COMPLEMENT COMPONENT iRNA COMPOSITIONS AND METHODS OF USE THEREOF

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 December 11, 2014, is named 121301-01120_SL.txt and is 266,080 bytes in size.

Related Applications

This application claims the benefit of priority to U.S. Provisional Patent Application No.: 61/915,210, filed December 12, 2013, the entire contents of which are incorporated herein by reference.

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).

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 and/or initiating pathology in many different diseases, including, for example, paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome, rheumatoid arthritis, ischemia-reperfusion injuries and neurodegenerative diseases.

To date, only one therapeutic that targets 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) and atypical hemolytic uremic syndrome (aHUS) 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 alternative therapies and combination therapies for subjects having a complement component-associated disease.

Summary

The present disclosure provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a CFB gene. The CFB gene may be within a cell, *e.g.,* a cell within a subject, such as a human.

The present disclosure also provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a C3 gene. The C3 gene may be within a cell, *e.g.,* a cell within a subject, such as a human.

In one aspect, the present disclosure refers to a double-stranded ribonucleic acid (dsRNA) for inhibiting expression of complement component C3 in a cell, wherein said dsRNA comprises a sense strand and an antisense strand, wherein each strand comprises 15-30 nucleotides and said strands form a double-stranded region, and wherein the antisense strand comprising at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the following antisense sequences:

a. GAGAGAAGACCUUGACCACGUAG (SEQ ID NO:282);

b. UAGAGAAGACCUUGACCACGUAG (SEQ ID NO:267);

c. AGAGAGAAGACCUUGACCACGUA (SEQ ID NO:266);

d. AGAGAAGACCUUGACCACGUAGG (SEQ ID NO:299); and wherein at least one nucleotide in said dsRNA comprises a modification.

In another aspect, the present disclosure refers to a cell containing the dsRNA as disclosed herein.

In another aspect, the present disclosure refers to a pharmaceutical composition for inhibiting expression of a complement component C3 gene comprising the dsRNA as disclosed herein.

In another aspect, the present disclosure refers to an *in vitro* method of inhibiting complement component 3 (C3) expression in a cell, the method comprising:

(a) contacting the cell with the dsRNA as disclosed herein or the pharmaceutical composition as disclosed herein; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a C3 gene, thereby inhibiting expression of the C3 gene in the cell.

In another aspect, the present disclosure refers to the use of the dsRNA as disclosed herein or the pharmaceutical composition as disclosed hereiin the manufacture of a medicament for treating a complement component-associated disease.

In adition, the present disclosure provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a C9 gene. The C9 gene may be within a cell, *e.g.,* a cell within a subject, such as a human.

The present disclosure also provides methods and combination therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a CFB, C3, and/or C9 gene, *e.g.,* a complement component-associated disease, such as paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS) using iRNA compositions which effect the RNA-induced silencing

complex (RISC)-mediated cleavage of RNA transcripts of a CFB, C3, and/or C9 gene for inhibiting the expression of a CFB, C3, and/or C9 gene.

Accordingly, in one example the present disclosure provides double-stranded ribonucleic acids (dsRNA) for inhibiting expression of complement factor B (CFB) in a cell, wherein the dsRNA comprises a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequence of SEQ ID NOs:1-5, 27, and 30, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequence of SEQ ID NOs:12-16, 33, and 36.

In another example the present disclosure provides double-stranded ribonucleic acids (dsRNA) for inhibiting expression of complement factor B (CFB) in a cell, wherein the dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences listed in Table 3 and 4.

In one example, the sense and antisense strands comprise sequences selected from the group consisting of AD-60304, AD-60331, and AD-60344 and any one of the agents listed in Tables 3 and 4.

In one example the region of complementarity consists of the nucleotide sequence of one of the antisense sequences of any one of Tables 3 and 4.

In one example, the dsRNA comprises a sense strand consisting of the nucleotide sequence of a sense strand sequence selected from the sequence of any one of Tables 3 and 4, and an antisense strand consisting of the nucleotide sequence of an antisense sequence selected from the sequences of any one of Tables 3 and 4.

In another example the present disclosure provides double-stranded ribonucleic acids (dsRNA) for inhibiting expression of complement component C3 in a cell, wherein the dsRNA comprises a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of

the nucleotide sequence of SEQ ID NOs:6-8, 28, and 31, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequence of SEQ ID NOs:17-19, 34, and 37.

In another example the present disclosure provides double-stranded ribonucleic acids (dsRNA) for inhibiting expression of complement component C3 in a cell, wherein the dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences listed in Table 5 and 6.

In one example, the sense and antisense strands comprise sequences selected from the group consisting of AD-60169 and any one of the agents listed in Tables 5 and 6.

In one example the region of complementarity consists of the nucleotide sequence of one of the antisense sequences of any one of Tables 5 and 6.

In one example, the dsRNA comprises a sense strand consisting of the nucleotide sequence of a sense strand sequence selected from the sequence of any one of Tables 5 and 6, and an antisense strand consisting of the nucleotide sequence of an antisense sequence selected from the sequences of any one of Tables 5 and 6.

In another example the present disclosure provides double-stranded ribonucleic acids (dsRNA)for inhibiting expression of complement component C9 in a cell, wherein the dsRNA comprises a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequence of SEQ ID NOs:9-11, 29, and 32, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequence of SEQ ID NOs:20-22, 35, and 38.

In another example the present disclosure provides double-stranded ribonucleic acids (dsRNA) for inhibiting expression of complement component C9 in a cell, wherein the dsRNA comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences listed in Table 7 and 8.

In one example, the sense and antisense strands comprise sequences selected from the group consisting of any one of the agents listed in Tables 7 and 8.

In one example the region of complementarity consists of the nucleotide sequence of one of the antisense sequences of any one of Tables 7 and 8.

In one example, the dsRNA comprises a sense strand consisting of the nucleotide sequence of a sense strand sequence selected from the sequence of any one of Tables 7 and 8, and an antisense strand consisting of the nucleotide sequence of an antisense sequence selected from the sequences of any one of Tables 7 and 8.

The dsRNA may include at least one modified nucleotide, *e.g.,*a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a deoxy-nucleotide, a 3'-

terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group, a 2'-deoxy-2'-fluoro modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, and a nucleotide comprising a 5'-phosphate mimic.

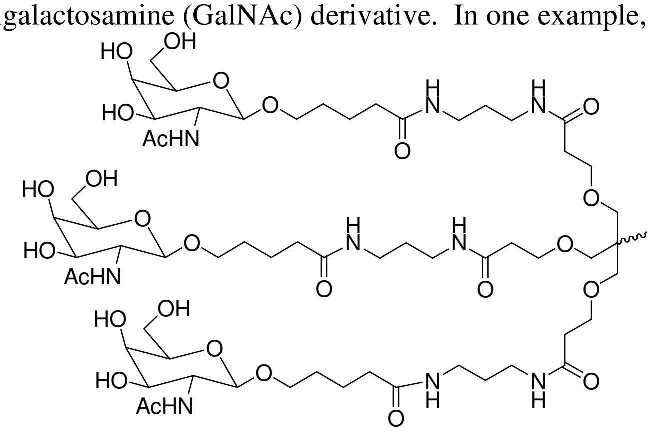
In one example, substantially all the nucleotides of the sense strand and the antisense strand are modified nucleotides. In another example, all the nucleotides of the sense strand and the antisense strand are modified nucleotides.

The the region of complementarity may be at least 17 nucleotides in length, such as 19 nucleotides in length, or no more than 30 nucleotides in length.

The region of complementarity may be between 19 and 21 nucleotides in length.

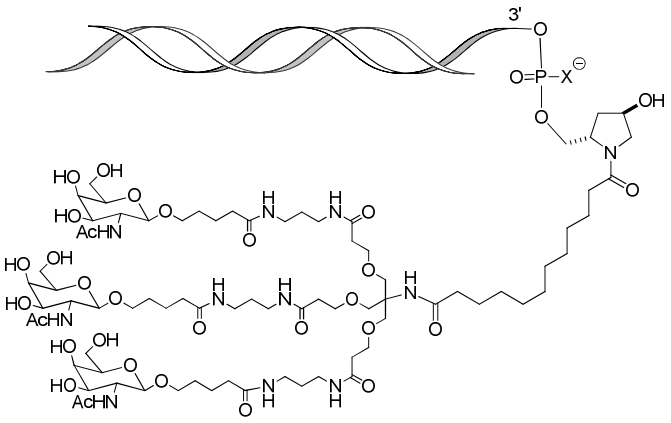
At least one strand of the dsRNA may include a 3' overhang of at least 1 nucleotide, or at least 2 nucleotides.

The dsRNA omay further include a ligand. In one example, the ligand is conjugated to the 3' end of the sense strand of the dsRNA. In one example, the ligand is an N-acetylgalactosamine (GalNAc) derivative. In one example, the ligand is



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



and, wherein X is O or S.

In one example, the X is O.

In another example, the present disclosure provides double stranded RNAi agents capable of inhibiting the expression of complement factor B (CFB) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CFB, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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

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;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand.

In another example, the present disclosure provides double stranded RNAi agents capable of inhibiting the expression of complement component 3 (C3) in a cell. The agents

include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C3, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

each N_a and N_a′ independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b′ independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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

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;

modifications on N_b differ from the modification on Y and modifications on N_b′ differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand.

In a further example, the present disclosure provides double stranded RNAi agents capable of inhibiting the expression of complement component 9 (C9) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C9, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

each N_a and N_a′ independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b′ independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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

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;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand.

In one example, i is 0; j is 0; i is 1; j is 1; both i and j are 0; or both i and j are 1.

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

In one example, XXX is complementary to X′X′X′, YYY is complementary to Y′Y′Y′, and ZZZ is complementary to Z′Z′Z′.

In one example, the YYY motif occurs at or near the cleavage site of the sense strand.

In one example, the Y′Y′Y′ motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end.

In one example, the Y′ is 2′-O-methyl.

In one example, formula (III) is represented by formula (IIIa):

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

antisense: 3' $n_{p'}$-$N_{a'}$- Y′Y′Y′- $N_{a'}$- $n_{q'}$ 5' (IIIa).

In one example, formula (III) is represented by formula (IIIb):

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

antisense: 3' $n_{p'}$-$N_{a'}$- Y′Y′Y′-$N_{b'}$-Z′Z′Z′- $N_{a'}$- $n_{q'}$ 5' (IIIb)

wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides.

In one example, formula (III) is represented by formula (IIIc):

sense: 5' n_p -N_a –X X X -N_b -Y Y Y -N_a - n_q 3'

antisense: 3' $n_{p'}$-$N_{a'}$- X′X′X′-$N_{b'}$- Y′Y′Y′- $N_{a'}$- $n_{q'}$ 5' (IIIc)

wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides.

In one example, formula (III) is represented by formula (IIId):

sense: 5' n_p -N_a –X X X- N_b -Y Y Y -N_b -Z Z Z -N_a - n_q 3'

antisense: 3' $n_{p'}$-$N_{a'}$- X′X′X′- $N_{b'}$-Y′Y′Y′-$N_{b'}$-Z′Z′Z′- $N_{a'}$- $n_{q'}$ 5'

(IIId)

wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides and each N_a and N_a' independently represents an oligonucleotide sequence comprising 2-10 modified nucleotides.

The double-stranded region may 15-30 nucleotide pairs in length, 17-23 nucleotide pairs in length, 17-25 nucleotide pairs in length, 23-27 nucleotide pairs in length, 19-21 nucleotide pairs in length, or 21-23 nucleotide pairs in length.

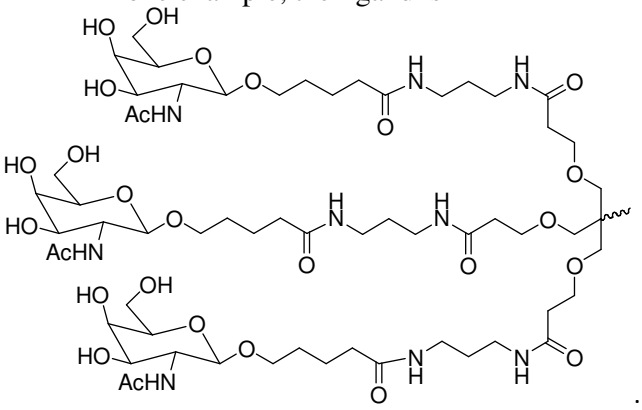
In one example, each strand has 15-30 nucleotides.

In one example, 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 combinations thereof.

In one example, the modifications on the nucleotides are 2′-O-methyl or 2′-fluoro modifications.

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

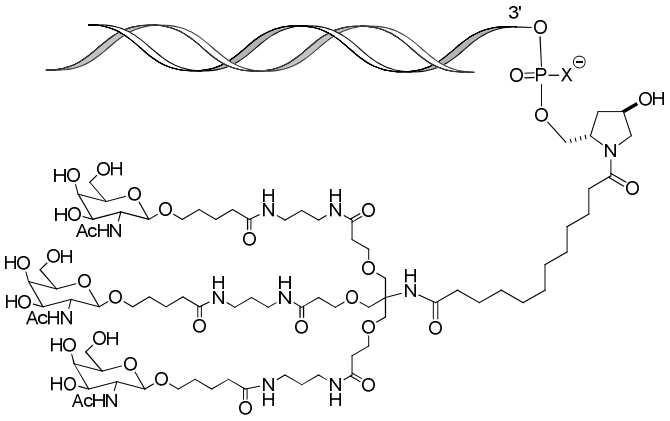
In one example, the ligand is



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In one example, the ligand is attached to the 3′ end of the sense strand.

In one example, the RNAi agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

In one example, the X is O.

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

In one example, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. In one example, the strand is the antisense strand. In another example, the strand is the sense strand.

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

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

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

In one example, the Y nucleotides contain a 2′-fluoro modification.

In one example, the Y′ nucleotides contain a 2′-O-methyl modification.

In one example, p′>0. In another example, p′=2.

In one example, q'=0, p=0, q=0, and p' overhang nucleotides are complementary to the target mRNA.

In another example, q'=0, p=0, q=0, and p' overhang nucleotides are non-complementary to the target mRNA.

In one example, the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

In one example, at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage. In another example, all n_p' are linked to neighboring nucleotides via phosphorothioate linkages.

In one example, the RNAi agent is selected from the group of RNAi agents listed in Tables 3 and 4. In one example, the RNAi agent is selected from the group of RNAi agents AD-60304, AD-60331, and AD-60344.

In another example, the RNAi agent is selected from the group of RNAi agents listed in Tables 5 and 6.

In yet another example, the RNAi agent is selected from the group of RNAi agents listed in Tables 7 and 8.

In one example, the present disclosure provides double stranded RNAi agents comprising the RNAi agents listed in any one of Tables 3, 5, and 7.

In one example, the present disclosure provides compositions comprising a modified antisense polynucleotide agent. The agents are capable of inhibiting the expression of Complement Factor B (CFB) in a cell, and include a sequence complementary to a sense sequence selected from the group of the sequences listed in Table 3, wherein the polynucleotide is about 14 to about 30 nucleotides in length.

In another example, the present disclosure provides compositions comprising a modified antisense polynucleotide agent. The agents are capable of inhibiting the expression of Complement Component 3 (C3) in a cell, and include a sequence complementary to a sense sequence selected from the group of the sequences listed in Table 5, wherein the polynucleotide is about 14 to about 30 nucleotides in length.

In yet another example, the present disclosure provides compositions comprising a modified antisense polynucleotide agent. The agents are capable of inhibiting the expression of Complement Component 9 (C9) in a cell, and include a sequence complementary to a sense sequence selected from the group of the sequences listed in Table 7, wherein the polynucleotide is about 14 to about 30 nucleotides in length.

In one example, the present disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Factor B (CFB) in a cell. The agent include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CFB, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

each N_a and N_a′ independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b′ independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b′ differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand.

In another example, the disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Factor B (CFB) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CFB, wherein each strand is about 14 to

about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand.

In another example, the present disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Factor B (CFB) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CFB, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-

25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In yet a further example, the present disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Factor B (CFB) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CFB, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′;

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In another example, the disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Factor B (CFB) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding CFB, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

antisense: 3' n_p′-N_a′- Y′Y′Y′- N_a′- n_q′ 5' (IIIa)

wherein:

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

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

n_p′ >0 and at least one n_p′ is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a′ independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

YYY and Y′Y′Y′ each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one example, the present disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Component 3 (C3) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C3, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand.

In another example, the present disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Component 3 (C3) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C3, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b'

differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand.

In another example, the disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Component 3 (C3) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C3, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

n_p′ >0 and at least one n_p′ is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a′ independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b′ independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b′ differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In yet another example, the present invemtion provides double stranded RNAi agents capable of inhibiting the expression of Complement Component 3 (C3) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C3, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′;

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one example, the present disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Component 3 (C3) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C3, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

antisense: 3' n_p'-N_a'- Y′Y′Y′- N_a'- n_q' 5' (IIIa)

wherein:

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

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

n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

YYY and Y′Y′Y′ each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In another example, the present disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Component 9 (C9) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C9, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

each N_a and N_a′ independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b′ independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b′ differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand.

In one example, the disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Component 9 (C9) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C9, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand.

In another example, the imvention provides double stranded RNAi agents capable of inhibiting the expression of Complement Component 9 (C9) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C9, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

n_p' >0 and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In another example, the disclosure provides double stranded RNAi agents capable of inhibiting the expression of Complement Component 9 (C9) in a cell. The agents include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C9, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

$n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

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, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y′;

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In a further example, the present disclosure provises double stranded RNAi agents capable of inhibiting the expression of Complement Component 9 (C9) in a cell. The agent include a sense strand complementary to an antisense strand, wherein the antisense strand comprises a region complementary to part of an mRNA encoding C9, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

antisense: 3' n_p′-N_a′- Y′Y′Y′- N_a′- n_q′ 5' (IIIa)

wherein:

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

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

n_p′ >0 and at least one n_p′ is linked to a neighboring nucleotide via a phosphorothioate linkage;

each N_a and N_a′ independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

YYY and Y′Y′Y′ each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2′-O-methyl or 2′-fluoro modifications;

wherein the sense strand comprises at least one phosphorothioate linkage; and

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In another example, the disclosure provides double stranded RNAi agents for inhibiting expression of complement factor B (CFB) in a cell, wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of any one of SEQ ID NOs:1-5, 27, and 30, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequence of SEQ ID NOs:12-16, 33, and 36, wherein substantially all of the nucleotides of the sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus, wherein substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the antisense strand comprises two phosphorothioate

internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and

wherein the sense strand is conjugated to one or more GalNAc derivatives attached through a branched bivalent or trivalent linker at the 3'-terminus.

In another example, the present disclosure provides double stranded RNAi agents for inhibiting expression of complement component C3 in a cell, wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of any one of SEQ ID NOs:6-8, 28, and 31, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequence of SEQ ID NOs:17-19, 34, and 37, wherein substantially all of the nucleotides of the sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus, wherein substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and wherein the sense strand is conjugated to one or more GalNAc derivatives attached through a branched bivalent or trivalent linker at the 3'-terminus.

In yet another example, the present disclosure provides double stranded RNAi agents for inhibiting expression of complement component C9 in a cell, wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of any one of SEQ ID NOs:9-11, 29, and 32, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequence of SEQ ID NOs:20-22, 35, and 38, wherein substantially all of the nucleotides of the sense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus, wherein substantially all of the nucleotides of the antisense strand comprise a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and

wherein the sense strand is conjugated to one or more GalNAc derivatives attached through a branched bivalent or trivalent linker at the 3'-terminus.

In one example, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In another example, the present disclosure provides cells containing the agents of the disclosure.

In one example, the disclosure provides vectors encoding at least one strand of the agents of the disclosure.

In another example, the disclosure provides cells comprising the vectors of the disclosure.

In one example, the present disclosure provides pharmaceutical compositions for inhibiting expression of a complement component factor B gene comprising the agents the disclosure.

In another example, the present disclosure provides pharmaceutical compositions for inhibiting expression of a complement component C3 gene comprising the agents of the disclosure.

In yet another example, the present disclosure provides pharmaceutical compositions for inhibiting expression of a complement component C9 gene comprising the agents of the disclosure.

In one example, the RNAi agent is administered in an unbuffered solution.

In one example, the unbuffered solution is saline or water.

In one example, the RNAi agent is administered with a buffer solution.

In one example, the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

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

In one example, the present disclosure provides methods of inhibiting complement factor B (CFB) expression in a cell. The methods include contacting the cell with the agent of athe disclosure or a pharmaceutical composition of the disclosure, and maintaining the cell produced for a time sufficient to obtain degradation of the mRNA transcript of a CFB gene, thereby inhibiting expression of the CFB gene in the cell.

In another example, the present disclosure provides methods of inhibiting complement component 3 (C3) expression in a cell. The methods include contacting the cell with the agent of athe disclosure or a pharmaceutical composition of the disclosure, and maintaining the cell produced for a time sufficient to obtain degradation of the mRNA transcript of a C3 gene, thereby inhibiting expression of the C3 gene in the cell.

In yet another example, the present disclosure provides methods of inhibiting complement component 9 (C9) expression in a cell. The methods include contacting the cell with the agent of athe disclosure or a pharmaceutical composition of the disclosure, and maintaining the cell produced for a time sufficient to obtain degradation of the mRNA transcript of a C9 gene, thereby inhibiting expression of the C9 gene in the cell.

In one example, the cell is within a subject.

In one example, the subject is a human.

In one example, the human subject suffers from a complement component-associated disease.

In one example, the complement component-associated disease is selected from the group consisting of paroxysmal nocturnal hemoglobinuria (PNH), asthma, rheumatoid arthritis, systemic lupus erythmatosis, glomerulonephritis, psoriasis, dermatomyositis bullous pemphigoid, atypical hemolytic uremic syndrome, Shiga toxin *E. coli*-related hemolytic uremic syndrome, myasthenia gravis, neuromyelistis optica, dense deposit disease, C3 neuropathy, age-related macular degeneration, cold agglutinin disease, anti-neutrophil cytoplasmic antibody-associated vasculitis, humoral and vascular transplant rejection, graft dysfunction, myocardial infarction, a sensitized recipient of a transplant, and sepsis.

In one example, the complement component-associated disease is paroxysmal nocturnal hemoglobinuria (PNH).

In another example, the complement component-associated disease is atypical hemolytic uremic syndrome (aHUS).

In one example, the CFB expression is inhibited by at least about 30%.

In one example, the C3 expression is inhibited by at least about 30%.

In one example, the C9 expression is inhibited by at least about 30%.

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

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

In one example, the agent is administered subcutaneously.

In another example, the agent is administered intravenously.

In one example, the present disclosure provides methods of treating a subject having a disorder that would benefit from reduction in complement factor B (CFB) expression. The methods include administering to the subject a therapeutically effective amount of the agent of the disclosure, thereby treating the subject.

In another example, the present disclosure provides methods of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in complement factor B (CFB) expression. The methods include administering to the subject a therapeutically effective amount of the agent of the disclosure, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in CFB expression.

In yet another example, the present disclosure provides methods of treating a subject having a disorder that would benefit from reduction in complement component C3 (C3) expression. The methods include administering to the subject a therapeutically effective amount of the agent of the disclosure, thereby treating the subject.

In one example, the present disclosure provides methods of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in complement component C3 (C3) expression. The methods include administering to the subject a therapeutically effective amount of the agent of the disclosure, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in C3 expression.

In another example, the present disclosure provides methods of treating a subject having a disorder that would benefit from reduction in complement component C9 (C9) expression. The methods include administering to the subject a therapeutically effective amount of the agent of the disclosure, thereby treating the subject.

In one example, the present disclosure provides methods of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in complement component C9 (C9) expression. The methods include administering to the subject a therapeutically effective amount of the agent of the disclosure, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in C9 expression.

In one example, the disorder is a complement component-associated disease.

In one example, the complement component-associated disease is selected from the group consisting of paroxysmal nocturnal hemoglobinuria (PNH), asthma, rheumatoid arthritis, systemic lupus erythmatosis, glomerulonephritis, psoriasis, dermatomyositis bullous pemphigoid, atypical hemolytic uremic syndrome, Shiga toxin *E. coli*-related hemolytic uremic syndrome, myasthenia gravis, neuromyelistis optica, dense deposit disease, C3 neuropathy, age-related macular degeneration, cold agglutinin disease, anti-neutrophil cytoplasmic antibody-associated vasculitis, humoral and vascular transplant rejection, graft dysfunction, myocardial infarction, a sensitized recipient of a transplant, and sepsis.

In one example, the complement component -associated disease is paroxysmal nocturnal hemoglobinuria (PNH).

In another example, the complement component-associated disease is atypical hemolytic uremic syndrome (aHUS).

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

In one example, the administration of the agent to the subject causes a decrease in hemolysis and/or a decrease in C3 protein accumulation.

In one example, the administration of the agent to the subject causes a decrease in hemolysis and/or a decrease in C9 protein accumulation.

In one example, the methods further include administration of eculizumab to the subject.

In another example, the methods further include administration of compstatin to the subject.

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

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

In yet another example, the agent is administered at a dose selected from the group consisting of 0.5 mg/kg 1 mg/kg, 1.5 mg/kg, 3 mg/kg, 10 mg/kg, and 30 mg/kg.

In one example, the agent is administered to the subject once a week.

In another example, the agent is administered to the subject twice a month.

In one example, the methods further include measuring LDH levels in the subject.

In one example, the present disclosure provides methods of inhibiting the expression of complement factor B (CFB) in a subject. The methods include administering to the subject a therapeutically effective amount of the agent of the disclosure, thereby inhibiting the expression of CFB in the subject.

In another example, the present disclosure provides methods of inhibiting the expression of complement component C3 (C3) in a subject. The methods include administering to the subject a therapeutically effective amount of the agent of the disclosure, thereby inhibiting the expression of C3 in the subject.

In yet another example, the present disclosure provides methods of inhibiting the expression of complement component C9 (C9) in a subject. The methods include administering to the subject a therapeutically effective amount of the agent of any one of the disclosure, thereby inhibiting the expression of C9 in the subject.

In one example, the methods further include administering eculizumab to the subject.

In another example, the methods further include administering compstatin to the subject.

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

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

In yet another example, the agent is administered at a dose selected from the group consisting of 1 mg/kg, 3 mg/kg, 10 mg/kg, and 30 mg/kg.

In one example, the agent is administered to the subject once a week.

In another example, the dsRNA agent is administered to the subject twice a month.

Brief Description of the Drawings

Figure 1 is a schematic of the three complement pathways: alternative, classical and lectin.

Figure 2 is a graph showing the percentage of complement factor B (CFB) mRNA remaining in C57BL/6 mice 96 hours after a single 1 mg/kg or 10 mg/kg dose of the indicated iRNAs.

Figure 3 is a graph showing the percentage of complement factor B (CFB) mRNA remaining in C57BL/6 mice 72 hours after a single 1.25 mg/kg, 2.5 mg/kg, or 10 mg/kg dose of AD-60331.

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 component gene, *i.e.,* a CFB, C3, or C9 gene. The gene may be within a cell, *e.g.,* a cell within a subject, such as a human.

The present invention also provides methods and combination therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a CFB, C9, and/or C3 gene, *e.g.,* a complement component-associated disease, such as paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS) using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a CFB, C3, and/or C9 gene.

The present invention also provides methods for preventing at least one symptom, *e.g.,* hemolysis, in a subject having a disorder that would benefit from inhibiting or reducing the expression of a CFB, C3, and/or C9 gene, *e.g.,* a complement component-associated disease, such as paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS).

The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is about 30 nucleotides or less in length, *e.g.*, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a CFB, C3, or C9 gene. The use of these iRNAs enables the targeted degradation of mRNAs of the correponding gene (CFB, C3, or C9 gene) in mammals. Very low dosages of the iRNAs of the invention, in particular, can specifically and efficiently mediate RNA interference (RNAi), resulting in significant inhibition of expression of the correponding gene (CFB, C3, or C9 gene). Using cell-based assays, the present inventors

have demonstrated that iRNAs targeting these complement component genes can mediate RNAi, resulting in significant inhibition of expression of a complement gene (*i.e.,* CFB, C3, or C9). Thus, methods and compositions including these iRNAs are useful for treating a subject having a complement component-associated disease, such as paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS).

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a complement gene (*i.e.*, CFB, C_3 or C9) as well as compositions, uses, and methods for treating subjects having diseases and disorders that would benefit from inhibition and/or reduction of the expression of these genes.

I. Definitions

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

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

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

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

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 (http://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:1-5, 27, and 30. SEQ ID NOs:12-16, 33, and 36 are the antisense sequences of SEQ ID NOs: 1-5, 27, and 30, 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.,* ncbi.nlm.nih.gov/snp).

As used herein, the term "Complement Component 3," used interchangeably with the term "C3," refers to the well-known gene and polypeptide, also known in the art as ARMD9, C3a Anaphylatoxin, ASP, Complement Component C3a, C3a, Complement Component C3b, C3b, prepro-C3, Acylation-Stimulating Protein Cleavage Product, CPAMD1, Complement C3, C3 And PZP-Like Alpha-2-Macroglobulin Domain-Containing Protein 1, Complement Component C3, and AHUS5. The term "C3" includes human C3, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:115298677; mouse C3, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:126518316; and rat C3, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:158138560. The term "C3" also includes *Macaca fascicularis* CFB, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:544508182 and in the entry for the gene, ENSP00000245907 (locus=chr19:6921416:6963034), in the *Macaca* genome project web site (http://macaque.genomics.org.cn/page/species/index.jsp). Additional examples of C3 mRNA sequences are readily available using, *e.g.*, GenBank, UniProt, OMIM, and the *Macaca* genome project web site.

Exemplary C3 nucleotide sequences may also be found in SEQ ID NOs:6-8, 28, and 31. SEQ ID NOs:17-19, 34, and 37 are the antisense sequences of SEQ ID NOs: 6-8, 28, and 31, respectively.

The term"C3," as used herein, also refers to naturally occurring DNA sequence variations of the C3 gene. Numerous seuqnce variations within the C3 gene have been

identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, ncbi.nlm.nih.gov/snp).

As used herein, the term "Complement Component 9," used interchangeably with the term "C9," refers to the well-known gene and polypeptide. The term "C9" includes human C9, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:187608340; mouse C9, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:15375311; and rat C9, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:16924005. The term "C9" also includes *Macaca fascicularis* CFB, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI:544436867 and in the entry for the gene, isotig05361 (isogroup03350 length=2955 numContigs=1), in the *Macaca* genome project web site (http://macaque.genomics.org.cn/page/species/index.jsp). Additional examples of C3 mRNA sequences are readily available using, *e.g.*, GenBank, UniProt, OMIM, and the *Macaca* genome project web site.

Exemplary C9 nucleotide sequences may also be found in SEQ ID NOs:9-11, 29, and 32. SEQ ID NOs:20-22, 35, and 38 are the antisense sequences of SEQ ID NOs: 9-11, 29, and 32, respectively.

The term"C9," as used herein, also refers to naturally occurring DNA sequence variations of the C9 gene. Numerous seuqnce variations within the C9 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, ncbi.nlm.nih.gov/snp).

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a CFB, C3, or C9 gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodiment, the target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a CFB, C3, or C9 gene.

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

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

"G," "C," "A," "T" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.,* Table 2). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the 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 the target 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 CFB, C3, or C9 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 CFB,

C3, or C9 gene. Accordingly, the term "siRNA" is also used herein to refer to an RNAi as described above.

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

In another embodiment, an "iRNA" for use in the compositions, uses, and methods of the invention is a double-stranded RNA and is referred to herein as a "double stranded RNAi agent," "double-stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA", refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.,* a CFB, C3, or C9 gene. In some embodiments of the invention, a double-stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.,* an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.In general, the majority of nucleotides of each strand of a dsRNA molecule are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, as used in this specification, an "RNAi agent" may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides.

As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, and/or a modified nucleobase. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.,* a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the 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 "RNAi agent" for the purposes of this specification and claims.

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

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

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

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

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of an iRNA, *e.g.*, a dsRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or *vice versa*, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

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

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

The term "antisense strand" or "guide strand" refers to the strand of an iRNA, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.,* a CFB, C3, or C9 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 CFB, C3, or C9 nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, 3, or 2 nucleotides of the 5'- and/or 3'-terminus of the iRNA.

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

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

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

complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

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

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

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

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding CFB, C3, or C9). For example, a polynucleotide is complementary to at least a part of a CFB mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding CFB.

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

include all types of modifications disclosed herein or known in the art. Any such modifications, as used in an iRNA molecule, are encompassed by "iRNA" for the purposes of this specification and claims.

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

The phrase "contacting a cell with an RNAi agent," such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an RNAi agent 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 RNAi agent may be put into physical contact with the cell by the individual performing the method, or alternatively, the RNAi agent may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the RNAi agent. Contacting a cell *in vivo* may be done, for example, by injecting the RNAi agent into or near the tissue where the cell is located, or by injecting the RNAi agent into another area, *e.g.*, the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the RNAi agent may contain and/or be coupled to a ligand, *e.g.*, GalNAc3, that directs the RNAi agent to a site of interest, *e.g.*, the liver. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. For example, a cell may also be contacted *in vitro* with an RNAi agent and subsequently transplanted into a subject.

As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, a horse, and a whale), or a bird (*e.g.*, a duck or a goose). In an embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder or condition that would benefit from reduction in CFB, C3, and/or C9 expression; a human at risk for a disease, disorder or condition that would benefit from reduction in CFB, C3, and/or C9 expression; a human having a disease, disorder or condition that would benefit

from reduction in CFB, C3, and/or C9 expression; and/or human being treated for a disease, disorder or condition that would benefit from reduction in CFB, C3, and/or C9 expression as described herein.

As used herein, the term "complement component-associated disease" is a disease or disorder that is caused by, or associated with complement activation. The term "complement component-associated disease" includes a disease, disorder or condition that would benefit from reduction in CFB (*i.e.,* a "CFB-associated disease"), C3 (*i.e.,* a "C3-associated disease"), and/or C9 (*i.e.,* a "C9-associated disease") expression. Such diseases are typically associated with inflammation and/or immune system activation, *e.g.,* membrane attack complex-mediated lysis, anaphylaxis, and/or hemolysis. Non-limiting examples of complement component-associated diseases include 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, a complement component-associated disease is paroxysmal nocturnal hemoglobinuria (PNH). The PNH may be classical PNH or PNH in the setting of another bone marrow failure syndrome and/or myelodysplastic syndromes (MDS), *e.g.*, cytopenias. In another embodiment, a complement component-associated disease is atypical hemolytic uremic syndrome (aHUS). In yet another embodiment, a complement component-associated disease is rheumatoid arthritis.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a complement component-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 or antibody, or antigen-binding fragment thereof, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an iRNA agent that, when administered to a subject having a complement component-associate disease but not yet (or currently) experiencing or displaying symptoms of the disease, and/or a subject at risk of developing a complement component-associated disease, *e.g.,* a subject having a graft and/or transplant, *e.g.,* a sensitized or allogenic recipient, a subject having sepsis, and/or a subject having a myocardial infarction, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the iRNA 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 local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. iRNA agents employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

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, urine, lymph, cerebrospinal fluid, ocular fluids, saliva, and the like. Tissue samples may include samples

from tissues, organs or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes). In preferred embodiments, a "sample derived from a subject" refers to blood or plasma drawn from the subject. In further embodiments, a "sample derived from a subject" refers to liver tissue (or subcomponents thereof) derived from the subject.

II. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of a complement component gene. In one embodiment, the iRNA agent 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 having a complement component-associated disease as described herein, *e.g.,* PNH. In another embodiment, the iRNA agent includes double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a C3 gene in a cell, such as a cell within a subject, *e.g.,* a mammal, such as a human having a complement component-associated disease as described herein, *e.g.,* PNH. In a further embodiment, the iRNA agent includes double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a C9 gene in a cell, such as a cell within a subject, *e.g.,* a mammal, such as a human having a complement component-associated disease as described herein, *e.g.,* PNH. The dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of a target gene, *i.e.,* CFB, C3, or C9 gene. The region of complementarity is about 30 nucleotides or less in length (*e.g.,* about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). Upon contact with a cell expressing the target gene, the iRNA inhibits the expression of the target gene (*e.g.,* a human, a primate, a non-primate, or a bird CFB, C3, or C9 gene) by at least about 10% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, Western Blotting or flowcytometric techniques.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of a CFB, C3, or C9 gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as

known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

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

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

In some embodiments, the dsRNA is between about 15 and about 20 nucleotides in length, or between about 25 and about 30 nucleotides in length. In one embodiment, an RNAi agent of the invention is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, *i.e.*, a CFB, C3, or C9 target mRNA sequence, to direct the cleavage of the target RNA. 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 9 to 36 base pairs, *e.g.*, about 10-36, 11-36, 12-36, 13-36, 14-36, 15-36, 9-35, 10-35, 11-35, 12-35, 13-35, 14-35, 15-35, 9-34, 10-34, 11-34, 12-34, 13-34, 14-34, 15-34, 9-33, 10-33, 11-33, 12-33, 13-33, 14-33, 15-33, 9-32, 10-32, 11-32, 12-32, 13-32, 14-32, 15-32, 9-31, 10-31, 11-31, 12-31, 13-32, 14-31, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-

25, 21-24, 21-23, or 21-22 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target CFB, C3, or C9 expression is not generated in the target cell by cleavage of a larger dsRNA.

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

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

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

In one aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence.

In one embodiment, a dsRNA of the invention targeting CFB includes a sense strand selected from the group of sequences provided in any one of Tables 3 and 4, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 3 and 4. 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 CFB 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 3 and 4, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 3 and 4. In one embodiment, the substantially complementary sequences of the dsRNA are contained on

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

In one embodiment, a dsRNA of the invention targeting C3 includes a sense strand selected from the group of sequences provided in any one of Tables 5 and 6, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 5 and 6. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a C3 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 5 and 6, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 5 and 6. In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

In one embodiment, a dsRNA of the invention targeting C9 includes a sense strand selected from the group of sequences provided in any one of Tables 7 and 8, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 7 and 8. 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 C9 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 7 and 8, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 7 and 8. In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It will be understood that, although some of the sequences in Tables 3-8 are described as modified and/or conjugated sequences, the RNA of the iRNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth in Tables 3-8 that is un-modified, un-conjugated, and/or modified and/or conjugated differently than described therein.

The skilled person is well aware that dsRNAs having a duplex structure of between about 20 and 23 base pairs, *e.g.,* 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, *EMBO* 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in any one of Tables 3-8 dsRNAs described herein can include at least

one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having one of the sequences of any one of Tables 3-8 minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences of any one of Tables 3-8, and differing in their ability to inhibit the expression of the taret gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs provided in any one of Tables 3 and 4 identify a site(s) in a CFB transcript that is susceptible to RISC-mediated cleavage. Similarly, the RNAs provided in any one of Tables 5 and 6 identify a site(s) in a C3 transcript that is susceptible to RISC-mediated cleavage, and the RNAs provided in any one of Tables 7 and 8 identify a site(s) in a C9 transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of these sites. As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 15 contiguous nucleotides from one of the sequences provided in any one of Tables 3-8 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in the target gene.

While a target sequence is generally about 15-30 nucleotides in length, there is wide variation in the suitability of particular sequences in this range for directing cleavage of any given target RNA. Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can also be taken in which a "window" or "mask" of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, *e.g.*, in silico) placed on the target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence "window" progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays as described herein or as known in the art) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with an iRNA agent, mediate the best inhibition of target gene expression. Thus, while the sequences identified, for example, in any one of Tables 3-8 represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively "walking the window" one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified, *e.g.*, in any one of Tables 3-8, further optimization could be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of iRNAs based on those target sequences in an inhibition assay as known in the art and/or as described herein can lead to further improvements in the efficiency of inhibition. Further still, such optimized sequences can be adjusted by, *e.g.*, the introduction of modified nucleotides as described herein or as known in the art, addition or changes in overhang, or other modifications as known in the art and/or discussed herein to further optimize the molecule (*e.g.*, increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes) as an expression inhibitor.

An iRNA as described herein can contain one or more mismatches to the target sequence. In one embodiment, an iRNA as described herein contains no more than 3 mismatches. If the antisense strand of the iRNA contains mismatches to a target sequence, it is preferable that the area of mismatch is not located in the center of the region of complementarity. If the antisense strand of the iRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, for a 23 nucleotide iRNA agent the strand which is complementary to a region of, *e.g.,* 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 iRNA containing a mismatch to a target sequence is effective in inhibiting the expression of a target gene, *e.g.,* a CFB, C3, or C9 gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of a target gene is important, especially if the particular region of complementarity in a target gene is known to have polymorphic sequence variation within the population.

III. Modified iRNAs of the Invention

In one embodiment, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is un-modified, and does not comprise, *e.g.,* chemical modifications and/or conjugations known in the art and described herein. In another embodiment, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain 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 of the invention are modified iRNAs of the invention in which "substantially all of the

nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

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

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6, 239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and US Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

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

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

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--CH_2-, --CH_2--N(CH_3)--O--CH_2--[known as a methylene (methylimino) or MMI backbone], --CH_2--O--N(CH_3)--CH_2--, --CH_2--N(CH_3)--N(CH_3)--CH_2-- and --N(CH_3)--CH_2--CH_2--[wherein the native phosphodiester backbone is represented as --O--P--O--CH_2--] of the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506.

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

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

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

azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the 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.

The RNA of an iRNA can also be modified to include one or more bicyclic sugar moities. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A"bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4′-carbon and the 2′-carbon of the sugar ring. Thus, in some embodiments an agent of the invention may include 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. Examples of such 4′ to 2′ bridged bicyclic nucleosides, include but are not limited to 4′-(CH2)—O-2′ (LNA); 4′-(CH2)—S-2′; 4′-(CH2)2—O-2′ (ENA); 4′-CH(CH3)—O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′-CH(CH2OCH3)—O-2′ (and analogs thereof; see, *e.g.*, U.S. Pat. No. 7,399,845); 4′-C(CH3)(CH3)—O-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,283); 4′-CH2—N(OCH3)-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,425); 4′-CH2—O—N(CH3)-2′ (see, e.g.,U.S. Patent Publication No. 2004/0171570); 4′-CH2—N(R)—O-2′, wherein R is H, C1-C12 alkyl, or a protecting group (see, *e.g.*, U.S. Pat. No. 7,427,672); 4′-CH2—C(H)(CH3)-2′ (see, *e.g.*, Chattopadhyaya *et al., J. Org. Chem.*, 2009, 74, 118-134); and 4′-CH2—C(=CH2)-2′ (and analogs thereof; see, *e.g.*, US Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

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

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

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

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

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

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

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, US Patent No. 8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference. Potentially stabilizing modifications to the ends of RNA molecules can include N- (acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-0-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"- phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

A. Modified iRNAs Comprising Motifs of the Invention

In certain aspects of the invention, the double-stranded RNAi agents of the invention include agents with chemical modifications as disclosed, for example, in U.S. Provisional Application No. 61/561,710, filed on November 18, 2011, or in PCT/US2012/065691, the entire contents of each of which are incorporated herein by reference.

As shown herein and in Provisional Application No. 61/561,710 or in PCT/US2012/065691, a superior result may be obtained by introducing one or more motifs of three identical modifications on three consecutive nucleotides into a sense strand and/or antisense strand of an RNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the RNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense and/or antisense strand. The RNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the sense strand. The resulting RNAi agents present superior gene silencing activity.

More specifically, it has been surprisingly discovered that when the sense strand and antisense strand of the double-stranded RNAi agent are completely modified to have one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of an RNAi agent, the gene silencing acitivity of the RNAi agent was superiorly enhanced.

Accordingly, the invention provides double-stranded RNAi agents capable of inhibiting the expression of a target gene (*i.e.*, a CFB, C3, or C9 gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may range from 12-30 nucleotides in length. For example, each strand may be between 14-30 nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

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

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

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

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

3'-end of the sense strand, antisense strand, or both strands. In one embodiment, this 3'-overhang is present in the antisense strand. In one embodiment, this 3'-overhang is present in the sense strand.

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

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

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

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

In one embodiment, the RNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand. When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate

internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In one embodiment, every nucleotide in the sense strand and the antisense strand of the RNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In one embodiment each residue is independently modified with a 2'-O-methyl or 3'-fluoro, *e.g.,* in an alternating motif. Optionally, the RNAi agent further comprises a ligand (preferably $GalNAC_3$).

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

In one embodiment, the RNAi agent comprises sense and antisense strands, wherein the RNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end; wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region region which is at least 25 nucleotides in length, and the second strand is sufficiently complemenatary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein dicer cleavage of the RNAi agent preferentially results in an siRNA comprising the 3' end of the second strand, thereby

reducing expression of the target gene in the mammal. Optionally, the RNAi agent further comprises a ligand.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

In one embodiment, the RNAi agent of the invention comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the

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

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

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

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

The RNAi agent may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand or antisense strand or both strands in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand and/or antisense strand; each internucleotide linkage modification may occur in an alternating

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

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

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

In one embodiment, the RNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mistmatch may occur in the overhang region or the duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is

preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

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

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

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

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

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

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

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

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

each n_p and n_q independently represent an overhang nucleotide;

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

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

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

In one embodiment, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at

positions 6, 7, 8, 7, 8, 9, 8, 9, 10, 9, 10, 11, 10, 11,12 or 11, 12, 13) of - the sense strand, the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end.

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

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

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

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

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

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

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

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

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

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

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

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

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

wherein:

k and l are each independently 0 or 1;

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

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

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

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

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

and

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand

is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X′, Y′ and Z′, in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

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

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

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

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

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

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

(III)

wherein:

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

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

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

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

wherein

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

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

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

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

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

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

(IIIa)

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

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

(IIIb)

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

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

(IIIc)

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

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

(IIId)

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

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

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

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

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

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

nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y′ nucleotides.

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

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

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

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

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

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

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

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

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

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

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

group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, *e.g.*, a ligand to the constituent ring.

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

In certain specific embodiments, the RNAi agent for use in the methods of the invention is an agent selected from the group of agents listed in any one of Tables 3-8. These agents may further comprise a ligand.

IV. iRNAs Conjugated to Ligands

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

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

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

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

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

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They

can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

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

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

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

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

In the ligand-conjugated oligonucleotides and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that

already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

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

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

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

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

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

vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents

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

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to 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: 23). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 24) 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: 25) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 26) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

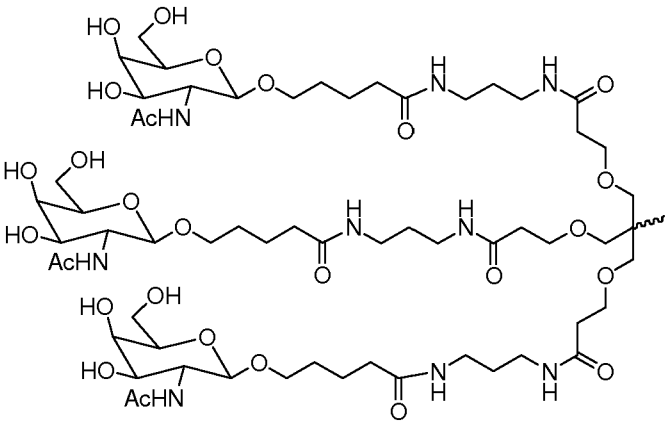
An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.,* glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics may include D-amino acids, as well as synthetic RGD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

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

C. Carbohydrate Conjugates

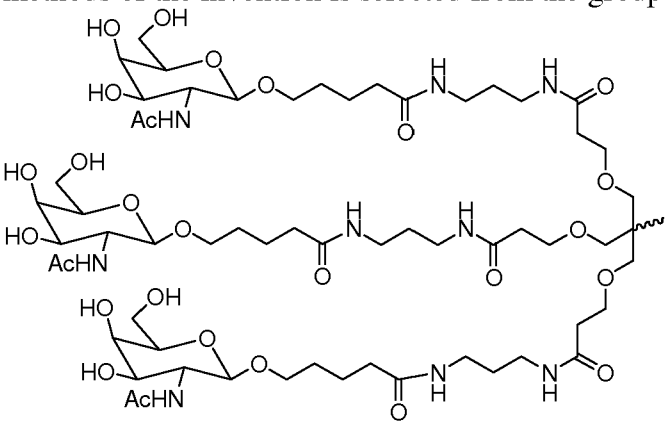
In some embodiments of the compositions and methods of the invention, an iRNA oligonucleotide further comprises a carbohydrate. The carbohydrate conjugated iRNA are advantageous for the *in vivo* delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein.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

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

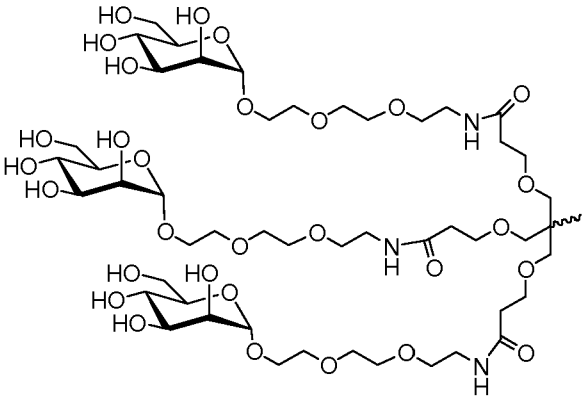


Formula II.

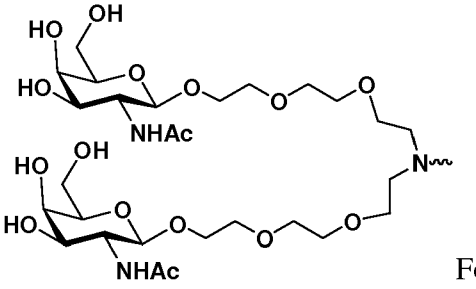
In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



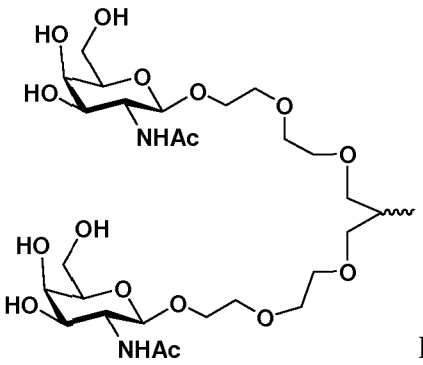
Formula II,



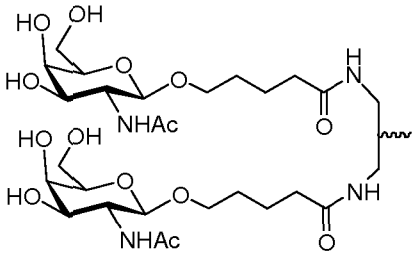
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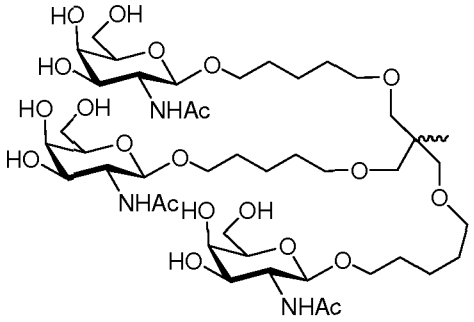
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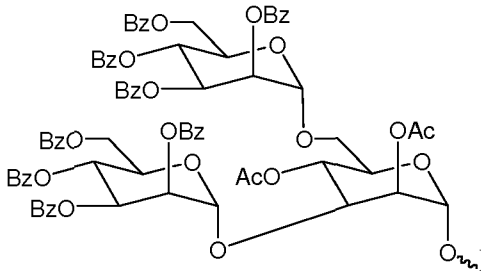
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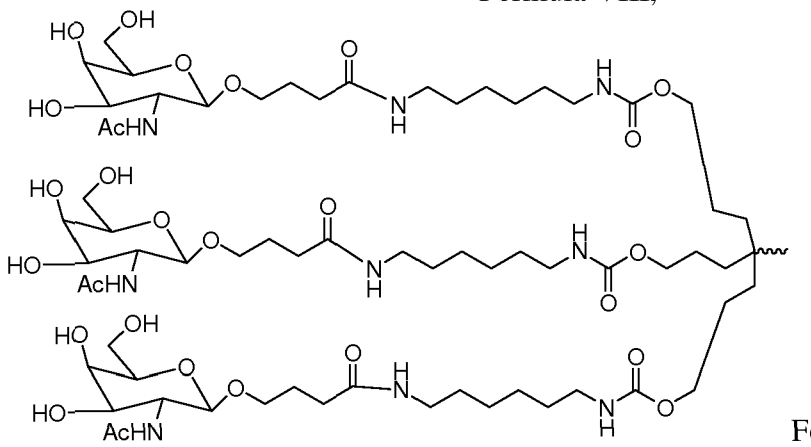
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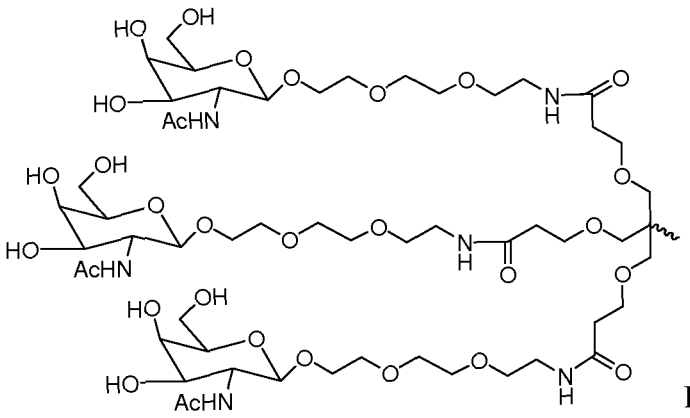
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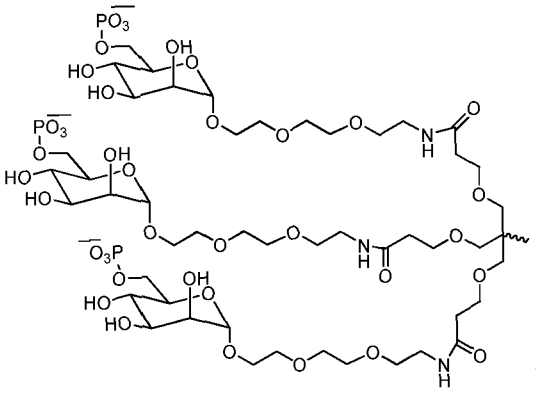
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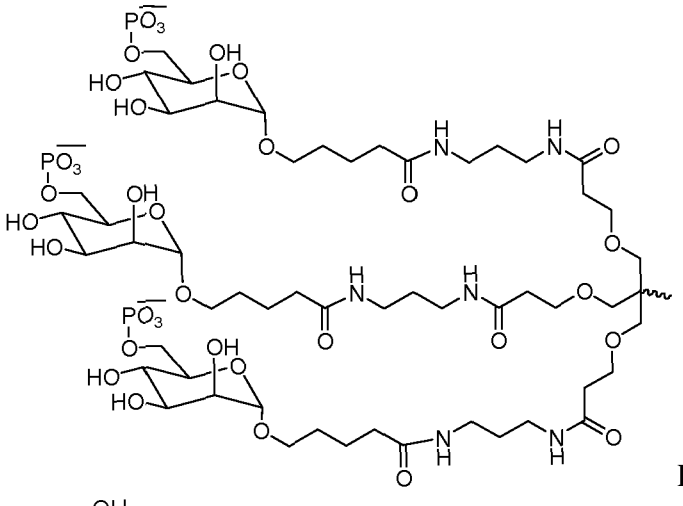
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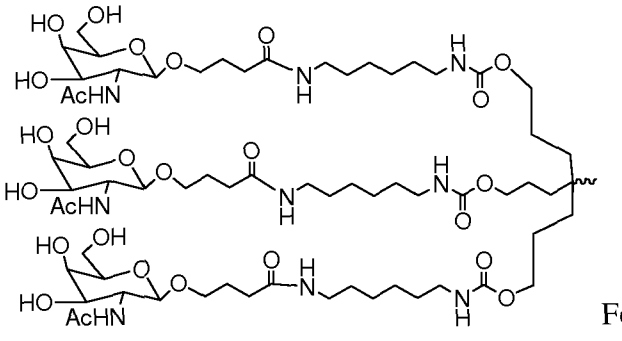
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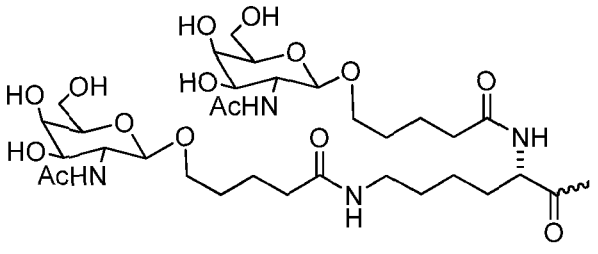
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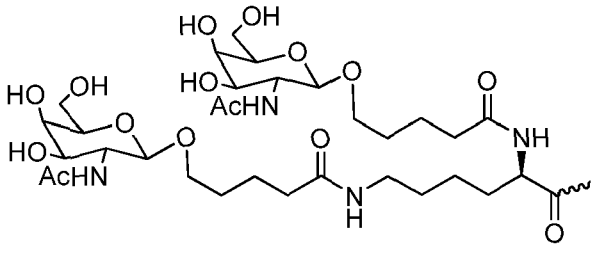
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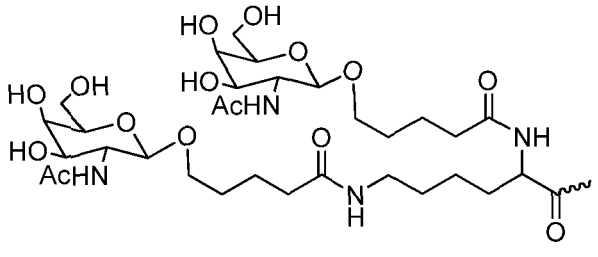
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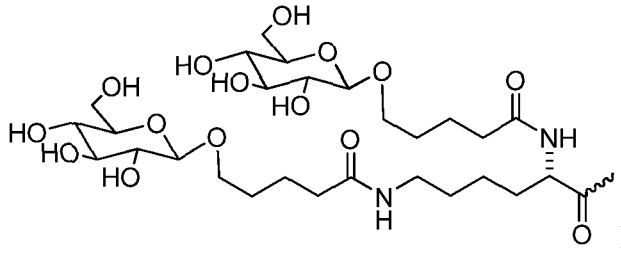
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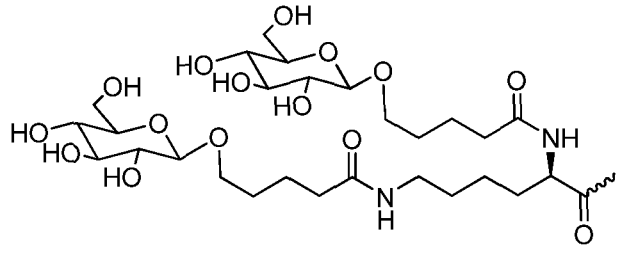
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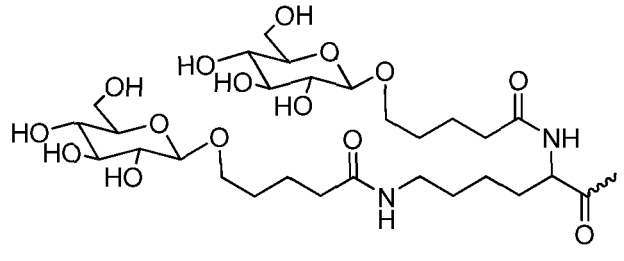
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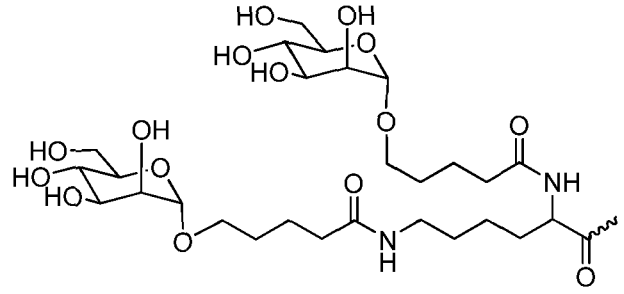
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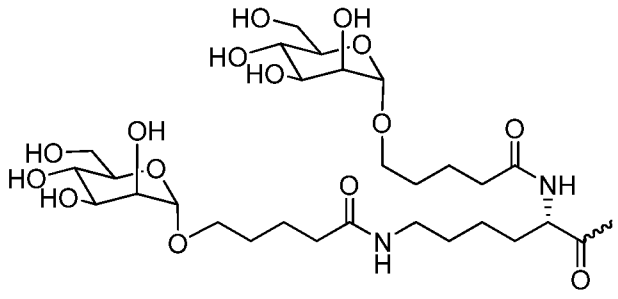
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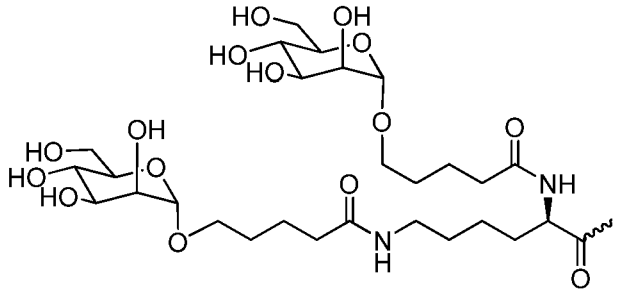
Formula XIX,



Formula XX,

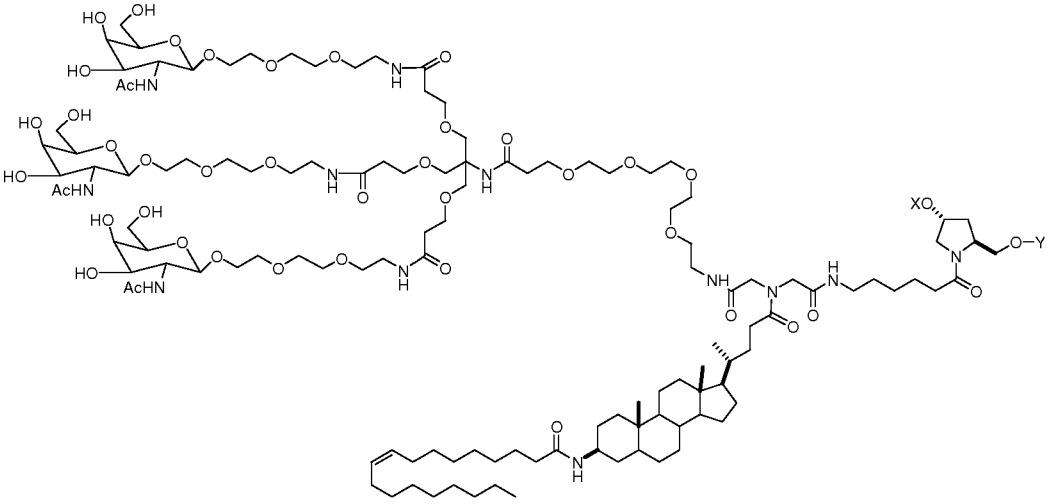


Formula XXI,



Formula XXII.

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



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

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

D. Linkers

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

The term "linker" or “linking group” means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl,

heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

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

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

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group

that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

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

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

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

i. Redox cleavable linking groups

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

conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

ii. Phosphate-based cleavable linking groups

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

iii. Acid cleavable linking groups

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

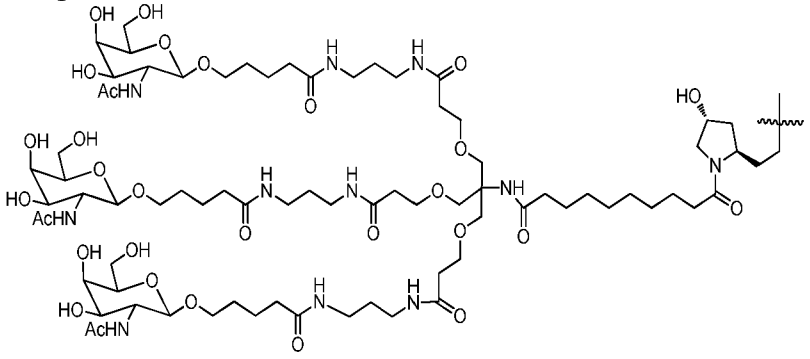
iv. Ester-based linking groups

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

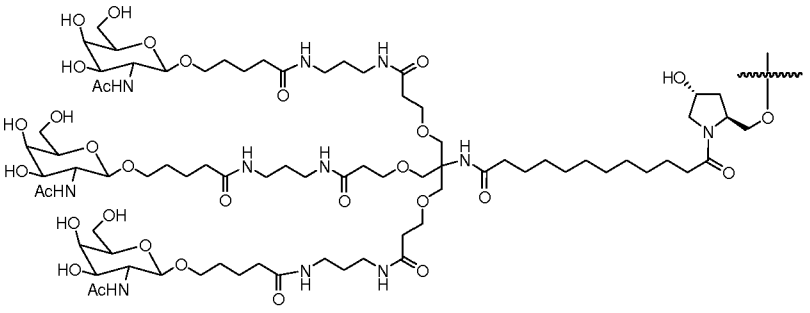
v. Peptide-based cleaving groups

In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides *etc.*) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula – NHCHRAC(O)NHCHRBC(O)-, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

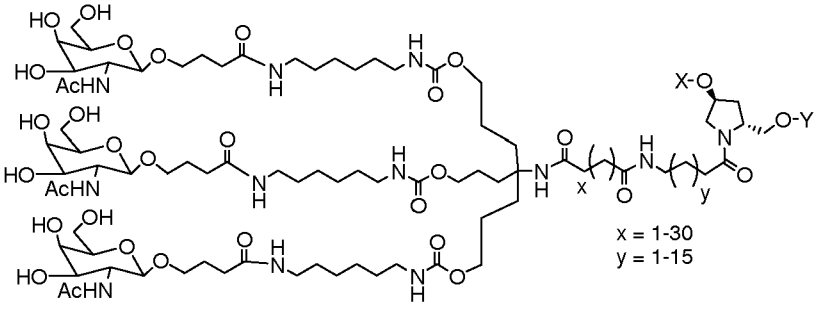
In one embodiment, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



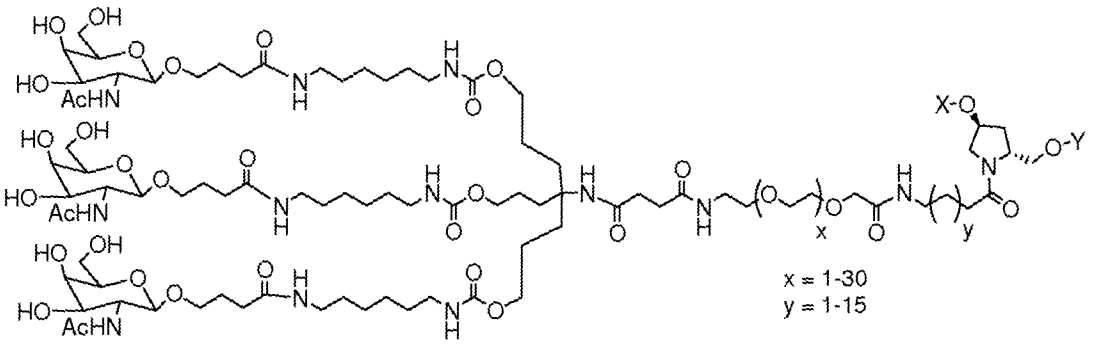
(Formula XXIV),



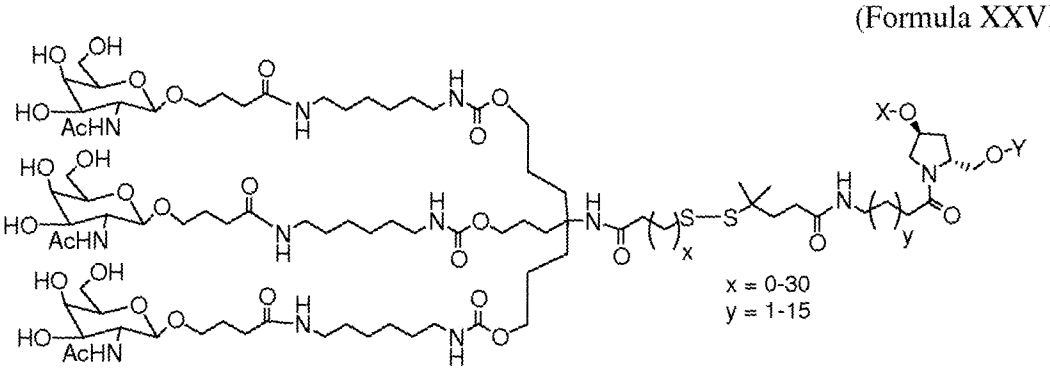
(Formula XXV),



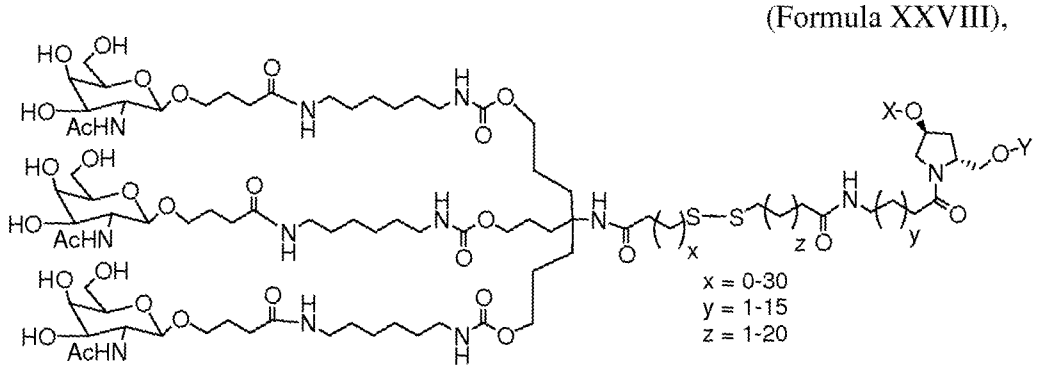
(Formula XXVI),



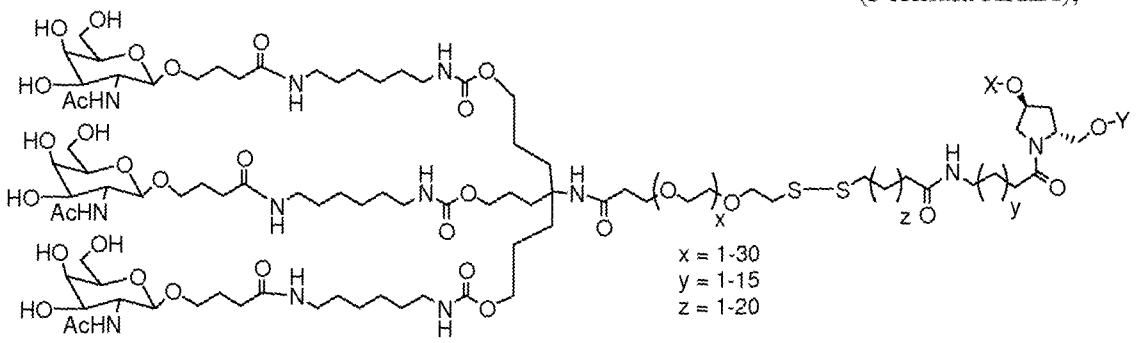
(Formula XXVII),



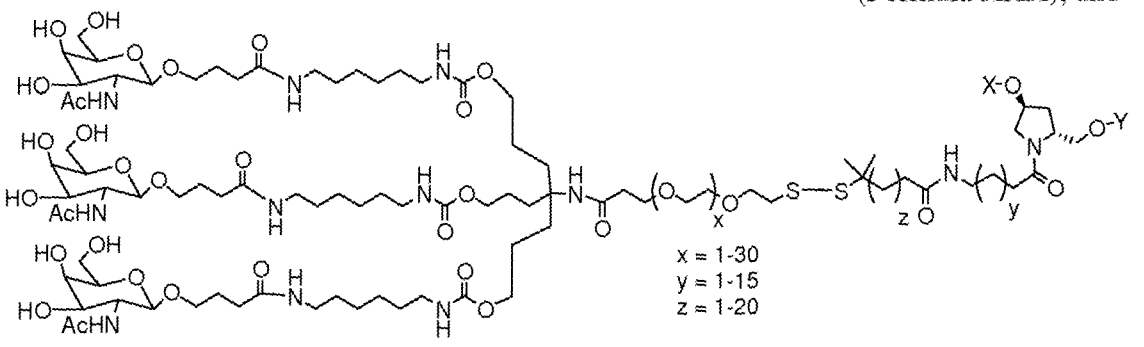
(Formula XXVIII),



(Formula XXIX),



(Formula XXX), and



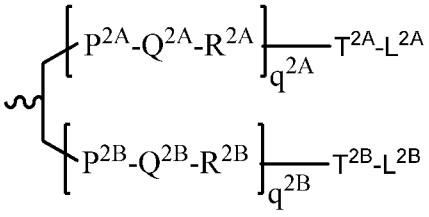
(Formula XXXI),

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

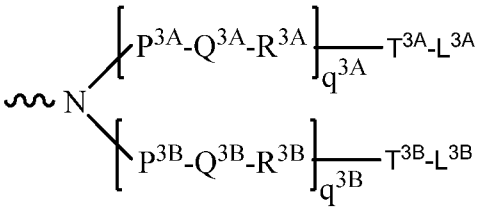
In certain embodiments of the compositions and methods of the invention, a ligand is one or more "GalNAc" (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

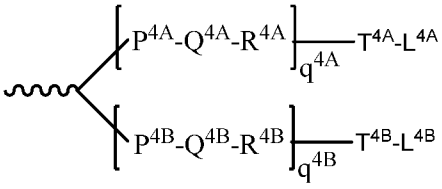
In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXII) – (XXXV):

Formula XXXII

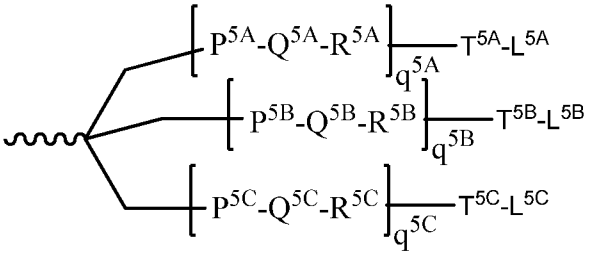


Formula XXXIII





, or



;

Formula XXXIV

Formula XXXV

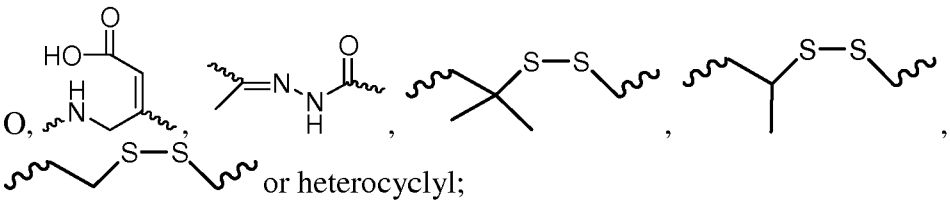
wherein:

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

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

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

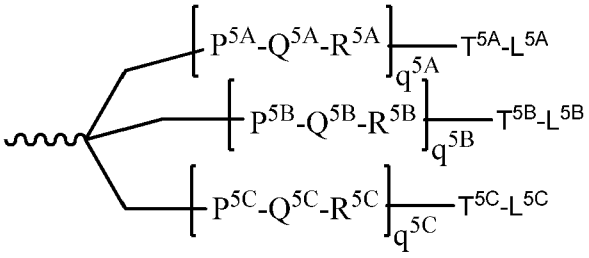
R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, -C(O)-$CH(R^a)$-NH-, CO, CH=N-



or heterocyclyl;

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

Formula XXXV



(VII)

,

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

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

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

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

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

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

solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

V. Delivery of an iRNA of the Invention

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

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an iRNA of the invention (see *e.g.*, Akhtar S. and Julian RL. (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. The non-specific effects of an iRNA can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the iRNA molecule to be administered. Several studies have shown successful knockdown of gene products when an iRNA is administered locally. For example, intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ., *et al* (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ., *et al* (2003) *Mol. Vis.* 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J., *et al* (2005) *Mol. Ther.*11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ., *et al* (2006) *Mol. Ther.* 14:343-350; Li, S., *et al* (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G., *et al.* (2004) *Nucleic Acids* 32:e49; Tan, PH., *et al* (2005) *Gene Ther.* 12:59-66; Makimura, H., *et al* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya,Y., *et al* (2005) *J. Neurophysiol.* 93:594-602) and to the lungs by intranasal administration (Howard, KA., *et al* (2006) *Mol. Ther.* 14:476-484; Zhang, X., *et al*

(2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V., *et al* (2005) *Nat. Med.* 11:50-55). For administering an iRNA systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases *in vivo*. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA composition to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J., *et al* (2004) *Nature* 432:173-178). Conjugation of an iRNA to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO., *et al* (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the iRNA can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim SH., *et al* (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al* (2003) *J. Mol. Biol* 327:761-766; Verma, UN., *et al* (2003) Clin. *Cancer Res.* 9:1291-1300; Arnold, AS *et al* (2007) *J. Hypertens.* 25:197-205, 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*), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS., *et al* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY., *et al* (2005) *Cancer Gene Ther.* 12:321-328; Pal, A., *et al* (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME., *et al* (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA., *et al* (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., *et al* (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in U.S. Patent No. 7,427,605, which is herein incorporated by reference in its entirety.

A. Vector encoded iRNAs of the Invention

iRNA targeting a CFB, C3, or C9 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. Pat. No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

The individual strand or strands of an iRNA can be transcribed from a promoter on an expression vector. Where two separate strands are to be expressed to generate, for example, a dsRNA, two separate expression vectors can be co-introduced (*e.g.*, by transfection or infection) into a target cell. Alternatively each individual strand of a dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as inverted repeat polynucleotides joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

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

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

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc.*; (c)

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

Vectors useful for the delivery of an iRNA will include regulatory elements (promoter, enhancer, *etc.*) sufficient for expression of the iRNA in the desired target cell or tissue. The regulatory elements can be chosen to provide either constitutive or regulated/inducible expression.

Expression of the iRNA can be precisely regulated, for example, by using an inducible regulatory sequence that is sensitive to certain physiological regulators, *e.g.*, circulating glucose levels, or hormones (Docherty *et al.*, 1994, *FASEB J.* 8:20-24). Such inducible expression systems, suitable for the control of dsRNA expression in cells or in mammals include, for example, regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 - thiogalactopyranoside (IPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the iRNA transgene.

Viral vectors that contain nucleic acid sequences encoding an iRNA can be used. For example, a retroviral vector can be used (see Miller *et al.*, *Meth. Enzymol.* 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding an iRNA are cloned into one or more vectors, which facilitate delivery of the nucleic acid into a patient. More detail about retroviral vectors can be found, for example, in Boesen *et al.*, *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, *J. Clin. Invest.* 93:644-651 (1994); Kiem *et al.*, *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993). Lentiviral vectors contemplated for use include, for example, the HIV based vectors described in U.S. Patent Nos. 6,143,520; 5,665,557; and 5,981,276, which are herein incorporated by reference.

Adenoviruses are also contemplated for use in delivery of iRNAs of the invention. Adenoviruses are especially attractive vehicles, *e.g.*, for delivering genes to respiratory

epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout *et al.*, *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, *Science* 252:431-434 (1991); Rosenfeld *et al.*, *Cell* 68:143-155 (1992); Mastrangeli *et al.*, *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, *et al.*, *Gene Therapy* 2:775-783 (1995). A suitable AV vector for expressing an iRNA featured in the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H *et al.* (2002), *Nat. Biotech.* 20: 1006-1010.

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

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

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

The pharmaceutical preparation of a vector can include the vector in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from

recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

VI. 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 treating a disease or disorder associated with the expression or activity of a CFB, C3, and/or C9 gene, *e.g.* a complement component-associated disease as descried herein. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration *via* parenteral delivery, *e.g.*, by subcutaneous (SC) or intravenous (IV) delivery. Another example is compositions that are formulated for direct delivery into the brain parenchyma, *e.g.*, by infusion into the brain, such as by continuous pump infusion. The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of the taregt gene. In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. For example, the dsRNA can be administered at about 0.01 mg/kg, about 0.05 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 3 mg/kg, about 10 mg/kg, about 20 mg/kg, about 30 mg/kg, about 40 mg/kg, or about 50 mg/kg per single dose.

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

In another embodiment, the dsRNA is administered at a dose of about 0.1 to about 50 mg/kg, about 0.25 to about 50 mg/kg, about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/mg, about 1.5 to about 50 mg/kb, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.1 to about 45 mg/kg, about 0.25 to about 45 mg/kg,

about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/mg, about 1.5 to about 45 mg/kb, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.1 to about 40 mg/kg, about 0.25 to about 40 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/mg, about 1.5 to about 40 mg/kb, about 2 to about 40 mg/kg, about 2.5 to about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.1 to about 30 mg/kg, about 0.25 to about 30 mg/kg, about 0.5 to about 30 mg/kg, about 0.75 to about 30 mg/kg, about 1 to about 30 mg/mg, about 1.5 to about 30 mg/kb, about 2 to about 30 mg/kg, about 2.5 to about 30 mg/kg, about 3 to about 30 mg/kg, about 3.5 to about 30 mg/kg, about 4 to about 30 mg/kg, about 4.5 to about 30 mg/kg, about 5 to about 30 mg/kg, about 7.5 to about 30 mg/kg, about 10 to about 30 mg/kg, about 15 to about 30 mg/kg, about 20 to about 30 mg/kg, about 20 to about 30 mg/kg, about 25 to about 30 mg/kg, about 0.1 to about 20 mg/kg, about 0.25 to about 20 mg/kg, about 0.5 to about 20 mg/kg, about 0.75 to about 20 mg/kg, about 1 to about 20 mg/mg, about 1.5 to about 20 mg/kb, about 2 to about 20 mg/kg, about 2.5 to about 20 mg/kg, about 3 to about 20 mg/kg, about 3.5 to about 20 mg/kg, about 4 to about 20 mg/kg, about 4.5 to about 20 mg/kg, about 5 to about 20 mg/kg, about 7.5 to about 20 mg/kg, about 10 to about 20 mg/kg, or about 15 to about 20 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

For example, the dsRNA may be administered at a dose of about 0..01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, or about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In another embodiment, the dsRNA is administered at a dose of about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/mg, about 1.5 to about 50 mg/kb, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5

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

For example, subjects can be administered, *e.g.,* subcutaneously or intravenously, a single therapeutic amount of iRNA, such as about 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275, 0.3, 0.325, 0.35, 0.375, 0.4, 0.425, 0.45, 0.475, 0.5, 0.525, 0.55, 0.575, 0.6, 0.625, 0.65, 0.675, 0.7, 0.725, 0.75, 0.775, 0.8, 0.825, 0.85, 0.875, 0.9, 0.925, 0.95, 0.975, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5,

20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 31, 32, 33, 34, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or about 50 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

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

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

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

administered on a less frequent basis. For example, after administration weekly or biweekly for three months, administration can be repeated once per month, for six months or a year or longer.

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

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

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

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as a disorder that would benefit from reduction in the expression of CFB, C3, or C9. Such models can be used for *in vivo* testing of iRNA, as well as for determining a therapeutically effective dose. Suitable mouse models are known in the art and include, for example, collagen-induced arthritis mouse model (Courtenay, J.S., *et al.* (1980) *Nature* 283, 666–668), myocardial ischemia (Homeister JW and Lucchesi BR (1994) *Annu Rev Pharmacol Toxicol* 34:17–40), ovalbumin induced asthma mouse models (*e.g.*, Tomkinson A., *et al.* (2001). *J. Immunol.* 166, 5792–5800), (NZB×NZW)F1, MRL/Fas^{lpr} (MRL/lpr) and BXSB mouse models (Theofilopoulos, A. N. and Kono, D. H. 1999. Murine lupus models: gene-specific and genome-wide studies. In Lahita R. G., ed., *Systemic Lupus Erythematosus*, 3rd edn, p. 145. Academic Press, San Diego, CA), mouse aHUS model

(Goicoechea de Jorge *et al.* (2011) *The development of atypical hemolytic uremic syndrome depeds on complement C5, J Am Soc Nephrol* 22:137-145.

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

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

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

A. iRNA Formulations Comprising Membranous Molecular Assemblies

An iRNA for use in the compositions and methods of the invention can be formulated for delivery in a membranous molecular assembly, *e.g.*, a liposome or a micelle. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, *e.g.*, one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an

aqueous interior. The aqueous portion contains the iRNA composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the iRNA composition, although in some examples, it may. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the iRNA are delivered into the cell where the iRNA can specifically bind to a target RNA and can mediate RNAi. In some cases the liposomes are also specifically targeted, *e.g.*, to direct the iRNA to particular cell types.

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

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

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

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

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

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

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

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

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the

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

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

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

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

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

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for

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

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

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

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

M. and Papahadjopoulos, D. *Meth. Enz.* 101:512-527, 1983; Wang, C. Y. and Huang, L., P*roc. Natl. Acad. Sci. USA* 84:7851-7855, 1987).

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

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

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

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

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the

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

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

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

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

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

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

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

A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of the siRNA composition, an alkali metal

C_8 to C_{22} alkyl sulphate, and a micelle forming compounds. Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

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

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

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

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

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

B. *Lipid particles*

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

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

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

The cationic lipid can be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I -(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 1,2-Dilinolenyloxy-N,N-

dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid can comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

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

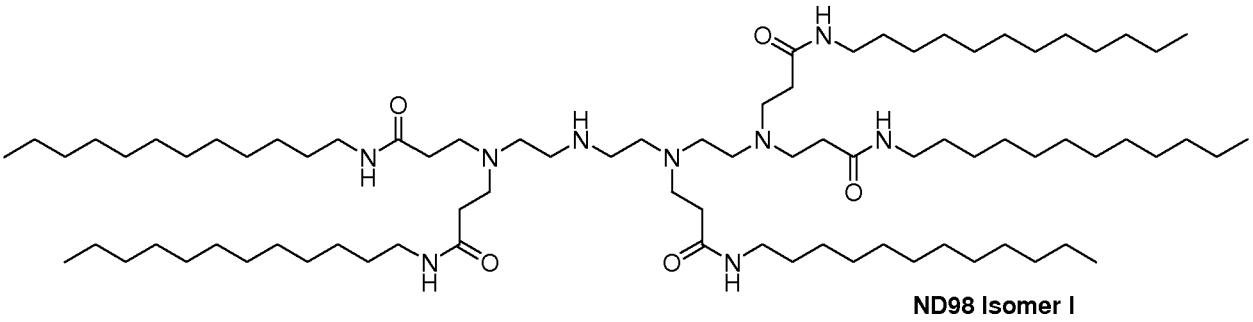
In one embodiment, the lipid-siRNA particle includes 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 siRNA/Lipid Ratio.

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

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

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

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



Formula 1

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

Additional exemplary lipid-dsRNA formulations are described in Table 1.

Table 1

	Ionizable/Cationic Lipid	**cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio**
SNALP-1	l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA ~ 7:1
2-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4

		lipid:siRNA ~ 7:1
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA ~ 11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 6:1
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA ~ 11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1)	Tech G1/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1

LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1
LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

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

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

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

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

XTC comprising formulations are described, *e.g.*, in U.S. Provisional Serial No. 61/148,366, filed January 29, 2009; U.S. Provisional Serial No. 61/156,851, filed March 2, 2009; U.S. Provisional Serial No. filed June 10, 2009; U.S. Provisional Serial No.

61/228,373, filed July 24, 2009; U.S. Provisional Serial No. 61/239,686, filed September 3, 2009, and International Application No. PCT/US2010/022614, filed January 29, 2010, which are hereby incorporated by reference.

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

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

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

Synthesis of ionizable/cationic lipids

Any of the compounds, *e.g.*, cationic lipids and the like, used in the nucleic acid-lipid particles of the invention can be prepared by known organic synthesis techniques, including the methods described in more detail in the Examples. All substituents are as defined below unless indicated otherwise.

"Alkyl" means a straight chain or branched, noncyclic or cyclic, saturated aliphatic hydrocarbon containing from 1 to 24 carbon atoms. Representative saturated straight chain alkyls include methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, and the like; while saturated branched alkyls include isopropyl, sec-butyl, isobutyl, tert-butyl, isopentyl, and the like. Representative saturated cyclic alkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like; while unsaturated cyclic alkyls include cyclopentenyl and cyclohexenyl, and the like.

"Alkenyl" means an alkyl, as defined above, containing at least one double bond between adjacent carbon atoms. Alkenyls include both cis and trans isomers. Representative straight chain and branched alkenyls include ethylenyl, propylenyl, 1-butenyl, 2-butenyl, isobutylenyl, 1-pentenyl, 2-pentenyl, 3-methyl-1-butenyl, 2-methyl-2-butenyl, 2,3-dimethyl-2-butenyl, and the like.

"Alkynyl" means any alkyl or alkenyl, as defined above, which additionally contains at least one triple bond between adjacent carbons. Representative straight chain and branched alkynyls include acetylenyl, propynyl, 1-butynyl, 2-butynyl, 1-pentynyl, 2-pentynyl, 3-methyl-1 butynyl, and the like.

"Acyl" means any alkyl, alkenyl, or alkynyl wherein the carbon at the point of attachment is substituted with an oxo group, as defined below. For example, -C(=O)alkyl, -C(=O)alkenyl, and -C(=O)alkynyl are acyl groups.

"Heterocycle" means a 5- to 7-membered monocyclic, or 7- to 10-membered bicyclic, heterocyclic ring which is either saturated, unsaturated, or aromatic, and which contains from

1 or 2 heteroatoms independently selected from nitrogen, oxygen and sulfur, and wherein the nitrogen and sulfur heteroatoms can be optionally oxidized, and the nitrogen heteroatom can be optionally quaternized, including bicyclic rings in which any of the above heterocycles are fused to a benzene ring. The heterocycle can be attached via any heteroatom or carbon atom. Heterocycles include heteroaryls as defined below. Heterocycles include morpholinyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, piperizynyl, hydantoinyl, valerolactamyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydroprimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, and the like.

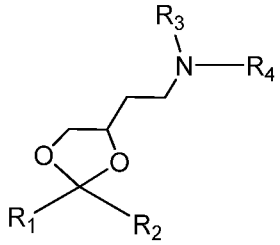
The terms "optionally substituted alkyl", "optionally substituted alkenyl", "optionally substituted alkynyl", "optionally substituted acyl", and "optionally substituted heterocycle" means that, when substituted, at least one hydrogen atom is replaced with a substituent. In the case of an oxo substituent (=O) two hydrogen atoms are replaced. In this regard, substituents include oxo, halogen, heterocycle, -CN, -ORx, -NRxRy, -NRxC(=O)Ry, -NRxSO2Ry, -C(=O)Rx, -C(=O)ORx, -C(=O)NRxRy, –SOnRx and -SOnNRxRy, wherein n is 0, 1 or 2, Rx and Ry are the same or different and independently hydrogen, alkyl or heterocycle, and each of said alkyl and heterocycle substituents can be further substituted with one or more of oxo, halogen, -OH, -CN, alkyl, -ORx, heterocycle, -NRxRy, -NRxC(=O)Ry, -NRxSO2Ry, -C(=O)Rx, -C(=O)ORx, -C(=O)NRxRy, -SOnRx and -SOnNRxRy.

"Halogen" means fluoro, chloro, bromo and iodo.

In some embodiments, the methods of the invention can require the use of protecting groups. Protecting group methodology is well known to those skilled in the art (see, for example, Protective Groups in Organic Synthesis, Green, T.W. *et al.*, Wiley-Interscience, New York City, 1999). Briefly, protecting groups within the context of this invention are any group that reduces or eliminates unwanted reactivity of a functional group. A protecting group can be added to a functional group to mask its reactivity during certain reactions and then removed to reveal the original functional group. In some embodiments an "alcohol protecting group" is used. An "alcohol protecting group" is any group which decreases or eliminates unwanted reactivity of an alcohol functional group. Protecting groups can be added and removed using techniques well known in the art.

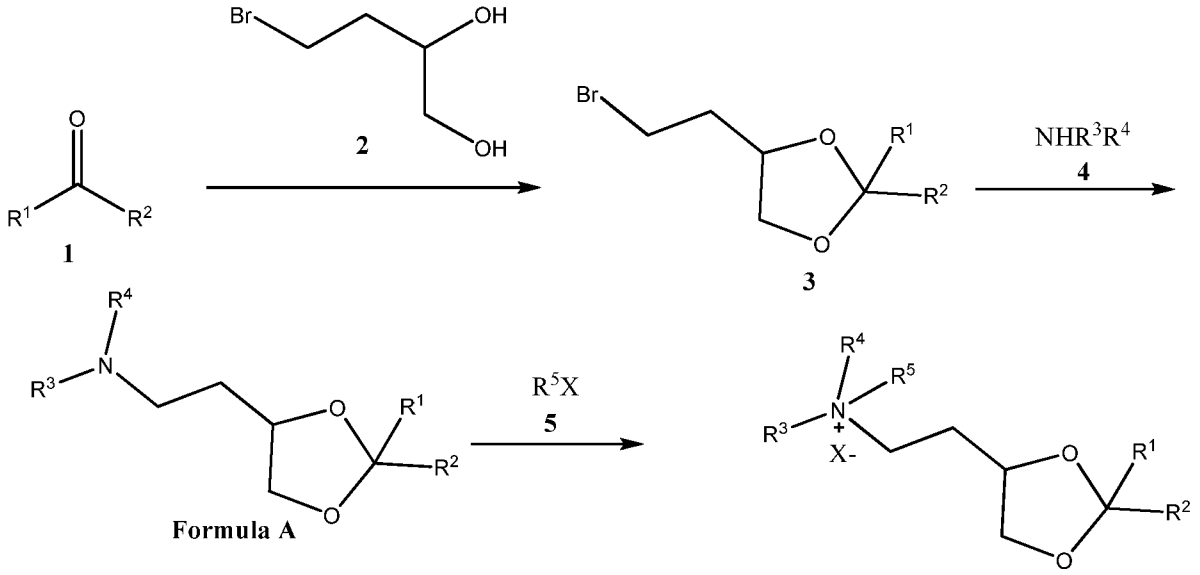
Synthesis of Formula A

In some embodiments, nucleic acid-lipid particles of the invention are formulated using a cationic lipid of formula A:



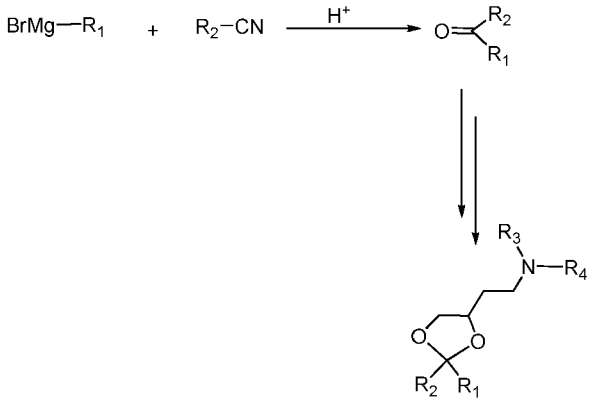
where R1 and R2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R3 and R4 are independently lower alkyl or R3 and R4 can be taken together to form an optionally substituted heterocyclic ring. In some embodiments, the cationic lipid is XTC (2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane). In general, the lipid of formula A above can be made by the following Reaction Schemes 1 or 2, wherein all substituents are as defined above unless indicated otherwise.

Scheme 1



Lipid A, where R1 and R2 are independently alkyl, alkenyl or alkynyl, each can be optionally substituted, and R3 and R4 are independently lower alkyl or R3 and R4 can be taken together to form an optionally substituted heterocyclic ring, can be prepared according to Scheme 1. Ketone 1 and bromide 2 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 1 and 2 yields ketal 3. Treatment of ketal 3 with amine 4 yields lipids of formula A. The lipids of formula A can be converted to the corresponding ammonium salt with an organic salt of formula 5, where X is anion counter ion selected from halogen, hydroxide, phosphate, sulfate, or the like.

Scheme 2

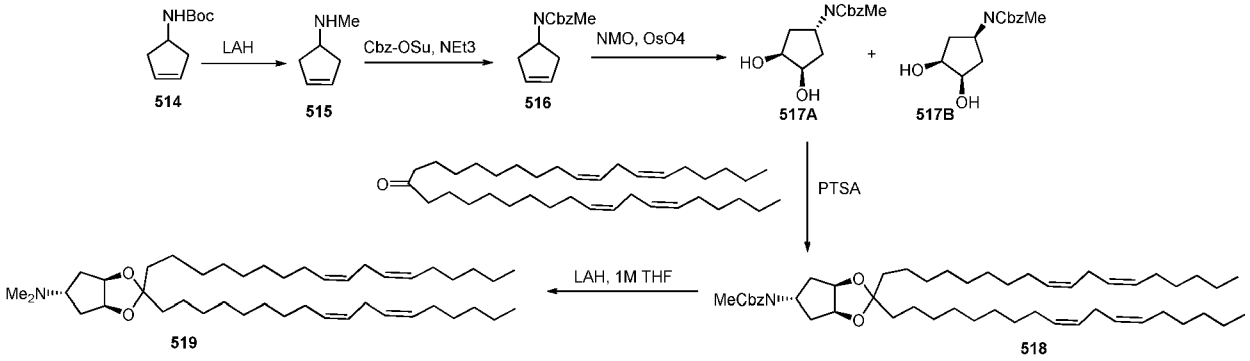


Alternatively, the ketone 1 starting material can be prepared according to Scheme 2. Grignard reagent 6 and cyanide 7 can be purchased or prepared according to methods known to those of ordinary skill in the art. Reaction of 6 and 7 yields ketone 1. Conversion of ketone 1 to the corresponding lipids of formula A is as described in Scheme 1.

Synthesis of MC3

Preparation of DLin-M-C3-DMA (*i.e.*, (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate) was as follows. A solution of (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (0.53 g), 4-N,N-dimethylaminobutyric acid hydrochloride (0.51 g), 4-N,N-dimethylaminopyridine (0.61g) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.53 g) in dichloromethane (5 mL) was stirred at room temperature overnight. The solution was washed with dilute hydrochloric acid followed by dilute aqueous sodium bicarbonate. The organic fractions were dried over anhydrous magnesium sulphate, filtered and the solvent removed on a rotovap. The residue was passed down a silica gel column (20 g) using a 1-5% methanol/dichloromethane elution gradient. Fractions containing the purified product were combined and the solvent removed, yielding a colorless oil (0.54 g). *Synthesis of ALNY-100*

Synthesis of ketal 519 [ALNY-100] was performed using the following scheme 3:



Synthesis of 515

To a stirred suspension of LiAlH4 (3.74 g, 0.09852 mol) in 200 ml anhydrous THF in a two neck RBF (1L), was added a solution of 514 (10g, 0.04926mol) in 70 mL of THF

slowly at 0 0C under nitrogen atmosphere. After complete addition, reaction mixture was warmed to room temperature and then heated to reflux for 4 h. Progress of the reaction was monitored by TLC. After completion of reaction (by TLC) the mixture was cooled to 0 0C and quenched with careful addition of saturated Na2SO4 solution. Reaction mixture was stirred for 4 h at room temperature and filtered off. Residue was washed well with THF. The filtrate and washings were mixed and diluted with 400 mL dioxane and 26 mL conc. HCl and stirred for 20 minutes at room temperature. The volatilities were stripped off under vacuum to furnish the hydrochloride salt of 515 as a white solid. Yield: 7.12 g 1H-NMR (DMSO, 400MHz): δ= 9.34 (broad, 2H), 5.68 (s, 2H), 3.74 (m, 1H), 2.66-2.60 (m, 2H), 2.50-2.45 (m, 5H).

Synthesis of 516

To a stirred solution of compound 515 in 100 mL dry DCM in a 250 mL two neck RBF, was added NEt3 (37.2 mL, 0.2669 mol) and cooled to 0 0C under nitrogen atmosphere. After a slow addition of N-(benzyloxy-carbonyloxy)-succinimide (20 g, 0.08007 mol) in 50 mL dry DCM, reaction mixture was allowed to warm to room temperature. After completion of the reaction (2-3 h by TLC) mixture was washed successively with 1N HCl solution (1 x 100 mL) and saturated NaHCO3 solution (1 x 50 mL). The organic layer was then dried over anhyd. Na2SO4 and the solvent was evaporated to give crude material which was purified by silica gel column chromatography to get 516 as sticky mass. Yield: 11g (89%). 1H-NMR (CDCl3, 400MHz): δ = 7.36-7.27(m, 5H), 5.69 (s, 2H), 5.12 (s, 2H), 4.96 (br., 1H) 2.74 (s, 3H), 2.60(m, 2H), 2.30-2.25(m, 2H). LC-MS [M+H] -232.3 (96.94%).

Synthesis of 517A and 517B

The cyclopentene 516 (5 g, 0.02164 mol) was dissolved in a solution of 220 mL acetone and water (10:1) in a single neck 500 mL RBF and to it was added N-methyl morpholine-N-oxide (7.6 g, 0.06492 mol) followed by 4.2 mL of 7.6% solution of OsO4 (0.275 g, 0.00108 mol) in tert-butanol at room temperature. After completion of the reaction (~ 3 h), the mixture was quenched with addition of solid Na2SO3 and resulting mixture was stirred for 1.5 h at room temperature. Reaction mixture was diluted with DCM (300 mL) and washed with water (2 x 100 mL) followed by saturated NaHCO3 (1 x 50 mL) solution, water (1 x 30 mL) and finally with brine (1x 50 mL). Organic phase was dried over an.Na2SO4 and solvent was removed in vacuum. Silica gel column chromatographic purification of the crude material was afforded a mixture of diastereomers, which were separated by prep HPLC. Yield: - 6 g crude

517A - Peak-1 (white solid), 5.13 g (96%). 1H-NMR (DMSO, 400MHz): δ= 7.39-7.31(m, 5H), 5.04(s, 2H), 4.78-4.73 (m, 1H), 4.48-4.47(d, 2H), 3.94-3.93(m, 2H), 2.71(s, 3H), 1.72- 1.67(m, 4H). LC-MS - [M+H]-266.3, [M+NH4 +]-283.5 present, HPLC-97.86%. Stereochemistry confirmed by X-ray.

Synthesis of 518

Using a procedure analogous to that described for the synthesis of compound 505, compound 518 (1.2 g, 41%) was obtained as a colorless oil. 1H-NMR (CDCl3, 400MHz): δ= 7.35-7.33(m, 4H), 7.30-7.27(m, 1H), 5.37-5.27(m, 8H), 5.12(s, 2H), 4.75(m,1H), 4.58-4.57(m,2H), 2.78-2.74(m,7H), 2.06-2.00(m,8H), 1.96-1.91(m, 2H), 1.62(m, 4H), 1.48(m, 2H), 1.37-1.25(br m, 36H), 0.87(m, 6H). HPLC-98.65%.

General Procedure for the Synthesis of Compound 519

A solution of compound 518 (1 eq) in hexane (15 mL) was added in a drop-wise fashion to an ice-cold solution of LAH in THF (1 M, 2 eq). After complete addition, the mixture was heated at 40oC over 0.5 h then cooled again on an ice bath. The mixture was carefully hydrolyzed with saturated aqueous Na2SO4 then filtered through celite and reduced to an oil. Column chromatography provided the pure 519 (1.3 g, 68%) which was obtained as a colorless oil. 13C NMR δ = 130.2, 130.1 (x2), 127.9 (x3), 112.3, 79.3, 64.4, 44.7, 38.3, 35.4, 31.5, 29.9 (x2), 29.7, 29.6 (x2), 29.5 (x3), 29.3 (x2), 27.2 (x3), 25.6, 24.5, 23.3, 226, 14.1; Electrospray MS (+ve): Molecular weight for C44H80NO2 (M + H)+ Calc. 654.6, Found 654.6.

Formulations prepared by either the standard or extrusion-free method can be characterized in similar manners. For example, formulations are typically characterized by visual inspection. They should be whitish translucent solutions free from aggregates or sediment. Particle size and particle size distribution of lipid-nanoparticles can be measured by light scattering using, for example, a Malvern Zetasizer Nano ZS (Malvern, USA). Particles should be about 20-300 nm, such as 40-100 nm in size. The particle size distribution should be unimodal. The total dsRNA concentration in the formulation, as well as the entrapped fraction, is estimated using a dye exclusion assay. A sample of the formulated dsRNA can be incubated with an RNA-binding dye, such as Ribogreen (Molecular Probes) in the presence or absence of a formulation disrupting surfactant, *e.g.*, 0.5% Triton-X100. The total dsRNA in the formulation can be determined by the signal from the sample containing the surfactant, relative to a standard curve. The entrapped fraction is determined by subtracting the "free" dsRNA content (as measured by the signal in the absence of surfactant) from the total dsRNA content. Percent entrapped dsRNA is typically >85%. For SNALP formulation, the particle size is at least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm, at least 100 nm, at least 110 nm, and at least 120 nm. The suitable range is typically about at least 50 nm to about at least 110 nm, about at least 60 nm to about at least 100 nm, or about at least 80 nm to about at least 90 nm.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In some embodiments, oral

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

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

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be

generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly preferred are formulations that target the liver when treating hepatic disorders such as hepatic carcinoma.

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

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

C. Additional Formulations

i. Emulsions

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

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

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

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

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties

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

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

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

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

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

Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

ii. Microemulsions

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

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

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

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

Microemulsions of the present invention can also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to

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

iii. Microparticles

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

iv. Penetration Enhancers

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

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

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

Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-

dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C_{1-20} alkyl esters thereof (*e.g.*, methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (*i.e.*, oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, *etc.*) (see *e.g.*, Touitou, E., *et al.* Enhancement in Drug Delivery, CRC Press, Danvers, MA, 2006; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri *et al.*, *J. Pharm. Pharmacol.*, 1992, 44, 651-654).

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

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

MA, 2006; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur *et al.*, *J. Control Rel.*, 1990, 14, 43-51).

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

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

UniFECTOR (B-Bridge International; Mountain View, CA, USA), SureFECTOR (B-Bridge International; Mountain View, CA, USA), or HiFect™ (B-Bridge International, Mountain View, CA, USA), among others.

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

v. Carriers

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

vi. Excipients

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

Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable

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

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

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

vii. Other Components

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

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

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA compounds and (b) one or more agents which function by a non-RNAi mechanism and which are useful in treating a hemolytic disorder. Examples of such agents include, but are not lmited to an anti-inflammatory agent, anti-steatosis agent, anti-viral, and/or anti-fibrosis agent. In addition, other substances commonly used to protect the liver, such as silymarin, can also be used in conjunction with the *iRNAs described herein.* Other agents useful for treating liver diseases include telbivudine, entecavir, and protease inhibitors such as telaprevir and other disclosed, for example, in Tung *et al.*, U.S. Application

Publication Nos. 2005/0148548, 2004/0167116, and 2003/0144217; and in Hale *et al.*, U.S. Application Publication No. 2004/0127488.

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

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC_{50} (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by CFB, C3, and/or C9 expression. In any event, the administering physician can adjust the amount and timing of iRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VII. Methods For Inhibiting Complement Component Expression

The present invention provides methods of inhibiting expression of a complement component as described herein. In one aspect, the present invention provides methods of inhibiting expression of CFB in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, a double stranded RNAi agent, in an amount effective to inhibit expression of the CFB in the cell, thereby inhibiting expression of the CFB in the cell.

The present invention also provides methods of inhibiting expression of C3 in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, a double stranded RNAi agent, in an amount effective to inhibit expression of the C3 in the cell, thereby inhibiting expression of the C3 in the cell.

In addition, the present invention provides methods of inhibiting expression of C9 in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, a double stranded RNAi agent, in an amount effective to inhibit expression of the C9 in the cell, thereby inhibiting expression of the C9 in the cell.

Contacting of a cell with a double stranded RNAi agent may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the RNAi agent includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the RNAi agent. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. Contacting may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a $GalNAc_3$ ligand, or any other ligand that directs the RNAi agent to a site of interest, *e.g.*, the liver of a subject.

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

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

"Inhibiting expression of a CFB gene" includes any level of inhibition of a CFB gene, *e.g.*, at least partial suppression of the expression of a CFB gene. The expression of the CFB gene may be assessed based on the level, or the change in the level, of any variable associated with CFB gene expression, *e.g.*, CFB mRNA level, CFB protein level, or, for example, CH_{50} activity as a measure of total hemolytic complement, AH_{50} to measure the hemolytic activity of the alternate pathway of complement, and/or lactate dehydrogenase (LDH) levels as a measure of intravascular hemolysis, and/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. 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).

The phrase "inhibiting expression of a C3" is intended to refer to inhibition of expression of any C3 gene (such as, *e.g.*, a mouse C3 gene, a rat C3 gene, a monkey C3 gene, or a human C3 gene) as well as variants or mutants of a C3 gene. Thus, the C3 gene may be a wild-type C3 gene, a mutant C3 gene, or a transgenic C3 gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of a C3 gene" includes any level of inhibition of a C3 gene, *e.g.*, at least partial suppression of the expression of a C3 gene. The expression of the C3 gene may be assessed based on the level, or the change in the level, of any variable associated with C3 gene expression, *e.g.*, C3 mRNA level, C3 protein level, or, for example, CH_{50} activity as a measure of total hemolytic complement, AH_{50} to measure the hemolytic activity of the alternate pathway of complement, and/or lactate dehydrogenase (LDH) levels as a measure of intravascular hemolysis, and/or hemoglobin levels. Levels of CFB, C9, C5, C5a, C5b, and soluble C5b-9 complex may also be measured to assess C3 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. 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).

The phrase "inhibiting expression of a C9" is intended to refer to inhibition of expression of any C9 gene (such as, *e.g.*, a mouse C9 gene, a rat C9 gene, a monkey C9 gene, or a human C9 gene) as well as variants or mutants of a C9 gene. Thus, the C9 gene may be a wild-type C9 gene, a mutant C9 gene, or a transgenic C9 gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of a C9 gene" includes any level of inhibition of a C9 gene, *e.g.*, at least partial suppression of the expression of a C9 gene. The expression of the C9 gene may be assessed based on the level, or the change in the level, of any variable associated with C9 gene expression, *e.g.*, C9 mRNA level, C9 protein level, or, for example, CH_{50} activity as a measure of total hemolytic complement, AH_{50} to measure the hemolytic activity of the alternate pathway of complement, and/or lactate dehydrogenase (LDH) levels as a measure of intravascular hemolysis, and/or hemoglobin levels. Levels of CFB, C3, C5, C5a, C5b, and soluble C5b-9 complex may also be measured to assess C9 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. 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 target gene, *e.g.,* CFB, C3, or C9 gene, is inhibited by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least

about 94%. at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

Inhibition of the expression of the target gene, *e.g.,* a CFB, C3, or C9, 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 target gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an RNAi agent of the invention, or by administering an RNAi agent of the invention to a subject in which the cells are or were present) such that the expression of a target gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s)). In preferred embodiments, the inhibition is assessed by expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

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

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

The level of CFB, C3, or C9 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 CFB, C3, and/or C9 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.,* mRNA of the CFB, C3, and/or C9 gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy RNA preparation kits (Qiagen) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays (Melton *et al.*, *Nuc. Acids Res.* 12:7035), Northern blotting, *in situ* hybridization, and microarray analysis.

In one embodiment, the level of expression of CFB, C3, and/or C9 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 CFB, C3, or C9. 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, *e.g.,* specifically hybridize, to CFB, C3, or C9 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 CFB, C3, and/or C9 mRNA.

An alternative method for determining the level of expression of CFB, C3, and/or C9 in a sample involves the process of nucleic acid amplification and/or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of CFB, C3, and/or C9 is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System).

The expression levels of CFB, C3, and/or C9 mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as Northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of PCSK9 expression level may also comprise using nucleic acid probes in solution.

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

The level of CFB, C3, and/or C9 protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, Western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

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

In some embodiments of the methods of the invention, the RNAi agent is administered to a subject such that the RNAi agent is delivered to a specific site within the subject. The inhibition of expression of CFB, C3, and/or C9 may be assessed using measurements of the level or change in the level of CFB, C3, and/or C9 mRNA and/or CFB, C3, and/or C9 protein in a sample derived from fluid or tissue from the specific site within the subject. In preferred embodiments, the site is sthe liver. The site may also be a subsection or subgroup of cells from any one of the aforementioned sites. The site may also include cells that express a particular type of receptor.

VIII. Methods for Treating or Preventing a Complement Component-Associated Disease

The present invention provides therapeutic and prophylactic methods which include administering to a subject having a complement component-associated disease, as described herein, *e.g.,* PNH or aHUS, an iRNA agent, pharmaceutical compositions comprising an iRNA agent, or vector comprising an iRNA of the invention.

It is to be understood, that any of the methods of the invention may be practiced with a single iRNA agent of the invention or a combination of iRNA agents of the invention. For example, in some aspects, the methods (and uses) of the invention include using an iRNA agent targeting a CFB gene and an iRNA agent targeting a C3 gene. In some aspects, the

methods (and uses) of the invention include using an iRNA agent targeting a CFB gene and an iRNA agent targeting a C9 gene. In some aspects, the methods (and uses) of the invention include using an iRNA agent targeting a C3 gene and an iRNA agent targeting a C9 gene. In other aspects, the methods (and uses) of the invention include using an iRNA agent targeting a CFB gene, an iRNA agent targeting a C3 gene, and an iRNA agent targeting a C9 gene. In some aspects of the invention, the methods which include either a single iRNA agent of the invention or a combination of iRNA agents, further include administering to the subject one or more additional therapeutic agents such as, for example, Soliris® (as further described below).

In one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in CFB expression, *e.g.,* "a complement component-associated disease," *e.g.,* PNH, aHUS, or rheumatoid arthritis. The treatment methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent targeting a CFB gene or a pharmaceutical composition comprising an iRNA agent targeting a CFB gene, thereby treating the subject having a disorder that would benefit from reduction in CFB expression.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in C3 expression, *e.g.,* "a complement component-associated disease," *e.g.,* PNH, aHUS, or rheumatoid arthritis. The treatment methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent targeting a C3 gene or a pharmaceutical composition comprising an iRNA agent targeting a C3 gene, thereby treating the subject having a disorder that would benefit from reduction in C3 expression.

In a further aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in C9 expression, *e.g.,* "a complement component-associated disease," *e.g.,* PNH, aHUS, or rheumatoid arthritis. The treatment methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent targeting a C9 gene or a pharmaceutical composition comprising an iRNA agent targeting a C9 gene, thereby treating the subject having a disorder that would benefit from reduction in C9 expression.

In one aspect, the invention provides methods of preventing at least one symptom in a subject having a disorder that would benefit from reduction in CFB expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis. The methods include administering to the subject a therapeutically effective amount of the iRNA agent, *e.g.,* dsRNA, or vector of the invention, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in CFB expression. For example, the invention provides methods for preventing hemolysis in a subject suffering from a

disorder that would benefit from reduction in CFB expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis.

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

In a further aspect, the present invention provides uses of a therapeutically effective amount of an iRNA agent of the invention for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of C3 expression.

In yet another aspect, the present invention provides uses of a therapeutically effective amount of an iRNA agent of the invention for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of C9 expression.

In yet another aspect, the present invention provides use of an iRNA agent, *e.g.,* a dsRNA, of the invention targeting a CFB gene or a pharmaceutical composition comprising an iRNA agent targeting a CFB gene in the manufacture of a medicament for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of CFB expression, such as a subject having a disorder that would benefit from reduction in CFB expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis.

In a further aspect, the present invention provides use of an iRNA agent, *e.g.,* a dsRNA, of the invention targeting a C3 gene or a pharmaceutical composition comprising an

iRNA agent targeting a C3 gene in the manufacture of a medicament for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of C3 expression, such as a subject having a disorder that would benefit from reduction in C3 expression, *e.g.*, a complement component-associated disease, *e.g.*, PNH, aHUS, or rheumatoid arthritis.

In yet a further aspect, the present invention provides use of an iRNA agent, *e.g.*, a dsRNA, of the invention targeting a C9 gene or a pharmaceutical composition comprising an iRNA agent targeting a C9 gene in the manufacture of a medicament for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of C9 expression, such as a subject having a disorder that would benefit from reduction in C9 expression, *e.g.*, a complement component-associated disease, *e.g.*, PNH, aHUS, or rheumatoid arthritis.

In another aspect, the invention provides uses of an iRNA, *e.g.*, a dsRNA, of the invention for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of CFB expression, such as a complement component-associated disease, *e.g.*, PNH, aHUS, or rheumatoid arthritis.

In another aspect, the invention provides uses of an iRNA, *e.g.*, a dsRNA, of the invention for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of C3 expression, such as a complement component-associated disease, *e.g.*, PNH, aHUS, or rheumatoid arthritis.

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In a further aspect, the present invention provides uses of an iRNA agent of the invention in the manufacture of a medicament for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of CFB expression, such as a a complement component-associated disease, *e.g.*, PNH, aHUS, or rheumatoid arthritis.

In a further aspect, the present invention provides uses of an iRNA agent of the invention in the manufacture of a medicament for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of C3 expression, such as a a complement component-associated disease, *e.g.*, PNH, aHUS, or rheumatoid arthritis.

In a further aspect, the present invention provides uses of an iRNA agent of the invention in the manufacture of a medicament for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of C9 expression, such as a a complement component-associated disease, *e.g.*, PNH, aHUS, or rheumatoid arthritis.

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

In some aspects, the additional therapeutic agent is an iRNA agent targeting a C5 gene, such as described in U.S. Provisional Patent Application No.: 61/782,531, filed on March 14, 2013, U.S. Provisional Patent Application No.: 61/837,3991, filed on June 20, 2013, and U.S. Provisional Patent Application No.: 61/904,579, filed on November 15, 2013, the entire contents of each of which are hereby incorporated herein by reference.

In other aspects, the additional therapeutic agent is an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab). 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.

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.

Accordingly, in one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in CFB expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis, which include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent targeting a CFB gene or a pharmaceutical composition comprising an iRNA agent targeting a CFB gene, and an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), thereby treating the subject having a disorder that would benefit from reduction in CFB expression.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in C3 expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis, which include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent targeting a C3 gene or a pharmaceutical composition comprising an iRNA agent targeting a C3 gene, and an additional therapeutic agent, such as an anti-complement

component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), thereby treating the subject having a disorder that would benefit from reduction in C3 expression.

In another aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in C9 expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis, which include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent targeting a C9 gene or a pharmaceutical composition comprising an iRNA agent targeting a C9 gene, and an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), thereby treating the subject having a disorder that would benefit from reduction in C9 expression.

In another aspect, the invention provides methods of preventing at least one symptom in a subject having a disorder that would benefit from reduction in CFB expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis. The methods include administering to the subject a therapeutically effective amount of the iRNA agent, *e.g.,* dsRNA, or vector of the invention, and an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in CFB expression.

In another aspect, the invention provides methods of preventing at least one symptom in a subject having a disorder that would benefit from reduction in C3 expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis. The methods include administering to the subject a therapeutically effective amount of the iRNA agent, *e.g.,* dsRNA, or vector of the invention, and an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in C3 expression.

In another aspect, the invention provides methods of preventing at least one symptom in a subject having a disorder that would benefit from reduction in C9 expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis. The methods include administering to the subject a therapeutically effective amount of the iRNA agent, *e.g.,* dsRNA, or vector of the invention, and an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,*

eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in C9 expression.

In another aspect, the present invention provides uses of a therapeutically effective amount of an iRNA agent of the invention and an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide nhibitor (*e.g.,* comstatin), for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of CFB expression.

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In another aspect, the present invention provides uses of an iRNA agent, *e.g.,* a dsRNA, of the invention targeting a CFB gene or a pharmaceutical composition comprising an iRNA agent targeting a CFB gene in the manufacture of a medicament for use in combination with an additional therapeutic agent, such as an anti-complement component CFB antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of CFB expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis.

In another aspect, the present invention provides uses of an iRNA agent, *e.g.,* a dsRNA, of the invention targeting a C3 gene or a pharmaceutical composition comprising an iRNA agent targeting a C3 gene in the manufacture of a medicament for use in combination with an additional therapeutic agent, such as an anti-complement component C3 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of C3 expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis.

In another aspect, the present invention provides uses of an iRNA agent, *e.g.,* a dsRNA, of the invention targeting a C9 gene or a pharmaceutical composition comprising an iRNA agent targeting a C9 gene in the manufacture of a medicament for use in combination with an additional therapeutic agent, such as an anti-complement component C9 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of C9 expression, *e.g.,* a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis.

In yet another aspect, the invention provides uses of an iRNA agent, *e.g.,* a dsRNA, of the invention, and an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of CFB expression, such as a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis.

In yet another aspect, the invention provides uses of an iRNA agent, *e.g.,* a dsRNA, of the invention, and an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of C3 expression, such as a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis.

In yet another aspect, the invention provides uses of an iRNA agent, *e.g.,* a dsRNA, of the invention, and an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of C9 expression, such as a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis.

In a further aspect, the present invention provides uses of an iRNA agent of the invention in the manufacture of a medicament for use in combination with an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of CFB expression, such as a a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis.

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In a further aspect, the present invention provides uses of an iRNA agent of the invention in the manufacture of a medicament for use in combination with an additional therapeutic agent, such as an anti-complement component C5 antibody, or antigen-binding fragment thereof (*e.g.,* eculizumab), an iRNA agent targeting complement component C5, and/or a C3 peptide inhibitor (*e.g.,* compstatin), for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of C9 expression, such as a a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis.

In one embodiment, an iRNA agent targeting CFB, C3, or C9 is administered to a subject having a complement component-associated disease as described herein such that CFB, C3, and/or C9 levels, *e.g.*, in a cell, tissue, blood, urine or other tissue or fluid of the subject are reduced by at least about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more and, subsequently, an additional therapeutic is administered to the subject.

The additional therapeutic may be an anti-complement component C5 antibody, or antigen-binding fragment or derivative thereof. In one embodiment, the anti-complement component C5 antibody is eculizumab (SOLIRIS®), or antigen-binding fragment or derivative thereof.

The methods of the invention comprising administration of an iRNA agent of the invention and eculizumab to a subject may further comprise administration of a meningococcal vaccine to the subject.

The additional therapeutic, *e.g.,* eculizumab and/or a meningococcal vaccine, may be administered to the subject at the same time as the iRNA agent targeting CFB, C3, and/or C9 (and/or C5) or at a different time.

Moreover, the additional therapeutic, *e.g.,* eculizumab, may be administered to the subject in the same formulation as the iRNA agent targeting CFB, C3, and/or C9 (and/or C5) or in a different formulation as the iRNA agent targeting CFB, C3, and/or C9 (and/or C5).

Eculizumab dosage regimens are described in, for example, the product insert for eculizumab (SOLIRIS®) and in U.S. Patent Application No. 2012/0225056, the entire contents of each of which are incorporated herein by reference. In exemplary methods of the invention for treating a complement component-associated disease, *e.g.,* PNH, aHUS, or rheumatoid arthritis, an iRNA agent targeting, *e.g.,* CFB, C3, or C9, is administered (*e.g.,* subcutaneously) to the subject first, such that the C5 levels in the subject are reduced (*e.g.,* by at least about 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more) and subsequently eculizumab is administered at doses lower than the ones described in the product insert for SOLIRIS®. For example, eculizumab may be adminsitered to the subject weekly at a dose less than about 600 mg for 4 weeks followed by a fifth dose at about one week later of less than about 900 mg, followed by a dose less than about 900 mg about every two weeks thereafter. Eculizumab may also be administered to the subject weekly at a dose less than about 900 mg for 4 weeks followed by a fifth dose at about one week later of less than about 1200 mg, followed by a dose less than about 1200 mg about every two weeks thereafter. If the subject is less than 18 years of age, eculizumab may be administered to the subject weekly at a dose less than about 900 mg for 4 weeks followed by a fifth dose at about one week later of less than about 1200 mg, followed by a dose less than about 1200 mg about every two weeks thereafter; or if the subject is less than 18 years of age, eculizumab may be administered to the subject weekly at a dose less than about 600 mg for 2 weeks followed by a third dose at about one week later of less than about 900 mg, followed by a dose less than about 900 mg about every two weeks thereafter; or if the subject is less than 18 years of age, eculizumab may be administered to the subject weekly at a dose less than about 600 mg for 2 weeks followed by a third dose at about one week later of less than about 600 mg, followed by a dose less than about 600 mg about every two weeks thereafter; or if the subject is less than 18 years of age, eculizumab may be administered to the subject weekly at a dose less than about 600 mg for 1 week followed by a second dose at about one week later of less than about 300 mg, followed by a dose less than about 300 mg about every two weeks thereafter; or if the subject is less than 18 years of age, eculizumab may be administered to the subject weekly at a dose less than about 300 mg for 1 week followed by a second dose at about one week later of less than about 300 mg, followed by a dose less than about 300 mg about every two weeks thereafter. If the subject is

receiving plamapheresis or plasma exchange, eculizumab may be administered to the subject at a dose less than about 300 mg (*e.g.,* if the most recent does of eculizumab was about 300 mg) or less than about 600 mg (*e.g.,* if the most recent does of eculizumab was about 600 mg or more). If the subject is receiving plasma infusion, eculizumab may be administered to the subject at a dose less than about 300 mg (*e.g.,* if the most recent does of eculizumab was about 300 mg or more). The lower doses of eculizumab allow for either subcutaneous or intravenous administration of eculizumab.

In the combination therapies of the present invention comprising eculizumab, eculizumab may be adminisitered to the subject, *e.g.,* subcutaneously, at a dose of about 0.01 mg/kg to about 10 mg/kg, or about 5 mg/kg to about 10 mg/kg, or about 0.5 mg/kg to about 15 mg/kg. For example, eculizumab may be administered to the subject, *e.g.,* subcutaneously, at a dose of 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4 mg/kg, 4.5 mg/kg, 5 mg/kg, 5.5 mg/kg, 6 mg/kg, 6.5 mg/kg, 7 mg/kg, 7.5 mg/kg, 8 mg/kg, 8.5 mg/kg, 9 mg/kg, 9.5 mg/kg, 10 mg/kg, 10.5 mg/kg, 11 mg/kg, 11.5 mg/kg, 12 mg/kg, 12.5 mg/kg, 13 mg/kg, 13.5 mg/kg, 14 mg/kg, 14.5 mg/kg, or15 mg/kg.

The methods and uses of the invention include administering a composition described herein such that expression of the target CFB, C3, and/or C9 (and/or C5) gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 18, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, or about 80 hours. In one embodiment, expression of the target gene is decreased for an extended duration, *e.g.*, at least about two, three, four, five, six, seven days or more, *e.g.*, about one week, two weeks, three weeks, or about four weeks or longer.

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

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of a hemolytic disorder may be assessed, for example, by periodic monitoring of LDH and CH_{50} levels. Comparisons of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of

parameters. In connection with the administration of an iRNA targeting CFB, C3, and/or C9, or pharmaceutical composition thereof, "effective against" a complement component-associated disease indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating a complement component-associated disease and the related causes.

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

Alternatively, the efficacy can be measured by a reduction in the severity of disease as determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading scale, as but one example the Rheumatoid Arthritis Severity Scale (RASS). Any positive change resulting in *e.g.*, lessening of severity of disease measured using the appropriate scale, represents adequate treatment using an iRNA or iRNA formulation as described herein.

Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg, 0.25 mg/kg, 0.3 mg/kg, 0.35 mg/kg, 0.4 mg/kg, 0.45 mg/kg, 0.5 mg/kg, 0.55 mg/kg, 0.6 mg/kg, 0.65 mg/kg, 0.7 mg/kg, 0.75 mg/kg, 0.8 mg/kg, 0.85 mg/kg, 0.9 mg/kg, 0.95 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, 2.0 mg/kg, 2.1mg/kg, 2.2mg/kg, 2.3 mg/kg, 2.4 mg/kg, 2.5 mg/kg dsRNA, 2.6 mg/kg dsRNA, 2.7 mg/kg dsRNA, 2.8 mg/kg dsRNA, 2.9 mg/kg dsRNA, 3.0 mg/kg dsRNA, 3.1 mg/kg dsRNA, 3.2 mg/kg dsRNA, 3.3 mg/kg dsRNA, 3.4 mg/kg dsRNA, 3.5 mg/kg dsRNA, 3.6 mg/kg dsRNA, 3.7 mg/kg dsRNA, 3.8 mg/kg dsRNA, 3.9 mg/kg dsRNA, 4.0 mg/kg dsRNA, 4.1 mg/kg dsRNA, 4.2 mg/kg dsRNA, 4.3 mg/kg dsRNA, 4.4 mg/kg dsRNA, 4.5 mg/kg dsRNA, 4.6 mg/kg dsRNA, 4.7 mg/kg dsRNA, 4.8 mg/kg dsRNA, 4.9 mg/kg dsRNA, 5.0 mg/kg dsRNA, 5.1 mg/kg dsRNA, 5.2 mg/kg dsRNA, 5.3 mg/kg dsRNA, 5.4 mg/kg dsRNA, 5.5 mg/kg dsRNA, 5.6 mg/kg dsRNA, 5.7 mg/kg dsRNA, 5.8 mg/kg dsRNA, 5.9 mg/kg dsRNA, 6.0 mg/kg dsRNA, 6.1 mg/kg dsRNA, 6.2 mg/kg dsRNA, 6.3 mg/kg dsRNA, 6.4 mg/kg dsRNA, 6.5 mg/kg dsRNA, 6.6 mg/kg dsRNA, 6.7 mg/kg dsRNA, 6.8 mg/kg dsRNA, 6.9 mg/kg dsRNA, 7.0 mg/kg dsRNA, 7.1 mg/kg dsRNA, 7.2 mg/kg

dsRNA, 7.3 mg/kg dsRNA, 7.4 mg/kg dsRNA, 7.5 mg/kg dsRNA, 7.6 mg/kg dsRNA, 7.7 mg/kg dsRNA, 7.8 mg/kg dsRNA, 7.9 mg/kg dsRNA, 8.0 mg/kg dsRNA, 8.1 mg/kg dsRNA, 8.2 mg/kg dsRNA, 8.3 mg/kg dsRNA, 8.4 mg/kg dsRNA, 8.5 mg/kg dsRNA, 8.6 mg/kg dsRNA, 8.7 mg/kg dsRNA, 8.8 mg/kg dsRNA, 8.9 mg/kg dsRNA, 9.0 mg/kg dsRNA, 9.1 mg/kg dsRNA, 9.2 mg/kg dsRNA, 9.3 mg/kg dsRNA, 9.4 mg/kg dsRNA, 9.5 mg/kg dsRNA, 9.6 mg/kg dsRNA, 9.7 mg/kg dsRNA, 9.8 mg/kg dsRNA, 9.9 mg/kg dsRNA, 9.0 mg/kg dsRNA, 10 mg/kg dsRNA, 15 mg/kg dsRNA, 20 mg/kg dsRNA, 25 mg/kg dsRNA, 30 mg/kg dsRNA, 35 mg/kg dsRNA, 40 mg/kg dsRNA, 45 mg/kg dsRNA, or about 50 mg/kg dsRNA. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In certain embodiments, for example, when a composition of the invention comprises a dsRNA as described herein and a lipid, subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 5 mg/kg, about 0.01 mg/kg to about 10 mg/kg, about 0.05 mg/kg to about 5 mg/kg, about 0.05 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 5 mg/kg, about 0.1 mg/kg to about 10 mg/kg, about 0.2 mg/kg to about 5 mg/kg, about 0.2 mg/kg to about 10 mg/kg, about 0.3 mg/kg to about 5 mg/kg, about 0.3 mg/kg to about 10 mg/kg, about 0.4 mg/kg to about 5 mg/kg, about 0.4 mg/kg to about 10 mg/kg, about 0.5 mg/kg to about 5 mg/kg, about 0.5 mg/kg to about 10 mg/kg, about 1 mg/kg to about 5 mg/kg, about 1 mg/kg to about 10 mg/kg, about 1.5 mg/kg to about 5 mg/kg, about 1.5 mg/kg to about 10 mg/kg, about 2 mg/kg to about about 2.5 mg/kg, about 2 mg/kg to about 10 mg/kg, about 3 mg/kg to about 5 mg/kg, about 3 mg/kg to about 10 mg/kg, about 3.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 5 mg/kg, about 4.5 mg/kg to about 5 mg/kg, about 4 mg/kg to about 10 mg/kg, about 4.5 mg/kg to about 10 mg/kg, about 5 mg/kg to about 10 mg/kg, about 5.5 mg/kg to about 10 mg/kg, about 6 mg/kg to about 10 mg/kg, about 6.5 mg/kg to about 10 mg/kg, about 7 mg/kg to about 10 mg/kg, about 7.5 mg/kg to about 10 mg/kg, about 8 mg/kg to about 10 mg/kg, about 8.5 mg/kg to about 10 mg/kg, about 9 mg/kg to about 10 mg/kg, or about 9.5 mg/kg to about 10 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

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

In other embodiments, for example, when a composition of the invention comprises a dsRNA as described herein and an N-acetylgalactosamine, subjects can be administered a therapeutic amount of iRNA, such as a dose of about 0.1 to about 50 mg/kg, about 0.25 to

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

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

In certain embodiments of the invention, for example, when a double-stranded RNAi agent includes a modification (*e.g.,* one or more motifs of three identical modifications on three consecutive nucleotides), including one such motif at or near the cleavage site of the agent, six phosphorothioate linkages, and a ligand, such an agent is administered at a dose of about 0.01 to about 0.5 mg/kg, about 0.01 to about 0.4 mg/kg, about 0.01 to about 0.3 mg/kg, about 0.01 to about 0.2 mg/kg, about 0.01 to about 0.1 mg/kg, about 0.01 mg/kg to about 0.09 mg/kg, about 0.01 mg/kg to about 0.08 mg/kg, about 0.01 mg/kg to about 0.07 mg/kg, about 0.01 mg/kg to about 0.06 mg/kg, about 0.01 mg/kg to about 0.05 mg/kg, about 0.02 to about 0.5 mg/kg, about 0.02 to about 0.4 mg/kg, about 0.02 to about 0.3 mg/kg, about 0.02 to about 0.2 mg/kg, about 0.02 to about 0.1 mg/kg, about 0.02 mg/kg to about 0.09 mg/kg, about 0.02 mg/kg to about 0.08 mg/kg, about 0.02 mg/kg to about 0.07 mg/kg, about 0.02 mg/kg to about 0.06 mg/kg, about 0.02 mg/kg to about 0.05 mg/kg, about 0.03 to about 0.5 mg/kg, about 0.03 to about 0.4 mg/kg, about 0.03 to about 0.3 mg/kg, about 0.03 to about 0.2 mg/kg, about 0.03 to about 0.1 mg/kg, about 0.03 mg/kg to about 0.09 mg/kg, about 0.03 mg/kg to about 0.08 mg/kg, about 0.03 mg/kg to about 0.07 mg/kg, about 0.03 mg/kg to about 0.06 mg/kg, about 0.03 mg/kg to about 0.05 mg/kg, about 0.04 to about 0.5 mg/kg, about 0.04 to about 0.4 mg/kg, about 0.04 to about 0.3 mg/kg, about 0.04 to about 0.2 mg/kg, about 0.04 to about 0.1 mg/kg, about 0.04 mg/kg to about 0.09 mg/kg, about 0.04 mg/kg to about 0.08 mg/kg, about 0.04 mg/kg to about 0.07 mg/kg, about 0.04 mg/kg to about 0.06 mg/kg, about 0.05 to about 0.5 mg/kg, about 0.05 to about 0.4 mg/kg, about 0.05 to about 0.3 mg/kg, about 0.05 to about 0.2 mg/kg, about 0.05 to about 0.1 mg/kg, about 0.05 mg/kg to about 0.09 mg/kg, about 0.05 mg/kg to about 0.08 mg/kg, or about 0.05 mg/kg to about 0.07 mg/kg. Values and ranges intermediate to the foregoing recited values are also intended to be part of this invention, *e.g.,*, the RNAi agent may be administered to the subject at a dose of about 0.015 mg/kg to about 0.45 mg/mg.

For example, the RNAi agent, *e.g.*, RNAi agent in a pharmaceutical composition, may be administered at a dose of about 0.01 mg/kg, 0.0125 mg/kg, 0.015 mg/kg, 0.0175 mg/kg, 0.02 mg/kg, 0.0225 mg/kg, 0.025 mg/kg, 0.0275 mg/kg, 0.03 mg/kg, 0.0325 mg/kg, 0.035

mg/kg, 0.0375 mg/kg, 0.04 mg/kg, 0.0425 mg/kg, 0.045 mg/kg, 0.0475 mg/kg, 0.05 mg/kg, 0.0525 mg/kg, 0.055 mg/kg, 0.0575 mg/kg, 0.06 mg/kg, 0.0625 mg/kg, 0.065 mg/kg, 0.0675 mg/kg, 0.07 mg/kg, 0.0725 mg/kg, 0.075 mg/kg, 0.0775 mg/kg, 0.08 mg/kg, 0.0825 mg/kg, 0.085 mg/kg, 0.0875 mg/kg, 0.09 mg/kg, 0.0925 mg/kg, 0.095 mg/kg, 0.0975 mg/kg, 0.1 mg/kg, 0.125 mg/kg, 0.15 mg/kg, 0.175 mg/kg, 0.2 mg/kg, 0.225 mg/kg, 0.25 mg/kg, 0.275 mg/kg, 0.3 mg/kg, 0.325 mg/kg, 0.35 mg/kg, 0.375 mg/kg, 0.4 mg/kg, 0.425 mg/kg, 0.45 mg/kg, 0.475 mg/kg, or about 0.5 mg/kg. Values intermediate to the foregoing recited values are also intended to be part of this invention.

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

Administration of the iRNA can reduce CFB, C3, and/or C9 (and/or C5) levels, *e.g.*, in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more.

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

Owing to the inhibitory effects on CFB, C3, and/or C9 expression, a composition according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life.

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

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

Subjects that would benefit from a reduction and/or inhibition of CFB, C3, and/or C9 gene expression are those having a complement component-associated disease or disorder as described herein. In one embodiment, a subject having a complement component-associated disease has paroxysmal nocturnal hemoglobinuria (PNH). In another embodiment, a subject having a complement component-associated disease has asthma. In another embodiment, a subject having a complement component-associated disease has rheumatoid arthritis. In yet another embodiment, a subject having a complement component-associated disease has systemic lupus erythmatosis. In one embodiment, a subject having a complement component-associated disease has glomerulonephritis. In another embodiment, a subject having a complement component-associated disease has psoriasis. In yet another embodiment, a subject having a complement component-associated disease has dermatomyositis bullous pemphigoid. In one embodiment, a subject having a complement component-associated disease has atypical hemolytic uremic syndrome. In another embodiment, a subject having a complement component-associated disease has Shiga toxin *E. coli*-related hemolytic uremic syndrome. In another embodiment, a subject having a complement component-associated disease has myasthenia gravis. In yet another embodiment, a subject having a complement component-associated disease has neuromyelistis optica. In one embodiment, a subject having a complement component-associated disease has dense deposit disease. In one embodiment, a subject having a complement component-associated disease has C3 neuropathy. In another embodiment, a subject having a complement component-associated disease has age-related macular degeneration. In another embodiment, a subject having a complement component-associated disease has cold agglutinin disease. In one embodiment, a subject having a complement component-associated disease has anti-neutrophil cytoplasmic antibody-associated vasculitis. In another embodiment, a subject having a complement component-associated disease has humoral and vascular transplant rejection. In one embodiment, a subject having a complement component -associated disease has graft dysfunction. In one embodiment, a subject having a complement component-associated disease has had a myocardial infarction. In another embodiment, a subject having a complement component-associated disease is a sensitized recipient of a transplant. In yet another embodiment, a subject having a complement component-associated disease has sepsis.

Treatment of a subject that would benefit from a reduction and/or inhibition of CFB, C3, and/or C9 gene expression includes therapeutic and prophylactic (*e.g.,* the subject is to undergo sensitized (or allogenic) transplant surgery treatment.

The invention further provides methods and uses of an iRNA agent or a pharmaceutical composition thereof (including methods and uses of an iRNA agent or a

pharmaceutical composition comprising an iRNA agent and an additional therapeutic agent, *e.g.* an anti-complement component C5 antibody, or antigen-binding fragment thereof) for treating a subject that would benefit from reduction and/or inhibition of a target gene of the invention, *e.g.,* CFB, C3, and C9, expression, *e.g.,* a subject having a complement component-associated disease, in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. For example, in certain embodiments, an iRNA targeting CFB is administered in combination with, *e.g.*, an agent useful in treating a complement component-associated disease as described elsewhere herein.

For example, additional therapeutics and therapeutic methods suitable for treating a subject that would benefit from reducton in CFB, C3, and/or C9 expression, *e.g.,* a subject having a complement component-associated disease, include plasmaphoresis, thrombolytic therapy (*e.g.,* streptokinase), antiplatelet agents, folic acid, corticosteroids; immunosuppressive agents; estrogens, methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine, chloroquinine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, cochicine, corticosteroids (oral, inhaled and local injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines, such as TNF-α or IL-1 (*e.g.,* IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TNFαconverting enzyme (TACE) inhibitors, T-cell signalling inhibitors, such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (*e.g.,* soluble p55 or p75 TNF receptors and the derivatives p75TNFRIgG (Enbrel™ and p55TNFRIgG (Lenercept)), sIL-1RI, sIL-1RII, and sIL-6R), antiinflammatory cytokines (*e.g.,* IL-4, IL-10, IL-11, IL-13 and TGFβ), celecoxib, folic acid, hydroxychloroquine sulfate, rofecoxib, etanercept, infliximonoclonal antibody, naproxen, valdecoxib, sulfasalazine, methylprednisolone, meloxicam, methylprednisolone acetate, gold sodium thiomalate, aspirin, triamcinolone acetonide, propoxyphene napsylate/apap, folate, nabumetone, diclofenac, piroxicam, etodolac, diclofenac sodium, oxaprozin, oxycodone hydrochloride , hydrocodone bitartrate/apap, diclofenac sodium/misoprostol, fentanyl, anakinra, human recombinant, tramadol hydrochloride, salsalate, sulindac, cyanocobalamin/folic acid/pyridoxine, acetaminophen, alendronate sodium, prednisolone, morphine sulfate, lidocaine hydrochloride, indomethacin, glucosamine sulf/chondroitin, amitriptyline

hydrochloride , sulfadiazine, oxycodone hydrochloride /acetaminophen, olopatadine hydrochloride , misoprostol, naproxen sodium, omeprazole, cyclophosphamide, rituximonoclonal antibody, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-18, Anti-IL15, BIRB-796, SCIO-469, VX-702, AMG-548, VX-740, Roflumilast, IC-485, CDC-801, Mesopram, cyclosporine, cytokine suppressive anti-inflammatory drug(s) (CSAIDs); CDP-571/BAY-10-3356 (humanized anti-TNFα antibody; Celltech/Bayer); cA2/infliximonoclonal antibody (chimeric anti-TNFα antibody; Centocor); 75 kdTNFR-IgG/etanercept (75 kD TNF receptor-IgG fusion protein; Immunex; see *e.g.,* (1994) Arthr. Rheum. 37: S295; (1996) J. Invest. Med. 44: 235A); 55 kdTNF-IgG (55 kD TNF receptor-IgG fusion protein; Hoffmann-LaRoche); IDEC-CE9.1/SB 210396 (non-depleting primatized anti-CD4 antibody; IDEC/SmithKline; see *e.g.,* (1995) Arthr. Rheum. 38: S185); DAB 486-IL-2 and/or DAB 389-IL-2 (IL-2 fusion proteins; Seragen; see *e.g.,* (1993) Arthrit. Rheum. 36: 1223); Anti-Tac (humanized anti-IL-2Rα; Protein Design Labs/Roche); IL-4 (anti-inflammatory cytokine; DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10, anti-inflammatory cytokine; DNAX/Schering); IL-4; IL-10 and/or IL-4 agonists (*e.g.*, agonist antibodies); IL-1RA (IL-1 receptor antagonist; Synergen/Amgen); anakinra (Kineret®/Amgen); TNF-bp/s-TNF (soluble TNF binding protein; see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement)): S284; (1995) Amer. J. Physiol. - Heart and Circ. Physiol. 268: 37-42); R973401 (phosphodiesterase Type IV inhibitor; see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S282); MK-966 (COX-2 Inhibitor; see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S81); Iloprost (see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S82); methotrexate; thalidomide (see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S282) and thalidomide-related drugs (*e.g.,* Celgen); leflunomide (anti-inflammatory and cytokine inhibitor; see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S131; (1996) Inflamm. Res. 45: 103-107); tranexamic acid (inhibitor of plasminogen activation; see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S284); T-614 (cytokine inhibitor; see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S282); prostaglandin E1 (see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S282); Tenidap (non-steroidal anti-inflammatory drug; see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S280); Naproxen (non-steroidal anti-inflammatory drug; see *e.g.,* (1996) Neuro. Report 7: 1209-1213); Meloxicam (non-steroidal anti-inflammatory drug); Ibuprofen (non-steroidal anti-inflammatory drug); Piroxicam (non-steroidal anti-inflammatory drug); Diclofenac (non-steroidal anti-inflammatory drug); Indomethacin (non-steroidal anti-inflammatory drug); Sulfasalazine (see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S281); Azathioprine (see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S281); ICE inhibitor (inhibitor of the enzyme interleukin-1β converting enzyme); zap-70 and/or lck inhibitor (inhibitor of the tyrosine kinase zap-70 or lck); VEGF inhibitor and/or VEGF-R inhibitor (inhibitors of vascular endothelial cell growth factor or vascular endothelial cell growth factor receptor; inhibitors of angiogenesis); corticosteroid anti-inflammatory drugs (*e.g.,* SB203580); TNF-convertase

inhibitors; anti-IL-12 antibodies; anti-IL-18 antibodies; interleukin-11 (see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S296); interleukin-13 (see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S308); interleukin -17 inhibitors (see *e.g.,* (1996) Arthr. Rheum. 39(9 (supplement): S120); gold; penicillamine; chloroquine; chlorambucil; hydroxychloroquine; cyclosporine; cyclophosphamide; total lymphoid irradiation; anti-thymocyte globulin; anti-CD4 antibodies; CD5-toxins; orally-administered peptides and collagen; lobenzarit disodium; Cytokine Regulating Agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); ICAM-1 antisense phosphorothioate oligo-deoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); prednisone; orgotein; glycosaminoglycan polysulphate; minocycline; anti-IL2R antibodies; marine and botanical lipids (fish and plant seed fatty acids; see *e.g.,* DeLuca *et al.* (1995) Rheum. Dis. Clin. North Am. 21: 759-777); auranofin; phenylbutazone; meclofenamic acid; flufenamic acid; intravenous immune globulin; zileuton; azaribine; mycophenolic acid (RS-61443); tacrolimus (FK-506); sirolimus (rapamycin); amiprilose (therafectin); cladribine (2-chlorodeoxyadenosine); methotrexate; bcl-2 inhibitors (see Bruncko, M. *et al.* (2007) J. Med. Chem. 50(4): 641-662); antivirals and immune-modulating agents, small molecule inhibitor of KDR, small molecule inhibitor of Tie-2; methotrexate; prednisone; celecoxib; folic acid; hydroxychloroquine sulfate; rofecoxib; etanercept; infliximonoclonal antibody; leflunomide; naproxen; valdecoxib; sulfasalazine; methylprednisolone; ibuprofen; meloxicam; methylprednisolone acetate; gold sodium thiomalate; aspirin; azathioprine; triamcinolone acetonide; propxyphene napsylate/apap; folate; nabumetone; diclofenac; piroxicam; etodolac; diclofenac sodium; oxaprozin; oxycodone hcl; hydrocodone bitartrate/apap; diclofenac sodium/misoprostol; fentanyl; anakinra, human recombinant; tramadol hcl; salsalate; sulindac; cyanocobalamin/fa/pyridoxine; acetaminophen; alendronate sodium; prednisolone; morphine sulfate; lidocaine hydrochloride; indomethacin; glucosamine sulfate/chondroitin; cyclosporine; amitriptyline hydrochloride; sulfadiazine; oxycodone hcl/acetaminophen; olopatadine hcl; misoprostol; naproxen sodium; omeprazole; mycophenolate mofetil; cyclophosphamide; rituximonoclonal antibody; IL-1 TRAP; MRA; CTLA4-IG; IL-18 BP; IL-12/23; anti-IL 18; anti-IL 15; BIRB-796; SCIO-469; VX-702; AMG-548; VX-740; Roflumilast; IC-485; CDC-801; mesopram, albuterol, salmeterol/fluticasone, montelukast sodium, fluticasone propionate, budesonide, prednisone, salmeterol xinafoate, levalbuterol hcl, albuterol sulfate/ipratropium, prednisolone sodium phosphate, triamcinolone acetonide, beclomethasone dipropionate, ipratropium bromide, azithromycin, pirbuterol acetate, prednisolone, theophylline anhydrous, methylprednisolone sodium succinate, clarithromycin, zafirlukast, formoterol fumarate, influenza virus vaccine, methylprednisolone, amoxicillin trihydrate, flunisolide, allergy injection, cromolyn sodium, fexofenadine hydrochloride, flunisolide/menthol, amoxicillin/clavulanate, levofloxacin, inhaler assist device, guaifenesin, dexamethasone sodium phosphate, moxifloxacin hcl, doxycycline hyclate, guaifenesin/d-

methorphan, p-ephedrine/cod/chlorphenir, gatifloxacin, cetirizine hydrochloride, mometasone furoate, salmeterol xinafoate, benzonatate, cephalexin, pe/hydrocodone/chlorphenir, cetirizine hcl/pseudoephed, phenylephrine/cod/promethazine, codeine/promethazine, cefprozil, dexamethasone, guaifenesin/pseudoephedrine, chlorpheniramine/hydrocodone, nedocromil sodium, terbutaline sulfate, epinephrine, methylprednisolone, metaproterenol sulfate, aspirin, nitroglycerin, metoprolol tartrate, enoxaparin sodium, heparin sodium, clopidogrel bisulfate, carvedilol, atenolol, morphine sulfate, metoprolol succinate, warfarin sodium, lisinopril, isosorbide mononitrate, digoxin, furosemide, simvastatin, ramipril, tenecteplase, enalapril maleate, torsemide, retavase, losartan potassium, quinapril hcl/mag carb, bumetanide, alteplase, enalaprilat, amiodarone hydrochloride, tirofiban hcl m-hydrate, diltiazem hydrochloride, captopril, irbesartan, valsartan, propranolol hydrochloride, fosinopril sodium, lidocaine hydrochloride, eptifibatide, cefazolin sodium, atropine sulfate, aminocaproic acid, spironolactone, interferon, sotalol hydrochloride, potassium chloride, docusate sodium, dobutamine hcl, alprazolam, pravastatin sodium, atorvastatin calcium, midazolam hydrochloride, meperidine hydrochloride, isosorbide dinitrate, epinephrine, dopamine hydrochloride, bivalirudin, rosuvastatin, ezetimibe/simvastatin, avasimibe, and cariporide.

The iRNA agent (and/or an anti-complement component C5 antibody) and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

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

EXAMPLES

Example 1. iRNA Synthesis

Source of reagents

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

Transcripts

siRNA design was carried out to identify siRNAs targeting human (*Homo sapiens*), cynomolgus monkey (*Macaca fascicularis*; henceforth "cyno"), mouse (*Mus musculus*), and rat (*Rattus norvegicus*) transcripts. In general, the design of duplexes used human, mouse, and rat transcripts from the NCBI RefSeq collection, annotated in the NCBI Gene database (http://www.ncbi.nlm.nih.gov/gene/). For cyno, the design used either transcripts downloaded from the *M. fascicularis* genome project (http://macaque.genomics.org.cn/page/species/download.jsp) and/or transcripts obtained from a liver-derived cDNA library.

Design of CFB siRNAs used the following transcripts from the NCBI RefSeq collection: Human - NM_001710; Cyno (from the *M. fascicularis* genome project) - ENSMMUP00000000985 (locus=scaffold3881:47830:53620) ; Mouse - NM_001142706 and NM_008198; and Rat – NM_212466.3.

Design of C3 siRNAs used the following transcripts from the NCBI RefSeq collection: Human - NM_000064; Cyno (from the *M. fascicularis* genome project) - ENSP00000245907 (locus=chr19:6921416:6963034); Mouse - NM_009778; and Rat – NM_016994

Design of C9 siRNAs used the following transcripts from the NCBI RefSeq collection: Human - NM_001737; Cyno (from liver cDNA library) - isotig05361; Mouse - NM_013485; AND Rat – NM_057146.

siRNA duplexes were designed in several separate batches, including but not limited to batches containing duplexes matching human transcripts only; human and cyno transcripts; human, cyno, and mouse transcripts; AND human, cyno, mouse, and rat transcripts. Most siRNA duplexes were designed that shared 100% identity with the listed human transcript and other species transcripts considered in each design batch (above). In some instances, however, when the antisense strand:target mRNA complementary basepair was a GC or CG pair, siRNA duplexes were designed with mismatches between duplex and mRNA target at the first antisense (last sense) position (see, *e.g.* Table 5, oligos with label G21U, G21A, C21A, G21A). In these cases, duplexes were designed with UA or AU basepairs at the first antisense:last sense pair. Thus the duplexes maintained complementarity but were mismatched with respect to target (U:C, U:G, A:C, or A:G).

siRNA Design, Specificity, and Efficacy Prediction

The predicted specificity of all possible 19mers was predicted from each sequence. Candidate 19mers were then selected that lacked repeats longer than 7 nucleotides.

The following sets of candidate siRNAs were used in comprehensive searches against the appropriate transcriptomes (defined as the set of NM_ and XM_ records within the human, mouse, or rat NCBI Refseq sets, and the cyno transcriptome set in NCBI nucleotide) using an exhaustive "brute-force" algorithm implemented in the python script'BruteForce.py'.

C3: 46 human/cyno/mouse/rat, 80 human/cyno/mouse, 2384 human/cyno.

C9: 7 human/cyno/mouse/rat, 12 human/cyno/mouse, 816 human/cyno.

CFB: 23 human/cyno/mouse, 1232 human/cyno.

The script next parsed the transcript-oligo alignments to generate a score based on the position and number of mismatches between the siRNA and any potential 'off-target' transcript. The off-target score is weighted to emphasize differences in the 'seed' region of siRNAs, in positions 2-9 from the 5'-end of the molecule.

Each oligo-transcript pair from the brute-force search was given a mismatch score by summing the individual mismatch scores; mismatches in the position 2-9 were counted as 2.8, mismatches in the cleavage site positions 10-11 were counted as 1.2, and mismatches in region 12-19 counted as 1.0. An additional off-target prediction was carried out by comparing the frequency of heptamers and octomers derived from 3 distinct, seed-derived hexamers of each oligo. The hexamers from positions 2-7 relative to the 5' start were used to create 2 heptamers and one octamer. 'Heptamer1' was created by adding a 3'-A to the hexamer; heptamer2 was created by adding a 5'-A to the hexamer; the octomer was created by adding an A to both 5'- and 3'-ends of the hexamer. The frequency of octamers and heptamers in the human, rhesus, mouse, or rat 3'-UTRome (defined as the subsequence of the transcriptome from NCBI's Refseq database where the end of the coding region, the 'CDS', is clearly defined) was pre-calculated. The octamer frequency was normalized to the heptamer frequency using the median value from the range of octamer frequencies. A 'mirSeedScore' was then calculated by calculating the sum of ((3 X normalized octamer count) + (2 X heptamer2 count) + (1 X heptamer1 count)).

Both siRNAs strands were assigned to a category of specificity according to the calculated scores: a score above 3 qualifies as highly specific, equal to 3 as specific and between 2.2 and 2.8 as moderately specific. The duplexes were sorted by the specificity of the antisense strand and those duplexes whose antisense oligos lacked GC at the first position, lacked G at both positions 13 and 14, and had 3 or more Us or As in the seed region were selected.

For GalNaC-conjugated duplexes, sense 21mer and antisense 23mer oligos were designed by extending antisense 19mers (described above) to 23 nucleotides of target-

complementary sequence. All species transcripts included in the design batch were checked for complementarity. Only 23mers that preserved 100% sequence complementarity in at least 2 species were used. For each duplex, the sense 21mer was specified as the reverse complement of the first 21 nucleotides of the antisense strand.

siRNA sequence selection

The following 21/23mer duplex sets for GalNac conjugate design were synthesized and formed into duplexes.

C3: twenty sense and 20 antisense derived human/cyno/mouse/rat oligo pairs, including 6 where the first antisense position was swapped to UA (above); 10 sense and 10 antisense derived human/cyno/mouse oligo pairs, including 3 where the first antisense position was swapped to UA (above); 12 sense and 12 antisense derived human/cyno oligo pairs.

C9: one sense and 1 antisense derived human/cyno/mouse/rat oligo pair; 2 sense and 2 antisense derived human/cyno/mouse oligo pairs; 1 sense and 1 antisense derived human/cyno/rat oligo pairs; 19 sense and 19 antisense derived human/cyno oligo pairs.

CFB: nine sense and 9 antisense derived human/cyno/mouse oligo pairs, including 4 where the first antisense position was swapped to UA (above); 23 sense and 23 antisense derived human/cyno oligo pairs.

A detailed list of CFB sense and antisense strand sequences is shown in Tables 3-4.

A detailed list of C3 sense and antisense strand sequences is shown in Tables 5-6.

A detailed list of C9 sense and antisense strand sequences is shown in Tables 7-8.

siRNA Synthesis

General Small and Medium Scale RNA Synthesis Procedure

RNA oligonucleotides were synthesized at scales between 0.2–500 μmol using commercially available 5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-t-butyldimethylsilyl-3'-*O*-(2-cyanoethyl-*N,N*-diisopropyl)phosphoramidite monomers of uridine, 4-*N*-acetylcytidine, 6-*N*-benzoyladenosine and 2-*N*-isobutyrylguanosine and the corresponding 2'-*O*-methyl and 2'-fluoro phosphoramidites according to standard solid phase oligonucleotide synthesis protocols. The amidite solutions were prepared at 0.1-0.15 M concentration and 5-ethylthio-1H-tetrazole (0.25-0.6 M in acetonitrile) was used as the activator. Phosphorothioate backbone modifications were introduced during synthesis using 0.2 M phenylacetyl disulfide (PADS) in lutidine:acetonitrile (1:1) (v;v) or 0.1 M 3-(dimethylaminomethylene) amino-3H-1,2,4-dithiazole-5-thione (DDTT) in pyridine for the oxidation step. After completion of synthesis, the sequences were cleaved from the solid support and deprotected using methylamine followed by triethylamine.3HF to remove any 2'-*O*-t-butyldimethylsilyl protecting groups present.

For synthesis scales between 5–500 µmol and fully 2' modified sequences (2'-fluoro and/ or 2'-*O*-methyl or combinations thereof) the oligonucleotides where deprotected using 3:1 (v/v) ethanol and concentrated (28-32%) aqueous ammonia either at 35°C 16 h or 55°C for 5.5 h. Prior to ammonia deprotection the oligonucleotides where treated with 0.5 M piperidine in acetonitrile for 20 min on the solid support. The crude oligonucleotides were analyzed by LC–MS and anion-exchange HPLC (IEX-HPLC). Purification of the oligonucleotides was carried out by IEX HPLC using: 20 mM phosphate, 10%-15% ACN, pH = 8.5 (buffer A) and 20 mM phosphate, 10%-15% ACN, 1 M NaBr, pH = 8.5 (buffer B). Fractions were analyzed for purity by analytical HPLC. The product-containing fractions with suitable purity were pooled and concentrated on a rotary evaporator prior to desalting. The samples were desalted by size exclusion chromatography and lyophilized to dryness. Equal molar amounts of sense and antisense strands were annealed in 1x PBS buffer to prepare the corresponding siRNA duplexes.

For small scales (0.2–1 µmol), synthesis was performed on a MerMade 192 synthesizer in a 96 well format. In case of fully 2'-modified sequences (2'-fluoro and/or 2'-*O*-methyl or combinations thereof) the oligonucleotides where deprotected using methylamine at room temperature for 30-60 min followed by incubation at 60°C for 30 min or using 3:1 (v/v) ethanol and concentrated (28-32%) aqueous ammonia at room temperature for 30-60 min followed by incubation at 40°C for 1.5 hours. The crude oligonucleotides were then precipitated in a solution of acetonitrile:acetone (9:1) and isolated by centrifugation and decanting the supernatant. The crude oligonucleotide pellet was re-suspended in 20 mM NaOAc buffer and analyzed by LC-MS and anion exchange HPLC. The crude oligonucleotide sequences were desalted in 96 deep well plates on a 5 mL HiTrap Sephadex G25 column (GE Healthcare). In each well about 1.5 mL samples corresponding to an individual sequence was collected. These purified desalted oligonucleotides were analyzed by LC-MS and anion exchange chromatography. Duplexes were prepared by annealing equimolar amounts of sense and antisense sequences on a Tecan robot. Concentration of duplexes was adjusted to 10 µM in 1x PBS buffer.

I. Synthesis of GalNAc-Conjugated Oligonucleotides for *In Vivo* Analysis

Oligonucleotides conjugated with GalNAc ligand at their 3'-terminus were synthesized at scales between 0.2–500 µmol using a solid support pre-loaded with a Y-shaped linker bearing a 4,4'-dimethoxytrityl (DMT)-protected primary hydroxy group for oligonucleotide synthesis and a GalNAc ligand attached through a tether.

For synthesis of GalNAc conjugates in the scales between 5–500 µmol, the above synthesis protocol for RNA was followed with the following adaptions: For polystyrene-based synthesis supports 5% dichloroacetic acid in toluene was used for DMT-cleavage during synthesis. Cleavage from the support and deprotection was performed as described above. Phosphorothioate-rich sequences (usually > 5 phorphorothioates) were synthesized

without removing the final 5'-DMT group ("DMT-on") and, after cleavage and deprotection as described above, purified by reverse phase HPLC using 50 mM ammonium acetate in water (buffer A) and 50 mM ammoniumacetate in 80% acetonitirile (buffer B). Fractions were analyzed for purity by analytical HPLC and/or LC-MS. The product-containing fractions with suitable purity were pooled and concentrated on a rotary evaporator. The DMT-group was removed using 20%-25% acetic acid in water until completion. The samples were desalted by size exclusion chromatography and lyophilized to dryness. Equal molar amounts of sense and antisense strands were annealed in 1x PBS buffer to prepare the corresponding siRNA duplexes.

For small scale synthesis of GalNAc conjugates (0.2–1 μmol), including sequences with multiple phosphorothioate linkages, the protocols described above for synthesis of RNA or fully 2'-F/2'-OMe-containing sequences on MerMade platform were applied. Synthesis was performed on pre-packed columns containing GalNAc-functionalized controlled pore glass support.

Example 2. *In vitro* screening

Cell culture and 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 (ATCC) supplemented with 10% FBS, streptomycin, and glutamine (ATCC) before being released from the plate by trypsinization. Cells were washed and re-suspended at $0.25x10^6$ cells/ml. During transfections, cells were plated onto a 96-well plate with about 20,000 cells per well.

Primary mouse hepatocytes (PMH) were freshly isolated from a C57BL/6 female mouse (Charles River Labortories International, Inc. Willmington, MA) less than 1 hour prior to transfections and grown in primary hepatocyte media. Cells were resuspended at $0.11x10^6$ cells/ml in InVitroGRO CP Rat (plating) medium (Celsis In Vitro Technologies, catalog number S01494). During transfections, cells were plated onto a BD BioCoat 96 well collagen plate (BD, 356407) at 10,000 cells per well and incubated at 37°C in an atmosphere of 5% CO_2.

For Hep3B and PMH, transfection was carried out by adding 14.8 μl of Opti-MEM plus 0.2 μl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. catalog number13778-150) 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 20 minutes. Eighty μl of complete growth media without antibiotic containing the appropriate cell number were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification.

Single dose experiments were performed at 1nM and 0.01nM final duplex concentration for GalNAc modified sequences. Dose response experiments were done at 3, 1, 0.3, 0.1, 0.037, 0.0123, 0.00412, and 0.00137 nM final duplex concentration for primary

mouse hepatocytes and at 3, 1, 0.3, 0.1, 0.037, 0.0123, 0.00412, 0.00137, 0.00046, 0.00015, 0.00005, and 0.000017 nM final duplex concentration for Hep3B cells.

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

Cells were harvested and lysed in 150 µl of Lysis/Binding Buffer then mixed for 5 minutes at 850 rpm using an Eppendorf Thermomixer (the mixing speed was the same throughout the process). Ten microliters of magnetic beads and 80 µl Lysis/Binding Buffer mixture were added to a round bottom plate and mixed for 1 minute. Magnetic beads were captured using a magnetic stand and the supernatant was removed without disturbing the beads. After removing the supernatant, the lysed cells were added to the remaining beads and mixed for 5 minutes. After removing the supernatant, magnetic beads were washed 2 times with 150 µl Wash Buffer A and mixed for 1 minute. The beads were capturedagain and the supernatant was removed. The beads were then washed with 150 µl Wash Buffer B, captured and the supernatant was removed. The beads were next washed with 150 µl Elution Buffer, captured and the supernatant removed. Finally, the beads were allowed to dry for 2 minutes. After drying, 50 µl of Elution Buffer was added and mixed for 5 minutes at 70°C. The beads were captured on magnet for 5 minutes. Forty-five µl of supernatant was removed and added to another 96 well plate.

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

A master mix of 2 µl 10X Buffer, 0.8 µl 25X dNTPs, 2 µl Random primers, 1 µl Reverse Transcriptase, 1 µl RNase inhibitor and 3.2 µl of H2O per reaction as prepared. Equal volumes master mix and RNA were mixed for a final volume of 12µl for *in vitro* screened or 20µl for *in vivo* screened samples. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal cycler (Hercules, CA) through the following steps: 25°C for 10 minutes, 37°C for 120 minutes, 85°C for 5 seconds, and 4°C hold.

Real time PCR

Two µl of cDNA were added to a master mix containing 2µl of H_2O, 0.5µl GAPDH TaqMan Probe (Life Technologies catalog number 4326317E for Hep3B cells, catalog number 352339E for primary mouse hepatocytes or custom probe for cynomolgus primary hepatocytes), 0.5µl of appropriate TaqMan probe (Life Technologies c catalog number Hs00156197_m1 for Hep3B cells or mm00439275_m1 for Primary Mouse Hepatoctyes or custom probe for cynomolgus primary hepatocytes) and 5µl Lightcycler 480 probe master mix (Roche catalog number 04887301001) per well in a 384 well plates (Roche catalog number 04887301001). Real time PCR was performed in an Roche LC480 Real Time PCR system (Roche) using the ΔΔCt(RQ) assay. For *in vitro* screening, each duplex was tested with two biological replicates unless otherwise noted and each Real Time PCR was performed in duplicate technical replicates. For *in vivo* screening, each duplex was tested in

one or more experiments (3 mice per group) and each Real Time PCR was run in duplicate technical replicates.

To calculate relative fold change in mRNA levels, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10 nM AD-1955, or mock transfected cells. IC_{50}s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 over the same dose range, or to its own lowest dose.

The sense and antisense sequences of AD-1955 are:

SENSE: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO: 39)

ANTISENSE: UCGAAGuACUcAGCGuAAGdTsdT (SEQ ID NO: 40).

Table 9 shows the results of a single dose screen in Hep3B cells transfected with the indicated CFB GalNAC conjugated iRNAs. Data are expressed as percent of message remaining relative to untreated cells.

Table 10 shows the results of a single dose screen in primary mouse hepatocytes ransfected with the indicated CFB GalNAC conjugated iRNAs. Data are expressed as percent of message remaining relative to untreated cells.

Table 11 shows the dose response in Hep3B cells transfected with the indicated CFB GalNAC conjugated iRNAs. The indicated IC_{50} values represent the IC_{50} values relative to untreated cells.

Table 12 shows the dose response in primary mouse hepatocytes transfected with the indicated CFB GalNAC conjugated iRNAs. The indicated IC_{50} values represent the IC_{50} values relative to untreated cells.

Table 13 shows the results of a single dose screen in primary mouse hepatocytes ransfected with the indicated C9 GalNAC conjugated iRNAs. Data are expressed as percent of message remaining relative to untreated cells.

Table 14 shows the results of a single dose screen in primary mouse hepatocytes ransfected with the indicated C3 GalNAC conjugated iRNAs. Data are expressed as percent of message remaining relative to untreated cells.

Table 15 shows the results of a single dose screen in Hep3B cells transfected with the indicated C3 GalNAC conjugated iRNAs. Data are expressed as percent of message remaining relative to untreated cells.

Table 16 shows the dose response in primary mouse hepatocytes transfected with the indicated C3 GalNAC conjugated iRNAs. The indicated IC_{50} values represent the IC_{50} values relative to untreated cells.

Table 17 shows the dose response in Hep3B cells transfected with the indicated C3 GalNAC conjugated iRNAs. The indicated IC_{50} values represent the IC_{50} values relative to untreated cells.

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

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

Abbreviation	Nucleotide(s)
s	phosphorothioate linkage
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3

Table 3. Complement Factor B (CFB) unmodified sequences

Human CFB Sequences						
Duplex ID	Sense ID	Sense Sequence (SEQ ID NOS: 41-71, respectively, in order of appearance)	Position in NM_001710.5	Antisense ID	Antisense Sequence (SEQ ID NOS: 72-102, respectively, in order of appearance)	Position in NM_001710.5
AD-60315.1	A-122021.1	AUUCCUGAAUUUUAUGACUAU	1987-2007	A-122022.1	AUAGUCAUAAAAUUCAGGAAUUC	1985-2007
AD-60326.1	A-122009.1	CCUGAUCAAGCUCAAGAAUAA	2016-2036	A-122010.1	UUAUUCUUGAGCUUGAUCAGGGC	2014-2036
AD-60303.1	A-122017.1	GAAGCAGGAAUUCCUGAAUUU	1978-1998	A-122018.1	AAAUUCAGGAAUUCCUGCUUCUU	1976-1998
AD-60331.1	A-121995.1	AGCAACAUGUGUUCAAAGUCA	1628-1648	A-121996.1	UGACUUUGAACACAUGUUGCUCA	1626-1648
AD-60344.1	A-122015.1	GCUGUGGUGUCUGAGUACUUU	1822-1842	A-122016.1	AAAGUACUCAGACACCACAGCCC	1820-1842
AD-60345.1	A-122031.1	AAGUGUCUAGUCAACUUAAUU	1153-1173	A-122032.1	AAUUAAGUUGACUAGACACUUUU	1151-1173
AD-60319.1	A-121991.1	AGCUGUGAGAGAGAUGCUCAA	2245-2265	A-121992.1	UUGAGCAUCUCUCUCACAGCUGC	2243-2265
AD-60308.1	A-122003.1	AGCCAAAAAGUGUCUAGUCAA	1146-1166	A-122004.1	UUGACUAGACACUUUUUGGCUCC	1144-1166
AD-60332.1	A-122011.1	UGUGAGUGAUGAGAUCUCUUU	648-668	A-122012.1	AAAGAGAUCUCAUCACUCACAUU	646-668
AD-60313.1	A-121989.1	AAUUGAGAAGGUGGCAAGUUA	1170-1190	A-121990.1	UAACUUGCCACCUUCUCAAUUAA	1168-1190
AD-60321.1	A-122023.1	CAACAUGUGUUCAAAGUCAAG	1630-1650	A-122024.1	CUUGACUUUGAACACAUGUUGCU	1628-1650
AD-60327.1	A-122025.1	UGUGAGAGAGAUGCUCAAUAU	2248-2268	A-122026.1	AUAUUGAGCAUCUCUCUCACAGC	2246-2268
AD-60302.1	A-122001.1	GUCUAGUCAACUUAAUUGAGA	1157-1177	A-122002.1	UCUCAAUUAAGUUGACUAGACAC	1155-1177
AD-60325.1	A-121993.1	UCCAAGAAAGACAAUGAGCAA	1612-1632	A-121994.1	UUGCUCAUUGUCUUUCUUGGAAG	1610-1632
AD-60337.1	A-121997.1	UGUGUUCAAAGUCAAGGAUAU	1635-1655	A-121998.1	AUAUCCUUGACUUUGAACACAUG	1633-1655
AD-60333.1	A-122027.1	AUUGAUGAGAUCCGGGACUUG	1486-1506	A-122028.1	CAAGUCCCGGAUCUCAUCAAUGA	1484-1506
AD-60314.1	A-122005.1	CUGUGAGAGAGAUGCUCAAUA	2247-2267	A-122006.1	UAUUGAGCAUCUCUCUCACAGCU	2245-2267
AD-60320.1	A-122007.1	GAGCCAAAAAGUGUCUAGUCA	1145-1165	A-122008.1	UGACUAGACACUUUUUGGCUCCU	1143-1165
AD-60339.1	A-122029.1	UCCAAGAUGAGGAUUUGGGUU	2549-2569	A-122030.1	AACCCAAAUCCUCAUCUUGGAGU	2547-2569
AD-60338.1	A-122013.1	CCCUUGAUAGUUCACAAGAGA	2386-2406	A-122014.1	UCUCUUGUGAACUAUCAAGGGGC	2384-2406
AD-60307.1	A-121987.1	CAAAGUCAAGGAUAUGGAAAA	1641-1661	A-121988.1	UUUUCCAUAUCCUUGACUUUGAA	1639-1661
AD-60309.1	A-122019.1	UAGUUCACAAGAGAAGUCGUU	2393-2413	A-122020.1	AACGACUUCUCUUGUGAACUAUC	2391-2413
AD-60343.1	A-121999.1	GGCCCCUUGAUAGUUCACAAG	2383-2403	A-122000.1	CUUGUGAACUAUCAAGGGGCCGC	2381-2403
AD-60324.1	A-121977.1	UGGUGCUAGAUGGAUCAGACA	1100-1120	A-121978.1	UGUCUGAUCCAUCUAGCACCAGG	1098-1120
AD-60318.1	A-121975.1	GCUAGAUGGAUCAGACAGCAU	1104-1124	A-121976.1	AUGCUGUCUGAUCCAUCUAGCAC	1102-1124

AD-60300.1	A-121969.1	UACCUGGUGCUAGAUGGAUCA	1096-1116	A-121970.1	UGAUCCAUCUAGCACCAGGUAGA	1094-1116
AD-60330.1	A-121979.1	GGUGCUAGAUGGAUCAGACAA	1101-1121 (G19A)	A-121980.1	UUGUCUGAUCCAUCUAGCACCAG	1099-1121 (G19A)
AD-60306.1	A-121971.1	UCUGAGUCUCUGUGGCAUGGU	1704-1724	A-121972.1	ACCAUGCCACAGAGACUCAGAGA	1702-1724
AD-60336.1	A-121981.1	GUGCUAGAUGGAUCAGACAGA	1102-1122 (C19A)	A-121982.1	UCUGUCUGAUCCAUCUAGCACCA	1100-1122 (C19A)
AD-60301.1	A-121985.1	CUACCUGGUGCUAGAUGGAUA	1095-1115 (C19A)	A-121986.1	UAUCCAUCUAGCACCAGGUAGAU	1093-1115 (C19A)
AD-60342.1	A-121983.1	ACCUGGUGCUAGAUGGAUCAA	1097-1117 (G19A)	A-121984.1	UUGAUCCAUCUAGCACCAGGUAG	1095-1117 (G19A)
Rodent CFB Sequences						
Duplex ID	Sense ID	Sense Sequence (SEQ ID NOS: 103-117, respectively, in order of appearance)	Position in NM_001142706.1	Antisense ID	Antisense Sequence (SEQ ID NOS: 118-132, respectively, in order of appearance)	Position in NM_001142706.1
AD-60334.1	A-122043.1	GCAAGCCAAGAUCUCAGUCAC	1888-1908	A-122044.1	GUGACUGAGAUCUUGGCUUGCCA	1886-1908
AD-60304.1	A-122033.1	GAUUGAGAAGGUGGCGAGUUA	1291-1311	A-122034.1	UAACUCGCCACCUUCUCAAUCAA	1289-1311
AD-60310.1	A-122035.1	CACAAGAGAAGCCGCUUCAUU	2515-2535	A-122036.1	AAUGAAGCGGCUUCUCUUGUGAA	2513-2535
AD-60328.1	A-122041.1	UUGUGAGAGAGAUGCUACAAA	2364-2384	A-122042.1	UUUGUAGCAUCUCUCUCACAACU	2362-2384
AD-60322.1	A-122039.1	UCCUUCAUGAAUGUUCCGGGA	193-213	A-122040.1	UCCCGGAACAUUCAUGAAGGAGG	191-213
AD-60316.1	A-122037.1	UCACAGAGAAGCUCAACCAAA	1407-1427	A-122038.1	UUUGGUUGAGCUUCUCUGUGACC	1405-1427
AD-60346.1	A-122047.1	CUCAACCAAAUCAGUUAUGAA	1418-1438	A-122048.1	UUCAUAACUGAUUUGGUUGAGCU	1416-1438
AD-60335.1	A-122059.1	CCCUGACAGAGACCAUCGAAG	1113-1133	A-122060.1	CUUCGAUGGUCUCUGUCAGGGAG	1111-1133
AD-60323.1	A-122055.1	GAGCAGAUUGCAUAAAAGGUU	261-281	A-122056.1	AACCUUUUAUGCAAUCUGCUCUG	259-281
AD-60340.1	A-122045.1	CUUCAUGAAUGUUCCGGGAAG	195-215	A-122046.1	CUUCCCGGAACAUUCAUGAAGGA	193-215
AD-60305.1	A-122049.1	CUUCAUUCAAGUUGGUGUGAU	2529-2549	A-122050.1	AUCACACCAACUUGAAUGAAGCG	2527-2549
AD-60317.1	A-122053.1	GAUUGAAGAGGUCCUGUUCCA	2050-2070	A-122054.1	UGGAACAGGACCUCUUCAAUCUC	2048-2070
AD-60329.1	A-122057.1	AUUUCUUUUCAAUGCUAUGAU	782-802	A-122058.1	AUCAUAGCAUUGAAAAGAAAUCU	780-802
AD-60341.1	A-122061.1	CCAGAGCAGAUUGCAUAAAAG	258-278	A-122062.1	CUUUUAUGCAAUCUGCUCUGGCA	256-278
AD-60311.1	A-122051.1	CACAGAGAAGCUCAACCAAAU	1408-1428	A-122052.1	AUUUGGUUGAGCUUCUCUGUGAC	1406-1428

Table 4. Complement Factor B (CFB) modified sequences

Human CFB Sequences				
Duplex ID	Sense ID	Sense Sequence (SEQ ID NOS 133-163, respectively, in order of appearance)	Antisense ID	Antisense Sequence (SEQ ID NOS 164-194, respectively, in order of appearance)
AD-60315.1	A-122021.1	AfsusUfcCfuGfaAfUfUfuUfaUfgAfcUfaUfL96	A-122022.1	asUfsaGfuCfaUfaAfaauUfcAfgGfaAfususc
AD-60326.1	A-122009.1	CfscsUfgAfuCfaAfGfCfuCfaAfgAfaUfaAfL96	A-122010.1	usUfsaUfuCfuUfgAfgcuUfgAfuCfaGfgsgsc
AD-60303.1	A-122017.1	GfsasAfgCfaGfgAfAfUfuCfcUfgAfaUfuUfL96	A-122018.1	asAfsaUfuCfaGfgAfauuCfcUfgCfuUfcsusu
AD-60331.1	A-121995.1	AfsgsCfaAfcAfuGfUfGfuUfcAfaAfgUfcAfL96	A-121996.1	usGfsaCfuUfuGfaAfcacAfuGfuUfgCfuscsa
AD-60344.1	A-122015.1	GfscsUfgUfgGfuGfUfCfuGfaGfuAfcUfuUfL96	A-122016.1	asAfsaGfuAfcUfcAfgacAfcCfaCfaGfcscsc
AD-60345.1	A-122031.1	AfsasGfuGfuCfuAfGfUfcAfaCfuUfaAfuUfL96	A-122032.1	asAfsuUfaAfgUfuGfacuAfgAfcAfcUfususu
AD-60319.1	A-121991.1	AfsgsCfuGfuGfaGfAfGfaGfaUfgCfuCfaAfL96	A-121992.1	usUfsgAfgCfaUfcUfcucUfcAfcAfgCfusgsc
AD-60308.1	A-122003.1	AfsgsCfcAfaAfaAfGfUfgUfcUfaGfuCfaAfL96	A-122004.1	usUfsgAfcUfaGfaCfacuUfuUfuGfgCfuscsc
AD-60332.1	A-122011.1	UfsgsUfgAfgUfgAfUfGfaGfaUfcUfcUfuUfL96	A-122012.1	asAfsaGfaGfaUfcUfcauCfaCfuCfaCfasusu
AD-60313.1	A-121989.1	AfsasUfuGfaGfaAfGfGfuGfgCfaAfgUfuAfL96	A-121990.1	usAfsaCfuUfgCfcAfccuUfcUfcAfaUfusasa
AD-60321.1	A-122023.1	CfsasAfcAfuGfuGfUfUfcAfaAfgUfcAfaGfL96	A-122024.1	csUfsuGfaCfuUfuGfaacAfcAfuGfuUfgscsu
AD-60327.1	A-122025.1	UfsgsUfgAfgAfgAfGfAfuGfcUfcAfaUfaUfL96	A-122026.1	asUfsaUfuGfaGfcAfucuCfuCfuCfaCfasgsc
AD-60302.1	A-122001.1	GfsusCfuAfgUfcAfAfCfuUfaAfuUfgAfgAfL96	A-122002.1	usCfsuCfaAfuUfaAfguuGfaCfuAfgAfcsasc
AD-60325.1	A-121993.1	UfscsCfaAfgAfaAfGfAfcAfaUfgAfgCfaAfL96	A-121994.1	usUfsgCfuCfaUfuGfucuUfuCfuUfgGfasasg
AD-60337.1	A-121997.1	UfsgsUfgUfuCfaAfAfGfuCfaAfgGfaUfaUfL96	A-121998.1	asUfsaUfcCfuUfgAfcuuUfgAfaCfaCfasusg
AD-60333.1	A-122027.1	AfsusUfgAfuGfaGfAfUfcCfgGfgAfcUfuGfL96	A-122028.1	csAfsaGfuCfcCfgGfaucUfcAfuCfaAfusgsa
AD-60314.1	A-122005.1	CfsusGfuGfaGfaGfAfGfaUfgCfuCfaAfuAfL96	A-122006.1	usAfsuUfgAfgCfaUfcucUfcUfcAfcAfgscsu
AD-60320.1	A-122007.1	GfsasGfcCfaAfaAfAfGfuGfuCfuAfgUfcAfL96	A-122008.1	usGfsaCfuAfgAfcAfcuuUfuUfgGfcUfcscsu
AD-60339.1	A-122029.1	UfscsCfaAfgAfuGfAfGfgAfuUfuGfgGfuUfL96	A-122030.1	asAfscCfcAfaAfuCfcucAfuCfuUfgGfasgsu
AD-60338.1	A-122013.1	CfscsCfuUfgAfuAfGfUfuCfaCfaAfgAfgAfL96	A-122014.1	usCfsuCfuUfgUfgAfacuAfuCfaAfgGfgsgsc
AD-60307.1	A-121987.1	CfsasAfaGfuCfaAfGfGfaUfaUfgGfaAfaAfL96	A-121988.1	usUfsuUfcCfaUfaUfccuUfgAfcUfuUfgsasa
AD-60309.1	A-122019.1	UfsasGfuUfcAfcAfAfGfaGfaAfgUfcGfuUfL96	A-122020.1	asAfscGfaCfuUfcUfcuuGfuGfaAfcUfasusc
AD-60343.1	A-121999.1	GfsgsCfcCfcUfuGfAfUfaGfuUfcAfcAfaGfL96	A-122000.1	csUfsuGfuGfaAfcUfaucAfaGfgGfgCfcsgsc
AD-60324.1	A-121977.1	UfsgsGfuGfcUfaGfAfUfgGfaUfcAfgAfcAfL96	A-121978.1	usGfsuCfuGfaUfcCfaucUfaGfcAfcCfasgsg
AD-60318.1	A-121975.1	GfscsUfaGfaUfgGfAfUfcAfgAfcAfgCfaUfL96	A-121976.1	asUfsgCfuGfuCfuGfaucCfaUfcUfaGfcsasc

AD-60300.1	A-121969.1	UfsasCfcUfgGfuGfCfUfaGfaUfgGfaUfcAfL96	A-121970.1	usGfsaUfcCfaUfcUfagcAfcCfaGfgUfasgsa
AD-60330.1	A-121979.1	GfsgsUfgCfuAfgAfUfGfgAfuCfaGfaCfaAfL96	A-121980.1	usUfsgUfcUfgAfuCfcauCfuAfgCfaCfcsasg
AD-60306.1	A-121971.1	UfscsUfgAfgUfcUfCfUfgUfgGfcAfuGfgUfL96	A-121972.1	asCfscAfuGfcCfaCfagaGfaCfuCfaGfasgsa
AD-60336.1	A-121981.1	GfsusGfcUfaGfaUfGfGfaUfcAfgAfcAfgAfL96	A-121982.1	usCfsuGfuCfuGfaUfccaUfcUfaGfcAfcscsa
AD-60301.1	A-121985.1	CfsusAfcCfuGfgUfGfCfuAfgAfuGfgAfuAfL96	A-121986.1	usAfsuCfcAfuCfuAfgcaCfcAfgGfuAfgsasu
AD-60342.1	A-121983.1	AfscsCfuGfgUfgCfUfAfgAfuGfgAfuCfaAfL96	A-121984.1	usUfsgAfuCfcAfuCfuagCfaCfcAfgGfusasg
Rodent CFB Sequences				
Duplex ID	Sense ID	Sense Sequence (SEQ ID NOS 195-209, respectively, in order of appearance)	Antisense ID	Antisense Sequence (SEQ ID NOS 210-224, respectively, in order of appearance)
AD-60334.1	A-122043.1	GfscsAfaGfcCfaAfGfAfuCfuCfaGfuCfaCfL96	A-122044.1	gsUfsgAfcUfgAfgAfucuUfgGfcUfuGfcscsa
AD-60304.1	A-122033.1	GfsasUfuGfaGfaAfGfGfuGfgCfgAfgUfuAfL96	A-122034.1	usAfsaCfuCfgCfcAfccuUfcUfcAfaUfcsasa
AD-60310.1	A-122035.1	CfsasCfaAfgAfgAfAfGfcCfgCfuUfcAfuUfL96	A-122036.1	asAfsuGfaAfgCfgGfcuuCfuCfuUfgUfgsasa
AD-60328.1	A-122041.1	UfsusGfuGfaGfaGfAfGfaUfgCfuAfcAfaAfL96	A-122042.1	usUfsuGfuAfgCfaUfcucUfcUfcAfcAfascsu
AD-60322.1	A-122039.1	UfscsCfuUfcAfuGfAfAfuGfuUfcCfgGfgAfL96	A-122040.1	usCfscCfgGfaAfcAfuucAfuGfaAfgGfasgsg
AD-60316.1	A-122037.1	UfscsAfcAfgAfgAfAfGfcUfcAfaCfcAfaAfL96	A-122038.1	usUfsuGfgUfuGfaGfcuuCfuCfuGfuGfascsc
AD-60346.1	A-122047.1	CfsusCfaAfcCfaAfAfUfcAfgUfuAfuGfaAfL96	A-122048.1	usUfscAfuAfaCfuGfauuUfgGfuUfgAfgscsu
AD-60335.1	A-122059.1	CfscsCfuGfaCfaGfAfGfaCfcAfuCfgAfaGfL96	A-122060.1	csUfsuCfgAfuGfgUfcucUfgUfcAfgGfgsasg
AD-60323.1	A-122055.1	GfsasGfcAfgAfuUfGfCfaUfaAfaAfgGfuUfL96	A-122056.1	asAfscCfuUfuUfaUfgcaAfuCfuGfcUfcsusg
AD-60340.1	A-122045.1	CfsusUfcAfuGfaAfUfGfuUfcCfgGfgAfaGfL96	A-122046.1	csUfsuCfcCfgGfaAfcauUfcAfuGfaAfgsgsa
AD-60305.1	A-122049.1	CfsusUfcAfuUfcAfAfGfuUfgGfuGfuGfaUfL96	A-122050.1	asUfscAfcAfcCfaAfcuuGfaAfuGfaAfgscsg
AD-60317.1	A-122053.1	GfsasUfuGfaAfgAfGfGfuCfcUfgUfuCfcAfL96	A-122054.1	usGfsgAfaCfaGfgAfccuCfuUfcAfaUfcsusc
AD-60329.1	A-122057.1	AfsusUfuCfuUfuUfCfAfaUfgCfuAfuGfaUfL96	A-122058.1	asUfscAfuAfgCfaUfugaAfaAfgAfaAfuscsu
AD-60341.1	A-122061.1	CfscsAfgAfgCfaGfAfUfuGfcAfuAfaAfaGfL96	A-122062.1	csUfsuUfuAfuGfcAfaucUfgCfuCfuGfgscsa
AD-60311.1	A-122051.1	CfsasCfaGfaGfaAfGfCfuCfaAfcCfaAfaUfL96	A-122052.1	asUfsuUfgGfuUfgAfgcuUfcUfcUfgUfgsasc

Table 5. C3 unmodified sequences

Duplex ID	Sense ID	Sense Sequence (SEQ ID NOS 225-265, respectively, in order of appearance)	Position in NM_000064.2	Antisense ID	Antisense Seqeunce (SEQ ID NOS 266-306, respectively, in order of appearance)	Position in NM_000064.2
AD-60149.1	A-121853.1	CGUGGUCAAGGUCUUCUCUCU	3309-3329	A-121854.1	AGAGAGAAGACCUUGACCACGUA	3307-3329
AD-60151.1	A-121885.1	ACGUGGUCAAGGUCUUCUCUA	3308-3324_C21A	A-121886.1	UAGAGAAGACCUUGACCACGUAG	3306-3324_C21A
AD-60152.1	A-121901.1	UUUGACCUCAUGGUGUUCGUG	1174-1194	A-121902.1	CACGAACACCAUGAGGUCAAAGG	1172-1194
AD-60153.1	A-121917.1	GGAGAAUUGCUUCAUACAAAA	4611-4631	A-121918.1	UUUUGUAUGAAGCAAUUCUCCUC	4609-4631
AD-60154.1	A-121933.1	UGUUAAAUGGCUGAUCCUGGA	3375-3395	A-121934.1	UCCAGGAUCAGCCAUUUAACAGC	3373-3395
AD-60155.1	A-121855.1	GACAGACAAGACCAUCUACAC	465-485	A-121856.1	GUGUAGAUGGUCUUGUCUGUCUG	463-485
AD-60156.1	A-121871.1	CCAGACAGACAAGACCAUCUA	462-482	A-121872.1	UAGAUGGUCUUGUCUGUCUGGAU	460-482
AD-60157.1	A-121887.1	CCAGAUCCACUUCACCAAGAA	1125-1141_C21A	A-121888.1	UUCUUGGUGAAGUGGAUCUGGUA	1123-1141_C21A
AD-60158.1	A-121903.1	UUGACCUCAUGGUGUUCGUGA	1175-1195	A-121904.1	UCACGAACACCAUGAGGUCAAAG	1173-1195
AD-60159.1	A-121919.1	CCCCUUCGAGGUCACAGUAAU	2523-2543	A-121920.1	AUUACUGUGACCUCGAAGGGGUC	2521-2543
AD-60160.1	A-121935.1	AUGAACAAAACUGUGGCUGUU	2878-2898	A-121936.1	AACAGCCACAGUUUUGUUCAUUC	2876-2898
AD-60161.1	A-121857.1	AGACAGACAAGACCAUCUACA	464-484	A-121858.1	UGUAGAUGGUCUUGUCUGUCUGG	462-484
AD-60162.1	A-121873.1	CCAGAUCCACUUCACCAAGAC	1125-1145	A-121874.1	GUCUUGGUGAAGUGGAUCUGGUA	1123-1145
AD-60163.1	A-121889.1	AGGGAUCUGUGUGGCAGACCA	2505-2521_C21A	A-121890.1	UGGUCUGCCACACAGAUCCCUUU	2503-2521_C21A
AD-60164.1	A-121905.1	GACAAGACCAUCUACACCCCU	469-489	A-121906.1	AGGGGUGUAGAUGGUCUUGUCUG	467-489
AD-60165.1	A-121921.1	GCUGAGGAGAAUUGCUUCAUA	4606-4626	A-121922.1	UAUGAAGCAAUUCUCCUCAGCAC	4604-4626
AD-60166.1	A-121859.1	ACGUGGUCAAGGUCUUCUCUC	3308-3328	A-121860.1	GAGAGAAGACCUUGACCACGUAG	3306-3328
AD-60167.1	A-121875.1	GGAUCUGUGUGGCAGACCCCU	2507-2527	A-121876.1	AGGGGUCUGCCACACAGAUCCCU	2505-2527
AD-60168.1	A-121891.1	ACAGACAAGACCAUCUACACA	466-482_C21A	A-121892.1	UGUGUAGAUGGUCUUGUCUGUCU	464-482_C21A

AD-60169.1	A-121907.1	AUCCAGACAGACAAGACCAUU	460-476_C21U	A-121908.1	AAUGGUCUUGUCUGUCUGGAUGA	458-476_C21U
AD-60170.1	A-121923.1	CUCCGUGUGGGUGGACGUCAA	1713-1733	A-121924.1	UUGACGUCCACCCACACGGAGUC	1711-1733
AD-60171.1	A-121861.1	UCCAGACAGACAAGACCAUCU	461-481	A-121862.1	AGAUGGUCUUGUCUGUCUGGAUG	459-481
AD-60172.1	A-121877.1	AGGGAUCUGUGUGGCAGACCC	2505-2525	A-121878.1	GGGUCUGCCACACAGAUCCCUUU	2503-2525
AD-60173.1	A-121893.1	CAAGAAAGGGAUCUGUGUGGA	2499-2515_C21A	A-121894.1	UCCACACAGAUCCCUUUCUUGUC	2497-2515_C21A
AD-60174.1	A-121909.1	UGACCUCAUGGUGUUCGUGAU	1176-1192_C21U	A-121910.1	AUCACGAACACCAUGAGGUCAAA	1174-1192_C21U
AD-60175.1	A-121925.1	GCAGCUAAAAGACUUUGACUU	3789-3809	A-121926.1	AAGUCAAAGUCUUUUAGCUGCAG	3787-3809
AD-60176.1	A-121863.1	CAUCCAGACAGACAAGACCAU	459-479	A-121864.1	AUGGUCUUGUCUGUCUGGAUGAA	457-479
AD-60177.1	A-121879.1	ACAGACAAGACCAUCUACACC	466-486	A-121880.1	GGUGUAGAUGGUCUUGUCUGUCU	464-486
AD-60178.1	A-121895.1	AUCCAGACAGACAAGACCAUC	460-480	A-121896.1	GAUGGUCUUGUCUGUCUGGAUGA	458-480
AD-60179.1	A-121911.1	UUUGACCUCAUGGUGUUCGUU	1174-1190_G21U	A-121912.1	AACGAACACCAUGAGGUCAAAGG	1172-1190_G21U
AD-60180.1	A-121927.1	GGAUGCCAAGAACACUAUGAU	4200-4220	A-121928.1	AUCAUAGUGUUCUUGGCAUCCUG	4198-4220
AD-60181.1	A-121865.1	AAGAAAGGGAUCUGUGUGGCA	2500-2520	A-121866.1	UGCCACACAGAUCCCUUUCUUGU	2498-2520
AD-60182.1	A-121881.1	CAAGAAAGGGAUCUGUGUGGC	2499-2519	A-121882.1	GCCACACAGAUCCCUUUCUUGUC	2497-2519
AD-60183.1	A-121897.1	UACGUGGUCAAGGUCUUCUCU	3307-3327	A-121898.1	AGAGAAGACCUUGACCACGUAGG	3305-3327
AD-60184.1	A-121913.1	CAGUUUCGAGGUCAUAGUGGA	756-776	A-121914.1	UCCACUAUGACCUCGAAACUGGG	754-776
AD-60185.1	A-121929.1	CGUGCCGGAAGGAAUCAGAAU	2859-2879	A-121930.1	AUUCUGAUUCCUUCCGGCACGAC	2857-2879
AD-60186.1	A-121867.1	GAAAGGGAUCUGUGUGGCAGA	2502-2522	A-121868.1	UCUGCCACACAGAUCCCUUUCUU	2500-2522
AD-60187.1	A-121883.1	GACAGACAAGACCAUCUACAA	465-481_C21A	A-121884.1	UUGUAGAUGGUCUUGUCUGUCUG	463-481_C21A
AD-60188.1	A-121899.1	UGACCUCAUGGUGUUCGUGAC	1176-1196	A-121900.1	GUCACGAACACCAUGAGGUCAAA	1174-1196
AD-60189.1	A-121915.1	UGUAAUAAAUUCGACCUCAAG	4138-4158	A-121916.1	CUUGAGGUCGAAUUUAUUACAGG	4136-4158
AD-60190.1	A-121931.1	AACUACAUGAACCUACAGAGA	3601-3621	A-121932.1	UCUCUGUAGGUUCAUGUAGUUGG	3599-3621

Table 6. C3 modified sequences

Duplex ID	Sense ID	Sense Sequence (SEQ ID NOS 308-347, respectively, in order of appearance)	Antisense ID	Antisense Sequence (SEQ ID NOS 348-388, respectively, in order of appearance)
AD-60149.1	A-121853.1	CfsgsUfgGfuCfaAfGfGfuCfuUfcUfcUfcUfL96	A-121854.1	asGfsaGfaGfaAfgAfccuUfgAfcCfaCfgsusa
AD-60151.1	A-121885.1	AfscsGfuGfgUfcAfAfGfgUfcUfuCfuCfuAfL96	A-121886.1	usAfsgAfgAfaGfaCfcuuGfaCfcAfcGfusasg
AD-60152.1	A-121901.1	UfsusUfgAfcCfuCfAfUfgGfuGfuUfcGfuGfL96	A-121902.1	csAfscGfaAfcAfcCfaugAfgGfuCfaAfasgsg
AD-60153.1	A-121917.1	GfsgsAfgAfaUfuGfCfUfuCfaUfaCfaAfaAfL96	A-121918.1	usUfsuUfgUfaUfgAfagcAfaUfuCfuCfcsusc
AD-60154.1	A-121933.1	UfsgsUfuAfaAfuGfGfCfuGfaUfcCfuGfgAfL96	A-121934.1	usCfscAfgGfaUfcAfgccAfuUfuAfaCfasgsc
AD-60155.1	A-121855.1	GfsasCfaGfaCfaAfGfAfcCfaUfcUfaCfaCfL96	A-121856.1	gsUfsgUfaGfaUfgGfucuUfgUfcUfgUfcsusg
AD-60156.1	A-121871.1	CfscsAfgAfcAfgAfCfAfaGfaCfcAfuCfuAfL96	A-121872.1	usAfsgAfuGfgUfcUfuguCfuGfuCfuGfgsasu
AD-60157.1	A-121887.1	CfscsAfgAfuCfcAfCfUfuCfaCfcAfaGfaAfL96	A-121888.1	usUfscUfuGfgUfgAfaguGfgAfuCfuGfgsusa
AD-60158.1	A-121903.1	UfsusGfaCfcUfcAfUfGfgUfgUfuCfgUfgAfL96	A-121904.1	usCfsaCfgAfaCfaCfcauGfaGfgUfcAfasasg
AD-60159.1	A-121919.1	CfscsCfcUfuCfgAfGfGfuCfaCfaGfuAfaUfL96	A-121920.1	asUfsuAfcUfgUfgAfccuCfgAfaGfgGfgsusc
AD-60160.1	A-121935.1	AfsusGfaAfcAfaAfAfCfuGfuGfgCfuGfuUfL96	A-121936.1	asAfscAfgCfcAfcAfguuUfuGfuUfcAfususc
AD-60161.1	A-121857.1	AfsgsAfcAfgAfcAfAfGfaCfcAfuCfuAfcAfL96	A-121858.1	usGfsuAfgAfuGfgUfcuuGfuCfuGfuCfusgsg
AD-60162.1	A-121873.1	CfscsAfgAfuCfcAfCfUfuCfaCfcAfaGfaCfL96	A-121874.1	gsUfscUfuGfgUfgAfaguGfgAfuCfuGfgsusa
AD-60163.1	A-121889.1	AfsgsGfgAfuCfuGfUfGfuGfgCfaGfaCfcAfL96	A-121890.1	usGfsgUfcUfgCfcAfcacAfgAfuCfcCfususu
AD-60164.1	A-121905.1	GfsasCfaAfgAfcCfAfUfcUfaCfaCfcCfcUfL96	A-121906.1	asGfsgGfgUfgUfaGfaugGfuCfuUfgUfcsusg
AD-60165.1	A-121921.1	GfscsUfgAfgGfaGfAfAfuUfgCfuUfcAfuAfL96	A-121922.1	usAfsuGfaAfgCfaAfuucUfcCfuCfaGfcsasc
AD-60166.1	A-121859.1	AfscsGfuGfgUfcAfAfGfgUfcUfuCfuCfuCfL96	A-121860.1	gsAfsgAfgAfaGfaCfcuuGfaCfcAfcGfusasg
AD-60167.1	A-121875.1	GfsgsAfuCfuGfuGfUfGfgCfaGfaCfcCfcUfL96	A-121876.1	asGfsgGfgUfcUfgCfcacAfcAfgAfuCfcscsu
AD-60168.1	A-121891.1	AfscsAfgAfcAfaGfAfCfcAfuCfuAfcAfcAfL96	A-121892.1	usGfsuGfuAfgAfuGfgucUfuGfuCfuGfuscsu

AD-60169.1	A-121907.1	AfsusCfcAfgAfcAfGfAfcAfaGfaCfcAfuUfL96	A-121908.1	asAfsuGfgUfcUfuGfucuGfuCfuGfgAfusgsa
AD-60170.1	A-121923.1	CfsusCfcGfuGfuGfGfGfuGfgAfcGfuCfaAfL96	A-121924.1	usUfsgAfcGfuCfcAfcccAfcAfcGfgAfgsusc
AD-60171.1	A-121861.1	UfscsCfaGfaCfaGfAfCfaAfgAfcCfaUfcUfL96	A-121862.1	asGfsaUfgGfuCfuUfgucUfgUfcUfgGfasusg
AD-60172.1	A-121877.1	AfsgsGfgAfuCfuGfUfGfuGfgCfaGfaCfcCfL96	A-121878.1	gsGfsgUfcUfgCfcAfcacAfgAfuCfcCfususu
AD-60173.1	A-121893.1	CfsasAfgAfaAfgGfGfAfuCfuGfuGfuGfgAfL96	A-121894.1	usCfscAfcAfcAfgAfuccCfuUfuCfuUfgsusc
AD-60174.1	A-121909.1	UfsgsAfcCfuCfaUfGfGfuGfuUfcGfuGfaUfL96	A-121910.1	asUfscAfcGfaAfcAfccaUfgAfgGfuCfasasa
AD-60175.1	A-121925.1	GfscsAfgCfuAfaAfAfGfaCfuUfuGfaCfuUfL96	A-121926.1	asAfsgUfcAfaAfgUfcuuUfuAfgCfuGfcsasg
AD-60176.1	A-121863.1	CfsasUfcCfaGfaCfAfGfaCfaAfgAfcCfaUfL96	A-121864.1	asUfsgGfuCfuUfgUfcugUfcUfgGfaUfgsasa
AD-60177.1	A-121879.1	AfscsAfgAfcAfaGfAfCfcAfuCfuAfcAfcCfL96	A-121880.1	gsGfsuGfuAfgAfuGfgucUfuGfuCfuGfuscsu
AD-60178.1	A-121895.1	AfsusCfcAfgAfcAfGfAfcAfaGfaCfcAfuCfL96	A-121896.1	gsAfsuGfgUfcUfuGfucuGfuCfuGfgAfusgsa
AD-60179.1	A-121911.1	UfsusUfgAfcCfuCfAfUfgGfuGfuUfcGfuUfL96	A-121912.1	asAfscGfaAfcAfcCfaugAfgGfuCfaAfasgsg
AD-60180.1	A-121927.1	GfsgsAfuGfcCfaAfGfAfaCfaCfuAfuGfaUfL96	A-121928.1	asUfscAfuAfgUfgUfucuUfgGfcAfuCfcsusg
AD-60181.1	A-121865.1	AfsasGfaAfaGfgGfAfUfcUfgUfgUfgGfcAfL96	A-121866.1	usGfscCfaCfaCfaGfaucCfcUfuUfcUfusgsu
AD-60182.1	A-121881.1	CfsasAfgAfaAfgGfGfAfuCfuGfuGfuGfgCfL96	A-121882.1	gsCfscAfcAfcAfgAfuccCfuUfuCfuUfgsusc
AD-60183.1	A-121897.1	UfsasCfgUfgGfuCfAfAfgGfuCfuUfcUfcUfL96	A-121898.1	asGfsaGfaAfgAfcCfuugAfcCfaCfgUfasgsg
AD-60184.1	A-121913.1	CfsasGfuUfuCfgAfGfGfuCfaUfaGfuGfgAfL96	A-121914.1	usCfscAfcUfaUfgAfccuCfgAfaAfcUfgsgsg
AD-60185.1	A-121929.1	CfsgsUfgCfcGfgAfAfGfgAfaUfcAfgAfaUfL96	A-121930.1	asUfsuCfuGfaUfuCfcuuCfcGfgCfaCfgsasc
AD-60186.1	A-121867.1	GfsasAfaGfgGfaUfCfUfgUfgUfgGfcAfgAfL96	A-121868.1	usCfsuGfcCfaCfaCfagaUfcCfcUfuUfcsusu
AD-60187.1	A-121883.1	GfsasCfaGfaCfaAfGfAfcCfaUfcUfaCfaAfL96	A-121884.1	usUfsgUfaGfaUfgGfucuUfgUfcUfgUfcsusg
AD-60188.1	A-121899.1	UfsgsAfcCfuCfaUfGfGfuGfuUfcGfuGfaCfL96	A-121900.1	gsUfscAfcGfaAfcAfccaUfgAfgGfuCfasasa
AD-60189.1	A-121915.1	UfsgsUfaAfuAfaAfUfUfcGfaCfcUfcAfaGfL96	A-121916.1	csUfsuGfaGfgUfcGfaauUfuAfuUfaCfasgsg
AD-60190.1	A-121931.1	AfsasCfuAfcAfuGfAfAfcCfuAfcAfgAfgAfL96	A-121932.1	usCfsuCfuGfuAfgGfuucAfuGfuAfgUfusgsg

Table 7: C9 unmodified sequences

Duplex ID	Sense ID	Sense Seqeunce (SEQ ID NOS 389-411, respectively, in order of appearance)	Position in NM_001737.3	Antisense ID	Antisense Seqeunce (SEQ ID NOS 412-434, respectively, in order of appearance)	Position in NM_001737.3
AD-59663.1	A-121046.1	UUUUGACAAUGAGUUCUACAA	606-626	A-121047.1	UUGUAGAACUCAUUGUCAAAAGG	604-626
AD-59664.1	A-121062.1	AUCAAUGAAUUUAGUGUAAGA	1597-1617	A-121063.1	UCUUACACUAAAUUCAUUGAUAU	1595-1617
AD-59665.1	A-121078.1	AGACAAAUGUUUCGUUCAAGA	268-288	A-121079.1	UCUUGAACGAAACAUUUGUCUGA	266-288
AD-59668.1	A-121048.1	CUUUUGACAAUGAGUUCUACA	605-625	A-121049.1	UGUAGAACUCAUUGUCAAAAGGU	603-625
AD-59669.1	A-121064.1	AACUUGGAAAGAGCCAUUGAAx	1570-1590	A-121065.1	UUCAAUGGCUCUUUCCAAGUUUU	1568-1590
AD-59670.1	A-121080.1	UACCUGAGAAGCUGAUUAACAx	2589-2609	A-121081.1	UGUUAAUCAGCUUCUCAGGUAGG	2587-2609
AD-59673.1	A-121050.1	ACCUUUUGACAAUGAGUUCUA	603-623	A-121051.1	UAGAACUCAUUGUCAAAAGGUGU	601-623
AD-59674.1	A-121066.1	GACUGCGGAAAUGACUUUCAA	391-411	A-121067.1	UUGAAAGUCAUUUCCGCAGUCAU	389-411
AD-59675.1	A-121082.1	GCCCAUUCAAAUUUGAGGGAA	1682-1702	A-121083.1	UUCCCUCAAAUUUGAAUGGGCAG	1680-1702
AD-59678.1	A-121052.1	UUUUGGAUAAAGCUUCCAUGA	1175-1195	A-121053.1	UCAUGGAAGCUUUAUCCAAAACA	1173-1195
AD-59679.1	A-121068.1	AACCAAAGGCGAGAAAAAUUU	708-728	A-121069.1	AAAUUUUUCUCGCCUUUGGUUUC	706-728
AD-59680.1	A-121084.1	CUUUGCCAACUACCUAUGAAA	1067-1087	A-121085.1	UUUCAUAGGUAGUUGGCAAAGCU	1065-1087
AD-59683.1	A-121054.1	CACCUUUUGACAAUGAGUUCU	602-622	A-121055.1	AGAACUCAUUGUCAAAAGGUGUG	600-622
AD-59684.1	A-121070.1	GAGAAGACAUCAAAUUUUAAU	781-801	A-121071.1	AUUAAAAUUUGAUGUCUUCUCUU	779-801
AD-59685.1	A-121086.1	GACAAUGAGUUCUACAAUGGA	610-630	A-121087.1	UCCAUUGUAGAACUCAUUGUCAA	608-630
AD-59688.1	A-121056.1	UUUGGAUAAAGCUUCCAUGAA	1176-1196	A-121057.1	UUCAUGGAAGCUUUAUCCAAAAC	1174-1196
AD-59689.1	A-121072.1	AUCUAUGAAACCAAAGGCGAG	700-720	A-121073.1	CUCGCCUUUGGUUUCAUAGAUCA	698-720
AD-59690.1	A-121088.1	AUAUCAAUGAAUUUAGUGUAA	1595-1615	A-121089.1	UUACACUAAAUUCAUUGAUAUAG	1593-1615
AD-59692.1	A-121058.1	CACACCUUUUGACAAUGAGUU	600-620	A-121059.1	AACUCAUUGUCAAAAGGUGUGCU	598-620
AD-59693.1	A-121074.1	UAGGGUCUGAGACCUUUUGAA	2648-2668	A-121075.1	UUCAAAAGGUCUCAGACCCUAAG	2646-2668

AD-59694.1	A-121090.1	CAAAACUUGGAAAGAGCCAUU	1567-1587	A-121091.1	AAUGGCUCUUUCCAAGUUUUGUU	1565-1587
AD-59696.1	A-121060.1	GCACACCUUUUGACAAUGAGUx	599-619	A-121061.1	ACUCAUUGUCAAAAGGUGUGCUU	597-619
AD-59697.1	A-121076.1	UGAAACCAAAGGCGAGAAAAA	705-725	A-121077.1	UUUUUCUCGCCUUUGGUUUCAUA	703-725

Table 8. C9 modified sequences

Duplex ID	Sense ID	Sense Sequence (SEQ ID NOS 435-457, respectively, in order of appearance)	Antisense ID	Antisense Sequence (SEQ ID NOS 458-480, respectively, in order of appearance)
AD-59663.1	A-121046.1	UfsusUfuGfaCfaAfUfGfaGfuUfcUfaCfaAfL96	A-121047.1	usUfsgUfaGfaAfcUfcauUfgUfcAfaAfasgsg
AD-59664.1	A-121062.1	AfsusCfaAfuGfaAfUfUfuAfgUfgUfaAfgAfL96	A-121063.1	usCfsuUfaCfaCfuAfaauUfcAfuUfgAfusasu
AD-59665.1	A-121078.1	AfsgsAfcAfaAfuGfUfUfuCfgUfuCfaAfgAfL96	A-121079.1	usCfsuUfgAfaCfgAfaacAfuUfuGfuCfusgsa
AD-59668.1	A-121048.1	CfsusUfuUfgAfcAfAfUfgAfgUfuCfuAfcAfL96	A-121049.1	usGfsuAfgAfaCfuCfauuGfuCfaAfaAfgsgsu
AD-59669.1	A-121064.1	AfsasCfuUfgGfaAfAfGfaGfcCfaUfuGfaAfL96	A-121065.1	usUfscAfaUfgGfcUfcuuUfcCfaAfgUfususu
AD-59670.1	A-121080.1	UfsasCfcUfgAfgAfAfGfcUfgAfuUfaAfcAfL96	A-121081.1	usGfsuUfaAfuCfaGfcuuCfuCfaGfgUfasgsg
AD-59673.1	A-121050.1	AfscsCfuUfuUfgAfCfAfaUfgAfgUfuCfuAfL96	A-121051.1	usAfsgAfaCfuCfaUfuguCfaAfaAfgGfusgsu
AD-59674.1	A-121066.1	GfsasCfuGfcGfgAfAfAfuGfaCfuUfuCfaAfL96	A-121067.1	usUfsgAfaAfgUfcAfuuuCfcGfcAfgUfcsasu
AD-59675.1	A-121082.1	GfscsCfcAfuUfcAfAfAfuUfuGfaGfgGfaAfL96	A-121083.1	usUfscCfcUfcAfaAfuuuGfaAfuGfgGfcsasg
AD-59678.1	A-121052.1	UfsusUfuGfgAfuAfAfAfgCfuUfcCfaUfgAfL96	A-121053.1	usCfsaUfgGfaAfgCfuuuAfuCfcAfaAfascsa
AD-59679.1	A-121068.1	AfsasCfcAfaAfgGfCfGfaGfaAfaAfaUfuUfL96	A-121069.1	asAfsaUfuUfuUfcUfcgcCfuUfuGfgUfususc
AD-59680.1	A-121084.1	CfsusUfuGfcCfaAfCfUfaCfcUfaUfgAfaAfL96	A-121085.1	usUfsuCfaUfaGfgUfaguUfgGfcAfaAfgscsu
AD-59683.1	A-121054.1	CfsasCfcUfuUfuGfAfCfaAfuGfaGfuUfcUfL96	A-121055.1	asGfsaAfcUfcAfuUfgucAfaAfaGfgUfgsusg
AD-59684.1	A-121070.1	GfsasGfaAfgAfcAfUfCfaAfaUfuUfuAfaUfL96	A-121071.1	asUfsuAfaAfaUfuUfgauGfuCfuUfcUfcsusu
AD-59685.1	A-121086.1	GfsasCfaAfuGfaGfUfUfcUfaCfaAfuGfgAfL96	A-121087.1	usCfscAfuUfgUfaGfaacUfcAfuUfgUfcsasa
AD-59688.1	A-121056.1	UfsusUfgGfaUfaAfAfGfcUfuCfcAfuGfaAfL96	A-121057.1	usUfscAfuGfgAfaGfcuuUfaUfcCfaAfasasc
AD-59689.1	A-121072.1	AfsusCfuAfuGfaAfAfCfcAfaAfgGfcGfaGfL96	A-121073.1	csUfscGfcCfuUfuGfguuUfcAfuAfgAfuscsa

AD-59690.1	A-121088.1	AfsusAfuCfaAfuGfAfAfuUfuAfgUfgUfaAfL96	A-121089.1	usUfsaCfaCfuAfaAfuucAfuUfgAfuAfusasg
AD-59692.1	A-121058.1	CfsasCfaCfcUfuUfUfGfaCfaAfuGfaGfuUfL96	A-121059.1	asAfscUfcAfuUfgUfcaaAfaGfgUfgUfgscsu
AD-59693.1	A-121074.1	UfsasGfgGfuCfuGfAfGfaCfcUfuUfuGfaAfL96	A-121075.1	usUfscAfaAfaGfgUfcucAfgAfcCfcUfasasg
AD-59694.1	A-121090.1	CfsasAfaAfcUfuGfGfAfaAfgAfgCfcAfuUfL96	A-121091.1	asAfsuGfgCfuCfuUfuccAfaGfuUfuUfgsusu
AD-59696.1	A-121060.1	GfscsAfcAfcCfuUfUfUfgAfcAfaUfgAfgUfL96	A-121061.1	asCfsuCfaUfuGfuCfaaaAfgGfuGfuGfcsusu
AD-59697.1	A-121076.1	UfsgsAfaAfcCfaAfAfGfgCfgAfgAfaAfaAfL96	A-121077.1	usUfsuUfuCfuCfgCfcuuUfgGfuUfuCfasusa

Table 9. CFB single dose screen in Hep3B Cells

	10nM	0.1nM	10nM SD	0.1nM SD
AD-60315.1	22.82	17.15	20.03	9.73
AD-60326.1	9.33	17.49	0.29	4.75
AD-60303.1	8.45	28.08	4.67	10.75
AD-60331.1	14.47	29.99	4.36	4.99
AD-60344.1	17.61	30.59	6.96	1.70
AD-60345.1	8.98	33.88	0.65	7.11
AD-60319.1	14.36	33.98	1.17	12.16
AD-60308.1	12.64	34.07	0.19	11.41
AD-60332.1	20.19	35.92	3.53	3.23
AD-60313.1	23.94	38.26	19.92	13.16
AD-60321.1	13.32	46.50	4.83	1.00
AD-60327.1	18.44	50.40	6.45	5.21
AD-60302.1	13.82	53.31	4.21	12.46
AD-60325.1	11.73	54.59	0.27	15.34
AD-60337.1	16.17	56.04	3.64	33.50
AD-60333.1	17.72	65.14	2.22	8.79
AD-60314.1	27.79	67.44	2.02	9.10
AD-60320.1	18.12	85.78	5.39	33.24
AD-60339.1	20.86	88.73	9.59	10.47
AD-60338.1	18.14	91.03	4.11	10.07
AD-60307.1	21.76	91.13	3.49	43.21
AD-60309.1	20.64	95.13	0.34	53.77
AD-60343.1	61.82	112.57	5.56	17.11
AD-60324.1	24.20	81.08	3.41	18.95
AD-60318.1	43.11	99.07	13.83	17.69
AD-60300.1	35.21	111.33	5.35	12.86
AD-60330.1	58.80	111.85	8.86	32.76
AD-60306.1	85.87	113.97	12.01	33.11
AD-60336.1	35.90	119.80	3.75	4.92
AD-60301.1	28.95	121.90	7.73	23.23
AD-60342.1	49.16	123.56	17.53	14.88
AD-60334.1	26.12	55.28	22.52	7.86
AD-60304.1	20.62	74.38	4.43	16.50
AD-60310.1	18.93	77.08	0.87	35.20
AD-60328.1	63.55	86.20	1.91	4.07
AD-60322.1	81.67	86.30	21.22	25.58
AD-60316.1	105.01	93.22	8.55	14.39
AD-60346.1	109.11	99.09	2.07	25.51
AD-60335.1	42.63	101.00	5.91	54.15
AD-60323.1	81.31	103.20	4.03	3.86

AD-60340.1	50.41	109.25	20.73	1.67
AD-60305.1	30.06	114.59	5.00	17.97
AD-60317.1	102.87	126.87	1.95	30.25
AD-60329.1	106.30	131.90	0.20	53.49
AD-60341.1	112.98	137.99	3.94	31.92
AD-60311.1	162.39	140.07	10.04	63.65

Table 10. CFB single dose screen in Primary Mouse Hepatocytes

	Avg 10nM	Avg 0.1nM	10nM SD	0.1nM SD
AD-60302.1	112.73	109.72	15.29	1.75
AD-60303.1	119.44	102.70	0.15	23.82
AD-60307.1	67.92	99.67	2.91	6.47
AD-60308.1	116.89	111.68	12.15	4.51
AD-60309.1	100.72	112.85	10.72	4.84
AD-60313.1	50.21	102.05	10.08	4.13
AD-60314.1	74.12	113.15	4.99	12.59
AD-60315.1	101.22	104.79	6.07	29.27
AD-60319.1	18.56	81.28	4.22	6.27
AD-60320.1	103.08	123.28	8.71	18.51
AD-60321.1	45.03	104.98	3.91	25.35
AD-60325.1	121.99	127.67	4.63	24.72
AD-60326.1	55.24	102.10	4.66	13.35
AD-60327.1	79.42	108.21	4.77	21.99
AD-60331.1	4.51	52.03	0.35	8.06
AD-60332.1	115.05	120.93	6.06	4.00
AD-60333.1	102.19	113.88	0.38	31.81
AD-60337.1	3.93	31.08	1.12	0.49
AD-60338.1	120.85	115.74	9.02	8.93
AD-60339.1	16.97	75.02	0.27	10.17
AD-60343.1	126.10	131.79	24.11	14.66
AD-60344.1	8.06	35.14	0.31	11.86
AD-60345.1	132.64	133.75	7.96	27.82
AD-60300.1	27.05	81.40	8.63	8.86
AD-60301.1	10.24	72.49	0.46	5.41
AD-60306.1	97.07	114.32	4.87	18.27
AD-60318.1	37.73	98.00	3.09	7.56
AD-60324.1	42.83	99.93	1.21	12.09
AD-60330.1	70.05	116.47	1.46	15.23
AD-60336.1	31.97	95.19	13.63	1.75
AD-60342.1	38.22	108.31	4.90	6.76
AD-60304.1	7.88	18.03	3.57	18.03
AD-60305.1	13.09	64.61	2.19	11.26
AD-60310.1	1.36	21.17	0.24	1.27

AD-60311.1	2.11	28.70	0.22	4.79
AD-60316.1	2.23	28.29	1.11	4.66
AD-60317.1	60.25	84.11	5.23	5.66
AD-60322.1	70.53	115.47	1.47	11.72
AD-60323.1	108.71	117.31	17.38	7.90
AD-60328.1	4.04	38.52	0.21	10.03
AD-60329.1	6.73	36.47	0.21	8.72
AD-60334.1	49.74	99.41	2.74	8.64
AD-60335.1	34.99	99.57	3.64	1.59
AD-60340.1	99.13	106.94	5.71	9.81
AD-60341.1	92.74	112.17	0.34	8.10
AD-60346.1	5.65	53.30	0.52	5.28

Table 11. CFB Dose response screen in Hep 3B cells

Duplex ID	Hep3B IC50(nM)
AD-60303.1	0.119
AD-60326.1	0.062
AD-60319.1	0.351
AD-60331.1	0.225
AD-60337.1	0.418
AD-60344.1	0.347
AD-60304.1	>10
AD-60324.1	7.039

Table 12. CFB Dose response screen in Primary Mouse Hepatocytes

Duplex ID	PrimaryMouse IC50(nM)
AD-60303.1	Not achieved
AD-60326.1	4.063
AD-60319.1	0.162
AD-60331.1	0.031
AD-60337.1	0.014
AD-60344.1	0.003
AD-60304.1	0.028
AD-60324.1	0.854

Table 13. C9 Single dose screen in Primary Mouse Hepatocytes

Duplex ID	Avg 10nM	Avg 0.1nM	SD 10nM	SD 0.1nM
AD-59663.1	5.92	27.33	2.13	16.40

AD-59664.1	83.71	76.56	42.80	21.75
AD-59665.1	91.76	85.56	20.62	26.31
AD-59668.1	30.66	49.06	4.23	13.47
AD-59669.1	95.36	64.74	18.69	19.30
AD-59670.1	96.91	103.65	26.38	7.23
AD-59673.1	22.34	31.20	7.34	20.44
AD-59674.1	12.16	45.36	5.13	14.79
AD-59675.1	93.18	109.59	3.77	8.45
AD-59678.1	47.33	47.23	14.22	6.86
AD-59679.1	98.53	30.06	12.88	32.30
AD-59680.1	33.75	86.68	1.20	28.07
AD-59683.1	25.81	44.31	9.78	23.12
AD-59684.1	58.89	96.75	16.45	21.05
AD-59685.1	68.90	115.36	8.17	6.36
AD-59688.1	32.69	41.63	6.49	21.72
AD-59689.1	86.86	102.46	24.47	0.38
AD-59690.1	101.98	131.95	4.87	0.16
AD-59692.1	33.98	36.81	9.73	3.38
AD-59693.1	84.70	75.60	35.91	16.09
AD-59694.1	108.88	132.73	2.53	45.43
AD-59696.1	32.87	45.82	9.72	15.79
AD-59697.1	110.00	120.20	1.21	3.98
AD-1955	109.44	92.04	24.08	32.14
AD-1955	105.93	104.33	4.54	6.01
AD-1955	87.62	93.01	6.11	3.30
AD-1955	90.95	117.91	3.90	29.31
AD-1955	91.04	93.49	6.80	8.35
AD-1955	106.63	107.78	1.44	9.89
AD-1955	95.33	82.10	9.45	2.92
AD-1955	123.15	121.27	44.13	11.42

Table 14. C3 Single dose screen in Primary Mouse Hepatocytes

Duplex ID	Avg 10nM	Avg 0.1nM	10nM SD	0.1nM SD
AD-60149.1	0.08	33.89	0.04	44.73
AD-60151.1	0.11	81.49	0.14	7.88
AD-60152.1	1.72	92.02	0.89	9.34
AD-60153.1	93.57	97.06	17.16	4.16
AD-60154.1	97.73	122.73	0.66	28.17
AD-60155.1	12.94	91.38	17.39	9.28
AD-60156.1	8.02	41.58	9.16	56.27
AD-60157.1	23.61	98.22	33.22	8.77

AD-60158.1	0.75	77.42	0.76	8.61
AD-60159.1	100.47	93.53	11.61	7.44
AD-60160.1	89.34	92.97	18.42	9.21
AD-60161.1	2.33	82.37	0.32	21.06
AD-60162.1	60.59	46.83	1.37	65.96
AD-60163.1	104.09	53.32	5.42	75.38
AD-60164.1	61.13	40.41	5.57	57.13
AD-60165.1	61.93	86.61	4.44	11.53
AD-60166.1	2.27	96.48	0.70	17.52
AD-60167.1	87.51	84.41	3.70	9.19
AD-60168.1	35.16	98.47	0.28	20.95
AD-60169.1	0.42	51.78	0.13	18.79
AD-60170.1	125.00	99.12	1.46	12.72
AD-60171.1	0.44	59.53	0.01	1.82
AD-60172.1	89.05	102.11	4.20	10.62
AD-60173.1	81.29	95.39	16.08	3.86
AD-60174.1	0.06	25.26	0.02	31.64
AD-60175.1	0.89	80.59	0.23	6.61
AD-60176.1	0.88	52.71	0.02	6.12
AD-60177.1	63.14	85.00	16.41	9.25
AD-60178.1	42.97	64.33	4.75	14.00
AD-60179.1	0.12	54.36	0.01	6.05
AD-60180.1	94.57	98.11	13.68	5.65
AD-60181.1	69.28	85.66	6.99	31.48
AD-60182.1	84.22	79.05	2.63	8.99
AD-60183.1	0.08	44.17	0.05	7.27
AD-60184.1	80.50	81.13	9.59	14.73
AD-60185.1	92.21	99.75	12.00	2.32
AD-60186.1	60.60	93.85	18.81	29.73
AD-60187.1	2.33	71.77	0.20	1.49
AD-60188.1	0.33	78.13	0.37	14.56
AD-60189.1	57.75	91.38	43.16	14.16
AD-60190.1	29.40	94.84	41.57	7.55
AD-1955	103.85	90.86	8.96	3.45
AD-1955	71.27	115.36	36.17	13.40
AD-1955	99.16	95.85	5.16	8.09
AD-1955	112.29	104.37	3.65	12.88
AD-1955	108.44	97.01	1.40	0.36
AD-1955	118.26	109.90	2.10	12.76
AD-1955	98.09	98.72	11.81	1.81

Table 15. C3 Single dose screen in Hep 3B cells

Duplex ID	Avg 10nM	Avg 0.1nM	10nM SD	0.1nM SD
AD-60149.1	7.49	55.90	7.75	4.41
AD-60151.1	24.05	101.65	14.22	8.27
AD-60152.1	16.58	112.51	10.66	19.82
AD-60153.1	20.13	22.40	22.87	3.76
AD-60154.1	24.21	112.90	8.93	25.58
AD-60155.1	20.48	68.97	2.10	1.73
AD-60156.1	18.22	66.39	0.80	1.67
AD-60157.1	29.07	125.72	5.80	8.08
AD-60158.1	81.03	105.18	14.03	14.20
AD-60159.1	27.58	92.91	4.77	2.22
AD-60160.1	11.49	60.48	4.68	11.60
AD-60161.1	27.49	80.57	10.88	16.13
AD-60162.1	49.58	89.22	3.76	6.06
AD-60163.1	91.18	99.19	5.14	21.40
AD-60164.1	33.93	85.93	4.07	1.00
AD-60165.1	5.54	13.05	0.43	2.69
AD-60166.1	35.21	81.66	21.31	14.48
AD-60167.1	106.64	115.02	8.09	39.17
AD-60168.1	26.91	92.99	2.50	5.86
AD-60169.1	10.66	49.63	6.66	17.36
AD-60170.1	52.73	104.43	2.71	22.03
AD-60171.1	23.77	60.35	7.94	7.27
AD-60172.1	143.57	99.22	8.09	11.58
AD-60173.1	100.25	108.80	12.25	44.49
AD-60174.1	16.68	92.68	0.45	45.25
AD-60175.1	24.94	42.14	4.74	7.68
AD-60176.1	17.30	66.19	8.83	13.81
AD-60177.1	50.71	116.18	20.19	1.49
AD-60178.1	22.65	90.84	5.82	15.23
AD-60179.1	15.21	85.30	3.55	23.07
AD-60180.1	45.91	93.35	16.19	28.54
AD-60181.1	63.50	109.82	10.07	14.56
AD-60182.1	110.82	121.62	1.09	6.78
AD-60183.1	13.82	69.24	8.64	3.35
AD-60184.1	26.47	97.94	9.64	9.88
AD-60185.1	41.42	103.45	7.77	2.47
AD-60186.1	72.24	88.39	6.37	51.31
AD-60187.1	9.49	51.15	3.28	11.65
AD-60188.1	55.44	95.66	7.05	30.36
AD-60189.1	52.59	89.41	4.25	20.79

AD-60190.1	16.67	95.38	1.22	11.83

Table 16. C3 Dose response screen in primary mouse hepatocytes

Duplex ID	PMH IC50(nM)
AD-60149.1	0.03
AD-60152.1	1.03
AD-60156.1	0.19
AD-60165.1	1.96
AD-60169.1	0.04
AD-60171.1	0.04
AD-60174.1	0.01
AD-60175.1	0.54
AD-60176.1	0.05
AD-60179.1	0.03
AD-60183.1	0.03
AD-60187.1	0.24

Table 17. C3 Dose response screen in Hep3B cells

Duplex ID	Hep3B IC50(nM)
AD-60149.1	0.88
AD-60152.1	2.87
AD-60156.1	2.06
AD-60165.1	0.08
AD-60169.1	0.41
AD-60171.1	5.51
AD-60174.1	2.60
AD-60175.1	0.48
AD-60176.1	2.29
AD-60179.1	1.70
AD-60183.1	0.94
AD-60187.1	1.65

Example 3. *In vivo* screening

A subset of three CFB GalNAC conjugated iRNAs was selected for further *in vivo* evaluation, AD-60304, AD-60331, and AD-60344. The nucleotide sequences of the sense and antisens strands of these iRNA agents are provided in Table 18. As indicated in Table

19, the nucleotide sequence of AD-60304 is a perfect match to the mouse and rat nucleotide sequences. The nucleotide sequence of AD-60331 and the nucleotide sequence of AD-60344 have nucleotide mismatches ("MM"; see bolded, underlined nucleotides) to the mouse gene but have activity in mouse hepatocytes.

C57BL/6 mice (N=3 per group) were injected subcutaneously with either 1 mg/kg or 10mg/kg of GalNAc conjugated duplexes or an equal volume of 1x Dulbecco's Phosphate-Buffered Saline (DPBS) (Life Technologies, Cat# 14040133). Ninety-six hours later, mice were euthanized and the livers were dissected and flash frozen in liquid nitrogen. Livers were ground in a 2000 Geno/Grinder (SPEX SamplePrep, Metuchen, NJ). Approximately 10mg of liver powder per sample was used for RNA isolation. Samples were first homogenized in a TissueLyserII (Qiagen Inc, Valencia, CA) and then RNA was extracted using a RNeasy 96 Universal Tissue Kit (Qiagen Inc, , Cat#74881) following manufacturer's protocol using vacuum/spin technology. RNA concentration was measured by a NanoDrop 8000 (Thermo Scientific, Wilmington, DE) and was adjusted to 100ng/µl. cDNA was prepared and RT-PCR were performed as described above.

Figure 2 demonstrates the efficacy of the CFB iRNAs to inhibit CFB mRNA at a dose of either 1 mg/kg or 10 mg/kg. At the 10 mg/kg dose, an average of about 80% silencing was observed for all three iRNAs tested. At the 1 mg/kg dose, an average of about 30% silencing was observed for AD-60331 and AD-60344.

The ability of AD-60331 to suppress expression of CFB mRNA *in vivo* was also assessed using a single dose of 1.25 mg/kg, 2.5 mg/kg, and 10 mg/kg. C57BL/6 mice were injected subcutaneously with the foregoing doses and seventy hours later, mice were euthanized. RNA isolation form the livers of the animals, cDNA preparation, and RT-PCR were performed as described above. Figure 3 demonstrates that AD-60331 reduces CFB mRNA in a dose responsive manner, with an ED_{50} of about 2.5 mg/kg. It is expected that when introduced into human subjects, these iRNAs will be even more effective given the design of the sequences.

Table 18.

Duplex	Sense Sequence (SEQ ID NOS 481-483, respectively, in order of appearance)	Antisense Sequence (SEQ ID NOS 484-486, respectively, in order of appearance)	species
AD-60304.1	GfsasUfuGfaGfaAfGfGfuGfgCfgAfgUfuAfL96	usAfsaCfuCfgCfcAfccuUfcUfcAfaUfcsasa	MR
AD-60331.1	AfsgsCfaAfcAfuGfUfGfuUfcAfaAfgUfcAfL96	usGfsaCfuUfuGfaAfcacAfuGfuUfgCfuscsa	HC
AD-60344.1	GfscsUfgUfgGfuGfUfCfuGfaGfuAfcUfuUfL96	asAfsaGfuAfcUfcAfgacAfcCfaCfaGfcscsc	HC

Table 19.

Duplex	Antisense MM to mouse (bold, underline) (SEQ ID NOS 487-489, respectively, in order of appearance)	Antisense MM to rat (bold, underline) (SEQ ID NOS 490-492, respectively, in order of appearance)	PrimaryMouse IC50(nM)	Hep3b IC50(nM)
AD-60304.1	UAACUCGCCACCUUCUCAAUCAA	UAACUCGCCACCUUCUCAAUCAA	0.028	2.876
AD-60331.1	UGACUUU**G**AACACAUG**U**UGCUCA	UGAC**U**UUGAACACAUG**U**UGCUCA	0.031	0.225
AD-60344.1	**A**AAGUACUCAGACACCAC**A**GCCC	**A**AAGUACUC**A**GACACCAC**A**GCCC	0.017	0.347

We claim:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting expression of complement component C3 in a cell, wherein said dsRNA comprises a sense strand and an antisense strand, wherein each strand comprises 15-30 nucleotides and said strands form a double-stranded region, and wherein the antisense strand comprising at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the following antisense sequences:

a. GAGAGAAGACCUUGACCACGUAG (SEQ ID NO:282);
b. UAGAGAAGACCUUGACCACGUAG (SEQ ID NO:267);
c. AGAGAGAAGACCUUGACCACGUA (SEQ ID NO:266);
d. AGAGAAGACCUUGACCACGUAGG (SEQ ID NO:299); and wherein at least one nucleotide in said dsRNA comprises a modification.

2. The double stranded RNAi of claim 1, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the following sense sequences:

a. ACGUGGUCAAGGUCUUCUCUC (SEQ ID NO:241);
b. ACGUGGUCAAGGUCUUCUCUA (SEQ ID NO:226);
c. CGUGGUCAAGGUCUUCUCUCU (SEQ ID NO:225); and
d. UACGUGGUCAAGGUCUUCUCU (SEQ ID NO:258).

3. The dsRNA of claim 1 or claim 2, wherein the double-stranded region is 15-23 nucleotide pairs in length.

4. The dsRNA of any one of claims 1-3, wherein the modifications on the nucleotides are selected from the group of modified nucleotides consisting of a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2′-fluoro modified nucleotide, a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group, a 2'-deoxy-2'-fluoro modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a

tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, and a nucleotide comprising a 5'-phosphate mimic.

5. The dsRNA of any one of claims 1-4, wherein the modifications on the nucleotides are 2′-O-methyl or 2′-fluoro modifications.

6. The dsRNA of any one of claims 1-5, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

7. The dsRNA of any one of claims 1-6, wherein the ligand is one or more GalNAc derivatives.

8. The dsRNA of any one of claims 1-7, wherein said dsRNA has a phosphorus atom in its internucleoside backbone.

9. The dsRNA of any one of claims 1-8, wherein the backbone includes one or more selected from 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'.

10. A cell containing the dsRNA of any one of claims 1-9.

11. A pharmaceutical composition for inhibiting expression of a complement component C3 gene comprising the dsRNA of any one of claims 1-9.

12. An *in vitro* method of inhibiting complement component 3 (C3) expression in a cell, the method comprising:

(a) contacting the cell with the dsRNA of any one of claims 1-9 or the pharmaceutical composition of claim 11; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a C3 gene, thereby inhibiting expression of the C3 gene in the cell.

13. Use of the dsRNA of any one of claims 1-9 or the pharmaceutical composition of claim 11 in the manufacture of a medicament for treating a complement component-associated disease.

14. The use of claim 13, wherein the complement component-associated disease is selected from the group consisting of paroxysmal nocturnal hemoglobinuria (PNH), asthma, rheumatoid arthritis, systemic lupus erythmatosis, glomerulonephritis, psoriasis, dermatomyositis bullous pemphigoid, atypical hemolytic uremic syndrome, Shiga toxin *E. coli*-related hemolytic uremic syndrome, myasthenia gravis, neuromyelistis optica, dense deposit disease, C3 neuropathy, age-related macular degeneration, cold agglutinin disease, anti-neutrophil cytoplasmic antibody-associated vasculitis, humoral and vascular transplant rejection, graft dysfunction, myocardial infarction, a sensitized recipient of a transplant, and sepsis.