGAGGUCUACAUC	411
AD-556920.1	gsascuccUfuCfAfUfaguacgacauL96	240	asGfsuguc(Cgn)uacauGfAfGfagaguscua	326	AAGACUCCUUAUGUACGACACC	412
AD-558078.1	asgsucguUfuCfAfUfuaagugguL96	241	asCfscaac(Tgn)ugaauGfAfAfcgacusc	327	GAAGUCGUUUAUUAAGUUGGU	413
AD-557861.1	ascsucgaGfeUfUfUfagagcuccuL96	242	asGfsgaag(Cgn)ucuaaGfeUfagagusc	328	CAACUCGAGCUUUGAGGCUUCCU	414
AD-557856.1	gsasacaaCfuCfGfAfagcuaagguL96	243	asCfscuca(Agn)agucgAfgUfugucscsc	329	GGGAACAACUCGAGCUUUGAGGC	415
AD-557041.1	usgsugucUfaGfAfUfagaucagacuL96	244	asGfsucug(Agn)uccauUfaGfaccasgsg	330	CCUGGUGCUAGAUGGAUCAGACA	416
AD-556874.1	gsusgucaGfgAfAfGfagugcuccuL96	245	asAfsagag(Cgn)cauccUfGfagacsgsu	331	ACGUGUCAGGAAGGUGGCUUUG	417
AD-557345.1	csusgaugGfaUfUfGfacaacauuL96	246	asCfsaagu(Tgn)guucaUfCfaucaugsusc	332	GACUGAUGGAUUGCACAACAUGG	418
AD-557839.1	csasgacuAfuCfAfGfagcuccauuL96	247	asCfsaaau(Ggn)ggccuGfAfAfucucgsc	333	GCCAGACUAUCAGGCCCAUUGU	419
AD-556962.1	gsasccauAfgAfAfGfagucgaugL96	248	asCfsaucg(Agn)uccuuCfuAfgucusc	334	GAGACCAUAGAAGGAGUCGACG	420
AD-557066.1	asgsagacCfaAfAfAfagugucuaL96	249	asCfsuagat(Cgn)uccuuUfGfucucsgsu	335	ACAGGAGCCAAAAGUGUCUAGU	421
AD-556961.1	asgsaccaUfaGfAfAfagagucgaauL96	250	asAfsucga(Cgn)uccuuUfaUfagucusc	336	AGAGACCAUAGAAGGAGUCGACG	422
AD-557226.1	asasguugAfaGfUfCfagagacuaauL96	251	asUfuaugu(Cgn)ccuagUfuCfauucsgsu	337	ACAAGUUGAAGUCAGGGACUAAC	423
AD-557865.1	gsasgcuUfGfAfGfCfucuccauL96	252	asUfsgag(Cgn)agccuCfaAfagucsgsa	338	UCGAGCUUUGAGGCUUCCUCCAA	424
AD-557209.1	uscsaauAfuGfAfAfagaccacauL96	253	asCfsuugu(Cgn)gucuuAfuAfaucgascu	339	AAUCAAUUAUGAAGACCACACAGU	425
AD-556918.1	asasgacuCfeUfUfCfauagacgacuL96	254	asGfsucgu(Agn)caugaGfGfAfucucsgsg	340	CCAAGACUCCUUAUGUACGACA	426
AD-557868.1	csusuuugaGfgCfUfUfucuccaauL96	255	asAfsuug(Cgn)aggaagCfeUfcaagcscu	341	AGCUUUGAGGCUUCCUCCAAACU	427
AD-557873.1	asgsccuuCfeUfCfCfauuaccuL96	256	asGfsuguu(Agn)guugaGfgAfagcuscua	342	UGAGGCUUCCUCCAAACUACCACU	428
AD-556787.1	asgsacagCfGfAfUfCfugagacacuL96	257	asGfsuugu(Cgn)acagauCfGfCfugucsgsc	343	GCAGACAGCGAUCUGUGACACACG	429
AD-558004.1	csasggcuAfuGfAfCfaagucacauL96	258	asCfsuuga(Cgn)uuuugUfuAfgcucsgsg	344	CCCAGGCUAUGACAAAAGUCAAGG	430
AD-557867.1	gsccsuugAfgGfCfUfUfuccuccaauL96	259	asGfsuug(Cgn)ggagCfuCfcaagcscu	345	GAGCUUUGAGGCUUCCUCCAAACU	431
AD-557788.1	usasugacUfaUfGfAfagucuccuL96	260	asAfsagg(Cgn)acuaUfaGfucuaasasa	346	UUUAUGACUAUGACGUUGCCUUG	432
AD-558106.1	uscsugcaAfaAfAfCfagaagcguL96	261	asCfscgcu(Tgn)cuuguUfuUfagagascua	347	UGUCUGCAAAAACACAGAACGGC	433
AD-556724.1	uscsuucuUfcCfAfCfugcuaugacuL96	262	asGfsucau(Agn)gcagugGfaAfagagascu	348	GAUCUCUUCCAUCUGCUAUGACG	434
AD-556739.1	asusgaagGfuUfaAfCfauucuccguL96	263	asCfscgga(Cgn)aguuaAfcCfcaagascg	349	CUAUGACGGUUAACACUCUCCGGG	435
AD-557417.1	csasaggGfGfAfUfuaucuggaauL96	264	asAfsucca(Tgn)auaauCfuCfcaucsgsg	350	CCCAAGGGGAUUAUCUCUGGAUG	436
AD-556790.1	csasgcgaUfcUfGfUfagacacgaauL96	265	asUfscgcu(Tgn)uguaGfAfUfagcucgusc	351	GACAGCGAUCUGUGACAACGGAG	437
AD-557872.1	gsasggcuUfcUfCfCfcauacacauL96	266	asUfsggna(Cgn)uuggagGfaAfagcucasa	352	UUGAGGCUUCCUCCAAACUACCAC	438
AD-556738.1	usasugacGfgUfUfAfcaucuccguL96	267	asCfsggag(Agn)guuaaCfcGfcauagsgc	353	GCUAUGACGGUUAACACUCUCCGG	439

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-557857.1	asascaacUfcGfAfGfcuungagguL96	268	asGfscuc(Agn)aagcucGfaGfuuuuscsc	354	GGAACAACUCGAGCUUUGAGGCU	440
AD-556726.1	uscstuuicCfaCfUfGfcauagacggL96	269	asCfscuc(Agn)uagcagUfgGfaaagsgsa	355	UCUCUUUCCACUGCUAUGACGGU	441
AD-557495.1	asascaugUfgUfUfCfaaagcaagL96	270	asCfsuuga(Cgn)uuuugaCfaCfauguusgsc	356	GCAACAUGUGUUCACAAAGUCAAGG	442
AD-557016.1	escstueaGfgCfUfCfcaugaaacauL96	271	asAfsuuu(Cgn)auggagCfcUfgaagsgsu	357	ACCCUUCAGGUCUCCAUUGAACAU	443
AD-558074.1	gsasgaagUfcGfUfUfcauucacauL96	272	asCfsuuga(Agn)ugaaacGfaCfuucuscusu	358	AAGAGAAGUCGCUUUCUUAUACAUG	444
AD-557604.1	gsgsguacCfGfAfUfUfaccacacagL96	273	asGfscuc(Tgn)gguaauCfGfuaaccscusu	359	AAGGGUACCGAUUACCAACAAGCA	445
AD-557346.1	usgsauggAfUfUfGfCfacaacagguL96	274	asCfscuag(Tgn)ugugcaAfUfCfaucacgsu	360	ACUGAUGGAUUGCACAACAUGGG	446
AD-557851.1	csgsagggAfAcfAfAfcaucagacuuL96	275	asAfsagcu(Cgn)ggauugUfuCfcaucgsgsu	361	ACCGAGGGAACAACUCGAGCUUU	447
AD-557786.1	ususuauagAfeUfAfUfcaugugcuL96	276	asGfsgcaal(Cgn)gucauaGfuCfaaasasu	362	AUUUAUGACUAUGACGUUGCCC	448

Table 4. Unmodified Sense and Antisense Strand Sequences of Complement Factor B dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)	Antisense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)
AD-558860.1	UGCCAAGACUCUCCUUAUGUAU	449	930-950	AUACAUGAAGGAGUCUUGGCAGG	520	928-950
AD-560018.1	AAGAGAAAGUCGUUUAUUAU	450	2397-2417	AUGAAUGAAACGACUUCUUGU	521	2395-2417
AD-560019.1	AGAGAAAGUCGUUUAUUAU	451	2398-2418	AUUGAAUGAAACGACUUCUUG	522	2396-2418
AD-559160.1	UAUGAAGACCACAAAGUUGAAU	452	1308-1328	AUUGAAUGUUGGUCUUAUUAU	523	1306-1328
AD-559921.1	CGGAAGGAGGUCUACAUCAAU	453	2202-2222	AUUGAUGUAGACUUCUCCGAG	524	2200-2222
AD-559755.1	AUCAAGCUCAAAGAAUAAGCUU	454	2016-2036	AAGCUUAUUCUUGAGCUUGAUA	525	2014-2036
AD-560017.1	CAAGAGAAGUCGUUUAUUAU	455	2396-2416	AGAUGAAACGACUUCUUGU	526	2394-2416
AD-559614.1	CUGUGGUCUGAGUACUUAU	456	1819-1839	AAAAGUACUCAGACACACAGCC	527	1817-1839
AD-559435.1	AUGAGCAACAUUGUUAUUAU	457	1621-1641	AUUUGAACACAUUGUUCUAUUG	528	1619-1641
AD-560016.1	ACAAGAGAAGUCGUUUAUUAU	458	2395-2415	AAUUGAAACGACUUCUUGA	529	2393-2415
AD-559451.1	CAAAAGUCAAGGAUAUGGAAU	459	1637-1657	AUUUCCAUUCCUUGACUUAUUA	530	1635-1657
AD-559617.1	UGGUGUCUGAGUACUUAUUGU	460	1822-1842	ACACAAAGUACUCAGACACACA	531	1820-1842
AD-560021.1	AGAAGUCGUUUAUUAUUAU	461	2400-2420	AACUUGAAUGAAACGACUUAU	532	2398-2420
AD-559449.1	UUCAAAGUCAAGGAUAUGGAU	462	1635-1655	AUCCAUUCCUUGACUUAUUA	533	1633-1655
AD-559450.1	UCAAAGUCAAGGAUAUGGAAU	463	1636-1656	AUUCCAUUCCUUGACUUAUUA	534	1634-1656
AD-559925.1	AGGAGGUCUACAUAAGAAU	464	2206-2226	AUUCUUGAUGAAGACCUUAU	535	2204-2226
AD-559440.1	CAACAUGUGUUAUUAUUAU	465	1626-1646	AUUGCUUUGAAGACUUAUUAU	536	1624-1646
AD-559788.1	ACUAUCAGGCCCAUUAUUAU	466	2049-2069	AAGACAAUUGGCCCUUAUUAU	537	2047-2069
AD-559437.1	GAGCAACAUGUGUUAUUAUUAU	467	1623-1643	AACUUUGAACACAUUAUUAU	538	1621-1643

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)	Antisense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)
AD-559369.1	AGGAUUUUCUGGAUGUCUAUU	468	1537-1557	AAUAGACAUCCAGAUAAUCCUCC	539	1535-1557
AD-559446.1	GUGUCAAAGUCAAGGAUUU	469	1632-1652	AAUUAUCCUUGAGCUUUUAAACACAU	540	1630-1652
AD-559924.1	AAGGAGGUCUACAUCAAGAAU	470	2205-2225	AUUCUUGAUUAGAGCCUCCUCC	541	2203-2225
AD-559965.1	UCAGGCUCCAUAGAACAUUU	471	1074-1094	AUAGUUUUUAGAGGAGCCUUAAG	542	1072-1094
AD-560045.1	GUGGAUGUCUGCAAAACCAU	472	2442-2462	AUGUUUUUUGAGACAUCCACUA	543	2440-2462
AD-559946.1	GUGAGAGAGAUUCUAUUU	473	2245-2265	AAUUAUUGAGCAUCUCUCACAG	544	2243-2265
AD-558697.1	AUCGCACCGCCAAAGUAAU	474	703-723	AAUUCACUUGGCGAGGUGCGAUUG	545	701-723
AD-559008.1	UCACAGGAGCCAAAAGUGUU	475	1135-1155	AACACUUUUUUGGCUCCUGUAG	546	1133-1155
AD-559357.1	AAACCCAAAGGAGGAUUUU	476	1525-1545	AAUUAUCCUCCUUGGGUUUUUG	547	1523-1545
AD-559020.1	AAAGUGUCUAGUCAUUUU	477	1147-1167	AUAAGUUGACUAGACAUUUUG	548	1145-1167
AD-559143.1	CAGCUCAAUAGAAUCAAUUU	478	1290-1310	AUAUUGAUUUCAUUUAGCUGCU	549	1288-1310
AD-559374.1	UAUCUGGAGUCUAUGUGUUU	479	1542-1562	AAACACAUAGACAUCCAGAUAAU	550	1540-1562
AD-560161.1	GGCGUGGGAUUGAAUAAAU	480	2594-2614	AUUUAUUUCAAUCCACGCCCC	551	2592-2614
AD-559947.1	UGAGAGAGAUUCUAUUUGU	481	2246-2266	ACAUUAUUGAGCAUCUCUCACAC	552	2244-2266
AD-559616.1	GUGGUGUCUGAGUACUUUGUU	482	1821-1841	AACAAAGUACUCAGACACACACAG	553	1819-1841
AD-559142.1	GCAGCUCAAUAGAAUCAAUUU	483	1289-1309	AAAUUGAUUUCAUUUAGCUGCUU	554	1287-1309
AD-558639.1	CGGUCUCCCUACUACAUGUU	484	627-647	AACAUUGUAGUAGGAGACCGGG	555	625-647
AD-560166.1	GGGAUUGAAUUAACAGCUU	485	2599-2619	AAGCUGUUUUAAUUAUCCAC	556	2597-2619
AD-559359.1	AACCAAGGAGGAUUUUCUU	486	1527-1547	AAGAUAAUCCUCCUUGGGUUUU	557	1525-1547
AD-558657.1	GUGAGUGAGAGAUUCUUUU	487	645-665	AAAAGAGAUUCUACUCACACAU	558	643-665
AD-559442.1	ACAUGUUAUCAAAGUCAAGGU	488	1628-1648	ACCUUGACUUUUGAACACAUUUU	559	1626-1648
AD-559023.1	AGUGUCUAGUCAACUUAUUU	489	1150-1170	AAUUAAGUUGACUAGACAUUU	560	1148-1170
AD-560160.1	GGCGUGGGAUUGAAUAAU	490	2593-2613	AUUUAUUUCAAUCCACGCCCC	561	2591-2613
AD-559398.1	CAAGUGAACAUCAUUGCUUU	491	1584-1604	AAAAGCAUUGAUUUCACUUGGU	562	1582-1604
AD-559722.1	AUUCUGAAUUUUUUGACUAAU	492	1983-2003	AUAGUCAUAAAUUUACAGGAUUC	563	1981-2003
AD-559146.1	CUCAAUGAAAUCAAUUUUGAU	493	1293-1313	AUCAUUAUUGAUUUUUAUUGAGCU	564	1291-1313
AD-558267.1	GGAUUUCGGGAAAGUGAUU	494	55-75	AAUCACUUUCCCGGAACAUCCAA	565	53-75
AD-559074.1	GAUAUGGUCUAGUGACAUUU	495	1201-1221	AUAUGUCACUAGACCAUUAUUU	566	1199-1221
AD-560162.1	GCGUGGGAUUGAAUUAUAAU	496	2595-2615	AGUUUAUUUCAAUCCACGCC	567	2593-2615
AD-559021.1	AAAGUGUCUAGUCAACUUAUU	497	1148-1168	AUUAAGUUGACUAGACAUUUUU	568	1146-1168
AD-559144.1	AGCUCAAUGAAAUCAAUUUU	498	1291-1311	AUAUUAUUGAUUUCAUUGCUGC	569	1289-1311
AD-559147.1	CAAUAGAAUCAAUUUUGAAGU	499	1295-1315	ACUUAUAAUUUUAUUUUAUUGAG	570	1293-1315
AD-560164.1	GUGGGAUUGAAUUAUAAACAGU	500	2597-2617	ACUGUUUUAAUUAUCCACACGC	571	2595-2617
AD-559714.1	AAGCAGGAUUUCCUGAAUUUU	501	1975-1995	AAAUAUUCAGGAUUUCCUGCUUCU	572	1973-1995

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)	Antisense Sequence 5' to 3'	SEQ ID NO:	Range (NM_001710.5)
AD-560165.1	UGGGAUUGAAUUAACACAGCU	502	2598-2618	AGCUGUUUAAUUCAAUCCACG	573	2596-2618
AD-559300.1	ACCCAAUACUGUCAUUGAUU	503	1468-1488	AAUCAUUGACAGUAAUUGGUCC	574	1466-1488
AD-559866.1	CCCUGCACAGGAUAUCAAAGU	504	2147-2167	ACUUUGAUUCCUGCAGGGAG	575	2145-2167
AD-559302.1	CCAAUUCUGUCAUUGAUGAU	505	1470-1490	AUCAUAAUGACAGUAAUUGGU	576	1468-1490
AD-560163.1	CGUGGAUUGAAUUAACAACAU	506	2596-2616	AUGUUUAAUUCAAUCCACGCC	577	2594-2616
AD-559718.1	AGGAUUCUGAAUUAUUGU	507	1979-1999	ACAUAAAUAUUCAGGAUUCUCC	578	1977-1999
AD-559721.1	AAUUCUGAAUUAUUGACUU	508	1982-2002	AAGUCAUAAAUAUUCAGGAUCC	579	1980-2002
AD-559026.1	GUCUAGUCAACUUAUUGAGU	509	1153-1173	ACUCAUUAAGUUGACUAGACAC	580	1151-1173
AD-559719.1	GGAAUUCUGAAUUAUUGAU	510	1980-2000	AUCAUAAAUAUUCAGGAUUCUCC	581	1978-2000
AD-559060.1	UGGUGUAGAGCCAAAGAUUGU	511	1187-1207	ACAUUUCUUGGCUUUCACCAUA	582	1185-1207
AD-559864.1	CUCCUGCACAGGAUAUCAAU	512	2145-2165	AUUGAUUCCUGUGCAGGGAGCA	583	2143-2165
AD-559059.1	AUGGUGAGAGCCAAAGAUUAU	513	1186-1206	AAUAUUCUUGGCUUUCACCAUA	584	1184-1206
AD-559865.1	UCCUGCACAGGAUAUCAAU	514	2146-2166	AUUGAUUCCUGUGCAGGGAGC	585	2144-2166
AD-559148.1	AAUGAAUCAAUUAUGAAGAU	515	1296-1316	AUUUCAUAAUUGAUUUCAUUGA	586	1294-1316
AD-559375.1	AUCUGGAUGUCUUAUGUGUUU	516	1543-1563	AAACACAUAGACAUCCAGAUAA	587	1541-1563
AD-559393.1	UGAACCAAGUGAACAUCAAUU	517	1579-1599	AAUUGAUUUCACUUGGUUACCC	588	1577-1599
AD-559717.1	CAGGAUUCUGAAUUAUUAU	518	1978-1998	AAUAAAUAUUCAGGAUUCUCCU	589	1976-1998
AD-559392.1	GUGAACCAAGUGAACAUCAAU	519	1578-1598	AUUGAUUUCACUUGGUUACCCA	590	1576-1598

Table 5. Modified Sense and Antisense Strand Sequences of Complement Factor B dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-558860.1	usgsccaaGfaCfuCfucuaugauL96	591	asUfsacaUfgAfAfaggagUfcUfuggcasgsg	662	CCUGCCAAAGACUCCUUAUUAUAC	733
AD-560018.1	asasgagaAfgUfCfGfuuucauL96	592	asUfsagaUfgAfAfagaCfuUfucuuusgu	663	ACAAGAGAAGUCGUUUAUUAUCAA	734
AD-560019.1	asgsagaaGfuCfGfUfuuucauL96	593	asUfsugaAfuGfAfaagAfeUfucuuusg	664	CAAGAGAAGUCGUUUAUUAUCAA	735
AD-559160.1	usasugaaGfaCfCfAfcagugauL96	594	asUfsucaAfcUfUfuggUfcUfucuuasau	665	AUUAUGAAGACCACAAGUUGAAG	736
AD-559921.1	csugsagGfaGfGfUfcuacauL96	595	asUfsugaUfgUfAfgaccUfcCfuuccgsag	666	CUCGGAAGGAGGUCUACAUAUCAA	737
AD-559755.1	asuscaagCfuCfAfAfgaauaageuL96	596	asAfsgeuUfaUfUfcuugAfgCfuugausca	667	UGAUCAAGCUCAAAGAAUUAAGCUG	738
AD-560017.1	csasagagAfaGfUfCfuuucauL96	597	asGfsauGfaAfAfcgacUfuCfuucuuusg	668	CACAAGAGAAGUCGUUUAUUAUCAA	739
AD-559614.1	csusgugUfgUfCfUfaguuuuuL96	598	asAfsaagUfaCfuUfcaGfaCfcacagscsc	669	GGCUGUGGUGUCUGAGUACUUAUUG	740

Duplex Name	Sense Sequence 5' to 3'	SEQ ID	Antisense Sequence 5' to 3'	SEQ ID	mRNA Target Sequence	SEQ ID
AD-559435.1	asusggagcAfaCfAfUfgunucaaauL96	NO: 599	asUfsuuGfAfaCfAfcaugUfuGfcucausug	NO: 670	CAAUGAGCAACAUGUGUUCAAAG	741
AD-560016.1	ascsaagaGfaAfAfGUfgeuucauuL96	600	asAfsaugAFaAfAfCfagacuUfgUfciuugsasa	671	UCACAAGAGAAAGCGUUUCAUUC	742
AD-559451.1	csasaaguCfaAfGfGFfauauggaauL96	601	asUfsuucCfaAfAfAfcuecuUfgAfcuunggsasa	672	UUCAAAGUCAAGGAUAUGGAAAA	743
AD-559617.1	usgsguguCfuGfAfGfAfcuuncuugul96	602	asCfisacaAfAfgUfAfcueAfAfcaccacsasa	673	UGUGGUCUGAGUACUUGGUGGC	744
AD-560021.1	asgsaaguCfGfUfUfUfcauacaguuL96	603	asAfscuucGfaAfUfGfaaaCfGfAfcuuccesu	674	AGAGAAGUCGUUUCAUUCAAGUU	745
AD-559449.1	ususcaaaGfuCfAfAfGfagauuggauL96	604	asUfsccaUfaUfCfueugAfCufuugaasesa	675	UGUUCAAAGUCAAGGAUAUGGAA	746
AD-559450.1	uscсаааgUfcAfAfGfGfauuggauL96	605	asUfsuuccAfuaAfUfCfueuGfaCfuungasasc	676	GUUCAAAGUCAAGGAUAUGGAAA	747
AD-559925.1	asgsaggUfcUfAfCfaucagaauuL96	606	asAfsuucUfuGfAfAfnugaGfaCfceuucisc	677	GAAGGAGGUCUACAUCAAGAAUG	748
AD-559440.1	csasacauGfuUfUfUfcaagucaaul96	607	asUfslugaCfuUfUfgaacAfAfcuungescu	678	AGCAACAUGUGUUCAAAGUCAAG	749
AD-559788.1	ascsuuacAfGfGfCfCfcaunugucul96	608	asAfsagacAfafAfUfgggcChuGfauguscusu	679	AGACUAUCAGGCCCAUUUGUCUC	750
AD-559437.1	gsasgcaaCfaUfGfUfgucacaaguuL96	609	asAfsucuUfcAfAfAcacaUfgUfgucucasasu	680	AUGAGCAACAUGUGUUCAAAGUC	751
AD-559369.1	asgsgauAfCuFtUFGfagucucuuL96	610	asAfsuagAfcAfUffccagAfUfAfaucuscsc	681	GGAGGAUUACUGGUAUGUCUAUG	752
AD-559446.1	gsusguucAfaAfGUfcaagzaauuL96	611	asAfsuauCfcUfUfgacuUfuGfiaccaasasu	682	GUGUGUUCAAAGUCAAGGAUAUG	753
AD-559924.1	asasggagGfuCfUFAfcaucagaauL96	612	asUfsucuUfgAfUfGaugAfCfcfuuccuscsc	683	GGAAGGAGGUCUACAUCAAGAAU	754
AD-558965.1	uscсaggUfcCfAfUfGaacaucuuL96	613	asUfsagaUfgUfUfGaugGfaGfcuugasasg	684	CUUCAGGCUCUCAUGAACAUCAUC	755
AD-560045.1	gsusggauGfuCfUFGfcaaaaecaul96	614	asUfsgguUfuUfUfgeagAfAfcuaccasusa	685	UAGUGGAUGUCUGCAAAAACCAG	756
AD-559946.1	gsusgagaGfaGfAfUfGfcauaauuL96	615	asAfsuauUfgAfGfcaucUfcUfcaucsasg	686	CUGUGAGAGAGAGUGCUCAAUAUG	757
AD-558697.1	asuseccaCfcUfGfCfcaugzauuL96	616	asAfsuucAfcUfUfggcaGfgUfGcgauusug	687	CAAUCCGACCUGCCAAGUGAAUG	758
AD-559008.1	uscсacagGfaGfCfCaanaaguguuL96	617	asAfscaeUfuUfUfuggcUfcCfugugasasg	688	CUUCACAGGAGGCCAAAAAGUGUC	759
AD-559357.1	asasaaccCfaAfGfGfaggaauuuuL96	618	asAfsuaaUfcCfUfcccUfGfGfguuuusug	689	CAAAAACCCAAAGGAGGAUUUAUC	760
AD-559020.1	asasaaguCfuCfUfAfGfcaacuuaul96	619	asUfsaagUfuGfAfcauUfcAfcuuiuusug	690	CAAAAAGUGUCUAGUCAACUUA	761
AD-559143.1	csasgcucAfaUfGfAfcauuaauuL96	620	asUfsauUfGfAfUfcauUfcGfiagucscsu	691	AGCAGCUCUAUGAAUCAAUUAU	762
AD-559374.1	usasugugGfaUfGUfcauuguuuL96	621	asAfsacaCfaAfAfagcaUfcCfagauasasu	692	AUUUAUCUGGAUGUCUAUGUGUUU	763
AD-560161.1	gsgscugGfGfAfUfUfGaauuaaaul96	622	asUfsuuUfaUfUfcaauCfcCfacgccscsc	693	GGGGCGUGGGAUUGAAUUAANAAC	764
AD-559947.1	usgsasagAfGfAfUfGfcauaauuL96	623	asCfisauaUfuGfAfGfcauCfuCfuciacesa	694	UGUGAGAGAGAGUGCUCAAUAUGC	765
AD-559616.1	gsusgugUfcUfGfAfGfcauucuuuL96	624	asAfsceaaAfGfUfAfcucaGfaCfacaccasag	695	CUGUGGUGUCUGAGUACUUIUGUG	766
AD-559142.1	gscsagcuCfaAfUfGfhaaucaauuL96	625	asAfsauuGfaUfUfucanUfgAfGcugcsesu	696	AAGCAGCUCUAUGAAAUCAAUUA	767
AD-558639.1	csgsguucCfcCfUfAfcauacaguuL96	626	asAfscauUfgUfAfGfuaGfGfAfgaccgsgsg	697	CCCCGUCUCCCUCACUACAAUGUG	768
AD-560166.1	gsrgsauuGfaAfUfUffaaacagcuuL96	627	asAfsgeuGfuUfUfuaauUfcAfaucecsasc	698	GUGGGAUUGAAUUAANAACAGCUG	769
AD-559359.1	asaseccaAfGfGfAfGfcauucuuL96	628	asAfsgauAfafUfCfenceCfuUfggguususu	699	AAACCCAAAGGAGGAUUAUCUCU	770
AD-558657.1	gsusgaguGfaUfGfAfGfcauucuuL96	629	asAfsaagAfGfAfUfcauUfcAfcuaccasasu	700	AUGUGAGUGAUGAGUCUCUUCUC	771
AD-559442.1	ascsaguGfuUfCfAfAfaugacaguuL96	630	asCfscuucGfaCfUfngaAfAfcAugususu	701	CAACAUGUGUUCAAAAGUCAAGGA	772
AD-559023.1	asgsugucUfaGfUfCfAfaucuaauuL96	631	asAfsauuAfAfgUfUfugacUfaGfaiaccsusu	702	AAAGUGUCUAGUCAACUUAUUG	773
AD-560160.1	gsrgsgcuGfGfGfAfUfugaauuaauL96	632	asUfsuuaAfufUfCfaucCfcAfcgcccsesu	703	AGGGCGUGGGAUUGAAUUAANA	774

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-559398.1	csasagugAfaCfaUfcauugcuuuuL96	633	asAfsaagCfaUfUfgaugUfuCfcauugsgsu	704	ACCAAGUGAACAACAUAUGCUUUG	775
AD-559722.1	asusuccuGfaAfUfUfuaugacuuL96	634	asUfsagUcfaUfAfaauUfcafgaauusuc	705	GAAUCCUGAAAAUUUAUGACUAU	776
AD-559146.1	csuscauGfaAfAfUfcauuuagauL96	635	asUfscuAfaUfUfgauUfcafuugagcsu	706	AGCUAAUGAAAAUCAUUUAUGAA	777
AD-559267.1	gsgsauUfcaGfGfGfaagugauL96	636	asAfsuaCfuUfUfcccGfcafaucacsasa	707	UUGGAUUGUUCCGGGAAAGAGUAUG	778
AD-559074.1	gssauaugGfuCfuUfAfgagacuuuL96	637	asAfsuauGfuCfaUfcaugAfcfaauacsu	708	AAGAUAUGGUUUAUGAGACUAUUG	779
AD-560162.1	gscsgugGfaUfUfGfauuuauuL96	638	asGfsuuUfcaUfUfcauUfcafcagcsuc	709	GGGCGUGGGAUUGAAUUAACAACA	780
AD-559021.1	asasagugUfcaUfGfcauacuuuL96	639	asUfsuaGfuUfGfcauGfcauucsu	710	AAAAAGUGUCUAGUCAACUUAU	781
AD-559144.1	asgscuAfaUfGfAfaucuuuuL96	640	asAfsuaUfuGfAfuuuUfUfgagcsuc	711	GCAGCUCAAUGAAAUCAUUAUG	782
AD-559147.1	csasaugaAfaUfCfAfauuuagauL96	641	asCfsuacAfaUfAfuugaUfuUfcauugsg	712	CUCAUUGAAAUCAUUAUGAAGA	783
AD-560164.1	gssuggaUfuGfAfauuuacagL96	642	asCfsuugUfuUfAfaucAfaUfcccagsg	713	GCGUGGGAUUGAAUUAACAAGC	784
AD-559714.1	asasgagGfaAfUfUfcauuuuuL96	643	asAfsauUfcaUfGfcauUfcafuucsu	714	AGAAAGCAGGAUUAUCCUGAAUUU	785
AD-560165.1	usgsggaUfGfAfaUfuaaacagL96	644	asGfscuUfuUfUfcauUfcafuuccsg	715	CGUGGGAUUGAAUUAACAACAGCU	786
AD-559300.1	ascscuUfuAfcUfGfcauugauL96	645	asAfsuaUfuGfAfcagUfUfgggucsc	716	GGACCCAAUUAUUGUUAUUGAUG	787
AD-559866.1	cscscuUfcaUfGfcauuaagL96	646	asCfsuauGfcaUfAfuucUfUfgaggsag	717	CUCCUGCACAGGAUUAUCAAAGC	788
AD-559302.1	cscsaauAfcUfGfUfcauugauL96	647	asUfscuUfcaUfUfgacaGfuAfauggsu	718	ACCCAAUUAUUGUUAUUGAUGAG	789
AD-560163.1	csgsugGfaUfUfGfAfauuuacauL96	648	asUfsguUfuAfaUfcaUfUfcccagsg	719	GCGUGGGAUUGAAUUAACAACAG	790
AD-559718.1	asgsgauUfcaUfGfcauuuuuL96	649	asCfsauAfaUfUfcaUfcafuucsg	720	GCAGGAUUAUUGUUAUUAUGA	791
AD-559721.1	asauuUfGfAfaUfuaugacuuL96	650	asAfsuacAfaUfAfaauUfcafuucsg	721	GGAAUUAUUGAUAUUAUGACUA	792
AD-559026.1	gssuUfcaUfAfaUfuaugacuuL96	651	asCfsuacAfaUfAfaauUfcafuucsg	722	GUGCUAGUCAAUUAUUAUGAGA	793
AD-559719.1	gsgsaauUfcaUfGfAfauuuagauL96	652	asUfscuAfaUfAfaauUfcafuucsg	723	CAGGAUUAUUGUUAUUAUGAC	794
AD-559060.1	usgsguUfcaUfGfcauuaugL96	653	asCfsauUfcaUfUfcauUfcafuucsg	724	UAUGGUGUAGGCAAGAAUUAUGG	795
AD-559864.1	csusccuUfcaUfGfcauuaauL96	654	asUfsguUfcaUfUfcauUfcafuucsg	725	UGUCCUGCACAGGAUUAUCAA	796
AD-559059.1	asusgugUfcaUfGfcauuaauL96	655	asAfsuauUfcaUfUfcauUfcafuucsg	726	UUAUGGUGUAGGCAAGAAUUAUG	797
AD-559865.1	uscscuUfcaUfGfcauuaauL96	656	asUfsguUfcaUfUfcauUfcafuucsg	727	GUUCCUGCACAGGAUUAUCAAAG	798
AD-559148.1	asasugaaUfcaUfAfaUfuaugauL96	657	asUfscuUfcaUfUfcauUfcafuucsg	728	UCAUUGAAUUAUUAUUAUGAAGAC	799
AD-559375.1	asuscuUfcaUfGfcauuaugauL96	658	asAfsuacAfaUfUfcauUfcafuucsg	729	UUAUUGGAGUUAUUAUGUUAUG	800
AD-559393.1	usgsaaccAfaUfGfcauuaauL96	659	asAfsuauUfcaUfUfcauUfcafuucsg	730	GGUGAACCAAGUGAACAUCAUUG	801
AD-559717.1	csasggaUfcaUfGfcauuaauL96	660	asAfsuauUfcaUfUfcauUfcafuucsg	731	AGCAGGAUUAUUAUUAUUAUG	802
AD-559392.1	gssugaaUfcaUfGfcauuaauL96	661	asUfsguUfcaUfUfcauUfcafuucsg	732	UGGUGAACCAAGUGAACAUCAU	803

Table 6. Unmodified Sense and Antisense Strand Sequences of Complement Factor B dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Sense Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Antisense Source Name
AD-560969.1	UGCCAAGAUUCCUUAUG UAU	804	NM_008198.2_1051-1071_s	AUACAUGAAGAAUUCUUGG CAGG	849	NM_008198.2_1049-1071_as
AD-561537.1	AUGUGUUUAAAAGUCAAGG AUU	805	NM_008198.2_1751-1771_A21U_s	AAUCCUTGACUUUAAACACA UGA	850	NM_008198.2_1749-1771_U1A_as
AD-562262.1	AAAGAUGAGGAUUUUGGGU UUU	806	NM_008198.2_2668-2688_s	AAAACCCCAAUCCUCAUCUU UGA	851	NM_008198.2_2666-2688_as
AD-561960.1	AAGGAUGUCAAAAGCUCUG UUU	807	NM_008198.2_2275-2295_s	AAACAGAGCUUUGACAUCU UUA	852	NM_008198.2_2273-2295_as
AD-561580.1	CUACCAAUGAUUGAUGA AAU	808	NM_008198.2_1794-1814_C21U_s	AUUUCATCAAUCAUUUUGGU AGAA	853	NM_008198.2_1792-1814_G1A_as
AD-561254.1	AUCAGUUUAUGAAGACCAC AAU	809	NM_008198.2_1423-1443_G21U_s	AUUGUGGUCUUAUAAACUG AUUU	854	NM_008198.2_1421-1443_C1A_as
AD-561578.1	UUCUACCAAUUGAUUUGAU GAU	810	NM_008198.2_1792-1812_A21U_s	AUCAUCAAUUUUGGUAG AAAA	855	NM_008198.2_1790-1812_U1A_as
AD-561538.1	UGUGUUUAAAAGUCAAGGA UAU	811	NM_008198.2_1752-1772_s	AUAUCCTUGACUUUAAACAC AUG	856	NM_008198.2_1750-1772_as
AD-561252.1	AAAUACAGUUUAUGAAGACC ACU	812	NM_008198.2_1421-1441_A21U_s	AGUGGUCUUAUAAACUGAU UUGG	857	NM_008198.2_1419-1441_U1A_as
AD-561963.1	GAUGUCAAAAGCUCUGUUU GUU	813	NM_008198.2_2278-2298_A21U_s	AACAAACAGAGCUUUGACA UCCU	858	NM_008198.2_2276-2298_U1A_as
AD-562027.1	CCAGUUGUGAGAGAGAUG CUU	814	NM_008198.2_2360-2380_A21U_s	AAGCAUCUCUCACACAACUG GCU	859	NM_008198.2_2358-2380_U1A_as
AD-561653.1	AGCCAAAGAUUCAGUCAC UCU	815	NM_008198.2_1887-1907_G21U_s	AGAGUGACUGAGAUUCUUG CUUG	860	NM_008198.2_1885-1907_C1A_as
AD-562137.1	CGCUUCAUUAAGUUGGU GUU	816	NM_008198.2_2527-2547_G21U_s	AACACCAACUUUGAUAUGAAG CGGC	861	NM_008198.2_2525-2547_C1A_as
AD-561536.1	CAUGUGUUUAAAAGUCAAG GAU	817	NM_008198.2_1750-1770_s	AUCCUUGACUUUAAACACA UGAU	862	NM_008198.2_1748-1770_as
AD-561253.1	AAUCAGUUUAUGAAGACCA CAU	818	NM_008198.2_1422-1442_A21U_s	AUGUGTGUUAUAAACUGA UUUG	863	NM_008198.2_1420-1442_U1A_as
AD-561959.1	GAAGGAUGUCAAAAGCUCU GUU	819	NM_008198.2_2274-2294_s	AACAGAGCUUUGACAUCU UCAC	864	NM_008198.2_2272-2294_as
AD-	AUGUUUUUACCAAUGA	820	NM_008198.2_1787-	AAAUCAUUGGUAGAGAAAAC	865	NM_008198.2_1785-

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Sense Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Antisense Source Name
561573.1	UUU		1807_G21U_s	AUUC		1807_C1A_as
AD-561573.1	CAAGCCAAAGAUUCACAGUC		NM_008198.2_1885-1905_s	AGUGACTGAGAUUUUGGCU		NM_008198.2_1883-1905_as
AD-561651.1	ACU	821	NM_008198.2_1279-1299_G21U_s	UGCC	866	NM_008198.2_1277-1299_C1A_as
AD-561148.1	ACCAACUUGAUUGAGAAG	822	NM_008198.2_2277-2297_s	GUGA	867	NM_008198.2_2275-2297_as
AD-561962.1	UGU	823	NM_001710.5_2549-2569_s	ACAAACAGAGCUUUUGACAU	868	NM_008198.2_2668-2690_as
AD-562237.1	AGAUAGGAAUUUGGGUUU	824	NM_008198.2_1888-1908_C21U_s	AGAAAACCCCAAUCCUCAUC	869	NM_008198.2_1886-1908_G1A_as
AD-561654.1	GCU	825	NM_008198.2_2526-2546_s	ACGAGUGACUGAGAUUUUG	870	NM_008198.2_2524-2546_as
AD-562136.1	CCGCUUCAUUAAGUUGG	826	NM_008198.2_2359-2379_s	ACACCAACUUGAAUGAAGC	871	NM_008198.2_2357-2379_as
AD-562026.1	GCU	827	NM_008198.2_1201-1221_G21U_s	AGCAUUCTCUCACAAACUGG	872	NM_008198.2_1199-1221_C1A_as
AD-561101.1	UUU	828	NM_008198.2_1753-1773_G21U_s	AACCAAGUAGAUUUUCAUG	873	NM_008198.2_1751-1773_C1A_as
AD-561539.1	AUU	829	NM_008198.2_2276-2296_G21U_s	AAUUAUCCUUGACUUUAAAC	874	NM_008198.2_2274-2296_C1A_as
AD-561961.1	UUU	830	NM_008198.2_1749-1769_A21U_s	CUUC	875	NM_008198.2_1747-1769_U1A_as
AD-561535.1	GGU	831	NM_008198.2_1886-1906_C21U_s	ACCUUGACUUUAAACACAU	876	NM_008198.2_1884-1906_G1A_as
AD-561652.1	CUU	832	NM_008198.2_2666-2686_s	UUGC	877	NM_008198.2_2664-2686_as
AD-562260.1	UUU	833	NM_001710.5_1096-1116_A21U_s	AGC	878	NM_001710.5_1094-1116_U1A_as
AD-557041.2	ACU	72	NM_008198.2_1889-1909_C21U_s	AGUCUGAUCCAUCUAGCACC	158	NM_008198.2_1887-1909_G1A_as
AD-561655.1	GCU	834	NM_001710.5_1098-1118_C21U_s	AGCGAGTGACUGAGAUUUUG	879	NM_001710.5_1096-1118_G1A_as
AD-557043.1	AGU	835	NM_008198.2_1815-1835_G21U_s	ACUGCTGAUCCAUCUAGCA	880	NM_008198.2_1813-1835_C1A_as
AD-561603.1	UUU	836		CCA	881	
				ACACAGAGACUCAGAGAUU		
				UGGU		

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Sense Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Antisense Source Name
AD-557044.1	UGCUGAUGGAGCAGACA GCU	837	NM_001710.5_1099-1119_A21U_s	AGCUGUCUGAUCCAUAGC ACC	882	NM_001710.5_1097-1119_U1A_as
AD-561266.1	GACCACAAAGCUGAAGUCA GGU	838	NM_008198.2_1435-1455_G21U_s	ACCUAGACUUCAGCUUGUGG UCUU	883	NM_008198.2_1433-1455_C1A_as
AD-561541.1	GUUUAAGUCAAGGAUUAU GGU	839	NM_008198.2_1755-1775_A21U_s	ACCAUATCCUUGAGCUUUAAA CAC	884	NM_008198.2_1753-1775_U1A_as
AD-562257.1	AGCUCAAAAGAGAGGAUUAU GGU	840	NM_008198.2_2663-2683_G21U_s	ACAAUCCUACUUCUUGAGC UUG	885	NM_008198.2_2661-2683_C1A_as
AD-560538.1	GAGGGAGUAGAGAUCAAAA GGU	841	NM_008198.2_517-537_C21U_s	ACCUUUGAUCUCUACUCCCU CCA	886	NM_008198.2_515-537_G1A_as
AD-561579.1	UCUACCAAAUGAUUGAUG AAU	842	NM_008198.2_1793-1813_A21U_s	AUUCAUCAAUCAUUUGGUA GAAA	887	NM_008198.2_1791-1813_U1A_as
AD-562259.1	CUCAAAAGAGAGGAUUUG GGU	843	NM_008198.2_2665-2685_s	ACCCAAAUCCUCAUCUUUGA GCU	888	NM_008198.2_2663-2685_as
AD-560968.1	CUGCCAAAGAUUCCUUCAU GUU	844	NM_008198.2_1050-1070_A21U_s	AACAUGAAGGAUUCUUGGC AGGA	889	NM_008198.2_1048-1070_U1A_as
AD-561647.1	AUGGCAAGCCAAGAUUCUC AGU	845	NM_008198.2_1881-1901_s	ACUGAGAUCUUGGCUUGGCC AUGG	890	NM_008198.2_1879-1901_as
AD-561469.1	CCUGGAGUGUGUAUGUGUU UGU	846	NM_008198.2_1665-1685_G21U_s	ACAAACACAUACACAUCCAG GUA	891	NM_008198.2_1663-1685_C1A_as
AD-561540.1	UGUUUAAAAGUCAAGGAUA UGU	847	NM_008198.2_1754-1774_G21U_s	ACAUUCCUUGACUUUAAA CACA	892	NM_008198.2_1752-1774_C1A_as
AD-562135.1	GCCGCUUCAUUCAAAGUUG GUU	848	NM_008198.2_2525-2545_G21U_s	AACCAACUUGAAUGAAGCG GCUU	893	NM_008198.2_2523-2545_C1A_as

Table 7. Modified Sense and Antisense Strand Sequences of Complement Factor B dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-560969.1	usgscacGfaUfUfCfucuaugauL96	894	asUfsacau(Ggn)aaggaaUfcUfuggcasg	939	CCUGCCAAAGAUCCUUCAUUAU	984
AD-561537.1	asusguguUfuAfaAfAfgueaagauL96	895	asAfsuccu(Tgn)gacuuAfaAfeacausga	940	UCAUGUGUUUAAAGUCAAGGAUA	985
AD-562262.1	asasagauGfaGfGfAfuuggguuuL96	896	asAfsaace(Cgn)aaauccUfcAfuuuusga	941	UCAAAGAUAGAGGAUUUGGUGUUU	986
AD-561960.1	asagggauGfuCfaAfAfgucucuuuL96	897	asAfsacag(Agn)gcuuugAfcAfuuuuscsa	942	UGAAGGAUGUCAAGCUCUGUUU	987
AD-561580.1	csusaccaAfaUfGfAfuugaugaauL96	898	asUfsuucca(Tgn)cauuccUfuUfuguagsasa	943	UUCUACCAAAUGAUUGAUGAAAC	988

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-561254.1	asuscauguUfaUfGfAfagaccacaauL96	899	asUfsugug(Ggn)uucuaUfaUfcaugaususu	944	AAAUCAUUUAUGAAGACCACCAAG	989
AD-561578.1	ususcuacCfaAfAfUfGfauugauL96	900	asUfscauc(Agn)aucuuUfGfuaugaasasa	945	UUUUUACCAAAUGAUUGAUGAA	990
AD-561538.1	usgsuguuUfaAfAfGfuaaggaauL96	901	asUfsauce(Tgn)uaguuUfaAfacaasug	946	CAUGUGUUUAAAAGUCAAAGGAUUAU	991
AD-561252.1	asasaucGfuUfAfUfGfuaagaccaculL96	902	asGfsugug(Cgn)uucuaAfeUfcauuusgsg	947	CCAAUACAGUUUAUGAAGACCACA	992
AD-561963.1	gsasugucAfaAfGfCfucuguuuuL96	903	asAfscaaa(Tgn)agagcuUfuGfacaucsu	948	AGGAUUCAAAAGCUCUGUUUGUA	993
AD-562027.1	cscsaguuGfuGfAfGfagagauuuL96	904	asAfsagcu(Cgn)ucucAfeAfacugscsu	949	AGCCAGUUGAGAGAGAUUGCUA	994
AD-561653.1	asgsccaaGfaUfCfUfagucaculL96	905	asGfsagug(Agn)cuagagUfcUfugcuscusg	950	CAAGCCAAAGAUUCAGUCACUCG	995
AD-562137.1	csfgscuucAfuUfCfAfaguuuguuL96	906	asAfscaae(Agn)aucuaAfuGfaagcgsgsc	951	GCCGUUCAUUAAGUUUGGUGUG	996
AD-561536.1	csasugugUfuUfAfAfagucaggaauL96	907	asUfscuu(Tgn)aucuuAfaCfaaugsasu	952	AUCAUGUGUUUAAAGUCAAGGAU	997
AD-561253.1	asasucagUfuUfUfGfagaccacaauL96	908	asUfsgug(Tgn)uucuaAfaCfugauiusug	953	CAAAUCAGUUUAUGAAGACCACAA	998
AD-561959.1	gsasaggaUfgUfCfAfagcucuguuL96	909	asAfscaag(Ggn)cuuugaCfaUfcaucasc	954	GUGAAGGAGUGUCAAGCUCUCUGUU	999
AD-561573.1	asusguuuUfeUfAfCfcaaaugauuuL96	910	asAfsauce(Tgn)uuuguaGfaAfaacausc	955	GAAUGUUUUUACCAAAUGAUUG	1000
AD-561651.1	csasagccAfaGfAfUfcaagacaculL96	911	asGfsugac(Tgn)gagauUfuGfgeuugscsc	956	GGCAAGCCAAGAUCUCAGUCACU	1001
AD-561148.1	ascscacUfuGfAfUfagagaguuL96	912	asAfsccuu(Cgn)ucauacAfaGfuugugsa	957	UCACCAACUUUAUGAAGAGAGGUG	1002
AD-561962.1	gsasaguuCfaAfAfGfucuguuuuL96	913	asCfsaaac(Agn)gagcuUfgAfcaucscsu	958	AAGGAGUUCAAAAGCUCUGUUUGU	1003
AD-562237.1	asgsaagaGfgAfUfUfuggguuuuL96	914	asGfsaaa(Cgn)ccaaUfcUfcaucsu	959	AAGAUGAGGAUUUGGGUUUUUCU	1004
AD-561654.1	gsccaaGfuUfUfCfagucaculL96	915	asCfsagug(Ggn)acugagAfuCfuugscsu	960	AAGCCAAGAUUCAGUCACUCGCG	1005
AD-562136.1	csccscuuCfaUfUfCfaguuuguuL96	916	asCfsacca(Agn)cuuugaUfgAfagcgscsu	961	AGCCGUUCAUUCAAGUUUGGUGU	1006
AD-562026.1	gsccscaguUfgUfGfAfagagaguuL96	917	asGfscauc(Tgn)cuucaCfaAfcaugcscsu	962	AAGCCAGUUGAGAGAGAGAGUGU	1007
AD-561101.1	uscscagAfaUfAfUfcaucuguuL96	918	asAfsccag(Ggn)uagauUfuCfauugagsgc	963	GCUCCAUGAAUAUCUACCUUGUG	1008
AD-561539.1	gsusguuuAfaAfGfUfcaaggaauuuL96	919	asAfsuauc(Cgn)uuagauUfuAfaacacsa	964	AUGUGUUUAAAAGUCAAGGAUAUG	1009
AD-561961.1	asgsaugUfeAfAfAfagcuguuuuL96	920	asAfsaaca(Ggn)agcuuGfaCfaucscsu	965	GAAGGAUGUCAAAAGCUCUGUUUG	1010
AD-561535.1	uscscaguGfuUfUfAfagucaguuL96	921	asCfsccug(Agn)cuuuaAfeAfcaugscsu	966	CAUCAUGUGUUUAAAGUCAAGGA	1011
AD-561652.1	asasgccaAfgAfUfCfagucacuuL96	922	asAfsugag(Cgn)uagagUfuUfgcucusc	967	GCAAGCCAAGAUUCAGUCACUC	1012
AD-562260.1	uscscaaagAfuGfAfGfaguuuguuL96	923	asAfsccca(Agn)aucucAfuCfuugagsgc	968	GCUCAAAAGAGAGGAUUUGGGUUU	1013
AD-557041.2	usgsugucUfaGfAfUfGfagacaguuL96	244	asGfsucug(Agn)uucuaUfaGfcaacsgsg	330	CCUGGUGCUAGAUGGAUCAGACA	416
AD-561655.1	csccsaagaUfeUfCfAfagucacuculL96	924	asGfsagag(Tgn)gacuaGfaUfcaugscsu	969	AGCCAAGAUUCAGUCACUCGCC	1014
AD-557043.1	gsusgcuAfaUfGfAfagacaguuL96	925	asCfsuguc(Tgn)gacuaUfeUfagcscsa	970	UGGUGCUAGAUGGAUCAGACAGC	1015
AD-561603.1	csasaucUfeUfGfAfagucucuguuL96	926	asCfsacag(Agn)gacuaGfaGfauuugsgu	971	ACCAAUUCUCUGAGUCUCUGUGG	1016
AD-557044.1	usgsuagAfuGfGfAfagacaguuL96	927	asGfsccug(Cgn)uagauAfuCfuagcscsc	972	GGUGCUAGAUGGAUCAGACAGCA	1017
AD-561266.1	gsasccacAfaGfCfUfagagacuuL96	928	asCfsccua(Tgn)uucuaAfuGfugscsu	973	AAGACCACAAGGUAAGACAGGG	1018
AD-561541.1	gsusuuAfaGfUfCfAfagaguuuuL96	929	asCfscaag(Cgn)cuugaCfuUfuaacsc	974	GUGUUUAAAAGUCAAGGAUAUGGA	1019
AD-562257.1	asgsucaAfaGfAfUfagaguuuuL96	930	asCfsaauc(Cgn)cucaUfuUfagcuscsg	975	CAAGCUCAAAAGAGAGGAUUUGG	1020
AD-560538.1	gsasgggaGfuAfGfAfagacaaaguuL96	931	asCfsccuu(Tgn)aucucAfeUfcccscsa	976	UGGAGGGAGUAGAGAUCAAAGGC	1021

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-561579.1	uscnuaccAfaAfUfGfaungangaauL96	932	asUfsucau(Cgn)aaucuuUfuGfngagasa	977	UUUCUACCAAAUGAUUGAUGAAA	1022
AD-562259.1	csuscaaaGfaUfGfAfGgaunuggguL96	933	asCfsccaa(Agn)uccucaUfcUfhuagsgcsu	978	AGCUCAAAAGAUGAGGAUUUUGGGU	1023
AD-560968.1	csusgccaAfgAfUfUfcccuauguuL96	934	asAfseaug(Agn)aggauuCfuUfpgcagsgsa	979	UCCUGCCAAGAUAUCCUUCUAUGUA	1024
AD-561647.1	asusggcaAfgCfCfAfagaueucaguL96	935	asCfsugag(Agn)ueuugCfuUfgecausgsg	980	CCAUGGCAAGCCAAGAUUCUCAGU	1025
AD-561469.1	cscsuggaUfgUfGfUfaunguunugulL96	936	asCfsaaac(Agn)cauacaCfaUfccaggsusa	981	UACCUGGAUGUGUAUGUGUUUUGG	1026
AD-561540.1	usgsuuuaAfaGfUfCfaaggauaugulL96	937	asCfsauau(Cgn)cuugacUfuUfaaacasca	982	UGUGUUUAAAGUCAAGGAUAUGG	1027
AD-562135.1	gscscgeuUfcAfUfUfcaagunugguuL96	938	asAfsecaa(Cgn)uuugaauGfaAfgcgcsusu	983	AAGCCGCUUCAUUAAGUUGGUG	1028

Table 8. Complement Factor B *In Vitro* Single Dose Screens in Hep3B cells

DuplexID	10 nM % of Message Remaining	1nM % of Message Remaining	0.1 nM % of Message Remaining
AD-557072.1	4.99	8.87	40.99
AD-558097.1	5.00	11.75	45.33
AD-557068.1	9.00	22.78	53.22
AD-557774.1	6.55	17.23	52.48
AD-557070.1	7.33	21.06	54.78
AD-558225.1	19.10	29.43	106.87
AD-558065.1	12.21	24.28	61.24
AD-557853.1	9.39	35.28	55.01
AD-556919.1	20.01	27.64	138.66
AD-557859.1	13.46	25.92	104.56
AD-557069.1	11.26	29.35	54.36
AD-558068.1	14.59	21.63	48.67
AD-557422.1	14.87	27.15	55.45
AD-558096.1	10.81	26.65	76.39
AD-557084.1	28.07	54.88	138.96
AD-558076.1	13.06	24.15	42.77
AD-558063.1	20.24	28.87	54.49
AD-558069.1	17.70	27.78	53.41
AD-558061.1	19.59	36.04	48.88
AD-558066.1	23.98	44.12	51.72
AD-556581.1	22.46	67.53	186.97
AD-557079.1	9.97	41.07	71.40
AD-558012.1	17.26	35.39	112.72
AD-556701.1	9.91	23.66	62.85
AD-557782.1	13.23	45.66	53.87
AD-557498.1	28.16	56.57	131.91
AD-556788.1	33.88	58.98	118.02
AD-557078.1	18.18	50.25	80.91
AD-557852.1	40.94	60.36	167.53
AD-557353.1	23.37	44.41	62.52
AD-557041.1	26.00	87.78	110.85
AD-556786.1	51.74	66.59	90.96
AD-556734.1	29.10	54.88	70.13
AD-557475.1	26.95	50.54	65.87
AD-557972.1	63.07	77.45	71.99
AD-556390.1	31.40	46.72	72.19
AD-556963.1	42.99	68.75	146.93
AD-558078.1	30.67	83.78	62.14
AD-557204.1	40.18	69.41	199.62
AD-556962.1	67.05	92.06	80.95

DuplexID	10 nM % of Message Remaining	1nM % of Message Remaining	0.1 nM % of Message Remaining
AD-556733.1	34.07	61.86	62.03
AD-556724.1	43.85	114.37	225.16
AD-557067.1	42.27	59.20	67.56
AD-557602.1	39.99	70.66	155.25
AD-557345.1	45.66	91.09	186.86
AD-557969.1	70.94	72.23	72.25
AD-557867.1	43.92	108.43	178.41
AD-557860.1	49.94	63.71	134.13
AD-557868.1	52.22	99.81	112.51
AD-558064.1	61.63	72.39	59.83
AD-556725.1	45.46	62.37	68.67
AD-556787.1	58.40	102.98	163.60
AD-558062.1	55.53	70.65	156.02
AD-556874.1	83.34	90.67	62.04
AD-557066.1	75.61	93.55	168.75
AD-556791.1	64.56	74.17	75.36
AD-557450.1	56.23	68.84	77.40
AD-557865.1	66.83	94.99	153.50
AD-556961.1	69.87	93.70	206.00
AD-556920.1	52.86	81.48	188.46
AD-558074.1	74.73	132.35	71.18
AD-556738.1	72.67	123.81	206.91
AD-558004.1	100.55	103.41	190.48
AD-556918.1	85.53	97.13	199.73
AD-557209.1	84.13	96.02	140.72
AD-557861.1	66.07	84.41	120.27
AD-557226.1	83.39	94.85	123.74
AD-557856.1	84.68	85.69	137.01
AD-557839.1	114.79	91.84	69.49
AD-557873.1	84.73	102.93	65.71
AD-557417.1	88.08	115.91	225.81
AD-558106.1	95.90	110.10	217.75
AD-557788.1	87.87	109.59	129.42
AD-556917.1	79.04	71.81	78.26
AD-556739.1	87.37	115.81	70.32
AD-556726.1	96.01	129.96	221.48
AD-556790.1	109.55	118.05	193.15
AD-557604.1	74.95	133.00	177.11
AD-557495.1	96.32	130.00	254.48
AD-557016.1	106.90	131.43	110.73
AD-558105.1	77.28	73.82	85.07
AD-557872.1	102.16	123.17	172.64

DuplexID	10 nM % of Message Remaining	1nM % of Message Remaining	0.1 nM % of Message Remaining
AD-557346.1	113.18	138.10	228.78
AD-557851.1	107.51	138.22	178.85
AD-557786.1	117.86	149.34	168.00
AD-557857.1	118.52	127.67	145.90

Table 9. Complement Factor B *In Vitro* Single Dose Screens in Hep3B cells

DuplexID	10 nM Dose % of Message Remaining	STDEV	1 nM Dose % of Message Remaining	STDEV	0.1 nM Dose % of Message Remaining	STDEV
AD-558657.1	4.81	0.55	6.98	1.27	24.75	5.46
AD-559020.1	5.18	0.75	7.21	1.31	17.95	3.88
AD-559023.1	7.71	0.93	9.85	2.87	32.65	5.12
AD-558860.1	8.35	1.69	14.34	1.97	33.70	7.41
AD-559143.1	8.57	1.09	11.19	1.02	28.52	7.22
AD-559021.1	8.65	3.21	13.97	4.56	42.99	5.93
AD-559144.1	9.25	0.94	16.75	3.32	33.52	2.97
AD-559435.1	10.47	0.85	13.57	1.00	38.94	5.33
AD-560019.1	10.57	0.67	12.96	1.19	17.67	1.23
AD-559722.1	11.24	1.35	19.04	1.63	42.16	2.85
AD-560016.1	11.42	1.30	17.54	2.39	28.72	3.63
AD-559008.1	11.51	3.34	17.60	0.78	45.58	15.60
AD-560018.1	11.76	1.89	16.87	2.51	28.83	4.60
AD-560045.1	12.13	1.71	28.22	11.60	53.32	14.16
AD-559375.1	12.45	1.28	21.86	1.78	61.75	7.34
AD-559142.1	13.01	2.52	20.98	3.88	43.82	6.89
AD-559160.1	13.06	3.15	19.02	1.35	47.19	7.99
AD-559374.1	13.17	3.28	27.58	5.37	72.78	11.80
AD-559369.1	13.75	0.79	25.72	0.58	62.74	6.62
AD-559148.1	13.81	1.72	25.39	1.60	53.03	5.36
AD-560163.1	14.11	0.87	20.31	1.31	49.05	4.99
AD-559398.1	14.23	3.97	18.11	2.34	46.53	6.07
AD-559060.1	14.39	3.41	32.15	3.07	62.25	5.65
AD-559392.1	14.69	3.59	21.68	2.40	55.88	8.59
AD-559451.1	14.84	3.18	25.50	3.19	43.71	5.05
AD-559721.1	15.23	3.73	23.03	3.34	51.33	10.51
AD-559146.1	15.26	5.05	28.14	1.99	71.88	8.41
AD-560165.1	15.74	2.70	25.08	2.14	65.45	14.31
AD-559921.1	15.99	1.86	19.91	4.59	38.78	9.90
AD-560021.1	16.02	3.62	37.73	4.08	58.59	3.73
AD-560164.1	16.23	2.00	22.73	3.67	63.13	4.12
AD-559614.1	16.27	4.70	31.75	4.73	49.34	6.16

DuplexID	10 nM Dose % of Message Remaining	STDEV	1 nM Dose % of Message Remaining	STDEV	0.1 nM Dose % of Message Remaining	STDEV
AD-559393.1	16.46	3.99	31.63	4.04	61.56	10.13
AD-559717.1	16.68	1.69	20.78	3.33	42.46	10.02
AD-560017.1	16.71	2.32	28.04	3.35	57.72	16.22
AD-559440.1	16.90	1.64	27.94	4.84	50.75	9.54
AD-560166.1	17.02	4.84	20.01	3.95	58.88	13.01
AD-559059.1	17.61	5.82	33.74	3.87	59.15	7.45
AD-559300.1	17.90	5.18	34.67	2.86	67.79	6.31
AD-559946.1	18.11	1.61	36.74	5.04	62.70	7.20
AD-559147.1	18.33	2.11	47.39	7.94	85.39	10.23
AD-559755.1	19.99	5.12	26.88	2.30	52.33	10.49
AD-559446.1	20.58	2.25	30.84	7.47	64.13	7.62
AD-559026.1	21.11	2.49	41.40	14.50	65.91	11.08
AD-559714.1	22.71	0.91	32.56	2.47	69.69	6.49
AD-560160.1	22.85	5.07	28.30	2.24	65.27	9.41
AD-559437.1	25.76	4.50	44.87	12.58	78.13	8.91
AD-560162.1	25.95	4.26	43.29	5.64	71.14	20.99
AD-560161.1	26.34	3.19	38.91	4.83	68.19	5.53
AD-559924.1	26.34	8.91	36.46	3.90	57.07	6.32
AD-559865.1	27.49	2.47	47.05	6.32	75.04	14.94
AD-559449.1	28.57	3.20	54.33	4.83	70.39	14.64
AD-559788.1	28.70	6.51	62.57	7.45	79.25	8.07
AD-559617.1	29.43	9.48	67.18	6.78	88.54	10.81
AD-559719.1	30.38	3.11	55.82	5.69	83.18	12.99
AD-559718.1	32.51	3.42	57.77	8.99	82.88	18.79
AD-558697.1	32.69	5.66	44.32	3.45	58.56	7.73
AD-559357.1	33.33	3.84	33.06	4.91	59.41	16.08
AD-558965.1	35.13	2.66	34.85	11.29	51.66	15.94
AD-558267.1	37.43	7.93	35.43	6.56	40.76	7.84
AD-558639.1	37.99	5.49	46.82	7.78	44.37	7.45
AD-559925.1	39.15	17.96	74.82	7.86	75.18	4.17
AD-559450.1	40.47	6.34	63.09	4.35	53.12	12.34
AD-559302.1	41.61	5.63	59.86	3.32	79.54	9.58
AD-559074.1	41.79	3.21	57.94	3.73	62.36	5.75
AD-559947.1	43.30	2.71	66.78	11.57	73.55	7.33
AD-559442.1	47.58	8.47	69.78	10.91	92.36	7.58
AD-559359.1	48.10	2.37	86.83	11.09	102.01	6.31
AD-559616.1	50.68	6.63	93.13	11.78	88.54	12.26
AD-559864.1	63.01	11.07	91.70	12.15	89.75	13.66
AD-559866.1	85.15	11.86	94.33	6.79	79.14	12.77

Table 10. Complement Factor B *In Vitro* Single Dose Screens in Primary Mouse Hepatocytes (PMH)

DuplexID	10 nM Dose % of Message Remaining	STDE V	1 nM Dose % of Message Remaining	STDE V	0.1 nM Dose % of Message Remaining	STDE V
AD-558860.1	10.33	2.38	40.10	5.03	94.64	11.07
AD-560018.1	13.28	3.25	64.35	11.23	114.97	8.99
AD-560019.1	18.44	3.65	75.46	18.65	118.42	14.85
AD-559160.1	19.42	3.01	47.55	4.18	125.03	7.48
AD-559921.1	20.12	3.89	67.51	6.36	123.87	9.76
AD-559755.1	20.65	5.45	77.24	11.02	117.00	9.54
AD-560017.1	22.11	5.43	82.72	11.41	123.23	4.98
AD-559614.1	23.18	7.36	67.15	7.61	123.18	19.54
AD-559435.1	25.13	3.68	66.19	8.46	118.78	11.85
AD-560016.1	25.40	5.70	91.81	22.08	128.76	16.87
AD-559451.1	27.47	9.44	71.89	12.25	137.27	20.14
AD-559617.1	29.70	10.00	84.66	4.39	140.37	6.69
AD-560021.1	32.74	4.65	91.51	4.21	126.40	12.31
AD-559449.1	37.90	5.63	82.95	9.33	130.52	19.04
AD-559450.1	38.55	8.96	87.87	22.39	129.90	7.64
AD-559925.1	38.62	3.39	90.35	10.90	104.75	5.55
AD-559440.1	39.15	5.87	82.63	10.21	124.84	7.74
AD-559788.1	42.67	7.18	114.25	12.73	138.37	4.54
AD-559437.1	45.26	10.40	94.10	7.91	130.12	11.84
AD-559369.1	47.64	10.56	83.98	9.74	136.96	5.52
AD-559446.1	49.32	10.27	104.76	13.05	141.33	23.45
AD-559924.1	56.47	10.68	101.50	14.50	131.17	5.61
AD-558965.1	58.30	14.89	94.61	10.27	109.17	5.25
AD-560045.1	68.96	6.99	94.93	5.17	98.24	5.15
AD-559946.1	74.67	7.51	116.30	7.12	108.08	6.00
AD-558697.1	79.21	8.58	103.00	11.53	141.24	23.63
AD-559008.1	79.41	8.78	103.11	8.12	103.48	5.21
AD-559357.1	81.03	4.45	95.92	5.13	110.83	8.24
AD-559020.1	82.26	11.63	98.50	12.69	102.20	4.21
AD-559143.1	82.37	15.28	97.44	5.47	104.94	5.93
AD-559374.1	85.24	7.46	89.59	3.03	130.94	10.46
AD-560161.1	85.46	17.44	91.49	7.11	106.52	4.01
AD-559947.1	86.06	13.19	115.94	7.06	118.65	5.89
AD-559616.1	86.75	7.61	115.52	9.47	134.57	3.86
AD-559142.1	87.22	10.07	88.47	5.77	96.92	5.92
AD-558639.1	87.64	13.26	99.95	4.27	122.33	12.04
AD-560166.1	88.71	22.04	104.01	13.19	97.42	5.90
AD-559359.1	89.05	14.80	104.69	7.55	123.70	6.43
AD-558657.1	89.59	11.42	95.66	6.61	109.56	9.31

DuplexID	10 nM Dose % of Message Remaining	STDE V	1 nM Dose % of Message Remaining	STDE V	0.1 nM Dose % of Message Remaining	STDE V
AD-559442.1	90.05	26.12	107.09	17.30	152.65	17.71
AD-559023.1	93.44	12.18	96.28	8.79	116.18	4.76
AD-560160.1	93.98	12.77	106.07	14.37	96.30	1.99
AD-559398.1	94.15	14.19	89.68	5.33	141.12	7.36
AD-559722.1	94.20	7.15	114.34	13.25	128.86	17.84
AD-559146.1	94.84	7.07	94.68	6.79	150.36	18.96
AD-558267.1	96.73	10.58	101.69	8.01	107.42	7.77
AD-559074.1	99.75	10.40	91.77	6.74	132.38	20.72
AD-560162.1	100.49	17.85	98.34	9.70	99.71	7.13
AD-559021.1	102.22	20.82	100.57	9.81	143.53	24.85
AD-559144.1	105.28	4.70	98.16	9.44	149.08	20.99
AD-559147.1	106.45	27.57	89.01	4.30	147.67	22.58
AD-560164.1	106.73	13.72	97.25	8.25	103.76	7.45
AD-559714.1	107.80	24.01	130.26	16.11	139.71	8.34
AD-560165.1	108.93	14.81	100.31	4.51	95.90	5.35
AD-559300.1	111.25	11.78	90.58	4.56	134.36	10.29
AD-559866.1	112.76	7.74	123.50	10.25	143.37	8.58
AD-559302.1	113.89	12.96	90.69	3.34	112.12	19.35
AD-560163.1	114.10	15.54	92.44	9.83	107.52	5.99
AD-559718.1	114.19	9.34	139.34	10.12	143.95	10.17
AD-559721.1	115.30	17.06	116.42	5.04	143.90	13.34
AD-559026.1	115.85	15.27	95.64	12.87	132.32	13.74
AD-559719.1	116.22	12.55	130.31	6.06	150.76	26.42
AD-559060.1	117.05	20.30	95.06	4.36	141.26	25.56
AD-559864.1	118.44	9.57	134.91	10.92	143.92	22.59
AD-559059.1	120.63	14.40	94.88	3.85	154.23	9.79
AD-559865.1	123.03	9.71	134.45	14.17	142.24	10.43
AD-559148.1	123.28	10.25	91.36	4.38	156.95	14.94
AD-559375.1	125.42	7.77	96.09	8.53	148.89	19.81
AD-559393.1	126.82	14.36	97.97	6.13	142.67	16.86
AD-559717.1	131.20	6.00	131.80	6.57	148.89	12.67
AD-559392.1	134.26	21.19	96.35	2.77	130.60	8.29

Table 11. Complement Factor B *In Vitro* Single Dose Screens in Primary Mouse Hepatocytes (PMH)

DuplexID	10 nM Dose % of Message Remaining	STDEV	1 nM Dose % of Message Remaining	STDEV	0.1 nM Dose % of Message Remaining	STDEV
AD-560969.1	1.76	0.44	12.85	2.32	72.08	7.11
AD-561537.1	1.97	0.30	13.11	1.38	57.66	1.85

DuplexID	10 nM Dose % of Message Remaining	STDEV	1 nM Dose % of Message Remaining	STDEV	0.1 nM Dose % of Message Remaining	STDEV
AD-562262.1	2.07	0.40	14.20	2.60	54.79	3.33
AD-561960.1	2.82	0.45	15.86	1.84	76.74	10.88
AD-561580.1	2.88	1.59	18.55	1.98	69.39	11.71
AD-561254.1	2.89	0.86	15.62	4.05	61.84	5.51
AD-561578.1	2.95	0.42	25.93	1.50	76.75	7.73
AD-561538.1	3.50	0.97	4.45	1.11	31.69	5.04
AD-561252.1	3.60	1.65	16.00	2.58	59.33	6.64
AD-561963.1	3.62	0.30	29.50	8.22	81.62	4.93
AD-562027.1	3.89	0.78	23.89	5.04	70.54	7.81
AD-561653.1	4.01	0.74	23.82	3.53	80.62	7.07
AD-562137.1	4.14	1.55	24.06	11.00	69.61	6.62
AD-561536.1	4.21	1.10	29.07	5.79	80.38	5.28
AD-561253.1	5.04	1.15	27.31	6.10	68.27	12.61
AD-561959.1	5.17	1.81	50.97	13.35	90.71	8.16
AD-561573.1	5.38	0.27	33.50	11.42	91.06	13.14
AD-561651.1	6.36	1.01	49.06	2.79	92.69	9.37
AD-561148.1	6.36	0.87	38.00	1.38	89.30	12.00
AD-561962.1	7.16	1.19	36.21	6.11	91.19	5.39
AD-562237.1	8.80	2.07	53.16	6.31	86.16	11.24
AD-561654.1	9.91	1.61	55.56	7.65	101.67	5.04
AD-562136.1	10.27	0.81	62.46	9.28	97.86	7.44
AD-562026.1	11.34	1.47	60.69	2.81	89.22	3.89
AD-561101.1	11.78	2.75	62.67	4.18	84.92	9.50
AD-561539.1	12.00	1.31	53.65	10.81	84.80	12.57
AD-561961.1	14.01	2.50	58.58	7.03	101.01	6.13
AD-561535.1	14.27	3.13	70.96	10.08	103.90	12.70
AD-561652.1	16.40	3.40	64.57	7.97	96.09	9.38
AD-562260.1	19.73	4.82	77.55	5.09	91.07	8.78
AD-557041.2	25.76	5.84	50.26	6.69	93.59	0.72
AD-561655.1	26.16	3.41	80.98	7.80	102.69	10.61
AD-557043.1	27.03	3.02	69.48	3.50	95.16	3.84
AD-561603.1	31.99	4.57	78.55	6.25	86.39	7.35
AD-557044.1	38.05	3.97	53.45	3.97	90.84	7.63
AD-561266.1	38.64	2.80	79.00	6.75	89.14	5.01
AD-561541.1	45.60	6.83	92.73	1.82	107.60	8.31
AD-562257.1	52.27	2.66	93.01	8.78	100.58	4.10
AD-560538.1	57.85	5.71	92.26	2.62	102.77	6.06
AD-561579.1	65.71	10.54	91.74	8.83	101.05	6.91
AD-562259.1	66.28	3.34	91.38	7.85	96.23	5.92
AD-560968.1	73.08	6.97	87.16	9.40	100.89	2.47
AD-561647.1	74.09	6.95	96.10	5.34	103.62	8.94

DuplexID	10 nM Dose % of Message Remaining	STDEV	1 nM Dose % of Message Remaining	STDEV	0.1 nM Dose % of Message Remaining	STDEV
AD-561469.1	78.98	10.38	91.17	7.11	102.25	6.72
AD-561540.1	84.14	6.35	95.72	4.13	104.96	9.90
AD-562135.1	87.86	10.90	102.43	11.38	102.47	11.43

Table 12. Complement Factor B *In Vitro* Single Dose Screens in Hep3B Cells

DuplexID	10 nM Dose % of Message Remaining	STDEV	1 nM Dose % of Message Remaining	STDEV	0.1 nM Dose % of Message Remaining	STDEV
AD-560969.1	29.34	1.71	71.22	4.40	88.98	14.10
AD-561537.1	86.96	7.93	84.92	6.56	97.67	11.01
AD-562262.1	19.99	1.52	43.30	9.67	77.20	2.33
AD-561960.1	101.89	7.12	93.06	6.78	86.95	11.28
AD-561580.1	77.04	4.88	72.79	13.33	99.51	7.87
AD-561254.1	47.27	3.23	59.31	9.89	80.05	5.60
AD-561578.1	17.62	1.52	54.39	5.50	86.17	9.50
AD-561538.1	14.90	6.97	24.45	3.87	42.19	9.11
AD-561252.1	93.27	11.00	96.17	6.74	78.24	13.72
AD-561963.1	40.07	4.40	63.17	10.68	84.66	8.57
AD-562027.1	77.41	4.07	84.74	1.64	95.86	11.49
AD-561653.1	44.48	8.74	68.46	11.39	101.86	10.10
AD-562137.1	12.14	2.49	35.01	5.57	63.19	12.92
AD-561536.1	81.78	6.45	107.20	6.41	72.90	5.20
AD-561253.1	83.43	9.54	84.78	15.70	70.37	8.01
AD-561959.1	78.06	6.19	70.68	2.99	89.62	9.10
AD-561573.1	62.25	8.08	74.47	7.81	69.74	13.65
AD-561651.1	65.86	6.99	94.78	14.92	86.37	10.00
AD-561148.1	85.70	6.64	93.17	12.36	96.96	10.73
AD-561962.1	73.24	4.15	104.85	7.06	105.02	11.38
AD-562237.1	35.58	3.42	62.94	2.85	82.87	12.89
AD-561654.1	68.27	6.59	84.86	8.20	88.86	16.82
AD-562136.1	43.50	5.85	96.79	6.07	97.96	8.53
AD-562026.1	78.57	11.58	92.78	9.79	100.53	12.79
AD-561101.1	80.42	8.87	88.94	8.58	78.42	5.79
AD-561539.1	73.75	9.48	70.46	13.02	81.45	11.45
AD-561961.1	93.29	14.09	102.04	3.89	82.92	14.39
AD-561535.1	90.31	7.87	100.89	9.09	89.58	10.53
AD-561652.1	99.83	14.12	86.03	11.59	99.32	15.07
AD-562260.1	72.75	4.48	93.46	5.11	91.71	18.08
AD-557041.2	18.62	0.85	50.98	12.96	78.19	10.26
AD-561655.1	81.07	2.83	92.73	6.74	73.97	11.80

DuplexID	10 nM Dose % of Message Remaining	STDEV	1 nM Dose % of Message Remaining	STDEV	0.1 nM Dose % of Message Remaining	STDEV
AD-557043.1	28.86	4.75	81.11	7.28	91.72	10.13
AD-561603.1	46.27	14.44	99.22	11.97	86.19	7.73
AD-557044.1	23.45	2.59	74.62	9.75	79.77	7.73
AD-561266.1	105.79	5.46	95.99	11.36	99.59	2.26
AD-561541.1	100.77	11.65	98.43	4.85	93.30	4.25
AD-562257.1	101.31	9.81	97.55	15.36	95.26	7.79
AD-560538.1	103.91	11.00	104.39	8.70	86.71	20.47
AD-561579.1	87.65	10.59	80.92	11.54	83.54	7.48
AD-562259.1	86.75	12.13	98.75	19.03	90.33	10.56
AD-560968.1	94.61	7.70	107.14	4.84	88.97	10.45
AD-561647.1	99.94	9.55	110.74	11.11	98.97	13.25
AD-561469.1	84.71	4.50	88.17	6.08	82.50	8.92
AD-561540.1	84.25	4.08	88.70	14.19	110.80	11.10
AD-562135.1	89.38	4.70	95.02	2.81	98.61	12.79

Example 3. *In vivo* screening of dsRNA Duplexes in Mice

Duplexes of interest, identified from the above *in vitro* studies, were evaluated *in vivo*.

- 5 In particular, 6-8-week old wild-type mice (C57BL/6) were administered 100 ml of a 2×10^{11} viral particles/ml solution of an adeno-associated virus 8 (AAV8) vector encoding human complement factor B (hCFB AAV) by intravenous tail vein injection at Day -14.

At day 0, mice were subcutaneously administered a single 2 mg/kg dose of a duplex of interest or PBS control (n=3/group). Table 13 provides the duplexes that were administered to the mice.

- 10 At day 0, 7, and 14 post dose, blood was collected and plasma was prepared for ELISA assay. At day14 post-dose, animals were sacrificed, liver samples were collected and snap-frozen in liquid nitrogen and tissue mRNA was extracted.

- 15 The level of human CFB protein was determined by quantitative sandwich enzyme immunoassay (AssayMax™, Human Complement Factor B ELISA Kit). Table 14 shows that the protein levels of human CFB were reduced upon treatment with a single dose of siRNA targeting hCFB at 2 mg/kg.

- 20 The level of human CFB expression was measured by RT-QPCR, as described above. Human CFB mRNA levels were compared to the mRNA level 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 were presented as mean plus standard deviation. As shown in Table 15, human CFB mRNA levels were reduced upon treatment with a single dose of siRNA targeting hCFB at 2 mg/kg.

Table 13. dsRNA Duplexes for *In Vivo* Screening

Duplex ID	Oligo ID	Strand	Sequence 5' to 3'	SEQ ID NO:	Range in NM_001710.5
AD-558657.2	A-1072067.1	sense	gsusgaguGfaUfGfAfgaucucuuuuL96	629	643-665
	A-1075535.1	antis	asAfsaagAfgAfUfcucaUfcAfcucacsasu	700	
AD-559020.2	A-1072793.1	sense	asasaaguGfuCfUfAfgucaacuauL96	619	1145-1167
	A-1075898.1	antis	asUfsaagUfuGfAfcuagAfcAfcuuuusug	690	
AD-559023.2	A-1072799.1	sense	asgsugucUfaGfUfCfaacuuuuuuL96	631	1148-1170
	A-1075901.1	antis	asAfsauuAfaGfUfugacUfaGfacacususu	702	
AD-558860.2	A-1072473.1	sense	usgsccaaGfaCfUfCfcuucaguauL96	591	928-950
	A-1075738.1	antis	asUfsacaUfgAfAfggagUfcUfuggcasgsg	662	
AD-560019.2	A-1074791.1	sense	asgsagaaGfuCfGfUfuucuuuauL96	593	2396-2418
	A-1076897.1	antis	asUfsugaAfuGfAfaacgAfcUfucucusug	664	
AD-560016.2	A-1074785.1	sense	ascsaagaGfaAfgUfCfuuuuuuuuL96	600	2393-2415
	A-1076894.1	antis	asAfsaugAfaAfcGfaguUfcUfcuugusgsa	671	
AD-559008.2	A-1072769.1	sense	uscsacagGfaGfCfCfaaaaaguguuL96	617	1133-1155
	A-1075886.1	antis	asAfsacUfuUfUfuggcUfcCfugugasasg	688	
AD-559717.2	A-1074187.1	sense	csasggaaUfuCfCfUfgaauuuuuuuL96	660	1976-1998
	A-1076595.1	antis	asAfsuaaAfaUfUfcaggAfaUfuccugscsu	731	
AD-557072.2	A-1072789.1	sense	csasaaaaGfuGfUfCfuagucacuL96	191	1143-1165
	A-1072790.1	antis	asAfsuug(Agn)cuagacAfcUfuuuugsgsc	277	
AD-558097.2	A-1074839.1	sense	usasguggAfuGfUfCfugcaaaaacuL96	192	2438-2460
	A-1074840.1	antis	asGfsuuuu(Tgn)gcagacAfuCfcacuasesu	278	
AD-557774.2	A-1074193.1	sense	gsasauucCfuGfAfauuuuuagacuL96	193	1979-2001
	A-1074194.1	antis	asGfsucau(Agn)aaaauucAfgGfaauucscsu	279	
AD-557070.2	A-1072785.1	sense	gscscaaaAfaGfUfGfucuaagucuuL96	194	1141-1163
	A-1072786.1	antis	asUfsugac(Tgn)agacacUfuUfuuggcsusc	280	
AD-558065.2	A-1074775.1	sense	asgsuucAfaGfAfgaagucguuuL96	199	2388-2410
	A-1074776.1	antis	asAfsacga(Cgn)uuucuuUfgUfgaacusasu	285	
AD-557853.2	A-1074351.1	sense	asgsggaaCfaAfcUfCfagcuuuuguL96	208	2078-2100
	A-1074352.1	antis	asCfsaaag(Cgn)ucgaguUfgUfuccucscsg	294	
AD-557079.2	A-1072803.1	sense	usgsucuaGfuCfAfaAfcuuuuuugauL96	211	1150-1172
	A-1072804.1	antis	asUfscaau(Tgn)aaguugAfcUfagacasesu	297	

Table 14. Human CFB siRNA *In Vivo* Screening

	ELISA Results D7		ELISA Results D14	
	Avg hCFB Protein Remaining	SD	Avg hCFB Protein Remaining	SD
PBS	110.13	22.47	104.31	17.38
Naïve	96.75	21.00	92.31	6.54
AD-558657.2	29.32	8.38	24.76	5.08
AD-559020.2	24.26	4.07	25.05	1.22
AD-559023.2	28.56	7.34	26.35	3.44
AD-558860.2	52.69	4.25	50.31	17.27
AD-560019.2	26.68	8.43	20.07	6.59
AD-560016.2	44.06	2.59	45.94	2.69
AD-559008.2	46.07	6.47	34.55	5.26
AD-559717.2	57.72	11.41	51.14	13.28
AD-557072.2	34.29	8.88	22.19	6.45

	ELISA Results D7		ELISA Results D14	
	Avg hCFB Protein Remaining	SD	Avg hCFB Protein Remaining	SD
AD-558097.2	31.01	9.59	29.55	10.60
AD-557774.2	70.26	6.03	64.26	12.89
AD-557070.2	91.58	21.23	71.11	18.76
AD-558065.2	120.96	14.77	82.66	44.55
AD-557853.2	94.22	24.50	81.47	18.23
AD-557079.2	101.05	17.40	97.09	15.44

Table 15. Human CFB siRNA *In Vivo* Screening

qPCR Results D14		
	% message remaining	SD
PBS	100.19	7.12
Naïve	88.59	6.21
AD-558657.2	37.82	11.34
AD-559020.2	56.19	2.64
AD-559023.2	37.50	3.28
AD-558860.2	65.89	10.68
AD-560019.2	22.01	1.70
AD-560016.2	68.31	5.22
AD-559008.2	53.91	12.03
AD-559717.2	63.13	7.55
AD-557072.2	39.39	1.21
AD-558097.2	43.35	11.63
AD-557774.2	81.10	4.79
AD-557070.2	83.09	13.83
AD-558065.2	86.18	9.28
AD-557853.2	84.42	6.60
AD-557079.2	98.00	15.34

Example 4. *In vivo* screening of dsRNA Duplexes in Mice

5 Additional duplexes of interest, identified from the above *in vitro* studies, were evaluated *in vivo*.

In particular, 6-8-week old wild-type mice (C57BL/6) were administered 100 ml of a 2×10^{11} viral particles/ml solution of an adeno-associated virus 8 (AAV8) vector encoding human complement factor B (hCFB AAV) by intravenous tail vein injection at Day -14.

10 At day 0, mice were subcutaneously administered a single 2 mg/kg dose of a duplex of interest or PBS control (n=3/group). Table 16 provides the duplexes that were administered to the mice.

At day 0, 7, and 14 post dose, blood was collected and plasma was prepared for ELISA assay. At day 14 post-dose, animals were sacrificed, liver samples were collected and snap-frozen in liquid nitrogen and tissue mRNA was extracted.

- The level of human CFB protein was determined by quantitative sandwich enzyme immunoassay (AssayMax™, Human Complement Factor B ELISA Kit). Table 17 shows that the protein levels of human CFB were reduced upon treatment with a single dose of siRNA targeting hCFB at 2 mg/kg.

The level of human CFB expression was measured by RT-QPCR, as described above. Human CFB mRNA levels were compared to the mRNA level 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 were presented as mean plus standard deviation. As shown in Table 18, human CFB mRNA levels were reduced upon treatment with a single dose of siRNA targeting hCFB at 2 mg/kg.

Table 16. dsRNA Duplexes for *In Vivo* Screening

DuplexID	OligoID	Strand	Sequence 5' to 3'	SEQ ID NO:	Range in NM_001710.5
AD-560018.2	A-1074789.1	sense	asasgagaAfgUfCfGfuuucauL96	592	2395-2417
	A-1076896.1	antis	asUfsgaaUfgAfAfacgaCfuUfcucuuusgsu	663	
AD-559375.2	A-1073503.1	sense	asuscuggAfuGfUfCfuauuguuuuL96	658	1541-1563
	A-1076253.1	antis	asAfsaacAfcAfUfagacAfuCfcagausasa	729	
AD-559160.2	A-1073073.1	sense	usasugaaGfaCfCfAfcagauuL96	594	1306-1328
	A-1076038.1	antis	asUfsucaAfcUfUfguggUfcUfucuuasasu	665	
AD-559374.2	A-1073501.1	sense	usasucugGfaUfGfUfcuauuguuuL96	621	1540-1562
	A-1076252.1	antis	asAfsacaCfaUfAfgacaUfcCfagauasasu	692	
AD-559060.2	A-1072873.1	sense	usgsuguuGfaAfgCfcaagauuL96	653	1185-1207
	A-1075938.1	antis	asCfsauaUfcUfUfgguUfcAfcaccasusa	724	
AD-559721.2	A-1074195.1	sense	asasuuccUfgAfAfUfuuuauagacuuL96	650	1980-2002
	A-1076599.1	antis	asAfsugcAfuAfAfaauuCfaGfgauuuscsc	721	
AD-559026.2	A-1072805.1	sense	gsuscuagUfcAfAfCfuauuugaguL96	651	1151-1173
	A-1075904.1	antis	asCfsucaAfuUfAfaguuGfaCfuagacsasc	722	
AD-558225.2	A-1075095.1	sense	usgsauuAfaAfAfCfagucgacuuL96	207	2602-2624
	A-1075096.1	antis	asGfsucgc(Agn)gcuguUfuAfauucasasu	293	
AD-557069.2	A-1072783.1	sense	asgsccaaAfaAfgUfgucuauguL96	206	1140-1162
	A-1072784.1	antis	asUfsgacu(Agn)gacuuUfuUfggucuscsc	292	
AD-558068.2	A-1074781.1	sense	uscsacaaGfaGfAfAfgucguuuL96	195	2391-2413
	A-1074782.1	antis	asUfsgaaa(Cgn)gacuuUfcUfugugasasc	281	
AD-557422.2	A-1073489.1	sense	gsasggauUfaUfCfUfgaugucuuL96	202	1534-1556
	A-1073490.1	antis	asUfsagac(Agn)uccagaUfaAfuccucscsc	288	
AD-558063.2	A-1074771.1	sense	asusaguuCfaCfAfAfgagaagucguL96	205	2386-2408
	A-1074772.1	antis	asCfsgacu(Tgn)cucuugUfgAfacuauscsa	291	
AD-558066.2	A-1074777.1	sense	gsusucacAfaGfAfGfaagucguuuL96	212	2389-2411
	A-1074778.1	antis	asAfsaacg(Agn)cuucucUfuGfugaacsusa	298	
AD-556701.2	A-1072047.1	sense	csusacuaCfaAfUfGfugagugaguL96	197	633-655
	A-1072048.1	antis	asCfsauca(Cgn)ucacauUfgUfaguagsgsg	283	

Table 17. Human CFB siRNA *In Vivo* Screening

	ELISA Results D7		ELISA Results D14	
	Avg hCFB Protein Remaining	SD	Avg hCFB Protein Remaining	SD
PBS	115.67	31.44	117.68	25.52
Naïve	110.11	4.46	108.16	15.25
AD-560018.2	16.29	2.97	15.23	4.63
AD-559375.2	42.43	8.09	44.31	14.32
AD-559160.2	39.66	10.78	49.87	3.52
AD-559374.2	43.88	10.14	41.19	9.12
AD-559060.2	63.43	15.73	50.37	6.51
AD-559721.2	30.89	2.80	28.32	5.76
AD-559026.2	70.20	7.55	53.71	3.75
AD-558225.2	42.06	1.30	41.12	6.52
AD-557069.2	94.78	13.32	71.48	6.01
AD-558068.2	94.83	9.22	97.31	20.15
AD-557422.2	74.07	1.97	63.49	9.48
AD-558063.2	77.64	3.66	81.77	4.84
AD-558066.2	76.19	5.77	65.77	8.00
AD-556701.2	69.44	3.28	62.51	21.07

Table 18. Human CFB siRNA *In Vivo* Screening

qPCR Results D14		
	% message remaining	SD
PBS	100.4	9.3
Naïve	77.4	13.2
AD-560018.2	20.0	1.7
AD-559375.2	66.4	10.1
AD-559160.2	60.4	6.4
AD-559374.2	38.3	12.3
AD-559060.2	67.7	4.4
AD-559721.2	38.6	7.2
AD-559026.2	69.5	22.8
AD-558225.2	58.7	12.7
AD-557069.2	74.2	20.7
AD-558068.2	56.4	18.2
AD-557422.2	92.5	10.5
AD-558063.2	91.9	26.6
AD-558066.2	62.3	8.2
AD-556701.2	58.4	3.2

Example 5. Design and Synthesis of Additional dsRNA Duplexes

Additional siRNAs were designed, synthesized and annealed using methods known in the art and described above in Example 1.

Detailed lists of the additional unmodified CFB sense and antisense strand nucleotide sequences are shown in Table 19. Detailed lists of the modified CFB sense and antisense strand nucleotide sequences are shown in Table 20.

In vitro and *in vivo* single dose screens of these agents in HepG2 cells were performed as described in the Examples above. Briefly, HepG2 cells were transfected by adding 5 µl of Opti-MEM plus 0.25 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 µl of each siRNA duplex to an individual well in a 96-well plate. The mixture was then incubated at room temperature for 15 minutes. Forty µl of Eagle's Minimum Essential Medium (ATCC Cat#30-2003) containing ~2 x10⁴ HepG2 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. The assays were performed as quadruplicates.

The results of the single dose screens of the dsRNA agents listed in Tables 19 and 20 in HepG2 cells are shown in Table 21. The results are presented as the mean percentage of message remaining.

Table 19. Unmodified Sense and Antisense Strand Sequences of Complement Factor B dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Start site in NM_001710.5	End site in NM_001710.5	Region	Exon
AD-558312	AAGGGAUUGAGACACAGGUCUU	1029	AAGACCGGUCACAUUCCCUUCC	1121	153	175	5'UTR	1
AD-558336	CUGGAGUUUCAGCUUGGACAU	43	AUGUCCAAGCUGAAACUCCAGAC	129	177	199	5'UTR	1
AD-558361	CCAAGCAGACAAAGCAU	1030	AUGCUUUGCUUGUCUGCUUGGCU	1122	202	224	5'UTR	1
AD-558382	GCCAGGACACACCAUCCUGCU	1031	AGCAGGAGUGGUGUCCUGGCUU	1123	223	245	5'UTR	1
AD-558389	CCAGCUUCUCUCCUGCCUUCU	1032	AGAAGGACAGGAGAGAAAGCUGGC	1124	250	272	5'UTR	1
AD-558407	UGCCUGAUGCCCUUUUAUCUUU	1033	AAAGUAAGGGCAUCAGGCAGA	1125	304	326	CDS	1
AD-558426	UGGGCCUUCUUGUCUGGAGGUU	1034	AACUCCAGACAAAGAGGCCAAG	1126	323	345	CDS	1-2
AD-558450	CCACCACUCCAUUGGUCUUUGU	1035	ACAAAGACCAUGGAGUGGUGUC	1127	347	369	CDS	2
AD-558482	UCCUCCGACUUCUCCAAAGAU	1036	AUCUUGGAGAAUCGGAAGGAGC	1128	418	440	CDS	2
AD-558507	AGGCACUGGAGUACGUGUGUU	1037	AACACACGUACUCCAGUGCCUGG	1129	443	465	CDS	2
AD-558522	UGUGUCCUUCUGGCUUCUACU	1038	AGUAGAACCCAGAAAGACACACG	1130	458	480	CDS	2
AD-558539	UACCCGUACCCUGUGCAGACU	1039	AGUCUGCACAGGUACGGGUAGA	1131	475	497	CDS	2
AD-558555	AGACACGUACCCUGCAGAUUU	1040	AAGAUUCGACAGUACGUGUCUGC	1132	491	513	CDS	2
AD-558579	AGACUCAAGACCAAAAGACUU	1041	AAGUCUUUUGGUCUUGAGUCUUC	1133	533	555	CDS	2
AD-558612	AGUGCAGAGCAUCCACUGUU	1042	AACAGUGGAUUGCUCUGCACUCU	1134	566	588	CDS	2-3
AD-558637	ACCACAGACUUCGAGAACGU	1043	ACGUUCUCGAAAGUCUGUGGUCU	1135	591	613	CDS	3
AD-558646	CCUACUACAAUGUGAGUGAUU	1044	AUUCACUCACAUUAGUAGGGA	1136	632	654	CDS	3
AD-558662	UGAUGAGAUUCUUUCCACUU	1045	AAGUGGAAAGAGAUUCACUACU	1137	648	670	CDS	3
AD-558679	ACUGCUAUGACGGUUACACUU	52	AAGUUAACCGUACUAGCAGUGG	138	665	687	CDS	3
AD-558701	CACUUGCCAAUGAAUUGGCCU	1046	AGGCCAUUCACUUGGCAGGUGCG	1138	705	727	CDS	3
AD-558737	AGCGAUUCUGUACAACGGAGU	66	ACUCCGUUGUCACAGAUCCGUGU	1139	741	763	CDS	3-4
AD-558747	CAUUGGCACAAGGAAGGUGGU	1047	ACCACCUUCCUUGUGCCAAUUGG	1140	789	811	CDS	4
AD-558778	CGCCUUGAAGACAGCGUCACU	1048	AGUGACGUCUGUCUCAAAGGCGGU	1141	820	842	CDS	4
AD-558813	GGCGAACGUGUCAGGAAGGUU	1049	AACUUCUCCAGACACGUUCGCCGC	1142	881	903	CDS	4
AD-558859	CUGCCAAAGACUCCUUAUGUU	1050	AACAUAGAGGAGUCUUGGCAGGA	1143	927	949	CDS	4-5
AD-558879	GCCGAAGCUUUCUUGUCUUCU	1051	AGAAGACAGGAAAGCUUCGGCCA	1144	967	989	CDS	5
AD-558896	UUCUCCGACAGAGACCAUAGU	1052	ACUAGGUCUCUGUCAGGGAAGA	1145	984	1006	CDS	5
AD-558918	GGAGUCGAUGCUGAGGAGUGGU	1053	ACCAUCCUCAGCAUCGACUCCUU	1146	1006	1028	CDS	5
AD-558935	CAACAGAAAGCGGAAGAUUGUU	1054	AACGAUCUCCCGCUUCUUGUUGU	1147	1042	1064	CDS	6
AD-558965	UCAGGCUCCAUAGAACAUCUUA	471	AUAGAUGUUAUGGAGCCUGAAG	542	1072	1094	CDS	6
AD-558993	UAGAUGGAUCACAGACGACUUU	1055	AAUAGCUGUCUGAUCCAUCAAGC	1148	1100	1122	CDS	6
AD-558998	GCCAGCAACUUCACAGGAGCU	1056	AGCUCCUGUGAAGUUGCUGGCC	1149	1123	1145	CDS	6

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Start site in NM_001710.5	End site in NM_001710.5	Region	Exon
AD-559023	AGUGUAGUAGUACUAAUUAU	489	AAUUUAGUUGACUAGACACUUU	560	1148	1170	CDS	6
AD-559040	AUUGAGAAGGUGGCAAGUUAU	1057	AUAACUUGCCACCUUCUCUAAUA	1150	1165	1187	CDS	6-7
AD-559059	AUGGUGUAGAGCCAAAGUUAU	513	AUAUUCUUGGCUUACACCAUAA	584	1184	1206	CDS	7
AD-559074	GAUUGGUCUAGUGACAUUAU	495	AUAUGUCACUAGACCAUUAUU	566	1199	1221	CDS	7
AD-559089	AUUUGGUCUAAAGUGUCUGAU	1058	AUCAGACACUUUGACCCAAUUU	1151	1234	1256	CDS	7
AD-559112	AGACAGCAGUAAUGCAGACUU	1059	AAGUCUGCAUUACUGCUGUCUGC	1152	1257	1279	CDS	7
AD-559143	CAGCUCUAAUGAAUUAUUAU	478	AUAUUGAUUUCAUUGAGCUGCU	549	1288	1310	CDS	7
AD-559163	GAAGACCACAAUGUUAAGUCU	1060	AGACUUCACUUUGUGGUCUUAU	1153	1309	1331	CDS	7-8
AD-559184	GGGACUAAACACCAAGAAGGCU	1061	AGCCUUCUUGGUGUUAUCCUUG	1154	1330	1352	CDS	8
AD-559208	CAGGCAGUGUACAGCAUGAUU	1062	AUAUCUGCUGUACACUGCCUGGA	1155	1354	1376	CDS	8
AD-559233	GGCCAGAUAGCUGCCUCCUU	1063	AAGGAGGACGUCUUAUUGGCCAG	1156	1379	1401	CDS	8
AD-559274	UGUCAUACUCCUAGUAGUCU	1064	ACAGUACAGGAGUAGUAGACAU	1157	1422	1444	CDS	8
AD-559290	ACUGAUGGAUUGCACAACAUU	1065	AAGUUGUGCAAUCCAUACAGUCA	1158	1438	1460	CDS	8-9
AD-559306	UUACUGUCAUUGAUGAGAUU	1066	AGAUCUCAUUAUAGACAGUAAUU	1159	1472	1494	CDS	9
AD-559329	GACUUGCUUAUACAUUGGCAU	1067	AUUGCCAAUGUAUAGCAAGUCCC	1160	1495	1517	CDS	9
AD-559359	AACCCAGGGAGGAUUAUCUU	486	AAGUAUUCUCCUUGGGUUUU	557	1525	1547	CDS	9
AD-559374	UAUCUGGAUGUCUUAUGUGUUU	479	AAACACAUAGACAUCCAGUAAU	550	1540	1562	CDS	9-10
AD-559383	GGGCCUUUGGUGAACCAAGUU	1068	AACUUGGUUACACCAAGGCCCGA	1161	1567	1589	CDS	10
AD-559398	CAAGUGAACAUCAUUGCUUUU	491	AAAAGCAUUGAUGUUAUUAUUGU	562	1582	1604	CDS	10
AD-559421	UCCCAAGAAAGACAAUAGAGCU	45	AGCUCUUGUCUUUUAUUGGAAGC	1162	1605	1627	CDS	10
AD-559438	AGCAACAUGUUAUCAAAGUCU	1069	AGACUUUGAACACAUUUGGUCA	1163	1622	1644	CDS	10
AD-559455	GUCAAGGAUUAUGGAAACCUU	1070	AAGGUUUUCCAUUCCUUGACUU	1164	1639	1661	CDS	10
AD-559476	GAAGAUGUUUUCUACCAAAU	1071	AAUUUGGUAGAAAACAUUUCCA	1165	1660	1682	CDS	10
AD-559497	AUCGAUGAAAGCCAGUCUCUU	1072	AAGAGACUGGGCUUUAUUCGAUCA	1166	1681	1703	CDS	10-11
AD-559512	UCUCUGAGUCUCUGUGGCAUU	1073	AAUGCCACAGAGACUCAGAGACU	1167	1696	1718	CDS	11
AD-559536	UGGGAACACAGGAAGGGAUACU	1074	AGUACCCUUCUUGUGUUCUCCAAA	1168	1720	1742	CDS	11
AD-559556	CGAUUACCAACAAAGCAACCAU	1075	AAUGGUUGCUUUGUGGUAAUCCGGU	1169	1740	1762	CDS	11
AD-559590	UCAGUCAUUCGGCCUUAUUAU	1076	AUUUGAAGGGCGAAUAGACUGAGA	1170	1774	1796	CDS	11-12
AD-559610	GGGACACGAGAGCUGUAUGGU	1077	ACCAUACAGCUCUCUGUCCUU	1171	1794	1816	CDS	12
AD-559616	GUGGUGUCUGAGUACUUUGUU	482	AACAAAGUACUCAGACACACACAG	553	1819	1841	CDS	12
AD-559641	CAGCAGCACAUUGUUUUCACUU	1078	AAGUGAAACAAUUGUGCUGCUGUC	1172	1844	1866	CDS	12

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Start site in NM_001710.5	End site in NM_001710.5	Region	Exon
AD-559670	AAGGAACACUCACAAUCAAAGGUU	1079	AACCUUGAUUGAGUGUUCUUGU	1173	1873	1895	CDS	12
AD-559704	UAGAAUAGUCCUAAUUUCACU	1080	AGUGAAUAGGACUACUUCUACU	1174	1925	1947	CDS	13
AD-559706	CAACUACAACAUAAUAGGGAU	1081	AUCCCAUAAUAGUUGUAGUUGG	1175	1947	1969	CDS	13
AD-559722	AUCCUGAAUUUUAUGACUAAU	492	AUAGUCAUAAAUUACAGAAUUC	563	1981	2003	CDS	13
AD-559740	UAUGACGUUGCCUGAUCAAU	1082	AUUGACAGGGCAACGUAUAGU	1176	1999	2021	CDS	13
AD-559760	GCUCAAGAAUAAAGCUGAAAUU	1083	AAUUUCAGCUUAAUUCUUGAGCUU	1177	2019	2041	CDS	13
AD-559788	ACUAUCAGGCCCAUUUUGUCUU	466	AAGACAAUUGGGCCUGAUAGUCU	537	2047	2069	CDS	13-14
AD-559799	AGGGAACAACUCGAGCUUUGU	36	ACAAAGCUCGAGUUGUUCUUCG	122	2078	2100	CDS	14
AD-559823	UUCUCCAAACUACCAUUGCU	1084	AGCAAGUGGUAUUGGAGGAAGC	1178	2102	2124	CDS	14
AD-559838	CUUGCCAGCAACAAAAGGAU	1085	AUUCUUUUUUGUCUGGCAAGUG	1179	2117	2139	CDS	14-15
AD-559873	CAGGAUAUCAAGCUCUGUUU	1086	AAACAGAGCUUUGAUUCCUGUG	1180	2152	2174	CDS	15
AD-559892	UUUGUCUGAGGAGGAGAAU	1087	AUUUCUCCUCCUCAGACACAAAC	1181	2171	2193	CDS	15
AD-559926	GGAGGUUACAUCAAAGAAUGU	1088	ACAUCUUGAUGUAGACUCCUU	1182	2205	2227	CDS	15
AD-559946	GUGAGAGAGUUCUCAAUAAU	473	AAUUAUGAGCAUCUCUCACAG	544	2243	2265	CDS	16
AD-559958	GACAAAGUCAAGGACAUUCU	37	AGAGAUUCUUGACUUGUUAU	123	2275	2297	CDS	16
AD-559973	UCGGUCCUUUGUACUGGAGU	1089	ACUCCAGUACAAAGGAACCGAGG	1183	2310	2332	CDS	16
AD-559993	GAGUAGUCCCUAUGCUGACU	1090	AGUCAGCAUAGGGACUACUCCU	1184	2330	2352	CDS	16
AD-559998	UACUUGCAGAGGUGAUUCUGU	1091	ACAGAAUCACCUCUGCAAGUAU	1185	2355	2377	CDS	16-17
AD-560011	AGUUCACAAGAGAGUGUUGU	27	AAACGACUUCUCUUGUGAACUAU	113	2388	2410	CDS	17
AD-560031	UCAUUCAAAGUUGGUGUAAUCU	1092	AGAUACACCAACUUGAAUGAAA	1186	2408	2430	CDS	17-18
AD-560040	GAGUAGUGGAGUGUCUGCAAU	1093	AUUUGCAGACAUCACUACUCCC	1187	2435	2457	CDS	18
AD-560060	AACCAAGAGCGGCAAAAGCAU	1094	AUGCUUUUGCCGUUCUGGUUUU	1188	2455	2477	CDS	18
AD-560099	GACUUUCACAUCAACCUCUUU	1095	AAAGAGGUUGAUGUAGAAAGUCU	1189	2494	2516	CDS	18
AD-560114	CUCUUUCAAAGUCUGCCUUGU	1096	ACAGGGCAGCAUGUAGAAAGAGU	1190	2509	2531	CDS	18
AD-560138	AAGGAGAAACUCCAAGAUAAU	1097	AUCAUCUUGGAGUUUCUCCUUA	1191	2533	2555	CDS	18
AD-560156	GAGGAUUUGGUGUUUUAUAAU	1098	AUAUAGAAACCCAAAUCCUCAU	1192	2551	2573	CDS	18
AD-560163	CGUGGAUUGAAUUAACAACAU	506	AUGUUUUAAUUAUCCACGCGC	577	2594	2616	3'UTR	18
AD-558378	GCAAGCCAGGACACACCAUCU	1099	AGAUGGUGUGUCCUGGUUGCUU	1193	219	241		
AD-558393	CUUCUCUCCUGCCUCCAAACU	1100	AGUUGGAAGGCAGGAGAGAAAGCU	1194	254	276		

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Start site in NM_0017 10.5	End site in NM_0017 10.5	Region	Exon
AD-558424	CUUGGGCCUUCUGUCUGGAGU	1101	ACUCCAGACAAAGAGGCCCAAGAU	1195	321	343		
AD-558466	AGAGAUCAAAGCGGCUCCUU	1102	AAGGACCGCCUUUGAUUCUAC	1196	402	424		
AD-558511	ACUGGAGUACGUGUGUCCUUU	1103	AAAGGACACAGUACUCCAGGUC	1197	447	469		
AD-558574	CCUGAAGACUCAAGACCAAAU	1104	AUUUGGUUUUGAUUCUACAGGU	1198	528	550		
AD-558595	GACUGACAGGAAGGACAGAUU	1105	AACUCUGCCUUCUGACAGUCUU	1199	549	571		
AD-558750	UGGCACAAAGGAAGGUGGGCAU	1106	AUGCCCAACCUUCCUUGGCCAAU	1200	792	814		
AD-558777	CCGCCUUUAAGACAGCGUCAU	1107	AUGACGCGUCUUAAGGCGGUA	1201	819	841		
AD-559105	CUGAAGCAGACAGCAGUAAUU	1108	AAUUCUGUCUGUCUUCAGAC	1202	1250	1272		
AD-559124	UGCAGACUGGGUACGAAAGCU	1109	AGCUUCGUGACCCAGUCUGCAU	1203	1269	1291		
AD-559189	UAAACCAAGGAAGGCCUCCU	1110	AGGAGGGCCUUCUUGGUGUAGU	1204	1335	1357		
AD-559226	AUGAGCUGGGCCAGAUAGCGUU	1111	AACGUAUCUGGCCAGCUCUAUA	1205	1372	1394		
AD-559330	ACUUGCUAUACAUUGGCAAGU	1112	ACUUGCCAAUGUAUAGCAAGUCC	1206	1496	1518		
AD-559486	UCUACCAAAUGAUCGAUGAAU	1113	AUUAUCGAUCAUUUGUAGAAA	1207	1670	1692		
AD-559532	GGUUUGGGAACACAGGAAGGU	1114	ACCUCCUGUGUUCUCCAAACCAU	1208	1716	1738		
AD-559573	CAUGGCAGGCCAAGAUCUCAU	1115	AUGAGAUUUUGGCCUGCCAUUGU	1209	1757	1779		
AD-559609	AGGGACACGAGAGCUGUAUGU	1116	ACAUACAGCUCUCUGUCCUUUU	1210	1793	1815		
AD-559668	ACAAGGAACACUCAAUCAAGU	1117	ACUUGAUUGAGUGUUCUUGUCA	1211	1871	1893		
AD-559688	AAGCGGACCCUGGAGAUAGAU	1118	AUCUAUCUCCAGGUCCCGCUUCU	1212	1909	1931		
AD-559882	AAAGCUCUGUUUGUCUGAU	1119	AUCAGACACAAACAGAGCUUUA	1213	2161	2183		
AD-560132	UGGCUGAAGGAGAAACUCCAU	1120	AUGGAGUUUCUCCUUCAGCCAGG	1214	2527	2549		

Table 20. Modified Sense and Antisense Strand Sequences of Complement Factor B dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-558312	asasgggaAfUfGfUfGfaccagguuuL96	1215	asAfsagCfuGfGfucAfUfUfcccuuisc	1307	GGAAGGGAATGTGACCAGGTCTA	1406
AD-558336	csusggagUfuUfCfAfgeuugagacauL96	215	asUfsgucCfaAfGfucgaAfaChuccagasc	1308	GTCGTGGAGTTTCAGCTTGGACAC	1407
AD-558361	cscsaagcAfgAfCfAfagcaagcauL96	1216	asUfsgcuUfuGfCfuuguCfuGfucggscsu	1309	AGCCAAAGCAGACAAGCAAAAGCAA	1408
AD-558382	gscscaggAfcAfCfAfccauccgeuL96	1217	asGfscagGfaUfGfugugGfuCfucggcsusu	1310	AAGCCAGGACACACCAATCCTGCC	1409
AD-558389	cscsagcuUfcUfCfUfcccugccuuL96	1218	asGfscagGfcAfGfaggaGfaAfcuugsgsc	1311	GCCCAGCTTCTCTCCTGCCCTTCC	1410
AD-558407	usgscgagAfuGfCfCfcmuuaucuuL96	1219	asAfsagaUfaAfAfgggcAfuCfagcgagga	1312	TCTGCCTGATGCCCTTTATCTTG	1411
AD-558426	usgsggccUfcUfUfGfucugggaguuL96	1220	asAfsccuCfcAfGfacaGfaGfcccacag	1313	CTTGGGCTCTTGTCTGTGGAGGTG	1412

Duplex Name	Sense Sequence 5' to 3'	SEQ ID	Antisense Sequence 5' to 3'	SEQ ID	mRNA Target Sequence	SEQ ID
AD-558450	cscsaccaCfuCfCAfUfgucuuuunL96	NO:	asCsaanaGfaCfCfauggAfGfUfguggsusc	1314	GACCACCATCCATGGTCTTTGG	1413
AD-558482	uscsccuucCfGfAfCfuCfucccaagauL96	1222	asUfsccuGfGfAfGfaaguCfGfGaaggasgsc	1315	GCTCCTTCAGACTTCTCCAAGAG	1414
AD-558507	asgsgeacUfGfAfCfGfuacuguguuL96	1223	asAfsceacAfGcGHufaceafCfGfGfucecusgsG	1316	CCAGGTACTGGAGTAGTACGTGTGC	1415
AD-558522	usgsugcuCfuUfCUfuggcuuacuL96	1224	asGfsuagAfaGfCfCaagaAfGfGfacacascg	1317	CGTGtGTCTTCTGGCTTCTACCC	1416
AD-558539	usascccGfUfaCfCfCfugucagacuL96	1225	asGfsuncGfcAfCfCaaggUfaCfGfgguasgsa	1318	TCTACCCGTACCCGTGTGCAGACA	1417
AD-558555	asgsasacGfuAfCfCfugcagacuL96	1226	asAfsganuCfuGfCfCaaguAfcGfGfucugsG	1319	GCAGACACGTACCTGCAGATCTA	1418
AD-558579	asgsasacAfaGfAfCfCfaaaagacuL96	1227	asAfsqncUfuUfUfGfGnuCfUfGfagucussc	1320	GAAGACTCAAGAACCAAAGAAGCTG	1419
AD-558612	asgsugaGfaGfCfAfaueacuunL96	1228	asAfsceagUfGfAfInuGCUfCfUfGcacussu	1321	AGAGTGCAGAGCAATCCACTGTCT	1420
AD-558637	aseseacaCfGfAfCfUfucgaagacuL96	1229	asCfsgnuCfuCfGfGaaguCfGfUfugugussu	1322	AGACCACACGACTTCGAGAACGG	1421
AD-558646	cscsuacuAfCfAfAFfGfagagauL96	1230	asAfsucaCfuCfAfaanuGfuAfguagsgsa	1323	TCCCTACTACAATGTGAGTGATG	1422
AD-558662	usgsaugaGfaUfCfUfGfucuccacuL96	1231	asAfsqngGfaAfAfGagaUfCfUfcaucassu	1324	AGTGTAGATCTCTTTTCCACTG	1423
AD-558679	asescguaFfuGfAfCfGfguuacuL96	224	asAfsqngAfFuAfCfGnuCfUfAfgcacussg	1325	CCTGTGTATGACGGTTACACTC	1424
AD-558701	csasccgCfcAfAfGfGfagagccuL96	1232	asGfsgccAfUfUfCfaccuGfGfCfagugssg	1326	CGCACCTGCCAAGTAATGGCCG	1425
AD-558737	asgsgeanCfuGHUfGfacaagacuL96	238	asCfsuccGfuUfGfHuacAfGfAfcucusgu	1327	ACAGCGATCTGTGACAAACGGAGC	1426
AD-558747	csasuungCfAcAfAfGfgaaggugguL96	1233	asCfscaeCfuUfCfGuugUfGfCfcaugsgsg	1328	CCCATTGGCACAAAGGAAGGTGGG	1427
AD-558778	csgsccuGfaAfGfAfcagegacuL96	1234	asGfsugaCfGfCUfGnuCfUfCfaggegsgsu	1329	ACCGCTTGAAGACAGCGTCAACC	1428
AD-558813	gsgsgeaaCfGfUfGHUfaggaagguL96	1235	asAfsccuUfCfUfGfacaCfGfUfucggcgsc	1330	GCGGGAAAGTGTCAAGGAAGGTG	1429
AD-558859	csusgccaAfGfAfCfUfccuacguL96	1236	asAfscauGfaAfGfGaguCfuUfGfcagsgsa	1331	TCCTGCCAAAGACTCCTTTCATGTA	1430
AD-558879	gscsegaAGfcUfUfUfGfGcuucuL96	1237	asGfsaagAfCfAfGfGaagGfcUfucggcsesa	1332	TGGCCGAAGCTTTCCTGCTTCC	1431
AD-558896	ususccuGfaCfAfGfAgacacuaL96	1238	asCfsuanGfGfUfCfueUGfUfCfaggaaisa	1333	CTTTCCTGTACAGAGACCATAGA	1432
AD-558918	gsgsagucGfaUfGHUfaggaagguL96	1239	asCfsceauCfUfUfGfageaUfCfGfauceussu	1334	AAGAGTCGATGTGAGATGGG	1433
AD-558935	csasacagAfaGfCfGfGfagacguL96	1240	asAfscegaUfCfUfUfGfceGfUfCfugunugsu	1335	AACAACAGACGCGGAAGATCGTC	1434
AD-558965	uscsagcgUfcCfAfAUfGzaacauL96	613	asUfsagaUfGUfUfGfauGfGfGfcugzasag	684	CTTCAGGCTCCATGAACATCTAC	1435
AD-558993	usasgaugGfaUfCfAfgacagcauL96	1241	asAfsaugCfuGHUfGfugaUfCfUfcaucasgsc	1336	GCTAGATGGATCAGACAGCATTTG	1436
AD-558998	gscseagcAfaCfUfUfcaagagguL96	1242	asGfsceucCfuGHUfGfagUfUfGfucggescsc	1337	GGCCAGCAACTTCACAGGAGCC	1437
AD-559023	asgsugucUfaGfUfCfcaucuaauL96	631	asAfsaanAfaGfUfugacUfaGfIracacusu	702	AAAGTGTCTAGTCAACTTAATTG	1438
AD-559040	asusugagAfaGfGHUfGfcaaguuL96	1243	asUfsaaecUfuGHfGfcaeeUfuCfuaaususa	1338	TAATTGAGAAGGTGGCAAGTTAT	1439
AD-559059	asusggugUfGfAfAfGfGcagaaauL96	655	asAfsuanCfuUfGfGfuuCfAcfIaacuasasa	726	TTATGGTGTGAAGCCAAGATATG	1440
AD-559074	gsasuaugGfuCfUFAfGfagacaaL96	637	asAfsuanGfCfAfCfuaagAfcCfIaaucusu	708	AAGATATGGTCTAGTGACATATG	1441
AD-559089	asusuuggCfuCfAfAfGfagucaguL96	1244	asUfscagAfuCfAfCfhuugAfcCfcaaausuu	1339	AAATTTGGGTCAAAGTGTCTGAA	1442
AD-559112	asgsacagCfaGfUfAfaugcagacuL96	1245	asAfsqneUfGcCfAfAuuaUfGfCfugucussc	1340	GCAGACACGACTAATGCAGACTG	1443
AD-559143	csasgeucAfaUfGfAfaaucuaauL96	620	asUfsaanUfGfAfUfmucaUfuGHfagugscsu	691	AGCAGCTCAATGAAAATCAATTAT	1444
AD-559163	gsasagacCfAcAfAfGfuaagaaguL96	1246	asGfsacuUfCfAfAfGfuuUfGfGfucucssu	1341	ATGAAGACCACAAAGTTGAAGTCA	1445
AD-559184	gsgsgacuAfaCfAfCfGfcaagaagguL96	1247	asGfsccuUfCfUfUfGfugUfUfAfguccesug	1342	CAGGGACTAACACGAAGAAGGCC	1446

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-559208	csasggcaGfuGfuAfcaagcauauL96	1248	asAfsucaUfgCfuGfuacAfeUfgccugsga	1343	TCCAGGCAGTGTACAGCATGATG	1447
AD-559233	gsgccagAfuGfuAfCfugccuccuuL96	1249	asAfsaggGfgGfaGfegcAfuCfugcccsag	1344	CTGGCCAGATGACGTCCCTCCTG	1448
AD-559274	usgsucauGfuGfuCfucagacugL96	1250	asCfsaguCfaUfGfaggauUfgAfuacacug	1345	CATGTATCATCTCTCATGACTGA	1449
AD-559290	ascstugauGfgAfuUfUfgcaacauL96	1251	asAfsuguUfgUfgfcaauCfeAfuacugesa	1346	TGATGTGATGGATTGCACAACATG	1450
AD-559306	ususcugUfcAfuUfUfgaagacauL96	1252	asGfsaucUfcAfuUfcauGfagauasusu	1347	AATTACTGTATGATGAGATCC	1451
AD-559329	gsascugCfuAfuAfcuaguccauL96	1253	asUfsugCfaAfuUfgauAfgCfaagucsc	1348	GGGACTGTCTATACATTTGGCAAG	1452
AD-559359	asascccaAfgGfgAfggaauuauL96	628	asAfsgauAfaUfCfuccCfuUfggguuusu	699	AAAACCCCAAGGAGGATTATCTG	1453
AD-559374	usascugGfaUfgUfUfcauuguuL96	621	asAfsacaCfaUfAfgacaUfcCfagauasusu	692	ATTATCTGGATGTCTATGTGTGT	1454
AD-559383	gsggcccUfuGfgUfgaaccaguuL96	1254	asAfsccuGfgUfUfcaacAfaAfgcccsag	1349	TCGGGCCTTTGGTGAACCAAGTG	1455
AD-559398	csasagugAfaCfaUfcauugcuuL96	633	asAfsaagCfaUfUfgaugUfhuCfauugsgu	704	ACCAAGTGAACATCAATGCTTTG	1456
AD-559421	ususcacaGfaAfaGfacaugagcuL96	217	asGfsucAfuUfGfucuuUfcUfuggaagsg	1350	GCTTCCAAGAAAAGACAATGAGCA	1457
AD-559438	asgscaacAfuGfuUfgfuaaagcuL96	1255	asGfsacuUfuGfaGfacaAfuGfuugcusa	1351	TGAGCAACATGTGTTCAAAGTCA	1458
AD-559455	gsuscaagGfaUfaUfUfgaaacuuL96	1256	asAfsaguUfuUfCfcauuUfcCfuagcusu	1352	AAGTCAAGGATATGGAAAACCTTG	1459
AD-559476	gsasagauGfuUfuUfUfcaaccuuL96	1257	asAfsuuuGfgUfaAfgaaaAfcAfuucscsa	1353	TGGAAGATGTTTTCTACCAAAATG	1460
AD-559497	asuscgaugGfaAfaGfaccagucuuL96	1258	asAfsagAfcUfgfgcuUfcAfuagucsa	1354	TGATCGATGAAAAGCCAGTCTCTG	1461
AD-559512	uscscugAfgUfCfuUfcugugcauL96	1259	asAfsugeCfaCfaGfagaCfuCfagagascu	1355	AGTCTGTAGTCTCTGTGGCATG	1462
AD-559536	usgsggaCfaCfaAfgfagagguuL96	1260	asGfsuacCfcUfUfuceugUfgUfuceasasa	1356	TTTGGGAACACAGGAAGGTACC	1463
AD-559556	csgsaunaCfeAfcAfgagcauuL96	1261	asAfsugUfuGfCfuuguGfgUfaucsgsu	1357	ACCGATTACCACAAGCAACCATG	1464
AD-559590	uscagucAfuUfCfGfcccuaauL96	1262	asUfsuugAfaGfGfgcgaAfuGfagugsga	1358	TCTCAGTCAATCGCCCTTCAAAG	1465
AD-559610	gsgsgacaCfgAfgGfAfgcugauugL96	1263	asCfscauAfcAfgfcuuCfGfugcccsusu	1359	AAGGACACGAGAGAGCTGTATGGG	1466
AD-559616	gsusggugUfcUfgGfAfguauuguuL96	624	asAfscaaAfgUfaAfcucaGfaCfaccacsag	695	CTGTGTGTCTGAGTACTTTGTG	1467
AD-559641	csasgcagCfaCfaUfUfguuuacuuL96	1264	asAfsugAfaAfcfaauUfgCfugcugusc	1360	GACAGCAGCACATGTTTTCACTG	1468
AD-559670	asasggaaCfaCfuUfcauacaguuL96	1265	asAfsccuUfgAfuUfgagUfgUfuccuugsu	1361	ACAAGGAACACATCAATCAAGGTC	1469
AD-559704	usasgaagUfaGfuUfCfcauauuacuuL96	1266	asGfsugaAfaUfaAfggacUfaCfuucuasusc	1362	GATAGAAGTAGTCTCTATTTTACC	1470
AD-559706	csasacuaCfaAfcAftuaauuggauL96	1267	asUfscccAfuUfaAfauguUfgUfaguugsgg	1363	CCCAACTACAACATTAATGGGAA	1471
AD-559722	asusuccuGfaAfuUfUfuaugacuuL96	634	asUfsaguCfaUfaAfaauUfcAfggaususc	705	GAATCTCTGAATTTTATGACTAT	1472
AD-559740	usaugacGfuUfgGfCfcuagacuuL96	1268	asUfsugaUfcAfgfgcgaAfcGfucuaugsu	1364	ACTATGACGTTCCTCTGATCAAG	1473
AD-559760	gscsucaaGfaAfuUfAfgcugaaauL96	1269	asAfsuuuCfaGfCfuauUfcUfugagcsusu	1365	AAGCTCAAGAATAAGCTGAAATA	1474
AD-559788	ascsuuacAfgGfCfcauugucuuL96	608	asAfsagAfaAfuUfgggCfuGfauugscu	679	AGACTATCAGGCCCATTTGTCTC	1475
AD-559799	asgsggaCfaAfcUfUfcagcuuugL96	208	asCfsaaaGfcUfCfagauUfgUfuccucsg	1366	CGAGGGAACAACCTGAGCTTTGA	1476
AD-559823	ususcucCfaAfcUfUfaccuugcuL96	1270	asGfscaaGfuGfGfuaguUfgGfagaagsg	1367	GCTTCTCTCCAACACTACCCTTGCC	1477
AD-559838	csusugccAfgCfaAfcfaaaaggaauL96	1271	asUfsuccUfuUfUfugCfuGfagaagsg	1368	CACTTGCCAGCAACAAGGAAG	1478
AD-559873	csasggauAfuCfaAfcagucuguuL96	1272	asAfsacaGfaGfCfuugAfuAfuucugsg	1369	CACAGGATATCAAAAGCTCTGTT	1479
AD-559892	usuguguCfuGfAfgfagaggaauL96	1273	asUfsuucUfcCfuUfccuAfgAfcacaasac	1370	GTTTGTGTCTGTGAGGAGGAGAAAA	1480

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-559926	ggsagguCfuAfCfaAuaaagauL96	1274	asCfsauCfuUfGfauuAfgAfccuacsusu	1371	AAGGAGGTCTACATCAAGAAATGG	1481
AD-559946	gsusgagaGfaGfAfUfgcaauauL96	615	asAfsuauUfgAfGfcauUfeUfcaacsag	686	CTGTGAGAGAGATGCTCAATATG	1482
AD-559958	gsascaaaCfuCfAfAfaggacauL96	209	asGfsagaUfgUfCfcuagAfeUfuaugcasu	1372	ATGACAAAAGTCAAGACATCTCA	1483
AD-559973	uscsgguuCfeUfUfUfguacuggagL96	1275	asCfsuacAfgUfAfaaaaGfgAfccagsgsg	1373	CCTCGGTTCCTTTGTTGATGGAGG	1484
AD-559993	gsasgugaGfuCfCfCfuauugcagL96	1276	asGfsucaGfeAfUfaggAfeUfcaucsu	1374	AGGAGTGAGTCCCTATGCTGACC	1485
AD-559998	usascuugCfaGfAfGfguagucugL96	1277	asCfsagaAfuCfAfccuUfgCfaaguasusu	1375	AATACTTGACAGAGGTGATCTGG	1486
AD-560011	asgsuuaCfaAfGfAfagaagucguuL96	199	asAfsacAfeUfUfcauUfgUfgaacsusu	1376	ATAGTTCAACAAGAGAAAGTCGTTT	1487
AD-560031	uscsaucAfaGfUfUfgguuaauL96	1278	asGfsauuAfeAfCfaacUfuGfaaugsasa	1377	TTTCATTCAAGTTGGTGTAAATCA	1488
AD-560040	gsasguagUfgGfAfUfguegcuaL96	1279	asUfsuugCfaGfAfaucCfaChiacuscsc	1378	GGGAGTAGTGGATGTCTGCAAAA	1489
AD-560060	asaccagAfaGfCfGfgcaaaagcaL96	1280	asUfsuugUfuUfGfcccUfuCfuguuusu	1379	AAAAACCAGAAAGCGGCAAAAGCAG	1490
AD-560099	gsascuuuCfaCfAfUfcaaccuuuL96	1281	asAfsagaGfgUfUfgauUfgAfaaguscsc	1380	GAGACTTTCACATCAACCTCTTT	1491
AD-560114	csuscuuuCfaAfGfUfGfgucuccuL96	1282	asCfsaggGfeAfGfcauUfgAfaagsgsu	1381	ACCTCTTTCAAGTGTGCTGCCCTGG	1492
AD-560138	asasggagAfaAfCfUfCfaaagauL96	1283	asUfscuuCfuUfGfgaguUfuChicuucsa	1382	TGAAGGAGAAACATCCAAAGATGAG	1493
AD-560156	gsasgauUfuGfGfGfuuuauauL96	1284	asUfsauaGfaAfAfaaccAfaAfuccuacsu	1383	ATGAGGATTTGGGTTTCTATAA	1494
AD-560163	csuguggAfuUfGfAfauuaaauL96	648	asUfsuuUfuAfaAfaaAfuCfcaagcsc	719	GGCGTGGGATTTGAATTAACACAG	1495
AD-558378	gscsaagCfaGfGfAfcaaccuL96	1285	asGfsuugGfuGfUfguUfgGfuuugcsusu	1384	AAGCAAGCAGGACACACCAUCC	1496
AD-558393	csusucueUfcCfuUfGfcauuccaL96	1286	asGfsuugGfaAfGfGcagGfaGfaagscsu	1385	AGCUUCUCUCCUGCCUCCAAACG	1497
AD-558424	csusuggGfCfUfCfUfugucugagL96	1287	asCfsuccAfgAfCfaaGfgCfcaagcsu	1386	AUCUUGGGCCUUCUUGUCUGGAGG	1498
AD-558466	asgsagauCfaAfAfGfgggccuL96	1288	asAfsaggGfcCfGfcauUfgAfuccuasc	1387	GUAGAGAUCAAAGGCGGCUCCU	1499
AD-558511	ascsuugaGfuAfCfGfugucuuL96	1289	asAfsaggAfeAfCfcauUfgUfcaagusc	1388	GCACUGGAGUACGUGUGUCCUUC	1500
AD-558574	cscsuagaGfaCfuUfCfaagaccuL96	1290	asUfsuugGfuCfuUfguUfgUfcaagsgsu	1389	ACCCUGAAGACUCAAGACCAAAA	1501
AD-558595	gsascuugCfaGfGfAfaggagauL96	1291	asAfsuccUfgCfCfuUfgUfcaagcsu	1390	AAGACUGUCAGGAAGGCAGAGUG	1502
AD-558750	usgsccacAfaGfGfAfagguggcL96	1292	asUfsgccCfaCfCfuUfgUfcaagcsu	1391	AUUGGCACAAAGGAAGGUGGCGAG	1503
AD-558777	cscsgcuUfgAfAfGfagcgcuaL96	1293	asUfsgacGfeUfGfuuuCfaAfgcggsu	1392	UACCGCCUUGAAGACAGCGUCAC	1504
AD-559105	csusgaagCfaGfAfCfagcaguuL96	1294	asAfsuuaCfuGfCfugueUfgCfuacgsac	1393	GUCUGAAGCAGACAGCAGUAAUG	1505
AD-559124	usgscaagCfuGfGfGfagcagcuL96	1295	asGfscuuCfUfGfagcAfgUfcaagcsu	1394	AAUGCAGACUGGGGUCACGAAGCA	1506
AD-559189	usasacacCfaAfGfAfaggccuL96	1296	asGfsgagGfgCfCfuUfgUfcaagcsu	1395	ACUAAACCAAGAAAGGCCCUCCA	1507
AD-559226	asusgagCfuGfCfCfagauagcuL96	1297	asAfsuugCfuUfCfugcCfaGfcauacsa	1396	UGAUGAGCUGGCCAGAUAGCGUC	1508
AD-559330	ascsuuugCfaUfAfCfauugcagcuL96	1298	asCfsuugCfeAfAfuuaUfaGfcaagusc	1397	GGACUUGCUAUACAUAUGGCAAGG	1509
AD-559486	uscnuaccAfaAfUfGfauagauL96	1299	asUfsucaUfeGfAfuuaUfuGfuaagasa	1398	UUUCUACCAAAUGAUCGAUAAA	1510
AD-559532	gsasuuugGfGfAfCfCfagcagcuL96	1300	asCfsuucCfeUfGfuuuUfuCfcaagcsu	1399	AUGGUUGGGAACACAGAAAGGG	1511
AD-559573	csasuggcAfgGfCfCfagauuL96	1301	asUfsgagAfuCfuUfgcCfuGfcaagsgsu	1400	ACCAUGGCAGGCCAAGAAUAGG	1512
AD-559609	asgsaggCfCfGfAfGfagcuauL96	1302	asCfsuuaCfaGfCfuucGfuGfuccuusu	1401	AAAGGGACACGAGAGAGCUUAGG	1513
AD-559668	ascsaaggAfaCfAfCfcauacagcuL96	1303	asCfsuugAfuUfGfagugUfuCfcaagcsu	1402	UGACAAAGGAACACACUCAAAGG	1514

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-559688	asasgcggGfaCfCfUfggagauagauL96	1304	asUfscuaUfcUfCfcaggUfcCfcgcuuscsu	1403	AGAAAGCGGGACCUUGGAGAUAGAA	1515
AD-559882	asasagcuChuGfUfUfugugucugauL96	1305	asUfscagAfcAfCfaaacAfgAfgcuuusgsa	1404	UCAAAGCUCUCUGUUUGUGUCUGAG	1516
AD-560132	usgscugAfaGfGfAfgaaacuccauL96	1306	asUfsggaGfuUfUfuceUfuCfagccasgsg	1405	CCUGGCUGAAGGAGAAACUCCAA	1517

Table 21. In Vitro Single Dose Screen in HepG2 cells

Duplex Name	CFB/gapdh		Target region in NM_001710.5
	10 nM		
	% average message remaining	SD	
AD-560132.1	9.927	1.092	2527-2549
AD-560099.1	9.213	1.335	2494-2516
AD-559998.1	11.297	1.165	2355-2377
AD-559993.1	11.852	1.080	2330-2352
AD-559973.1	53.866	5.431	2310-2332
AD-559882.1	10.290	1.623	2161-2183
AD-559706.1	11.537	1.805	1947-1969
AD-559704.1	15.239	3.563	1924-1947
AD-559688.1	38.443	9.924	1909-1931
AD-559668.1	10.169	0.593	1871-1893
AD-559641.1	29.195	3.820	1844-1866
AD-559609.1	88.678	6.099	1793-1815
AD-559590.1	35.152	4.022	1774-1796
AD-559573.1	38.212	6.701	1757-1779
AD-559532.1	74.766	13.877	1716-1738
AD-559486.1	24.644	9.120	1670-1692
AD-559330.1	95.265	4.050	1496-1518
AD-559274.1	13.751	2.733	1422-1444
AD-559226.1	102.524	5.658	1372-1394
AD-559208.1	16.066	2.069	1354-1376
AD-559189.1	16.100	2.031	1335-1357
AD-559124.1	15.423	2.069	1269-1291
AD-559105.1	9.673	1.193	1250-1272
AD-559089.1	10.663	2.329	1234-1256
AD-558935.1	13.757	1.222	1042-1064
AD-558879.1	14.752	3.406	967-989
AD-558777.1	14.623	2.074	819-841
AD-558750.1	16.390	2.306	792-814
AD-558637.1	24.702	7.767	591-613
AD-558612.1	12.506	1.594	566-588
AD-558595.1	11.413	1.630	549-571
AD-558574.1	12.253	1.051	528-550
AD-558555.1	12.550	1.821	491-513
AD-558511.1	10.434	4.050	447-469
AD-558482.1	47.746	10.594	418-440
AD-558466.1	17.794	3.389	402-424
AD-558450.1	11.521	2.865	347-369
AD-558424.1	23.751	5.256	321-343
AD-558407.1	14.394	1.584	304-326
AD-558393.1	14.017	1.825	254-276

Duplex Name	CFB/gapdh		
	10 nM		
	% average message remaining	SD	Target region in NM_001710.5
AD-558378.1	18.296	0.945	219-241
AD-558361.1	15.926	1.921	202-224
AD-558312.1	27.703	7.359	153-175

Example 6. Combinations of dsRNA agents targeting combinations of complement components

In order to determine whether hemolytic activity can be strongly suppressed using a combination of a dsRNA agent targeting complement component C3 (C3) and a dsRNA agent targeting complement component C5 (C5) or a dsRNA agent targeting complement component factor B (CFB) as compared to use of a single dsRNA targeting C3, C5, or CFB alone, *in vitro* double reconstitution studies were performed.

Briefly, *in vitro* complement combination modeling was conducted using sera depleted of two complement components and adding individual proteins back at various concentrations. All reagents were purchased from Complement Technology (Tyler, Texas), unless otherwise stated. An Alternative Hemolysis (AH) assay was performed by reconstituting C3 and CFB Complement component depleted human sera with a range of concentrations of C3 and CFB protein. Ten percent reconstituted serum was added to GVBE and 5 mM MgEGTA with 25% rabbit erythrocytes (Er). Samples were incubated at 37°C for one hour, with shaking. Hemolysis was stopped with addition of GVBE at a 1:1 ratio to samples. Samples were centrifuged, supernatants were transferred, and absorbance measured at 541 nm. Hemolytic activity was calculated by subtracting negative control sample and normalizing to positive control samples, where negative control was buffer and Er only and positive control was water and Er only.

The results of dual targeting of C3 and CFB are depicted in Figure 2A. In particular, the hemolytic activity (alternative hemolysis, AH) in human sera depleted of C3 and CFB is shown as a heatmap with higher hemolysis levels being medium gray (top left corner, “normal range”) and lower being darker gray. The concentration of C3 was plotted on the Y axis and the concentration of CFB on the X axis.

Normal levels of CFB and C3 yielded full hemolytic activity. Decreasing either C3 or CFB level decreased hemolytic activity. siRNA administration in non-human primates showed that C3 levels could be suppressed to 200 µg/ml levels; a similar reduction is expected in human sera. Assuming 200 µg/ml as the initial level of C3, Figure 2A demonstrates that suppression of CFB to about 40 µg/ml (about 80% silencing) reduces hemolytic activity to levels below 10%. Therefore, dual targeting of C3 and CFB can achieve near-complete suppression of AH.

It should be noted that CFB suppression does not impact Classical Hemolysis (CH), therefore no combination data was generated for CH.

Figure 2B depicts the results of dual dose response of C3 and C5 in depleted serum reconstituted with varying levels of C3 and C5 and assayed for AH as described above. C3 was plotted on the Y-axis and C5 on the X-axis. Normal levels of both C3 and C5 produced high levels of hemolytic activity; decreasing C5 levels lowered the AH. A dose response for C3 was also observed.

- 5 Although it is known that administration of a dsRNA agent targeting C5, cemdisiran, to human subjects can achieve C5 silencing down to the range of about 1 - 3 µg/ml, further decrease in hemolytic activity can be achieved by concurrently decreasing the level of C3 protein.

- The effect of dual targeting C3 and C5 on classical hemolytic activity was also determined using a Classical Hemolysis (CH) assay. C3 and C5 depleted human sera was reconstituted with a range of concentrations of C3 and C5 proteins. Reconstituted serum (0.7%) was added to GVB⁺⁺ with 13.4% antibody sensitized sheep erythrocytes (EA). Samples were incubated at 37°C for one hour, with shaking. Samples were centrifuged, supernatants were transferred, and absorbance measured at 541 nm. Hemolytic activity was calculated by subtracting negative control sample and normalizing to positive control samples, where negative control was buffer and Er only and positive control was water and Er only.

- Figure 2C depicts the results of C3 and C5 CH reconstitution experiments; it demonstrates that targeting both C3 and C5 resulted in benefit on CH. The observed effect of C3 suppression on CH activity when C5 is ≤ 3 µg/ml could not be resolved by determining the level of active C5b-9 formation to assess classical hemolysis activity using the Wieslab[®] Complement Classical Pathway (CCP) assay (Figure 2D). The CCP assay (Wieslab[®] COMPL CP310, IBL America) was conducted according to manufacturer's protocol. Briefly, in vitro complement combination modeling was conducted using sera depleted of two complement components and adding individual proteins back at various concentrations. C3 and C5 Complement component depleted human sera was reconstituted with a range of concentrations of C3 and C5 protein. Reconstituted depleted sera were diluted to a final sera concentration of 1:101. Samples were added to wells and incubated for 1 hour at 37°C. Plates were washed three times and then 100 µl of conjugate solution was added to each well. Plates were incubated for 30 minutes at room temperature then washed three times. Substrate solution (100 µl) was added to each well and incubated for 30 minutes at room temperature. Reaction was stopped with 100 µl of 5 mM EDTA and absorbance was read at 405 nm. Activity was calculated by subtracting blank control from all values and then normalizing to positive control.

- The ability of a combination of a dsRNA agent targeting complement component C3 (C3) and a dsRNA agent targeting complement component C5 (C5) or complement component factor B (CFB) to further suppress hemolytic activity as compared to use of a single dsRNA targeting C3, C5, or CFB alone was also assessed *in vivo* in non-human primates (NHPs), cynomolgus monkeys (*Macaca fascicularis*).

Cynomolgus monkeys were subcutaneously administered a single 6 mg/kg dose of a dsRNA agent targeting C3; or a dsRNA agent targeting CFB; or a dsRNA agent targeting C5; or a single 6

mg/kg dose of a dsRNA agent targeting C3 and 6 mg/kg dose of a dsRNA agent targeting CFB; or a single 6 mg/kg dose of a dsRNA agent targeting C3 and 6 mg/kg dose of a dsRNA agent targeting C5; or a single 6 mg/kg dose of a dsRNA agent targeting CFB and 6 mg/kg dose of a dsRNA agent targeting C5 on Day 1. The study design is depicted in the Table below.

Group	Target(s)	Test Article	Dose Route	Dose (mg/kg)	Number of Male Animals	Dose Regimen	End of Study
2	C3	AD-570714	SubQ	6	3	Day 1	Day 29
3	CFB	AD-560018	SubQ	6	3	Day 1	Day 29
4	C5	AD-61679	SubQ	6	3	Day 1	Day 29
5	C3 CFB	AD-570714 AD-560018	SubQ	6 6	3	Day 1	Day 29
6	C3 C5	AD-570714 AD-61679	SubQ	6 6	3	Day 1	Day 29
7	CFB C5	AD-560018 AD-61679	SubQ	6 6	3	Day 1	Day 29

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On Day -6, 1 pre-dose, and Days 8, 15, 22, and 29 post-dose, serum samples were obtained from the NHPs and the levels of C3, C5, and CFB protein and the alternative and classical hemolytic activities and the Wieslab® CAP and CCP activities were determined.

10 The level of C3 protein was determined by ELISA. Briefly, C3 protein was measured by a cynomolgus cross-reactive ELISA (C3 Human ELISA, Hycult HK366) according to the manufacturer's protocol. Serum was diluted by 1:40,000. C3 levels were normalized to individual animal's pre-dose levels to determine percent of C3 remaining.

15 The level of C5 protein was also determined by ELISA. Briefly, C5 was measured by a cynomolgus cross-reactive ELISA (Human Complement C5 ELISA Kit, Abcam ab125963) according to the manufacturer's protocol. Serum was diluted by 1:20,000 for pre-dose and day 8 samples and 1:5,000 for silenced samples days 12, 22, and 29. C5 levels were normalized to individual animal's pre-dose levels to determine % C5 remaining.

20 Serum CFB was measured at 1:20 dilution by quantitative analysis of western blot, using 4-12% Bis-Tris gels and imaged on Li-Cor Odyssey CLx. (1°: ProteinTech 10170-1-AP 1:50, 2°: Goat anti-Rabbit HRP).

The alternative and classical hemolytic activities were determined. The NHP alternative hemolysis was performed as briefly described. Serum (5.6%) was added to GVB° (Complement Technology, Tyler, Texas) and 5 mM MgEGTA with 25% rabbit erythrocytes (Er, Complement Technology, Tyler, Texas). Samples were incubated at 37°C for 1 hour, with shaking. Hemolysis was

stopped with addition of GVB_E (Complement Technology, Tyler, Texas) at a 1:1 ratio to samples. Samples were centrifuged, supernatants were transferred, and absorbance measured at 541 nm. Hemolytic activity was calculated by subtracting negative control sample and normalizing to positive control samples, where negative control was buffer and Er only and positive control was water and Er only. Then individual animal samples were normalized to their average pre-dose samples. The NHP classical pathway hemolysis was performed as briefly described. 1.77% serum was added to GVB++ (Complement Technology, Tyler, Texas) with 13.4% antibody sensitized sheep erythrocytes (EA, Complement Technology, Tyler, Texas). Samples were incubated at 37°C for 1 hour, with shaking. Samples were centrifuged, supernatants were transferred, and absorbance measured at 541 nm.

Hemolytic activity was calculated by subtracting negative control sample and normalizing to positive control samples, where negative control was buffer and Er only and positive control was water and EA only. Then individual animal samples were normalized to their average pre-dose samples.

The level of active C5b-9 formation to assess alternative hemolysis activity and classical hemolysis activity was also determined using the Wieslab® Complement Classical Pathway (CCP) assay as described above and the Wieslab® Complement Alternative Pathway (CAP) assay. The CAP assay (COMPL AP330 RUO, IBL America) was conducted according to manufacturer's protocol. Sera were diluted to a final sera concentration of 1:18. Samples were added to wells and incubated for 1 hour at 37°C. Plates were washed 3 times and then 100 µl of conjugate solution was added to each well. Plates were incubated for 30 minutes at room temperature then washed 3 times. Substrate solution (100 µl) was added to each well and incubated for 30 minutes at room temperature. Reaction was stopped with 100 µl of 5mM EDTA and absorbance was read at 405 nm. Activity was calculated by subtracting blank control from all values and then normalizing to positive control. For both CAP and CCP activity values were then normalized to individual animals' average pre-dose.

The results of these assays are depicted in Figures 3A-3E. In particular, Figure 3A demonstrates that a single 6 mg/kg dose of a dsRNA agent targeting C3 suppressed C3 protein up to 90% (~110 µg/ml) in serum. As expected, silencing CFB with a dsRNA agent targeting CFB caused C3 protein levels to slightly increase. Figure 3A also demonstrates that silencing C5 with a dsRNA agent targeting C5 left C3 protein levels unaffected and that neither a dsRNA agent targeting C3 nor a dsRNA agent targeting CFB affected C5 protein levels.

Figure 3B demonstrates that a single 6 mg/kg dose of a dsRNA agent targeting C3 or a single 6 mg/kg dose of a dsRNA agent targeting CFB showed similar suppression of alternative hemolysis (about 60% suppression) and the combination of a single 6 mg/kg dose of a dsRNA agent targeting C3 and a 6 mg/kg dose of a dsRNA agent targeting CFB suppressed alternative hemolysis by about 90%. Figure 3C demonstrates that the combination of a single 6 mg/kg dose of a dsRNA agent targeting C3 and 6 mg/kg dose of a dsRNA agent targeting C5 have the greatest impact on classical hemolysis.

As depicted in Figure 3D, using the Wieslab® Complement alternative Pathway (CAP) assay, silencing either CFB or C3 or silencing CFB and C3, CFB and C5, and C3 and C5 inhibited alternative pathway activity. Silencing C5 alone had an intermediate effect in the Wieslab® CAP assay.

5 Figure 3E demonstrates that C3 or CFB suppression did not confer a benefit on silencing classical pathway activity beyond that of C5 silencing.

 In summary, the combination of a dsRNA agent targeting C3 and a dsRNA agent targeting CFB effectively suppressed alternative hemolysis activity to less than about 10%, while the combination of a dsRNA agent targeting C3 and a dsRNA agent targeting C5 effectively suppresses
10 classical hemolysis activity.

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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.

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