



(12) **United States Patent**
Hinkle et al.

(10) **Patent No.:** **US 11,866,701 B2**
(45) **Date of Patent:** **Jan. 9, 2024**

(54) **COMPLEMENT COMPONENT C3 IRNA COMPOSITIONS AND METHODS OF USE THEREOF**

(71) Applicant: **Alnylam Pharmaceuticals, Inc.**,
Cambridge, MA (US)

(72) Inventors: **Gregory Hinkle**, Cambridge, MA (US);
Anna Borodovsky, Cambridge, MA (US)

(73) Assignee: **Alnylam Pharmaceuticals, Inc.**,
Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 114 days.

(21) Appl. No.: **16/760,593**

(22) PCT Filed: **Nov. 1, 2018**

(86) PCT No.: **PCT/US2018/058705**

§ 371 (c)(1),

(2) Date: **Apr. 30, 2020**

(87) PCT Pub. No.: **WO2019/089922**

PCT Pub. Date: **May 9, 2019**

(65) **Prior Publication Data**

US 2021/0261959 A1 Aug. 26, 2021

Related U.S. Application Data

(60) Provisional application No. 62/580,030, filed on Nov. 1, 2017.

(51) **Int. Cl.**

C12N 15/113 (2010.01)

A61K 31/7115 (2006.01)

(52) **U.S. Cl.**

CPC **C12N 15/113** (2013.01); **A61K 31/7115** (2013.01); **C12N 2310/14** (2013.01); **C12N 2310/315** (2013.01); **C12N 2310/321** (2013.01); **C12N 2310/322** (2013.01); **C12N 2310/3235** (2013.01); **C12N 2310/351** (2013.01)

(58) **Field of Classification Search**

CPC C12N 15/113; C12N 2310/14; C12N 2310/315; C12N 2310/321; C12N 2310/322; C12N 2310/3235; C12N 2310/351; C12N 2310/3125; A61K 31/7115

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

10,465,194 B2 11/2019 Borodovsky et al.
2003/0096775 A1 5/2003 Graham et al.
2007/0088154 A1 4/2007 Khvorova et al.
2007/0123484 A1 5/2007 Bhat
2008/0113351 A1 5/2008 Naito et al.

2009/0239814 A1 9/2009 Manoharan et al.
2009/0306178 A1 12/2009 Bhat et al.
2013/0217756 A1 8/2013 Cancilla et al.
2013/0281511 A1 10/2013 Bettencourt et al.
2016/0222389 A1 8/2016 Grossman
2020/0263183 A1 8/2020 Borodovsky et al.
2020/0339998 A1 10/2020 Borodovsky et al.

FOREIGN PATENT DOCUMENTS

EP 1752536 A1 2/2007
WO WO-2006/047673 A2 5/2006
WO WO-2007/064846 A2 6/2007
WO WO-2007/089375 A2 8/2007
WO WO-2008/036841 A2 3/2008
WO WO-2010/048352 A2 4/2010
WO WO-2012/037254 A1 3/2012
WO WO-2013/067076 A2 5/2013
WO WO-2013/074974 A2 5/2013
WO WO-2014/107763 A1 7/2014
WO WO-2015/038939 A2 3/2015
WO WO-2015/089368 A2 6/2015
WO WO-2017/040078 A1 3/2017
WO WO-2018075373 A1 * 4/2018 A61K 31/713
WO WO-2019/089922 A1 5/2019
WO WO-2021/081026 A1 4/2021
WO WO-2021/178607 A1 9/2021

OTHER PUBLICATIONS

Lawson, V. and Arnold, W.D., Multifocal motor neuropathy: a review of pathogenesis, diagnosis, and treatment, 2014, *Neuropsychiatric Disease and Treatment*, 10, 567-576. (Year: 2014).
Cleveland Clinic Foundation, "Lupus (Systemic Lupus Erythematosus)", <https://my.clevelandclinic.org/health/diseases/4875-lupus>, Accessed Nov. 8, 2022, publication reviewed Apr. 19, 2021 (Year: 2021).
PCT/US2018/058705, Nov. 1, 2018, WO 2019/089922, Published.
U.S. Appl. No. 15/176,231 U.S. Pat. No. 10,465,194, filed Jun. 8, 2016 Nov. 5, 2019, US 20160298124, Granted.
U.S. Appl. No. 16/574,158 U.S. Pat. No. 11,186,842, filed Sep. 18, 2019 Nov. 30, 2021, US 20200263183, Granted.
U.S. Appl. No. 16/925,463, filed Jul. 10, 2020, US 20200339998, Abandoned.
U.S. Appl. No. 17/405,199, filed Aug. 18, 2021, Pending.

(Continued)

Primary Examiner — Ram R Shukla

Assistant Examiner — Keyur A Vyas

(74) *Attorney, Agent, or Firm* — McCarter & English, LLP; Maria Laccotripe Zacharakis; Deborah L. Nagle

(57) **ABSTRACT**

The invention relates to iRNA, e.g., double stranded ribonucleic acid (dsRNA), compositions targeting the complement factor C3 gene, and methods of using such iRNA, e.g., dsRNA, compositions to inhibit expression of a C3 gene and to treat subjects having a complement component C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), and C3 glomerulonephritis.

24 Claims, 1 Drawing Sheet

Specification includes a Sequence Listing.

(56)

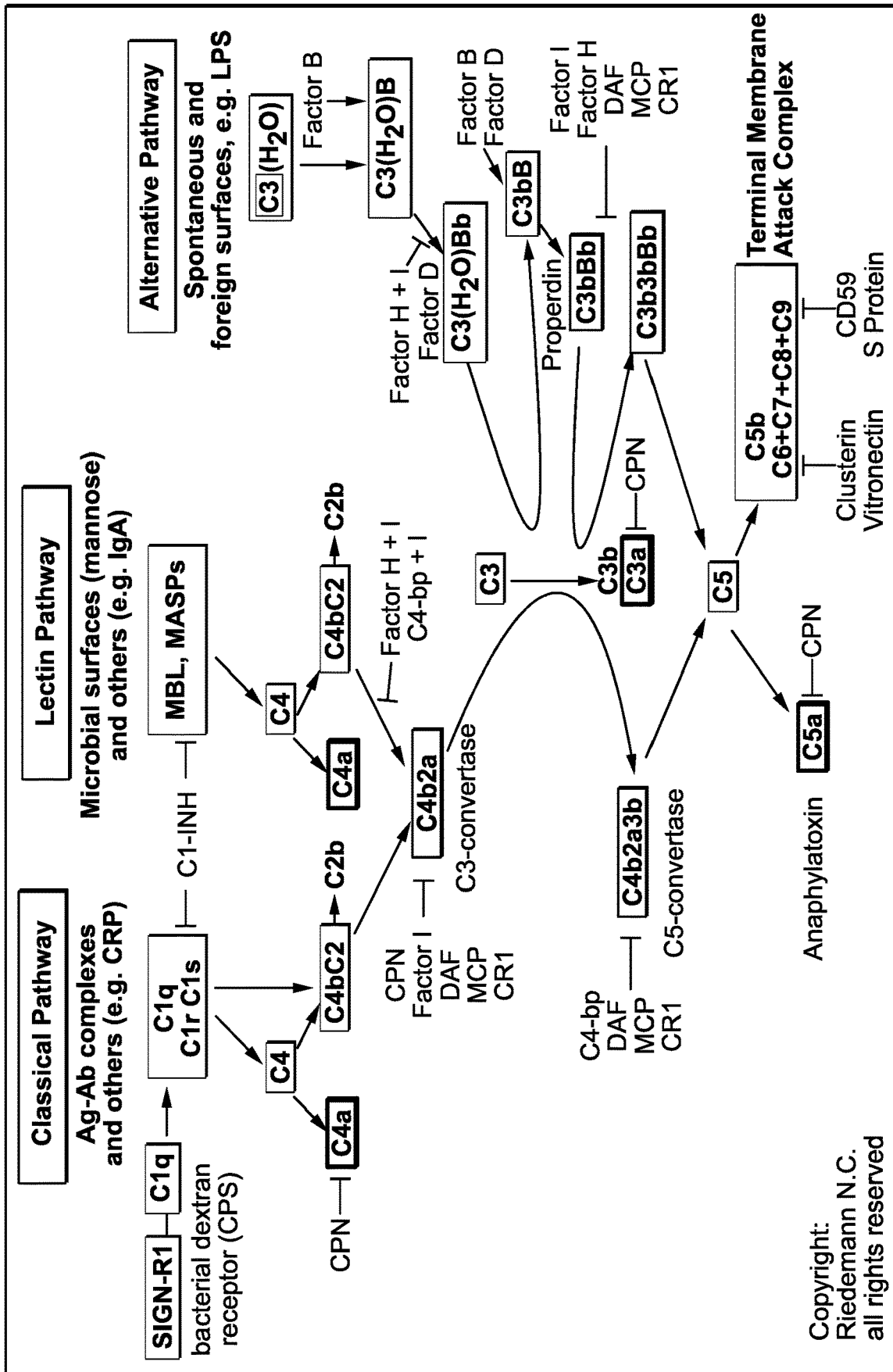
References Cited

OTHER PUBLICATIONS

PCT/US2020/056563, Oct. 21, 2020, WO 2021/081026, Published.
PCT/US2021/020777, Mar. 4, 2021, WO 2021/178607, Published.
Bora et al., Complement activation via alternative pathway is critical in the development of laser-induced choroidal neovascularization: role of factor B and factor H. *J Immunol.* Aug. 1, 2006;177(3):1872-8.
Borodovsky et al., Development of RNAi Therapeutics Targeting the Complement Pathway. *Blood.* 2013;122(21):2471.
Cheng et al., [Effect of C5-siRNA silencing receptor C5 on myocardial ischemia injury in rats]. *Nan Fang Yi Ke Da Xue Xue Bao.* Jun. 2010;30(6):1486-8.
International Search Report and Written Opinion for Application No. PCT/US2014/069951, dated Jul. 6, 2015.
Zheng et al., "Preventing Renal Ischemia-Reperfusion Injury Using Small Interfering RNA by Targeting Complement 3 Gene," *American Journal of Transplantation* 2006; 6: 2099-2108.

Zheng et al., "Protection of Renal Ischemia Injury using Combination Gene Silencing of Complement 3 and Caspase 3 Genes," *Transplantation* 2006;82: 1781-1786.
Reynolds et al., "Rational siRNA design for RNA interference," *Nature Biotechnology*, vol. 22, pp. 326-330, 2004.
International Search Report and Written Opinion from PCT/US2018/058705, dated Mar. 1, 2019.
International Search Report and Written Opinion from PCT/US2020/056563, dated Mar. 22, 2021.
International Search Report and Written Opinion from PCT/US2021/020777, dated Aug. 16, 2021.
Vickers et al., "Efficient Reduction of Target RNAs by Small Interfering RNA and RNase H-dependent Antisense Agents", *The Journal of Biological Chemistry*, 2003, 278:7108-7118).
Watts et al., "Gene silencing by siRNAs and antisense oligonucleotides in the laboratory and the clinic", *J Pathol* 2012; 226: 365-379.

* cited by examiner



**COMPLEMENT COMPONENT C3 IRNA
COMPOSITIONS AND METHODS OF USE
THEREOF**

RELATED APPLICATIONS

This application is a 35 U.S.C. § 371 national stage filing of International Application No. PCT/US2018/058705, filed on Nov. 1, 2018, which in turn claims the benefit of priority to U.S. Provisional Patent Application No. 62/580,030, filed on Nov. 1, 2017. The entire contents of each of the foregoing applications are incorporated herein by reference.

This application is related to U.S. Provisional Patent Application No. 61/915,210, filed on Dec. 12, 2013, to International Application No. PCT/US2014/069951, filed on Dec. 12, 2014, and to U.S. patent application Ser. No. 15/176,231, filed on Jun. 8, 2016. The entire contents of each of the foregoing applications are hereby incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Mar. 25, 2022, is named 121301_06603_SeqList.txt and is 538,920 bytes in size.

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 J R and Song W C. (2010) *Cell Res.* 20:34; Molina H, et al. (1996) *Proc Natl Acad Sci USA.* 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 M M. (2010) *Nat Rev Drug Discov.* 9:43).

Complement activation is known to occur through three different pathways: alternate, classical and lectin (FIG. 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 (PNH), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, systemic lupus erythematosus, rheumatoid arthritis, ischemia-reperfusion injuries and neurodegenerative diseases.

To date, only one therapeutic that targets the C5-05a axis is available for the treatment of complement component C3-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 C3-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 C3-associated disease.

SUMMARY OF THE INVENTION

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated

3

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. The use of these iRNA agents enables the selective targeted degradation of mRNAs of the corresponding gene (the C3 gene) in mammals.

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 C3 gene, e.g., a complement component C3-associated disease, such as paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosis using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a C3 gene for inhibiting the expression of a C3 gene.

Accordingly, in one aspect the present invention 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 SEQ ID NO:1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2.

In another aspect the present invention 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 any one of Tables 3, 4, 6, 7, and 9.

In one embodiment the region of complementarity consists of the nucleotide sequence of one of the antisense sequences of any one of Tables 3, 4, 6, 7, and 9.

In one embodiment, the dsRNA comprises a sense strand comprising, or consisting of, the nucleotide sequence of a sense strand sequence selected from the sequence of any one of Tables 3, 4, 6, 7, and 9, and an antisense strand comprising, or consisting of, the nucleotide sequence of an antisense sequence selected from the sequences of any one of Tables 3, 4, 6, 7, and 9.

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-

4

allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxyl-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.

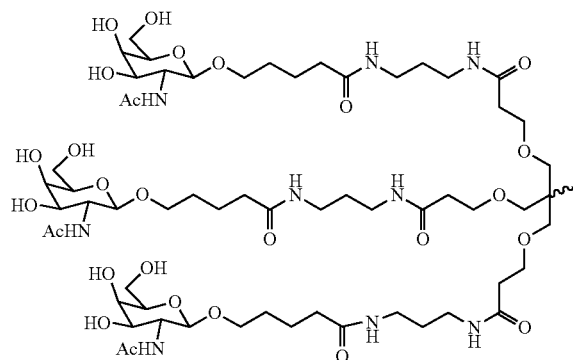
In one embodiment, substantially all the nucleotides of the sense strand and the antisense strand are modified nucleotides. In another embodiment, all the nucleotides of the sense strand and the antisense strand are modified nucleotides.

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.

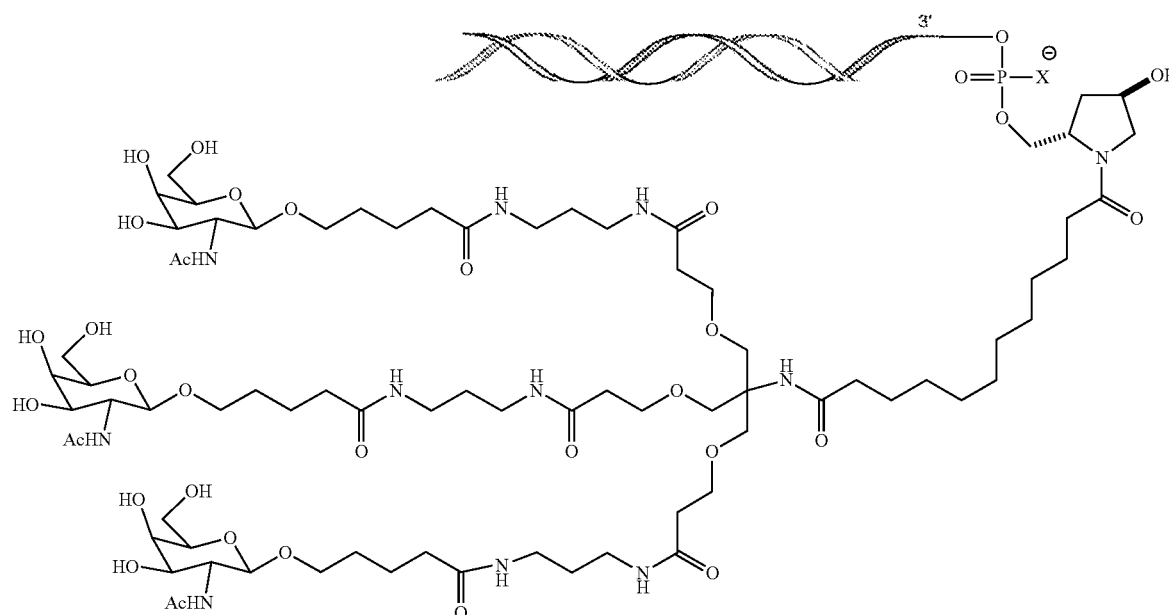
The dsRNA may further include a ligand. In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA. In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative. In one embodiment, the ligand is



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

5

6



and, wherein X is O or S.

In one embodiment, the X is O.

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

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

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

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

In another embodiment, the RNAi agent is selected from the group of RNAi agents listed in any one of Tables 3, 4, 6, 7, and 9. In one embodiment, the RNAi agent is AD-80806. In another embodiment, the RNAi agent is AD-80807.

In another aspect, the present invention provides cells containing the agents of the invention.

In one aspect, the invention provides vectors encoding at least one strand of the agents of the invention.

In another aspect, the invention provides cells comprising the vectors of the invention.

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

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

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

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

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

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

In one aspect, the present invention provides methods of inhibiting complement component 3 (C3) expression in a cell. The methods include contacting the cell with the agent of the invention or a pharmaceutical composition of the invention, 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 one embodiment, the cell is within a subject.

In one embodiment, the subject is a human.

In one embodiment, the human subject suffers from a complement component C3-associated disease.

In one embodiment, the complement component C3-associated disease is selected from the group consisting of paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, and systemic lupus erythematosus.

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

In one embodiment, 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 one embodiment, the agent is administered subcutaneously.

In another embodiment, the agent is administered intravenously.

In one aspect, the present invention 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 invention, thereby treating the subject.

In one aspect, the present invention provides methods of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in

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

In one embodiment, the disorder is a complement component C3-associated disease.

In one embodiment, the complement component C3-associated disease is selected from the group consisting of paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, and systemic lupus erythematosus. In one embodiment, the complement component C3-associated disease is systemic lupus erythematosus.

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

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

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

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

In one aspect, the present invention 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 invention, thereby inhibiting the expression of C3 in the subject.

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

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

In one embodiment, 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.

BRIEF DESCRIPTION OF THE DRAWINGS

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

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides iRNA compositions, which selectively effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a complement component gene, i.e., a C3 gene. The gene may be within a cell, e.g., a cell within a subject, such as a human. The use of these iRNAs enables the selective targeted degradation of mRNAs of the corresponding gene (the C3 gene) in mammals.

The RNAi agents of the invention have been designed to potently and selectively target the corresponding human C3 gene. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing properties and the specific target sites and/or the specific modifications in these RNAi agents confer to the RNAi agents of the invention improved efficacy, stability, potency, durability, and safety.

The iRNAs of the invention may 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 human C3 gene.

In certain embodiments, the iRNAs of the invention include an RNA strand (the antisense strand) which can include longer lengths, for example up to 66 nucleotides, e.g., 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of a C3 gene. These iRNAs with the longer length antisense strands preferably include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

Using in vitro assays, the present inventors have demonstrated that iRNAs targeting a C3 gene can potently mediate RNAi, resulting in significant inhibition of expression of a C3 gene. Thus, methods and compositions including these iRNAs are useful for treating a subject having a complement component C3-associated disease or disorder, e.g., paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, C3-associated disease, or systemic lupus erythematosus.

Accordingly, the present invention provides methods and combination therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a C3 gene, e.g., a complement component C3-associated disease, such as paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosus, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a C3 gene.

The present invention also provides methods for preventing at least one symptom in a subject having a disorder that would benefit from inhibiting or reducing the expression of a C3 gene, e.g., a complement component C3-associated disease, such as paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosus. For example, in a subject having MG the methods of the present invention may prevent at least one symptom in the subject including, e.g., hemolysis, MAC deposition, inflammation (e.g., chronic inflammation), complement activation and destruction of muscle membrane morphology; in a subject having NMO the methods of the present invention may prevent at least one symptom in the subject including, e.g., hemolysis, inflammation (e.g., chronic inflammation), and MAC tissue damage; in a subject having GN the methods of the present invention may prevent at least one symptom in the subject including, e.g., hemolysis, inflammation (e.g., chronic inflammation), and proteinuria; in a subject having aHUS

the methods of the present invention may prevent at least one symptom in the subject including, e.g., hemolysis, inflammation (e.g., chronic inflammation), hypertension, proteinuria, uremia, lethargy/fatigue, irritability, thrombocytopenia, microangiopathic hemolytic anemia, and renal function impairment (e.g., acute renal failure).

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a complement C3 gene, as well as compositions, uses, and methods for treating subjects having diseases and disorders that would benefit from inhibition and/or reduction of the expression of a C3 gene.

I. Definitions

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

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

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

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

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

The term “at least” prior to a number or series of numbers is understood to include the number adjacent to the term “at least”, and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, “at least 18 nucleotides of a 21 nucleotide nucleic acid molecule” means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that “at least” can modify each of the numbers in the series or range.

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

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

Various embodiments of the invention can be combined as determined appropriate by one of skill in the art.

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. NM_000064.3 (GI:726965399); mouse C3, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. NM_009778.3 (GI:773669943); and rat C3, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. NM_016994.2 (GI:158138560).

The term “C3” also includes *Macaca fascicularis* C3, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. m, butM_005587719.2 (GI:982312947) and in the entry for the gene, ENSP00000245907 (locus=chr19:6921416:6963034), in the *Macaca* genome project web site (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:1-8. SEQ ID NOs:5-8 are the antisense sequences of SEQ ID NOs:1-4, respectively.

Further information on C3 is provided, for example in the NCBI Gene database at www.ncbi.nlm.nih.gov/gene/718.

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

The term “C3,” as used herein, also refers to naturally occurring DNA sequence variations of the C3 gene. Numerous sequence variations within the C3 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, e.g., www.ncbi.nlm.nih.gov/snp?LinkName=gene_snp&from_uid=718, the entire contents of which is incorporated herein by reference as of the date of filing this application).

As used herein, “target sequence” refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a C3 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 C3 gene. In one embodiment, the target sequence is within the protein coding region of a C3 gene. In another embodiment, the target sequence is within the 3' UTR of a C3 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. In some embodiments, the target sequence is about 19 to about 30 nucleotides in length. In other embodiments, the target sequence is about 19 to about

25 nucleotides in length. In still other embodiments, the target sequence is about 19 to about 23 nucleotides in length. In some embodiments, the target sequence is about 21 to about 23 nucleotides in length. Ranges and lengths 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 a C3 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 RNAi that interacts with a target RNA sequence, e.g., a C3 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 double stranded short interfering RNAs (siRNAs) comprising a sense strand and an antisense strand by a Type III endonuclease known as Dicer (Sharp et al. (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes these dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, et al., (2001) *Nature* 409:363). These 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 (ssRNA) (the antisense strand of an siRNA duplex) generated within a cell and which promotes the formation of a RISC complex to

effect silencing of the target gene, i.e., a C3 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 RNA 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 RNAs are described in U.S. Pat. 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 C3 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. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modification, as used in an RNAi agent are encompassed by iRNA for the purposes of the specification and claims. In some embodiments, an RNAi agent includes 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. In some embodiments, the hairpin loop can be 10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised 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. In one embodiment of the RNAi agent, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In one embodiment, an RNAi agent of the invention is a dsRNA agent, each strand of which comprises 19-23 nucleotides that interacts with a target RNA sequence, i.e., a C3 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, 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). 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 C3 target mRNA sequence, to direct the cleavage of the target RNA.

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of 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 of the dsRNA, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotide, e.g., 0-3, 1-3, 2-4, 2-5, 4-10, 5-10, e.g., a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end 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.

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

"Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the double stranded 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 C3 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 C3 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, 2, or 1 nucleotides of the 5'- and/or 3'-terminus of the iRNA. In one embodiment, a double stranded RNAi agent of the invention includes a nucleotide mismatch in the anti sense strand. In another embodiment, a double stranded RNAi agent of the invention includes a nucleotide mismatch in the sense strand. In one embodiment, the nucleotide mismatch is, for example, within 5, 4, 3, 2, or 1 nucleotides from the 3'-terminus of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide 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 Hoogsteen base pairing.

The terms “complementary,” “fully complementary” and “substantially complementary” herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between 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 (i.e., a C3 gene). For example, a polynucleotide is complementary to at least a part of a C3 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding C3.

Accordingly, in some embodiments, the sense strand polynucleotides and the antisense polynucleotides disclosed herein are fully complementary to the target C3 gene sequence.

In one embodiment, the antisense polynucleotides disclosed herein are fully complementary to the target C3 sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target C3 sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NO:1, or a fragment of any one of SEQ ID NO:1, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target C3 sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 3, 4, 6, 7, and 9, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 3, 4, 6, 7, and 9, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In one embodiment, an RNAi agent of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target C3 sequence and comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 3, 4, 6, 7, and 9, or a fragment of any one of the sense strand nucleotide

sequences in any one of Tables 3, 4, 6, 7, and 9, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In 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.

As used herein, the term “complement component C3-associated disease” is a disease or disorder that is caused by, or associated with complement activation. The term “complement component C3-associated disease” includes a disease, disorder or condition that would benefit from reduction in C3 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 C3-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 (IHUS); dense deposit disease (DDD); neuromyelitis optica (NMO), multifocal motor neuropathy (MMN); multiple sclerosis (MS); macular degeneration (e.g., age-related macular degeneration (AMD)); systemic lupus erythematosus; 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).

“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 C3-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-associated disease but not yet (or currently) experiencing or displaying symptoms of the disease, and/or a subject at risk of developing a complement component C3-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.

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

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 C3 gene in a cell, such as a cell within a subject, e.g., a mammal, such as a human having a complement component C3-associated disease as described herein, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosis. 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., C3 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 selectively inhibits the expression of the target gene (e.g., a human, a primate, a non-primate, or a bird C3 gene) by at least about 10%, by at least 30%, preferably at least 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting or flow cytometric techniques. In preferred embodiments, inhibition of expression is determined by the qPCR method provided in the examples. For in vitro assessment of activity, percent inhibition is determined using the methods provided in Example 2 at a single dose at a 10 nM duplex final concentration. For in vivo studies, the level after treatment can be compared to, for example, an appropriate historical control or a pooled population sample control to determine the level of reduction, e.g., when a baseline value is no available for the subject.

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 C3 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 nucleo-

tides 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 sense and antisense strands of the dsRNA are each independently about 15 to about 30 nucleotides in length, or about 25 to about 30 nucleotides in length, e.g., each strand is independently 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. 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 C3 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, 13-31, 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 C3 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. In certain embodiments, longer, extended overhangs are possible.

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 antisense sequence.

In one embodiment, the sense strand is selected from the group of sequences provided in any one of Tables 3, 4, 6, 7, and 9, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 3, 4, 6, 7, and 9. 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 3, 4, 6, 7, and 9 and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 3, 4, 6, 7, and 9. 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 any one of Tables 3, 4, 6, 7, and 9 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 any one of Tables 3, 4, 6, 7, and 9 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 any one of Tables 3, 4, 6, 7, and 9 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, 4, 6, 7, and 9 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, 4, 6, 7, and 9, and differing in their ability to inhibit the expression of the target 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, 4, 6, 7, and 9 identify a site(s) in a C3 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, 4, 6, 7, and 9 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, 4, 6, 7, and 9 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, 4, 6, 7, and 9, 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 C3 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 C3 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, N.Y., 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 aminoal-

kylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. In some embodiments of the invention, the dsRNA agents of the invention are in a free acid form. In other embodiments of the invention, the dsRNA agents of the invention are in a salt form. In one embodiment, the dsRNA agents of the invention are in a sodium salt form. In certain embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for substantially all of the phosphodiester and/or phosphorothioate groups present in the agent. Agents in which substantially all of the phosphodiester and/or phosphorothioate linkages have a sodium counterion include not more than 5, 4, 3, 2, or 1 phosphodiester and/or phosphorothioate linkages without a sodium counterion. In some embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for all of the phosphodiester and/or phosphorothioate groups present in the agent.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6,239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat. No. 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 CH₂ component parts.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. 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 hybrid-

ization 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. Pat. 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 $-\text{CH}_2-\text{NH}-\text{CH}_2-$, $-\text{CH}_2-\text{N}(\text{CH}_3)-\text{O}-\text{CH}_2-$ [known as a methylene (methylimino) or MMI backbone], $-\text{CH}_2-\text{O}-\text{N}(\text{CH}_3)-\text{CH}_2-$, $-\text{CH}_2-\text{N}(\text{CH}_3)-\text{N}(\text{CH}_3)-\text{CH}_2-$ and $-\text{N}(\text{CH}_3)-\text{CH}_2-\text{CH}_2-$ [wherein the native phosphodiester backbone is represented as $-\text{O}-\text{P}-\text{O}-\text{CH}_2-$] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Pat. 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 $\text{O}[(\text{CH}_2)_n\text{O}]_m\text{CH}_3$, $\text{O}(\text{CH}_2)_n\text{OCH}_3$, $\text{O}(\text{CH}_2)_n\text{NH}_2$, $\text{O}(\text{CH}_2)_n\text{CH}_3$, $\text{O}(\text{CH}_2)_n\text{ONH}_2$, and $\text{O}(\text{CH}_2)_n\text{ON}[(\text{CH}_2)_m\text{CH}_3]_2$, 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, aralkyl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3 , OCN, Cl, Br, CN, CF_3 , OCF_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 ($2'-\text{O}-\text{CH}_2\text{CH}_2\text{OCH}_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chica. Acta*, 1995, 78:486-504) i.e., an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminoxyethoxy, i.e., a $\text{O}(\text{CH}_2)_2\text{ON}(\text{CH}_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethyl aminoethoxyethyl or 2'-DMAEOE), i.e., $2'-\text{O}-\text{CH}_2-\text{O}-\text{CH}_2-\text{N}(\text{CH}_3)_2$.

Other modifications include 2'-methoxy (2'- OCH_3), 2'-aminopropoxy (2'- $\text{OCH}_2\text{CH}_2\text{CH}_2\text{NH}_2$) 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.

5 The entire contents of each of the foregoing are hereby incorporated herein by reference.

The RNA of an iRNA of the invention 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 deoxythymine (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. Pat. 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.

60 The RNA of an iRNA can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects

(Elmen, J. et al., (2005) *Nucleic Acids Research* 33(1):439-447; Mook, O R. et al., (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. et al., (2003) *Nucleic Acids Research* 31(12):3185-3193).

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

The RNA of an iRNA can also be modified to include one or more bicyclic sugar moieties. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A "bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain 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, O R. 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., U.S. Pat. No. 8,278,283); 4'-CH₂-N(OCH₃)-2' (and analogs thereof; see e.g., U.S. Pat. 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., U.S. Pat. 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. Pat. 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)-O-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "5-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.

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

Other modifications of the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, e.g., a 5'-terminal phosphate or phosphate mimic on the antisense strand of an RNAi agent. Suitable phosphate mimics are disclosed in, for example US Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

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, 4, 6, 7, and 9. 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. Acad. Sci. USA*, 1989, 86: 6553-6556), cholic acid (Manoharan et al., *Biorg. Med. Chem. Lett.*, 1994, 4:1053-1060), a thioether, e.g., beryl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan et al., *Biorg. Med. Chem. Lett.*, 1993, 3:2765-2770), a thiocholesterol (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 hexylaminocarbonyloxycholesterol 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-glycolid) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

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

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

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 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: 1349). An RFGF analogue (e.g., amino acid sequence AALLPVLLAAP (SEQ ID NO: 1350) 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: 1351) and the *Drosophila* Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 1352) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam et al., Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

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

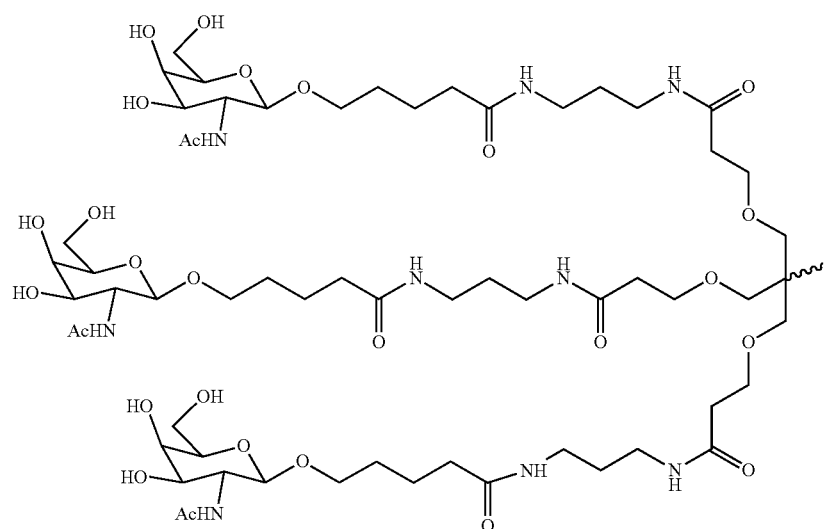
33

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

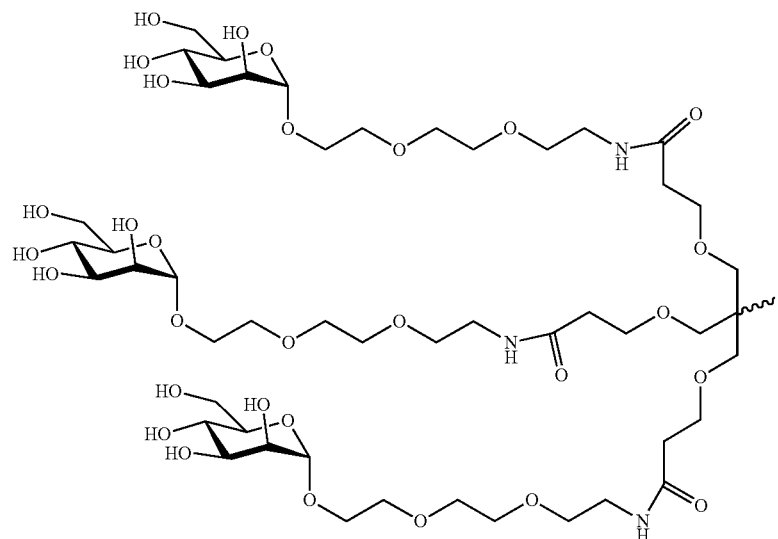
34

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



Formula II



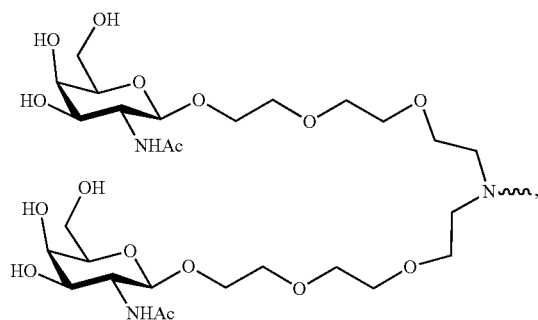
Formula III

35

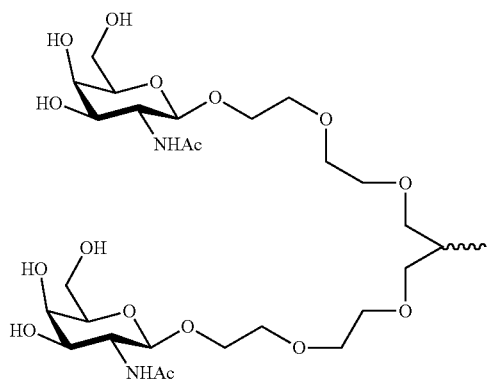
36

-continued

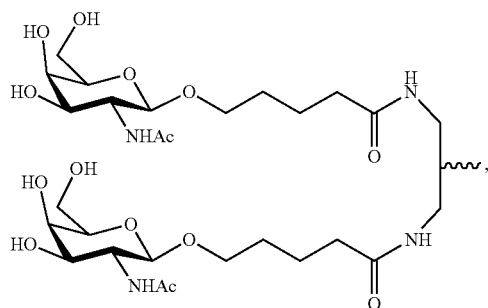
Formula IV



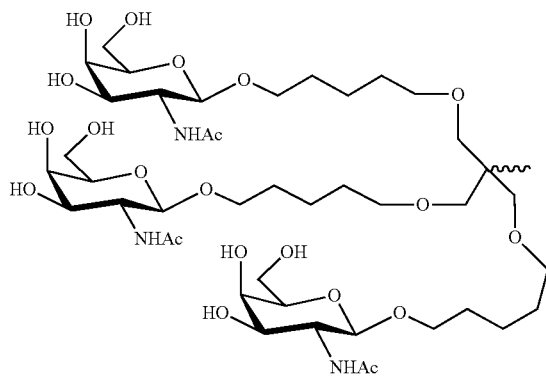
Formula V



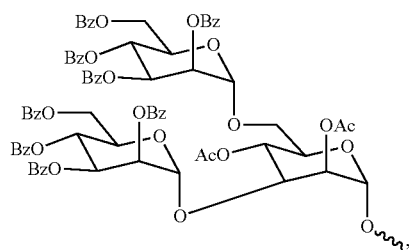
Formula VI



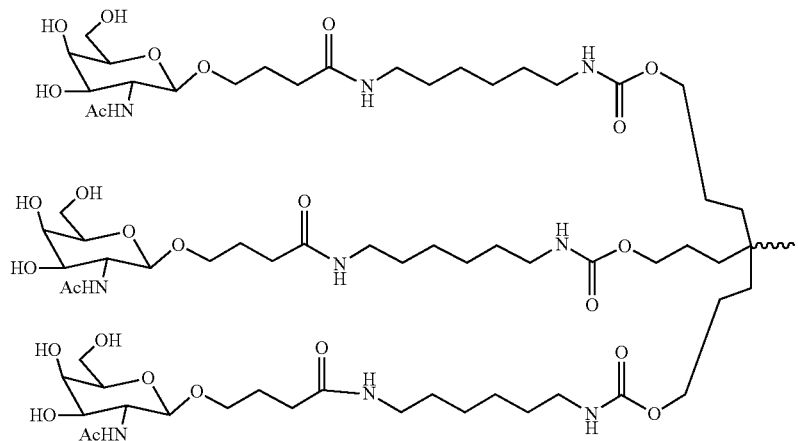
Formula VII



Formula VIII

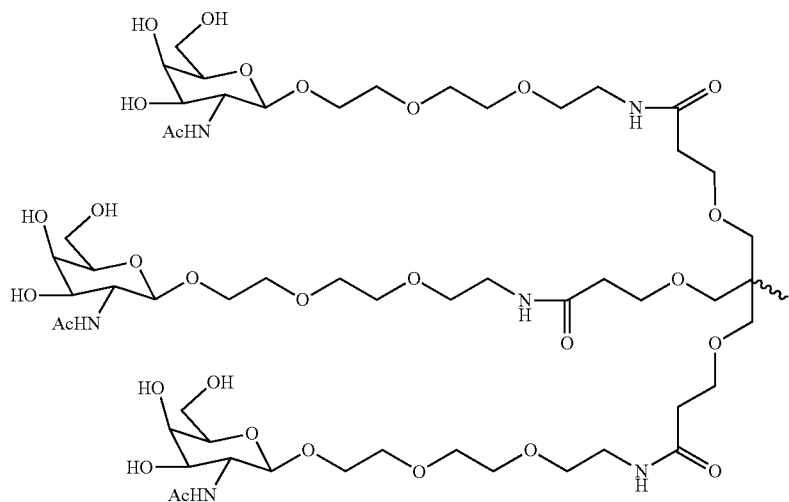


Formula IX

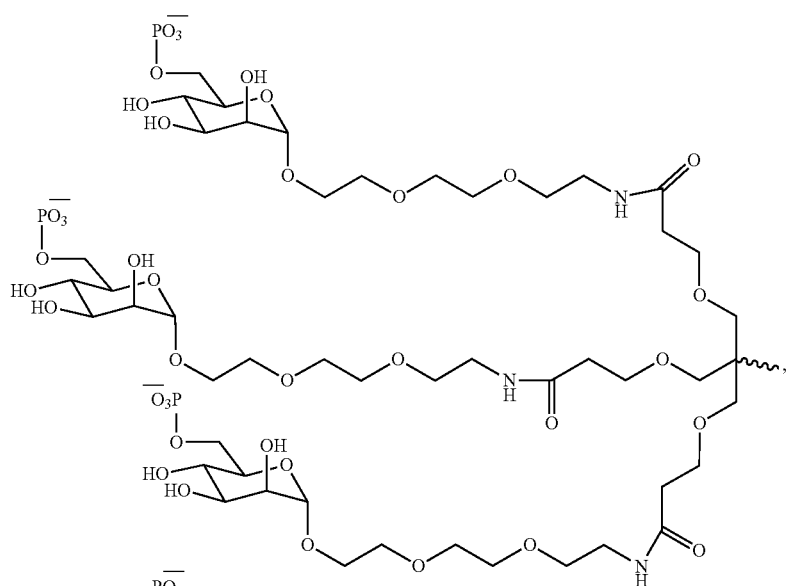


-continued

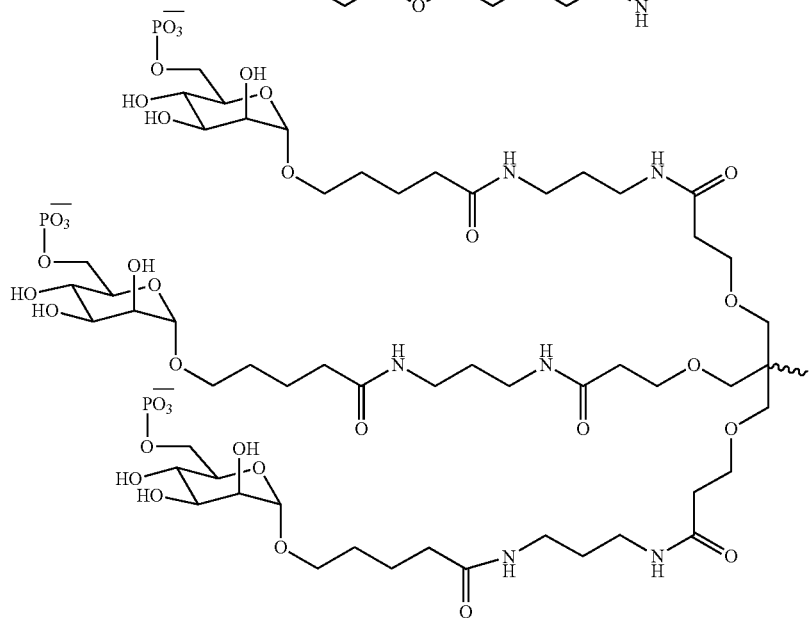
Formula X



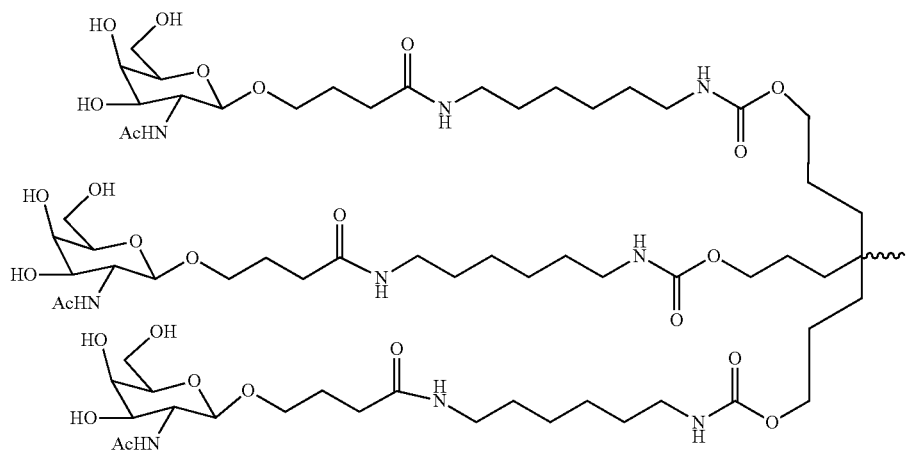
Formula XI



Formula XII

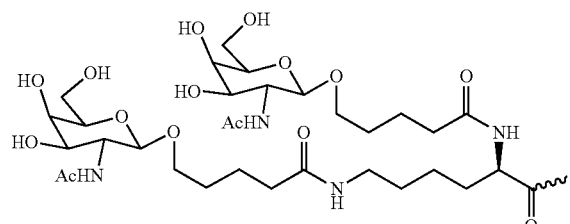
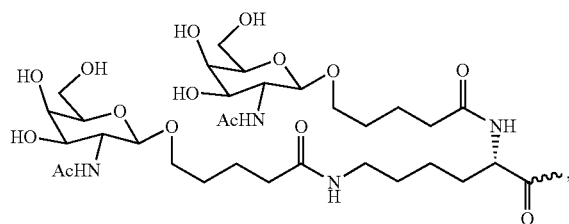


Formula XIII



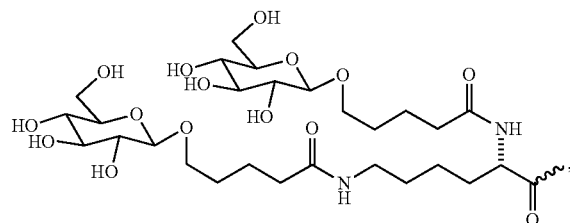
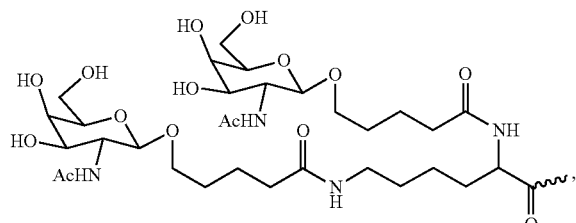
Formula XIV

Formula XV



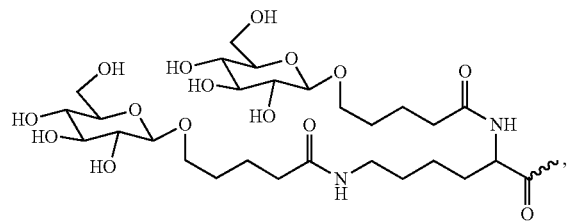
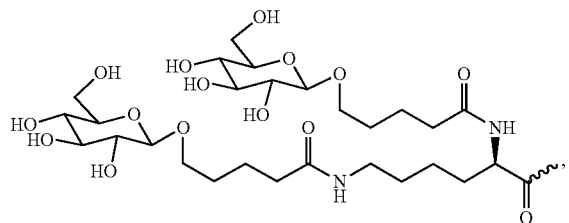
Formula XVI

Formula XVII



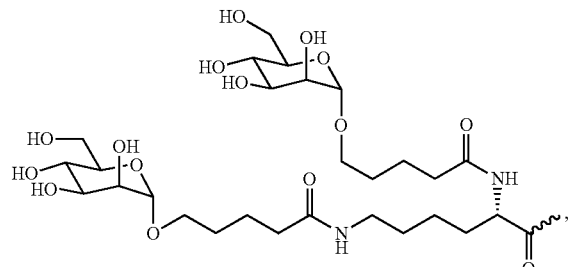
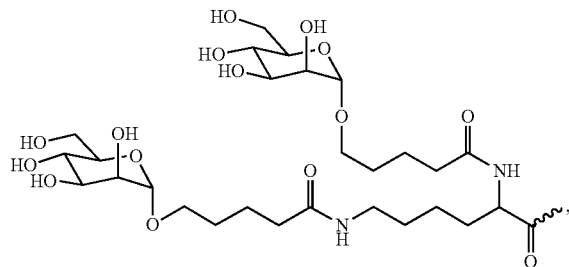
Formula XIX

Formula XVIII



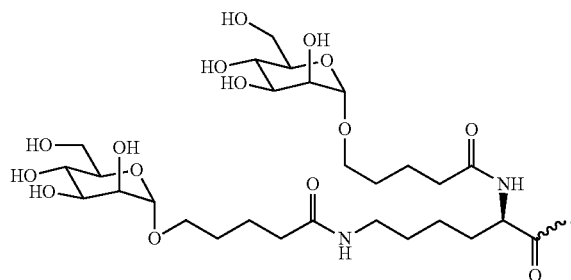
Formula XX

Formula XXI

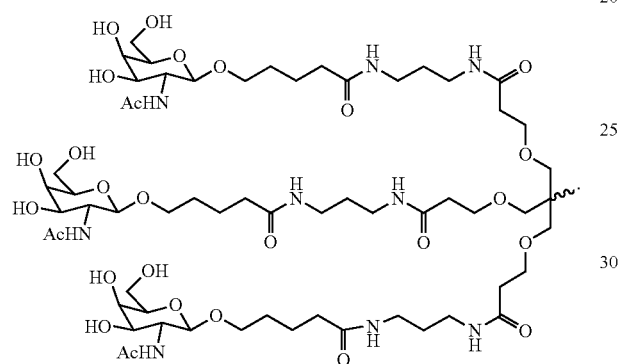


-continued

Formula XXII

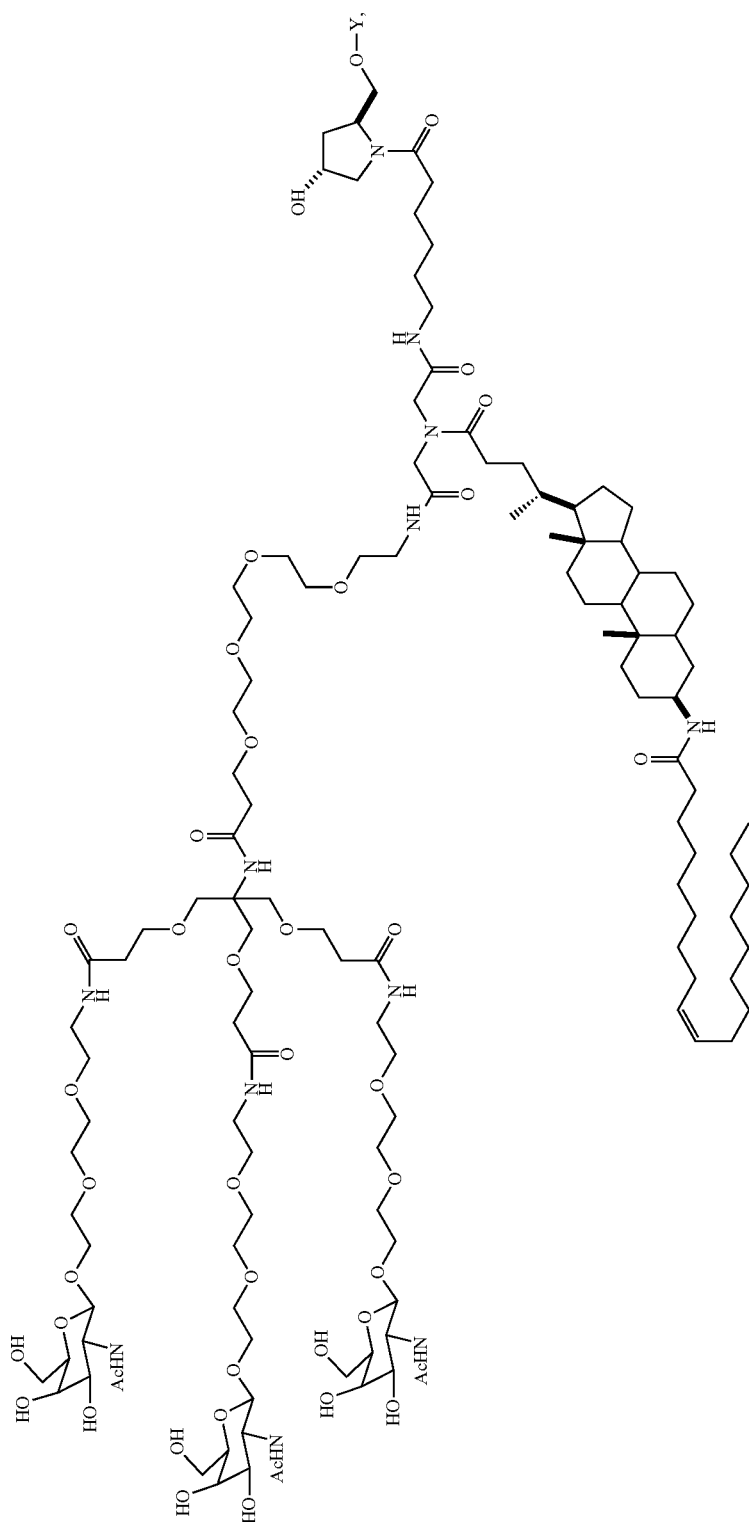


In one embodiment, the monosaccharide is an N-acetyl-¹⁵galactosamine, such as

Formula II ²⁰

Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,³⁵

(Formula XXIII)



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

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

In one embodiment, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In another embodiment, the double stranded RNAi agents of the invention comprise a plurality (e.g., 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

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

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

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

D. Linkers

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

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, e.g., covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO₂, SO₂NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclalkyl, heterocyclalkenyl, heterocyclalkynyl, aryl, heteroaryl, heterocycl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkyl aryl alkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclalkyl, alkylheterocyclalkenyl, alkylheterocyclalkynyl, alkenylheterocyclalkyl, alkenylhet-

erocyclalkenyl, alkenylheterocyclalkynyl, alkynylheterocyclalkyl, alkynylheterocyclalkenyl, alkynylheterocyclalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylheteroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO₂, N(R8), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, 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 known in the art, which mimic the rate of cleavage which would be observed in a cell, e.g., a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood (or under in vitro conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

ii. Phosphate-Based Cleavable Linking Groups

In 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 $-O-P(O)(ORk)-O-$, $-O-P(S)(ORk)-O-$, $-O-P(S)(SRk)-O-$, $-S-P(O)(ORk)-O-$, $-O-P(O)(ORk)-S-$, $-S-P(O)(ORk)-S-$, $-O-P(S)(ORk)-S-$, $-S-P(S)(ORk)-O-$, $-O-P(O)(Rk)-O-$, $-O-P(S)(Rk)-O-$, $-S-P(O)(Rk)-O-$, $-S-P(S)(Rk)-O-$, $-S-P(O)(Rk)-S-$. 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 acid 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

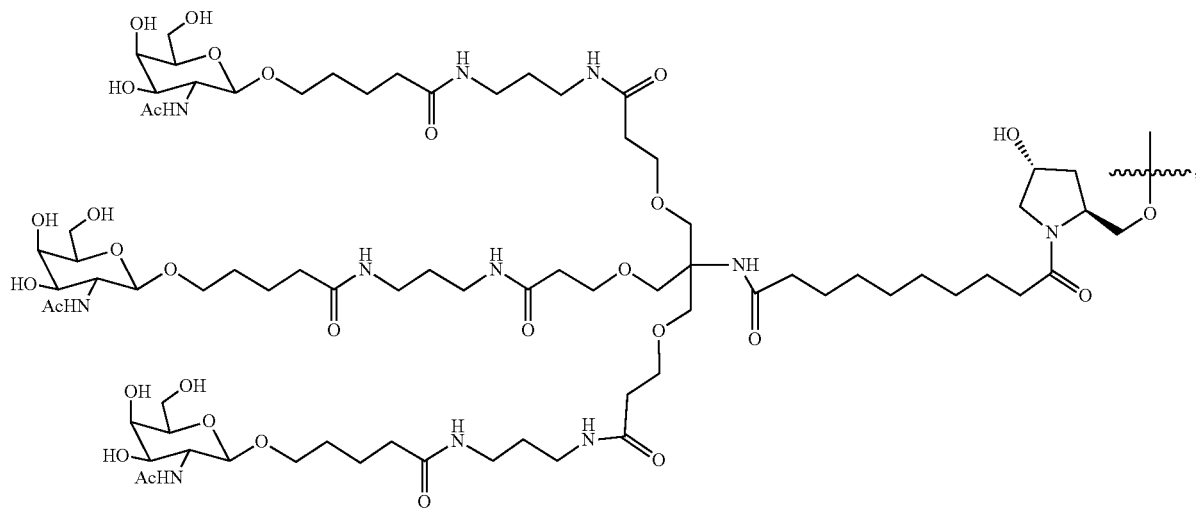
In another embodiment, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene 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

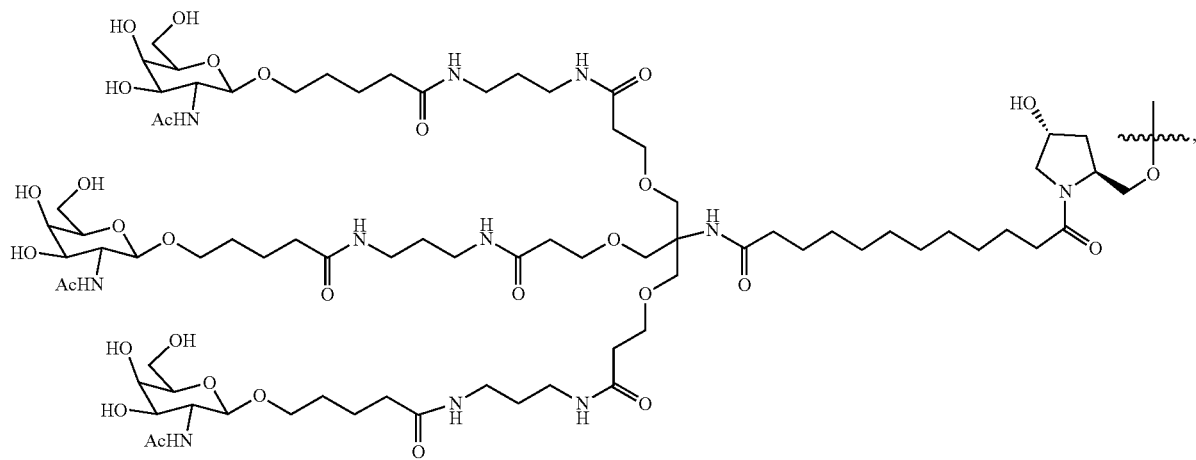
In yet another embodiment, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides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

In 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,

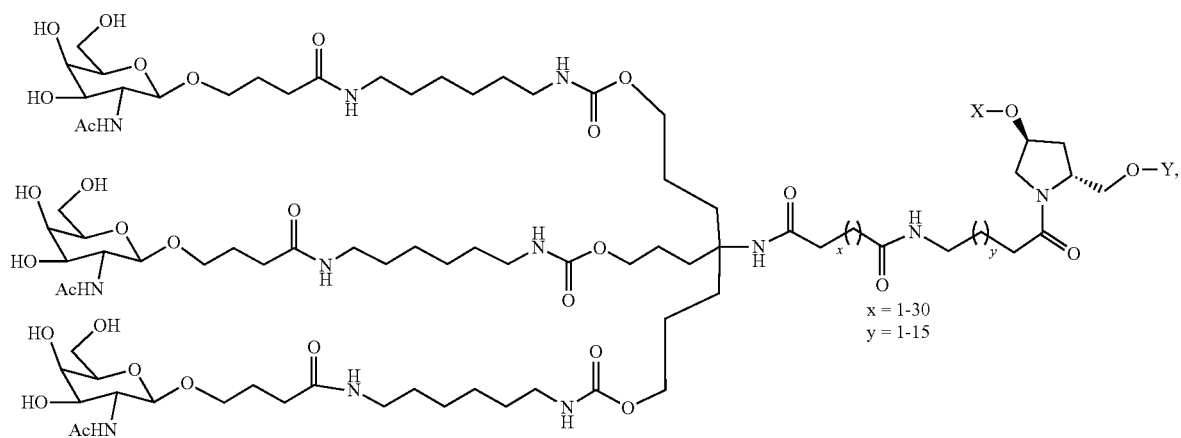
(Formula XXIV)



(Formula XXV)



(Formula XXVI)

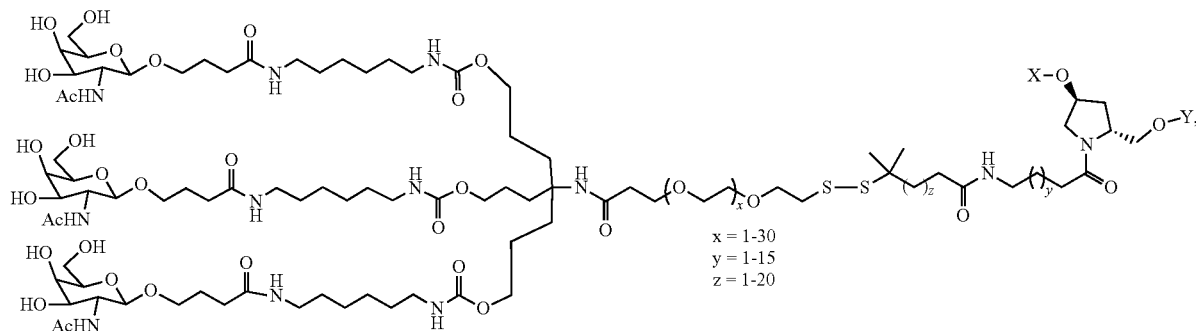


53

54

-continued

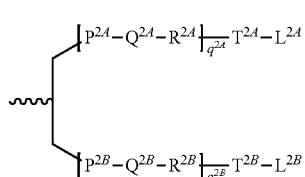
(Formula XXXI)



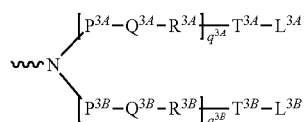
when one of X or Y is an oligonucleotide, the other is a hydrogen.

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.

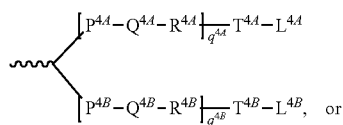
In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXII)-(XXXV):



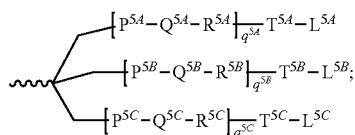
Formula XXXII



Formula XXXIII



Formula XXXIV

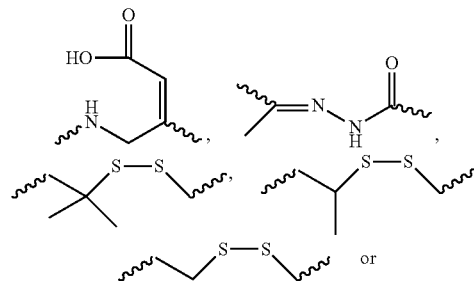


Formula XXXV

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), CH₂, CH₂NH or CH₂O; 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, substi-

tuted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO₂, N(R^N), C(R')=C(R''), C≡C or C(O); R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH₂, C(O)O, C(O)NH, NHCH(R^a)C(O), —C(O)—CH(R^a)—NH—, CO, CH=N—O,

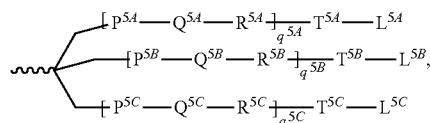


or heterocyclyl;

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

(VII)

Formula XXXV



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. Lett.*, 1993, 3:2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10:111; Kabanov et al., *FEBS Lett.*, 1990, 259:327; Svinarchuk et al., *Biochimie*, 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36:3651; Shea et al., *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan et al., *Tet-*

rahedron Lett., 1995, 36:3651), a palmitoyl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carboxyloxycholesterol 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 C3-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 R L. (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 knock-down of gene products when an iRNA is administered locally. For example, intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, M J., et al (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, S J., 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.* 0.11:267-274) and can prolong survival of tumor-bearing mice (Kim, W J., 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, P H., et al (2005) *Gene Ther.* 12:59-66; Makimura, H., et al (2002) *BMC Neurosci.* 3:18; Shishkina, G T., et al (2004) *Neuroscience* 129:521-528; Thakker, E R., 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, K A., 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, J O., 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 S H., 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, D R., et al (2003) *J. Mol. Biol* 327:761-766; Verma, U N., et al (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, A S 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, D R., et al (2003), supra; Verma, U N., et al (2003), supra), oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, T S., et al (2006) *Nature* 441:111-114), cardioliipin (Chien, P.Y., et al (2005) *Cancer Gene Ther.* 12:321-328; Pal, A., et al (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet M E., et al (2008) *Pharm. Res.* August 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, D A., 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. Pat. No. 7,427,605, which is herein incorporated by reference in its entirety.

A. Vector encoded iRNAs of the Invention

iRNA targeting a C3 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-KO'). 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. Pat. 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); Mastangeli 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. Nos. 5,252,479; 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 invention 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 C3 gene, e.g. a complement component C3-associated disease as described 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), intramuscular (IM), 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 target gene.

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).

In some embodiments, the pharmaceutical compositions of the invention are suitable for intramuscular administration to a subject. In other embodiments, the pharmaceutical compositions of the invention are suitable for intravenous administration to a subject. In some embodiments of the invention, the pharmaceutical compositions of the invention are suitable for subcutaneous administration to a subject, e.g., using a 29 g or 30 g needle.

The pharmaceutical compositions of the invention may include an RNAi agent of the invention in an unbuffered solution, such as saline or water, or in a buffer solution, such as a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

In one embodiment, the pharmaceutical compositions of the invention, e.g., such as the compositions suitable for subcutaneous administration, comprise an RNAi agent of the invention in phosphate buffered saline (PBS). Suitable concentrations of PBS include, for example, 1 mM, 1.5 mM, 2 mM, 2.5 mM, 3 mM, 3.5 mM, 4 mM, 4.5 mM, 5 mM, 6.5 mM, 7 mM, 7.5 mM, 9 mM, 8.5 mM, 9 mM, 9.5 mM, or about 10 mM PBS. In one embodiment of the invention, a pharmaceutical composition of the invention comprises an RNAi agent of the invention dissolved in a solution of about 5 mM PBS (e.g., 0.64 mM NaH₂PO₄, 4.36 mM Na₂HPO₄, 85 mM NaCl). Values intermediate to the above recited ranges and values are also intended to be part of this invention. In addition, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included.

The pH of the pharmaceutical compositions of the invention may be between about 5.0 to about 8.0, about 5.5 to about 8.0, about 6.0 to about 8.0, about 6.5 to about 8.0, about 7.0 to about 8.0, about 5.0 to about 7.5, about 5.5 to about 7.5, about 6.0 to about 7.5, about 6.5 to about 7.5, about 5.0 to about 7.2, about 5.25 to about 7.2, about 5.5 to about 7.2, about 5.75 to about 7.2, about 6.0 to about 7.2, about 6.5 to about 7.2, or about 6.8 to about 7.2. Ranges and values intermediate to the above recited ranges and values are also intended to be part of this invention.

The osmolality of the pharmaceutical compositions of the invention may be suitable for subcutaneous administration, such as no more than about 400 mOsm/kg, e.g., between 50 and 400 mOsm/kg, between 75 and 400 mOsm/kg, between 100 and 400 mOsm/kg, between 125 and 400 mOsm/kg, between 150 and 400 mOsm/kg, between 175 and 400 mOsm/kg, between 200 and 400 mOsm/kg, between 250 and 400 mOsm/kg, between 300 and 400 mOsm/kg, between 50 and 375 mOsm/kg, between 75 and 375 mOsm/kg, between 100 and 375 mOsm/kg, between 125 and 375 mOsm/kg, between 150 and 375 mOsm/kg, between 175 and 375 mOsm/kg, between 200 and 375 mOsm/kg, between 250 and 375 mOsm/kg, between 300 and 375 mOsm/kg, between 50 and 350 mOsm/kg, between 75 and 350 mOsm/kg, between 100 and 350 mOsm/kg, between 125 and 350 mOsm/kg, between 150 and 350 mOsm/kg, between 175 and 350 mOsm/kg, between 200 and 350 mOsm/kg, between 250 and 350 mOsm/kg, between 50 and 325 mOsm/kg, between 75 and 325 mOsm/kg, between 100 and 325 mOsm/kg, between 125 and 325 mOsm/kg, between 150 and 325 mOsm/kg, between 175 and 325 mOsm/kg, between 200 and 325 mOsm/kg, between 250 and 325 mOsm/kg, between 300 and 325 mOsm/kg,

between 300 and 350 mOsm/kg, between 50 and 300 mOsm/kg, between 75 and 300 mOsm/kg, between 100 and 300 mOsm/kg, between 125 and 300 mOsm/kg, between 150 and 300 mOsm/kg, between 175 and 300 mOsm/kg, between 200 and 300 mOsm/kg, between 250 and 300, between 50 and 250 mOsm/kg, between 75 and 250 mOsm/kg, between 100 and 250 mOsm/kg, between 125 and 250 mOsm/kg, between 150 and 250 mOsm/kg, between 175 and 350 mOsm/kg, between 200 and 250 mOsm/kg, e.g., about 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 295, 300, 305, 310, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, or about 400 mOsm/kg. Ranges and values intermediate to the above recited ranges and values are also intended to be part of this invention.

The pharmaceutical compositions of the invention comprising the RNAi agents of the invention, may be present in a vial that contains about 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or about 2.0 mL of the pharmaceutical composition. The concentration of the RNAi agents in the pharmaceutical compositions of the invention may be about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 130, 125, 130, 135, 140, 145, 150, 175, 180, 185, 190, 195, 200, 205, 210, 215, 230, 225, 230, 235, 240, 245, 250, 275, 280, 285, 290, 295, 300, 305, 310, 315, 330, 325, 330, 335, 340, 345, 350, 375, 380, 385, 390, 395, 400, 405, 410, 415, 430, 425, 430, 435, 440, 445, 450, 475, 480, 485, 490, 495, or about 500 mg/mL. In one embodiment, the concentration of the RNAi agents in the pharmaceutical compositions of the invention is about 100 mg/mL. Values intermediate to the above recited ranges and values are also intended to be part of this invention.

The pharmaceutical compositions of the invention may comprise a dsRNA agent of the invention in a free acid form. In other embodiments of the invention, the pharmaceutical compositions of the invention may comprise a dsRNA agent of the invention in a salt form, such as a sodium salt form. In certain embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for substantially all of the phosphodiester and/or phosphorothioate groups present in the agent. Agents in which substantially all of the phosphodiester and/or phosphorothioate linkages have a sodium counterion include not more than 5, 4, 3, 2, or 1 phosphodiester and/or phosphorothioate linkages without a sodium counterion. In some embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for all of the phosphodiester and/or phosphorothioate groups present in the agent.

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, distearoylphosphatidyl choline) negative (e.g., dimyristoylphosphatidyl glycerol DMPG), and cationic (e.g., dioleoyltetramethyl-

aminopropyl 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, tricaprinate, monoolein, dilaurin, glyceryl 1-monocaprinate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C1-20 alkyl ester (e.g., isopropylmyristate IPM), monoglyceride or diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in U.S. Pat. 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 an 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 be adjusted to favor condensation.

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, e.g., WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Felgner, P. L. et al., *Proc.*

Natl. Acad. Sci., USA 8:7413-7417, 1987; U.S. Pat. Nos. 4,897,355; 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, et 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. Nos. 5,283,185; 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 cyclosporin 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-dioleoyloxy)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, Ind.) 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, Wis.) 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, Calif.) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Md.). 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., *Proc. 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. Transfersomes 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 transfersomes 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 PCT Publication No. WO 2008/042973.

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 quater-

nary 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 amphiphathic 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 C₈ to C₂₂ 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. Pat. 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-dioleoyl-N,N-dimethylammonium chloride (DODAC), N,N-distearoyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleoyloxypropylamine (DODMA), 1,2-Dilinoleoyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleoxy-3-(dimethylamino)acetoxyp propane (DLin-DAC), 1,2-Dilinoleoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleoxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleoxy-3-trimethylaminopropane chloride salt (DLin-TMA-Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP-Cl), 1,2-Dilinoleoxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleoxylo-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-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediy)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 U.S. provisional patent application No. 61/107,998 filed on Oct. 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-1-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-phosphatidylethanolamine (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-dialkylxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (C₂), a PEG-dimyristyloxypropyl (C₄), a PEG-dipalmityloxypropyl (C₆), or a PEG-distearoyloxypropyl (C₈). 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 Ser. No. 12/056,230, filed Mar. 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

71

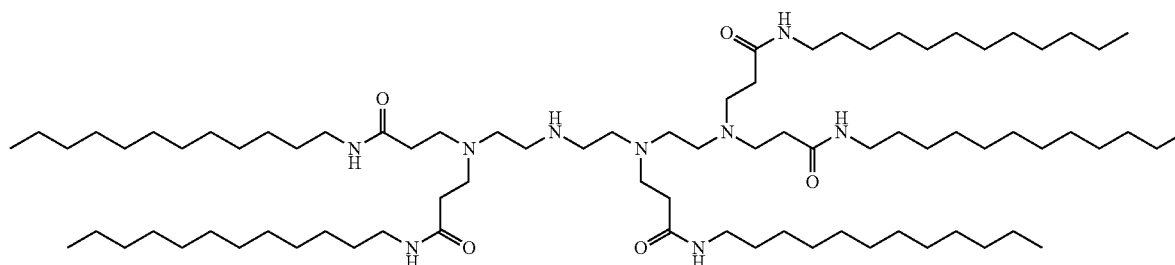
sodium acetate concentration is about 100-300 mM. Lipid-dsRNA nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (e.g., 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can

72

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.

ND98 Isomer I

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	1,2-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-hydroxydo decyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediy)l)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

TABLE 1-continued

Ionizable/Cationic Lipid		cationic lipid/non-cationic lipid/cholesterol/ PEG-lipid conjugate Lipid:siRNA ratio
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 (1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed Apr. 15, 2009, which is hereby incorporated by reference.

XTC comprising formulations are described in PCT Publication No. WO 2010/088537, the entire contents of which are hereby incorporated herein by reference.

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

ALNY-100 comprising formulations are described in PCT Publication No. WO 2010/054406, the entire contents of which are hereby incorporated herein by reference.

C12-200 comprising formulations are described in PCT Publication No. WO 2010/129709, the entire contents of which are hereby incorporated herein by reference.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitables. 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 ursodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, gluchoic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium

25 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 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(isohexylcyanoacrylate), 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. Pat. No. 6,887,906, US Publ. No. 20030027780, and U.S. Pat. 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 0.1 μm in diameter (see e.g., Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; 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 antioxidants 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, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; 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, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; 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, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; 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, atapulgit, 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, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; 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, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; 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, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; 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, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; 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 (M0310), hexaglycerol monooleate (P0310), hexaglycerol pentaoleate (P0500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (M0750), 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. Pat. 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. Pat. 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, N.Y., 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. Such compounds are well known in the art.

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 complement component C3-associated disease or disorder, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosus.

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 C3 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 C3 Expression

The present invention provides methods of inhibiting expression of a complement component C3 gene as described herein. In one aspect, the present invention 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.

Contacting of a cell with an RNAi agent, e.g., 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 a cell or a group of cells are also possible. Contacting a cell or a group of cells may be direct or indirect. 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. Furthermore, contacting a cell or a group of cells 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 GalNAc₃ ligand, or any other ligand that directs the RNAi agent to a site of interest, e.g., the liver of a subject.

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.

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

The phrase "inhibiting expression of a C3 gene" 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 C3 gene) as well as variants or mutants of a C3 gene.

The phrase "inhibiting expression of a C3 gene" 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 (such as 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 the severity of a C3-associated disease. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

In some embodiments of the methods of the invention, expression of a target gene, e.g., C3 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%, or to below the level of detection of the assay. In some embodiments, the inhibition of expression of a C3 gene results in normalization of the level of the C3 gene such that the difference between the level before treatment and a normal control level is reduced by at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%. In some embodiments, the inhibition is a clinically relevant inhibition.

Inhibition of the expression of the target gene, e.g., a C3 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 the rtPCR method provided in Example 2, with in vitro assays being performed in an appropriately matched cell line with the duplex at a 10 nM concentration, and expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

Alternatively, inhibition of the expression of a C3 gene may be assessed in terms of a reduction of a parameter that is functionally linked to C3 gene expression, e.g., C3 protein expression. C3 gene silencing may be determined in any cell expressing a C3 gene, either constitutively or by genomic engineering, and by any assay known in the art.

Inhibition of the expression of a complement component C3 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 inhibition of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

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. In alternative embodiments, the level may be compared to an appropriate control sample, e.g., a known population control sample.

The level of C3 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 C3 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, e.g., mRNA of the C3 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. Circulating C3 mRNA may be detected using methods described in PCT Publication No. WO 2012/177906, the entire contents of which are hereby incorporated herein by reference.

In one embodiment, the level of expression of C3 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 C3. 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 C3 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 C3 mRNA.

An alternative method for determining the level of expression of C3 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 C3 is determined by quantitative fluorogenic RT-PCR (i.e., the TaqMan™ System).

The expression levels of C3 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 C3 expression level may also comprise using nucleic acid probes in solution.

In preferred embodiments, the level of C3 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 C3 protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, Western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

In some embodiments, the efficacy of the methods of the invention can be monitored by detecting or monitoring a reduction in a symptom of a C3-associated disease. Symptoms may be assessed in vitro or in vivo using any method known in the art.

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), the retina or parts or parts of the retina (e.g., retinal pigment epithelium), the central nervous system or parts of the central nervous system (e.g., ventricles or choroid plexus), or the pancreas or certain cells or parts of the pancreas. In preferred embodiments, a “sample derived from a subject” refers to blood drawn from the subject or plasma derived therefrom. In further embodiments, a “sample derived from a subject” refers to liver tissue (or subcomponents thereof) or retinal tissue (or subcomponents thereof) 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 C3 may be assessed using measurements of the level or change in the level of C3 mRNA and/or C3 protein in a sample derived from fluid or tissue from the specific site within the subject. In preferred embodiments, the site is the 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 C3-Associated Disease or Disorder

The present invention provides therapeutic and prophylactic methods which include administering to a subject having a complement component C3-associated disease, as described herein, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosis, an iRNA agent, pharmaceutical composition comprising an iRNA agent, or vector comprising an iRNA of the invention.

In one 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 C3-associated disease,” e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), or C3 glomerulonephritis, or systemic lupus erythematosis. 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 one 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 C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosis. 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 C3 expression. For example, the invention provides methods for preventing hemolysis in a subject suffering from a disorder that would benefit from reduction in C3 expression, e.g., a complement component C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosis.

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 C3 expression.

In yet 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 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 C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosus.

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 C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosus.

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 complement component C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosus.

In one embodiment, an iRNA agent targeting C3 is administered to a subject having C3-associated disease such that the expression of a C3 gene, e.g., in a cell, tissue, blood 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, or to a level below the level of detection of the assay, when the dsRNA agent is administered to the subject.

The methods and uses of the invention include administering a composition described herein such that expression of the target C3 gene is decreased for an extended duration, e.g., at least one month, preferably at least three months.

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 C3-associated disease. By "reduction" in this context is meant a statistically or clinically 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 hemolysis, 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. Comparison of the later readings with the initial readings, or historically relevant population controls, 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 a C3 or pharmaceutical composition thereof, "effective against" a C3-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 C3-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.

Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg. Values and ranges intermediate to the recited values are also intended to be part of this invention.

The iRNA can be administered by intravenous infusion over a period of time, on a regular basis, e.g., once per month, once every other month, once per quarter.

In certain embodiments, one or more loading doses is administered.

Administration of the iRNA can reduce the presence of C3 protein, 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, or below the level of detection of the assay method used.

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 C3 gene 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, where the modified or unmodified iRNA agent is directly suspended in aqueous or suitable buffer

solvent, as a “free iRNA.” A free iRNA is administered in the absence of a pharmaceutical composition. The free 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 C3 gene expression are those having a C3-associated disease or disorder as described herein.

Treatment of a subject that would benefit from a reduction and/or inhibition of C3 gene expression includes therapeutic and prophylactic treatment.

Subjects that would benefit from a reduction and/or inhibition of C3 gene expression are those having a complement component C3-associated disease or disorder as described herein.

In one embodiment, a complement component C3-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 C3-associated disease is atypical hemolytic uremic syndrome (aHUS).

In another embodiment, a complement component C3-associated disease is neuromyelitis optica (NMO). NMO is the co-occurrence of optic neuritis with myelitis. In some embodiments, a subject having NMO may also have multiple sclerosis (MS); acute disseminated encephalomyelitis (ADEM); systemic lupus erythematosus (SLE); Sjögren syndrome; a viral infection; or a bacterial infection.

In yet another embodiment, a complement component C3-associated disease is multifocal motor neuropathy (MMN).

In another embodiment, a complement component C3-associated disease is myasthenia gravis (MG). The MG may be an ocular form of MG, a generalized form of MG, or a combination of ocular and generalized MG.

In another embodiment, a complement component C3-associated disease is C3 glomerulonephritis.

In one embodiment, a complement component C3-associated disease is systemic lupus erythematosus.

The invention further provides methods and uses of an iRNA agent or a pharmaceutical composition thereof for treating a subject that would benefit from reduction and/or inhibition of C3 gene expression, e.g., a subject having a C3-associated disease, in combination with other pharmaceuticals and/or other therapeutic methods, e.g., with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders.

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

The iRNA agent and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, e.g., parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

For example, additional therapeutics and therapeutic methods suitable for treating a subject that would benefit from reduction in C3 expression, e.g., a subject having a complement component C3-associated disease, include plasmapheresis, thrombolytic therapy (e.g., streptokinase), antiplatelet agents, folic acid, corticosteroids; immunosuppressive agents; estrogens, methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine, chloroquine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, cochlincine, 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, adenosine 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-1 β converting enzyme inhibitors, TNF α converting 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 p75TNFRlgG (Enbrel™ and p55TNFRlgG (Lenercept)), sIL-1RI, sIL-1RII, and sIL-6R), antiinflammatory cytokines (e.g., IL-4, IL-10, IL-11, IL-13 and TGF β), celecoxib, folic acid, hydroxychloroquine sulfate, rofecoxib, etanercept, infliximonoelonal antibody, naproxen, valdecoxib, sulfasalazine, methylprednisolone, meloxicam, methylprednisolone acetate, gold sodium thiomalate, aspirin, triamcinolone acetoneide, 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, rituximonoelonal 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-TNF α antibody; Celltech/Bayer); cA2/infliximonoelonal antibody (chimeric anti-TNF α 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-2R α ; 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)): 5284; (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): 5282) and thalidomide-related drugs (e.g., Celgen); leflunomide (anti-inflammatory and cytokine inhibitor; see e.g., (1996) *Arthr. Rheum.* 39(9 (supplement): 5131; (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-113 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 oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); prednisone; orgoten; glycosaminoglycan polysulfate; 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; infliximonal antibody; leflunomide; naproxen; valdecoxib; sulfasalazine; methylprednisolone; ibuprofen; meloxicam; methylprednisolone acetate; gold sodium thiomalate; aspirin; azathioprine; triamcinolone acetonide; propoxyphene 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; rituximonal antibody; IL-1 TRAP; MRA; CTLA4-IG; IL-18 BP; IL-12/23; anti-IL 18; anti-IL 15; BIRB-796; SC10-469; 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, cephalixin, 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.

In some aspects, the additional therapeutic agent is an iRNA agent targeting a C5 gene, such as described in U.S. Pat. No. 9,249,415, U.S. Provisional Patent Application Nos. 62/174,933, filed on Jun. 12, 2015, 62/263,066, filed on Dec. 4, 2015, 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 CSb, thereby inhibiting the generation of the terminal complement com-

plex C5b-9. Eculizumab is described in U.S. Pat. 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. Pat. 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 C3 expression, e.g., a complement component C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosis, 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 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 C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosis. 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 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 inhibitor (e.g., compstatin), for treating a subject, e.g., a subject that would benefit from a reduction and/or inhibition of C3 expression.

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 C5 antibody, or antigen-binding fragment thereof (e.g., eculizumab), an iRNA agent targeting complement compo-

nent 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 C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosis.

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 C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosis.

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 C3 expression, such as a complement component C3-associated disease, e.g., paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), atypical hemolytic uremic syndrome (aHUS), neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), myasthenia gravis (MG), C3 glomerulonephritis, or systemic lupus erythematosis.

In one embodiment, an iRNA agent targeting C3 is administered to a subject having a complement component C3-associated disease as described herein such that C3 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 C3 (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 C3 (and/or C5) or in a different formulation as the iRNA agent targeting C3 (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 C3-associated disease, e.g., PNH, aHUS, rheumatoid arthritis, or systemic lupus erythematosus, an iRNA agent targeting, e.g., C3, 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 administered 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 administered 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, or 15 mg/kg.

The methods and uses of the invention include administering a composition described herein such that expression of the target C3 (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.

The present invention also provides methods of using an iRNA agent of the invention and/or a composition containing an iRNA agent of the invention to reduce and/or inhibit C3 expression in a subject.

In other aspects, use of an iRNA of the invention and/or a composition comprising an iRNA of the invention for the manufacture of a medicament for reducing and/or inhibiting C3 gene expression in a subject are provided.

Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of C3 may be determined by determining the mRNA expression level of C3 using methods routine to one of ordinary skill in the art, e.g., Northern blotting, qRT-PCR, by determining the protein level of C3 using methods routine to one of ordinary skill in the art, such as Western blotting, immunological techniques, flow cytometry methods, ELISA, and/or by determining a biological activity of C3.

C3 gene expression may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or about 100%, or below the level of detection of the assay method used.

C3 protein production may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, 99%, or about 100%, or below the level of detection of the assay method used.

The in vivo methods and uses of the invention may include administering to a subject a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the C3 gene of the mammal to be treated. When the organism to be treated is a human, the composition can be administered by any means known in the art including, but not limited to subcutaneous, intravenous, oral, intraperitoneal, or parenteral routes, including intracranial (e.g., intraventricular, intraparenchymal and intrathecal), intramuscular, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by subcutaneous or intravenous infusion or injection. In one embodiment, the compositions are administered by subcutaneous injection. In some embodiments, the compositions are administered by intravenous infusion.

In some embodiments, the administration is via a depot injection. A depot injection may release the iRNA in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, e.g., a desired inhibition of C3, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

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

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

In one aspect, the present invention also provides methods for inhibiting the expression of a C3 gene in a mammal, e.g., a human. The present invention also provides a composition comprising an iRNA, e.g., a dsRNA, that targets a C3 gene in a cell of a mammal for use in inhibiting expression of the C3 gene in the mammal. In another aspect, the present invention provides use of an iRNA, e.g., a dsRNA, that targets a C3 gene in a cell of a mammal in the manufacture of a medicament for inhibiting expression of the C3 gene in the mammal.

The methods and uses include administering to the mammal, e.g., a human, a composition comprising an iRNA, e.g., a dsRNA, that targets a C3 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the C3 gene, thereby inhibiting expression of the C3 gene in the mammal.

In one embodiment, verification of RISC mediated cleavage of target in vivo following administration of iRNA agent is done by performing 5'-RACE or modifications of the protocol as known in the art (Lasham A et al., (2010) *Nucleic Acid Res.*, 38 (3) p-e19) (Zimmermann et al. (2006) *Nature* 441: 111-4).

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

A set of siRNAs targeting human C3 (human NCBI refseqID: NM_000064; NCBI GeneID: 718) were designed using custom R and Python scripts. The human C3 REFSEQ mRNA has a length of 5148 bases.

A detailed list of the unmodified C3 sense and antisense strand sequences is shown in Tables 3 and 6. A detailed list of the modified C3 sense and antisense strand sequences is shown in Tables 4 and 7.

Example 2. In Vitro Screening

Cell Culture and Transfections

Hep3b cells were transfected by adding 4.9 μ l of Opti-MEM plus 0.1 μ l of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad Calif. cat #13778-150) to 5 μ l of siRNA duplexes per well into a 384-well plate and incubated at room temperature for 15 minutes. Forty μ l of EMEM containing 5×10^3 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10 nM final duplex concentration.

Total RNA Isolation Using DYNABEADS mRNA Isolation Kit:

RNA was isolated using an automated protocol on a BioTek-EL406 platform using DYNABEADS (Invitrogen, cat #61012). Briefly, 50 μ l of Lysis/Binding Buffer and 25 μ l of lysis buffer containing 3 μ l of magnetic beads were added to the plate with cells. Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured and the supernatant was removed. Bead-bound RNA was then washed 2 times with 150 μ l Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150 μ l Elution Buffer, re-captured and supernatant removed.

cDNA Synthesis Using ABI High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, Calif., Cat #4368813):

Ten μ l of a master mix containing 1 μ l 10 \times Buffer, 0.4 μ l 25 \times dNTPs, 1 μ l 10 \times Random primers, 0.5 μ l Reverse Transcriptase, 0.5 μ l RNase inhibitor and 6.6 μ l of H₂O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 hours 37 $^{\circ}$ C.

Real Time PCR

Two µl of cDNA were added to a master mix containing 0.5 µl of GAPDH TaqMan Probe (Hs99999905), 0.5 µl C3 (Hs00163811_m1) and 5 µl Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well plates (Roche cat #04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche) using the ΔΔCt(RQ) assay. Each duplex was tested in four independent transfections.

To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 20 nM AD-1955, or mock transfected cells.

Tables 5 and 8 shows the results of a single dose screen in Hep3B cells transfected with the indicated C3 iRNAs. Data are expressed as percent of message remaining 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

TABLE 2-continued

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)
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
s	phosphorothioate linkage
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl]-4-hydroxyprolinol
	Hyp-(GalNAc-alkyl)3

TABLE 3

Complement Component C3 unmodified sequences

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000064.3 5' to 3'	Antisense Sequence	SEQ ID NO:
AD-76619	UCACUCCUCCCCAUCCUCU	9	31-49	AGAGGAUGGGGAGGAGUGA	277
AD-76864	UCUGUCCUCUGUCCUCUCU	10	53-71	AGAGGGACAGAGGGACAGA	278
AD-76867	CAGCACCAUGGGACCCACA	11	87-105	UGUGGGUCCCAUGGUGCUG	279
AD-76667	CCUCAGGUCCAGCCUGCU	12	104-122	AGCAGGCUGGGACCUGAGG	280
AD-76602	CCAGCCUGCUGUCCUGCU	13	113-131	AGCAGGAGCAGCAGGCUGG	281
AD-76892	UACUAAACCACCUCCCCU	14	131-149	AGGGGGAGGUGGGUAGUA	282
AD-76665	UACUCUAUCAUACCCCCA	15	169-187	UGGGGGUGAUGAUAGAGUA	283
AD-76900	CAACAUCUUGCGGCGGAA	16	186-204	UUCAGCCGCAAGAUGUUG	284
AD-76868	AGAGCGAGGAGACCAUGGU	17	203-221	ACCAUGGUCCUCGCUCU	285
AD-76671	UUCCAGUCACUGUUAUCUGU	18	251-269	ACAGUAACAGUGACUGGAA	286
AD-76564	UUCCCAGGCAAAAAACUAA	19	277-295	UUAGUUUUUGCCUGGGAA	287
AD-76600	AGUGCUGUCCAGUGAGAAA	20	294-312	UUUCUCACUGGACAGCACU	288
AD-76676	AGACUGUGCUGACCCUGA	21	311-329	UCAGGGGUCAGCACAGUCU	289
AD-76915	CAGGGAGUUCAGUCAGAA	22	372-390	UUCUGACUUGAACUCCUG	290
AD-76577	UUCAAGUCAGAAAAGGGGA	23	379-397	UCCCCUUUCUGACUUGAA	291
AD-76560	UUCGUGACCCUGCAGGCCA	24	406-424	UGGCCUGCACGGUCACGAA	292
AD-76664	CAGGCCACCUUCGGGACCA	25	418-436	UGGUCCGAAGUGGCCUG	293
AD-76889	CAAGUGGUGGAGAAGGUGA	26	436-454	UCACCUUCCACCACUUG	294

TABLE 3-continued

Complement Component C3 unmodified sequences					
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000064.3 5' to 3'	Antisense Sequence	SEQ ID NO:
AD-76718	GUGCUGGUCAGCCUGCAGA	27	454-472	UCUGCAGGCUGACCAGCAC	295
AD-76606	AGCGGGUACCUCUCAUCA	28	472-490	UGAUGAAGAGGUACCCGCU	296
AD-76685	CAGACAGACAAGACCAUCU	29	490-508	AGAUGGUCUUGUCUGUCUG	297
AD-76599	CUACACCCUGGCUCACACA	30	507-525	UGUGGAGCCAGGGGUGUAG	298
AD-76678	ACAGUUCUCUAUCGGAUCU	31	523-541	AGAUCCGAUAGAGAACUGU	299
AD-76670	UUCACCCGUAACCACAAGA	32	541-559	UCUUGUGGUUGACGGUGAA	300
AD-76609	GGACGGUCAUGGUCAACAU	33	575-593	AUGUUGACCAUGACCGUCC	301
AD-76616	UUGAGAACC CGAAGGCAU	34	593-611	AUGCCUUCGGGUUCUCAA	302
AD-76629	AUCCCGGUAAGCAGGACU	35	610-628	AGUCCUGCUUGACCGGGAU	303
AD-76850	CUCCUUGUCUUCUCAGAAA	36	627-645	UUUCUGAGAAGACAAGGAG	304
AD-76847	UUGUCUUCUCAGAACCAGA	37	631-649	UCUGGUUCUGAGAAGACAA	305
AD-76668	GCUUGGCUCUUGCCCUUA	38	648-666	UAAGGGCAAGACGCCAAGC	306
AD-76920	UGCCUUGUCUUGGGACAU	39	659-677	AUGUCCCAAGACAAGGGCA	307
AD-76637	AUGGGCCAGUGGAAGAUCA	40	694-712	UGAUCUCCACUGGCCCAU	308
AD-76627	GCCAGUGGAAGAUCCGAGA	41	698-716	UCUCGGAUUCUCCACUGGC	309
AD-76708	CCUACUAUGAAAACUCACA	42	716-734	UGUGAGUUUUCUAUGUAGG	310
AD-76594	CACUGAGUUUGAGGUGAAA	43	750-768	UUUCACCUCAAAUCAGUG	311
AD-76858	UUUGAGGUGAAGGAGUACA	44	757-775	UGUACUCCUCCACUCUAAA	312
AD-76731	CGUGCUGCCAGUUUCGAA	45	774-792	UUCGAAACUGGGCAGCACG	313
AD-76660	AGGUCAUAGUGGAGCCUAA	46	791-809	UUAGGCUCCACUAUGACCU	314
AD-76729	CAGAGAAAUUCUACUACAU	47	809-827	AUGUAGUAGAAUUUCUCUG	315
AD-76657	UCUAUAACGAGAAGGGCCU	48	827-845	AGGCCUUCUCGUUAUAGA	316
AD-76882	CUGGAGGUCACCAUCACCA	49	844-862	UGGUGAUGGUGACCUCAG	317
AD-76674	GCCAGGUUCCUCACGGGA	50	862-880	UCCCGUAGAGGAACCUGGC	318
AD-76581	AAGAAAGUGGAGGGAACUA	51	880-898	UAGUCCUCCACUUCUUCU	319
AD-76684	GGAAUCUGCCUUUGUCAUCU	52	892-910	AGAUGACAAAGGCAGUUC	320
AD-76633	UUCGGGAUCCAGGAUGGCA	53	910-928	UGCCAUCUGGAUCCCGAA	321
AD-76902	CGAACAGAGGAUUUCCCUA	54	927-945	UAGGGAAAUCCUCUGUUCG	322
AD-76921	UUUCCUGCCUGAAUCCCU	55	938-956	AGGGAUUCAGGCAGGGAAA	323
AD-76691	CUCAAGCGCAUUCCGAUUA	56	955-973	UAAUCGGAAUGCGCUUGAG	324
AD-76592	UGAGGAUGGCUCGGGGGAA	57	972-990	UUCCCCAGGCCAUCCUCA	325
AD-76642	AGGUUGUGCUGAGCCGGAA	58	989-1007	UUCGGCUCAGCACAAACCU	326
AD-76869	UGCUGAGCCGGAAGGUACU	59	995-1013	AGUACCUUCGGCUCAGCA	327
AD-76716	GGUACUGCUGGACGGGGUA	60	1008-1026	UACCCCGUCCAGCAGUACC	328
AD-76689	GCAGAACCCCGAGCAGAA	61	1026-1044	UUCUGCUCGGGGUUCUGC	329
AD-76679	UACGUGUCUGCACCCGCUA	62	1066-1084	UGACGGUGGCAGACACGUA	330
AD-76563	UUGCACUCAGGCAGUGACA	63	1087-1105	UGUCACUGCCUGAGUGCAA	331

TABLE 3-continued

Complement Component C3 unmodified sequences					
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000064.3	Antisense Sequence 5' to 3'	SEQ ID NO:
AD-76568	AGGCAGAGCGCAGCGGGAU	64	1112-1130	AUCCCGCUGCGCUCUGCCU	332
AD-76574	UUCACCAAGACACCCAAGU	65	1162-1180	ACUUGGGUGUCUUGGUGAA	333
AD-76875	ACCAAGACACCCAAGUACU	66	1165-1183	AGUACUUGGGUGUCUUGGU	334
AD-76677	UUCAAACAGGAAUGCCCU	67	1183-1201	AGGGCAUUCUGGUUUGAA	335
AD-76861	UCGUGACGAACCCUGAUGA	68	1217-1235	UCAUCAGGGUUCGUACGA	336
AD-76672	UCCAGGGCGAGGACACUGU	69	1265-1283	ACAGUGUCCUCGCCUGGA	337
AD-76908	GAGGACACUGUCAGUCUA	70	1273-1291	UAGACUGCACAGUGCCUC	338
AD-76625	CUAACCCAGGGAGAUGGCA	71	1291-1309	UGCCAUCUCCUGGGUUG	339
AD-76571	CGUGGCCAAACUCAGCAUA	72	1308-1326	UAUGCUGAGUUUGGCCACG	340
AD-76580	UCAACACACACCCAGCCA	73	1325-1343	UGGCUGGGGUGUGUUGA	341
AD-76673	CAGAAGCCCUUGAGCAUCA	74	1342-1360	UGAUGCUC AAGGGCUUCUG	342
AD-76641	UUGAGCAUCACGGUGCGCA	75	1351-1369	UGCACCCGUGAUGCUCAA	343
AD-76695	GAGCUCUCGGAGGCAGAGA	76	1381-1399	UCUCUGCCUCCGAGAGCUC	344
AD-76666	CAGGCUACCAGGACCAUGA	77	1399-1417	UCAUGGUCUGGUGAGCCUG	345
AD-76853	GCAGGCUCUGCCUACAGA	78	1416-1434	UCUGUAGGGCAGAGCCUGC	346
AD-76639	UACAGCACCGUGGGCAACU	79	1429-1447	AGUUGCCCACGGUGCUGUA	347
AD-76872	CUCCAACAAUACCUGCAU	80	1446-1464	AUGCAGGUAUUGUUGGAG	348
AD-76680	CAGUGCUACGUACAGAGCU	81	1469-1487	AGCUCUGUACGUAGCACUG	349
AD-76709	UCAGACCCGGGAGACCCU	82	1487-1505	AGGGUCUCCCCGGGUCUGA	350
AD-76860	GCGCCCACGAGGCCAAGAU	83	1535-1553	AUCUUGGCCUCUGGGGCGC	351
AD-76610	UCCGCUACUACACCUUACCU	84	1553-1571	AGGUAGGUGUAGUAGCGGA	352
AD-76608	CUGAUC AUGAAC AAGGGCA	85	1570-1588	UGCCCUUGUUC AUGAUCAG	353
AD-76662	CAGGCGUUGAAGGCGGGA	86	1587-1605	UCCCGCCUUCACAGCCUG	354
AD-76899	GACGCCAGGUGCGAGAGCA	87	1604-1622	UGCUCUCGCACCUGGCGUC	355
AD-76640	CCCGGCCAGGACCUUGGUGA	88	1621-1639	UCACCAGGUCUGGCGGG	356
AD-76705	UUCGCCUGGUGGCGUACU	89	1678-1696	AGUACGCCACCAGGCGGAA	357
AD-76866	CUACACGCUGAUCGGUGCA	90	1695-1713	UGCACCGAUCAGCGUGUAG	358
AD-76652	UGGGUGGACGUCAAGGACU	91	1747-1765	AGUCCUUGACGUCCACCCA	359
AD-76838	CCUGCGUGGGCUCGUGGU	92	1766-1784	ACCAGCGAGCCACGCAGG	360
AD-76636	UGGUAAAAGCGCCAGUA	93	1784-1802	UACUGGCCCGUUUUACCA	361
AD-76848	CAGAAGACCGGCAGCCUGU	94	1802-1820	ACAGGCUGCCGGUCUUCUG	362
AD-76655	CCUGGGCAGCAGAUGACCA	95	1822-1840	UGGUCAUCUGUCGCCAGG	363
AD-76700	CUGAAGAUAGAGGGUGACA	96	1840-1858	UGUCACCCUCUAUCUUCAG	364
AD-76583	CCACGGGGCCCGGGUGGUA	97	1857-1875	UACCACCCGGGGCCCGUGG	365
AD-76919	ACUGUGGCGGUGGACAAA	98	1875-1893	UUUGUCCACGGCCACCAGU	366
AD-76681	UAAGAAGAACAAACUGACA	99	1911-1929	UGUCAGUUUGUUCUUCUUA	367
AD-76724	CGCAGAGUAAGAUCUGGGA	100	1928-1946	UCCAGAUUCUACUCUGCG	368
AD-76696	GACGUGGUGGAGAAGGCAA	101	1945-1963	UUGCCUUCUCCACCACGUC	369

TABLE 3-continued

Complement Component C3 unmodified sequences					
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000064.3	Antisense Sequence 5' to 3'	SEQ ID NO:
AD-76890	ACCCCGGGCAGUGGGAAGA	102	1975-1993	UCUCCACUGCCGGGGU	370
AD-76638	GAUUACGCCGGUGUCUUCU	103	1993-2011	AGAAGACACCGCGUAAUC	371
AD-76883	UCCGACGCAGGGCUGACCU	104	2011-2029	AGGUCAGCCUGCGUCGGA	372
AD-76839	UUCACGAGCAGCAGUGGCA	105	2029-2047	UGCCACUGCUGCUGUGAA	373
AD-76590	CCAGCAGACGCCCAGAGA	106	2046-2064	UCUCUGGGCGGUCUGCUGG	374
AD-76721	CCGCCCAGAGGGCAGAACU	107	2054-2072	AGUUCUGCCUCUGGGCGG	375
AD-76646	UUCAGUGCCCGCAGCCAGA	108	2072-2090	UCUGGCUGCGGGCACUGAA	376
AD-76605	GCCGCCCGCCGACGCCGUU	109	2089-2107	AACGGCGUCGGCGGGCGGC	377
AD-76719	ACGGAGAAGCGAAUGGACA	110	2119-2137	UGUCCAUUCGCUUCUCCGU	378
AD-76624	AAAGUCGGAAGUACCCCA	111	2137-2155	UGGGGUACUUGCCGACUUU	379
AD-76626	CAAGGAGCUGCGCAAGUGA	112	2154-2172	UCACUUGCGCAGCUCCUUG	380
AD-76603	GCUCGAGGACGGCAUGCA	113	2171-2189	UGCAUGCCGUCCUCGCAGC	381
AD-76846	CCAUGAGGUUCUGUGCCA	114	2198-2216	UGGCACGAGAACCUCAUUG	382
AD-76635	UUCUCGUGCCAGCGCCGGA	115	2206-2224	UCCGGCGUGGCACGAGAA	383
AD-76893	CGUUUCAUCUCCUUGGGCA	116	2227-2245	UGCCCAGGAGAUGAAACG	384
AD-76923	GAGGCGUGCAAGAAGGUCU	117	2245-2263	AGACCUUCUUGCACGCCUC	385
AD-76736	UUCUGGACUGCUGCAACU	118	2263-2281	AGUUGCAGCAGUCCAGGAA	386
AD-76566	CUACAUCACAGAGCUGCGA	119	2280-2298	UCGAGCUCUGUGAUGUAG	387
AD-76656	ACGCGCGGCCAGCCACCU	120	2306-2324	AGGUGGCUGGCCCGCGCU	388
AD-76596	UGGGCCUGGCCAGGAGUAA	121	2324-2342	UUACUCCUGGCCAGGCCCA	389
AD-76562	ACCUGGAUGAGGACAUCAU	122	2342-2360	AUGAUGUCCUCAUCCAGGU	390
AD-76607	UUGCAGAAGAGAACAUCGU	123	2360-2378	ACGAUGUUCUCUUCUGCAA	391
AD-76702	GUUUCCGAAGUGAGUUCA	124	2377-2395	UGAACUCACUUCGGGAAAC	392
AD-76879	UUCCCAGAGAGCUGGCUGU	125	2392-2410	ACAGCCAGCUCUCUGGGAA	393
AD-76663	GUGGAACGUUGAGGACUUA	126	2409-2427	UAAGUCCUCAACGUUCCAC	394
AD-76913	UUGAGGACUUGAAAGAGCA	127	2417-2435	UGCUCUUUUAAGUCCUCAA	395
AD-76909	AGGACUUGAAAGAGCCACA	128	2420-2438	UGUGGCUCUUUAAGUCCU	396
AD-76843	CGAAAAAUGGAAUCUCUAA	129	2438-2456	UUAGAGAUUCCAUUUUUCG	397
AD-76597	CUCUACGAAGCUCAUCAAU	130	2451-2469	AUUCAUGAGCUUCGUAGAG	398
AD-76732	UAUAUUUUUGAAAGACUCA	131	2469-2487	UGAGUCUUUCAAUAUAUA	399
AD-76871	CCAUCACCACGUGGAGAU	132	2486-2504	AUCUCCACGUGGUGAUGG	400
AD-76643	AUUCUGGCUGAGCAUGU	133	2503-2521	ACAUGCUCACAGCCAGAAU	401
AD-76621	UCGGACAAGAAAGGGAUUCU	134	2521-2539	AGAUCUUUCUUGUCCGA	402
AD-76884	GCAGACCCUUCGAGGUCA	135	2545-2563	UGACCUUGAAGGGGUCUGC	403
AD-76570	UUCAUCGACCGCGCUAA	136	2581-2599	UUAGCCGAGGUCGAUGAA	404
AD-76612	ACCCUACUCUGUUGUUCGA	137	2598-2616	UCGAACAACAGAGUAGGGU	405
AD-76582	CUGUUGUUCGAAACGAGCA	138	2606-2624	UGCUCGUUCGAAACAACAG	406

TABLE 3-continued

Complement Component C3 unmodified sequences					
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000064.3	Antisense Sequence 5' to 3'	SEQ ID NO:
AD-76841	CAGGUGGAAAUCCGAGCCA	139	2623-2641	UGGCUCGGAUUCCACCUG	407
AD-76620	CGUUCUCUACAAUACC GA	140	2640-2658	UCGGUAAUUGUAGAGAACG	408
AD-76694	AAGAGCUC AAGGUGAGGGU	141	2666-2684	ACCCUCACC UUGAGCUCUU	409
AD-76632	GUGGAACUACUCCACAAUA	142	2683-2701	UAUUGUGGAGUAGUCCAC	410
AD-76591	UACUCCACA AUCCAGCCUU	143	2690-2708	AAGGCUGGAUUGUGGAGUA	411
AD-76593	UUCUGCAGCCUGGCCACCA	144	2707-2725	UGGUGGCCAGGCUGCAGAA	412
AD-76713	CACCAAGAGGCGUCACCAA	145	2724-2742	UUGGUGACGCCUCUUGGUG	413
AD-76726	AGCAGACCGUAACCAUCCA	146	2741-2759	UGGAUGGUUACGGUCUGCU	414
AD-76693	UCCUCGUUGUCGUUCCA U	147	2767-2785	AUGGAACGGACAACGAGGA	415
AD-76876	UAUGUCAUCGUGCCGCUAA	148	2785-2803	UUAGCGGCACGAUGACAU A	416
AD-76589	AGACCGGCCUGCAGGAAGU	149	2804-2822	ACUUC CUGCAGGCCGGUCU	417
AD-76891	GUGGAAGUCAAGGCUGCUA	150	2821-2839	UAGCAGCCUUGACUCCAC	418
AD-76907	UUCAUCAGUGACGGUGUCA	151	2851-2869	UGACACCGUCACUGAUGAA	419
AD-76613	CAGGAAGUCCUGAAGGUA	152	2868-2886	UACCUUCAGGGACUUC CUG	420
AD-76572	AAUCAGAAUGAACA AAAACU	153	2898-2916	AGUUUUGUUC AUUCUGAUU	421
AD-76873	CUGUGGCUGUUCGCACCCU	154	2915-2933	AGGGUGCGAACAGCCACAG	422
AD-76654	CUGGAUC CAGAACGCCUGA	155	2932-2950	UCAGGC GUUCUGGAUCCAG	423
AD-76578	AAGGAGUGCAGAAAGAGGA	156	2957-2975	UCCUCUUUCUGCACUCCUU	424
AD-76894	GUGACCAAGUCCCGACAA	157	2996-3014	UUGUCCGGGACUUGGUCAC	425
AD-76897	CCGAGUCUGAGACCAGAAU	158	3014-3032	AUUCUGGUCUCAGACUCGG	426
AD-76628	UUCUCCUGCAAGGGACCCA	159	3032-3050	UGGGUCCUUGCAGGAGAA	427
AD-76688	CCAGUGGCCAGAU GACAA	160	3049-3067	UUGUCAUCUGGGCCACUGG	428
AD-76651	CGUCGACCGGAACGGCUA	161	3075-3093	UAGCCGUUCCGCGUCGACG	429
AD-76692	CGGGAAACAGACAUGAUA	162	3123-3141	UAUCAUGUUCUGUUC CCG	430
AD-76588	UCGGCAUGACGCCACGGU	163	3140-3158	ACCGUGGGCGUCAUGCCGA	431
AD-76916	UACCUUGAUGAAACGGAGA	164	3172-3190	UCUCCGUUUC AUCCAGGUA	432
AD-76918	UGGAUGAAAACGGAGCAGUA	165	3176-3194	UACUGCUCGGUUC AUCCA	433
AD-76698	GGGAGAAGUUCGGCCUAGA	166	3194-3212	UCUAGGCCGAACUUCUCCC	434
AD-76723	AGAAGCGGCAGGGGCCUU	167	3212-3230	AAGGCCCCUGCCGCUUCU	435
AD-76735	UUGGAGCUC AUCAAGAAGA	168	3229-3247	UCUUCUUGAUGAGCUCCAA	436
AD-76710	GAGCUCAUCAAGAGGGGU	169	3232-3250	ACCCUUCUUGAUGAGCUC	437
AD-76852	UACACCCAGCAGCUGGCCU	170	3250-3268	AGGCCAGCUGCUGGGUGUA	438
AD-76840	CUUCAGACAACCCAGCUCU	171	3267-3285	AGAGCUGGGUUGUCUGAAG	439
AD-76727	AGCUCUGCCUUUGCGGCCU	172	3280-3298	AGGCCGCAAAGGCAGAGCU	440
AD-76573	UUCGUGAAACGGGCACCCA	173	3298-3316	UGGGUGCCCGUUUCACGAA	441
AD-76878	CAGCACCUGGCUGACCGCA	174	3315-3333	UGCGGUCAGCCAGGUGCUG	442
AD-76618	UACGUGGUC AAGGUCUUCU	175	3334-3352	AGAAGACCUUGACCACGUA	443
AD-76730	CUCUCUGGCUGUCAACCUA	176	3351-3369	UAGGUUGACAGCCAGAGAG	444

TABLE 3-continued

Complement Component C3 unmodified sequences					
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000064.3	Antisense Sequence 5' to 3'	SEQ ID NO:
AD-76715	UGGCUGAUCCUGGAGAAGA	177	3409-3427	UCUUCUCCAGGAUCAGCCA	445
AD-76733	CAGAAGCCCACGGGGUCU	178	3427-3445	AGACCCCGUCGGGUUCUG	446
AD-76706	GUCUCCAGGAGGAUGCGA	179	3442-3460	UCGCAUCCUCCUGGAAGAC	447
AD-76561	CCCUGUAUACACCAAGAAA	180	3460-3478	UUUCUUGGUGUAUCACGGG	448
AD-76669	AUGAUUGGUGAUUACGGA	181	3478-3496	UCCGUAUCCACCAAUCAU	449
AD-76845	AACAACAACGAGAAAGACA	182	3496-3514	UGUCUUUCUGUUGUUGUU	450
AD-76859	UUUGUUCUCAUCUCGCGA	183	3529-3547	UCAGCGAGAUGAGAACAAA	451
AD-76888	CAGGAGGCUAAAGAUUUUU	184	3547-3565	AAAUAUCUUUAGCCUCCUG	452
AD-76701	UUGCGAGGAGCAGGUCAAA	185	3564-3582	UUUGACCUGCUCUCGCAA	453
AD-76725	ACAGCCUGCCAGGCAGCAU	186	3581-3599	AUGCUGCCUGGCAGGCGUGU	454
AD-76728	GCCUGCCAGGCAGCAUCAA	187	3584-3602	UUGAUGCUGCCUGGCAGGC	455
AD-76653	CUAAAGCAGGAGACUUCU	188	3602-3620	AGGAAGUCUCCUGCUUUG	456
AD-76687	CUUGAAGCCAACUACAUGA	189	3619-3637	UCAUGUAGUUGGCUUCAAG	457
AD-76595	UACAUGAACCUACAGAGAU	190	3631-3649	AUCUCUGUAGGUUCAUGUA	458
AD-76617	AUCCUACACUGUGGCCAUU	191	3648-3666	AAUGGCCACAGUGUAGGAU	459
AD-76874	UUGCUGGCUAUGCUCUGGA	192	3665-3683	UCCAGAGCAUAGCCAGCAA	460
AD-76565	GAAGGGGCCUCUCUUAAA	193	3699-3717	UUUAAGAAGAGGCCCUUC	461
AD-76849	CAAAUUUCUGACCACAGCA	194	3717-3735	UGCUGUGGUCAGAAAUUG	462
AD-76587	CAAAGAUAAAGAACCGCGA	195	3735-3753	UCAGCGGUUCUUAUCUUUG	463
AD-76567	GGGAGGACCCUGGUAAGCA	196	3752-3770	UGC UUACCAGGGUCCUCCC	464
AD-76686	GACCUGGUAAGCAGCUCU	197	3757-3775	AGAGCUGCUUACCAGGGUC	465
AD-76911	UACAACGUGGAGGCCACAU	198	3775-3793	AUGUGGCCUCCACGUUGUA	466
AD-76895	CCUAUGCCUCUUGGCCCU	199	3794-3812	AGGGCCAAGAGGGCAUAGG	467
AD-76712	UACUGCAGCUAAAAGACUU	200	3812-3830	AAGUCUUUAGCUGCAGUA	468
AD-76634	CGUCUGCGUUGGCUCAAU	201	3846-3864	AUUGAGCCAACGCACGACG	469
AD-76558	CAAUGAACAGAGAUACUAA	202	3861-3879	UUAGUAUCUCUGUUAUUG	470
AD-76863	ACGGUGGUGGCUAUGGCUA	203	3878-3896	UAGCCAUAGCCACCACCGU	471
AD-76901	UCUACCCAGGCCACCUUCA	204	3895-3913	UGAAGGUGGCCUGGGUAGA	472
AD-76898	UUCAUGGUGUCCAAAGCCU	205	3910-3928	AGGCUUGGAACCAUGAA	473
AD-76647	CUUGGCUCAAUACCAAAAG	206	3927-3945	CUUUGGUAUUGAGCCAAG	474
AD-76722	AGGACGCCCCUGACCACCA	207	3944-3962	UGGUGGUCAGGGGCGUCCU	475
AD-76585	CAGGAACUGAACCUUGAUA	208	3961-3979	UAUCAAGGUUCAGUCCUG	476
AD-76586	UUGAUGUGUCCUCCAACU	209	3974-3992	AGUUGGAGGGACACAUCAA	477
AD-76857	CUGCCCAGCCGAGCUCCA	210	3991-4009	UGGAGCUGCGGUGGGCAG	478
AD-76862	CGCAGCUCCAAGAUACCA	211	4000-4018	UGGUGAUCUUGGAGCUGCG	479
AD-76614	CCACCGUAUCCACUGGGAA	212	4017-4035	UUCCAGUGGAUACGGUGG	480
AD-76851	CCGUAUCCACUGGGAAUCU	213	4020-4038	AGA UCCAGUGGAUACGG	481

TABLE 3-continued

Complement Component C3 unmodified sequences					
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000064.3	Antisense Sequence 5' to 3'	SEQ ID NO:
AD-76569	UGCCAGCCUCCUGCGAUCA	214	4038-4056	UGAUCGCAGGAGGCUGGCA	482
AD-76644	AGAAGAGACCAAGGAAAUAU	215	4056-4074	AUUUUCCUUGGUCUCUUCU	483
AD-76914	AUGAGGGUUUCACAGUCAAA	216	4073-4091	UUGACUGUGAAAACCCUCAU	484
AD-76877	ACAGCUGAAGGAAAAGGCA	217	4090-4108	UGCCUUUUCCUUCAGCUGU	485
AD-76881	CCAAGGCACCUUGUCGGUA	218	4107-4125	UACCGACAAGGUGCCUUGG	486
AD-76912	UUGUCGGUGGUGACAAGU	219	4117-4135	ACAUUGUCACCAACCGACAA	487
AD-76896	GUACCAUGCUAAGGCCAAA	220	4134-4152	UUUGGCCUUGAGCAUGGUAC	488
AD-76576	CUAAGGCCAAAGAUCAACU	221	4142-4160	AGUUGAUCUUUGGCCUUG	489
AD-76584	CAACUCACCCUGUAUAAAUAU	222	4156-4174	AUUUAUUACAGGUGAGUUG	490
AD-76714	UUCGACCUCAAGGUCACCA	223	4174-4192	UGGUGACCUCUGAGGUCGAA	491
AD-76904	UCACCAUAAAACAGCACACA	224	4187-4205	UGUGCUGUUUUUUGGUGA	492
AD-76648	CGGAAACAGAAAAGAGGCA	225	4205-4223	UGCCUCUUUUCUGUUUCCG	493
AD-76906	UCAGGAUGCCAAAGAACACU	226	4224-4242	AGUGUUCUUGGCAUCCUGA	494
AD-76623	UAUGAUCUUGAGAUUCUGU	227	4242-4260	ACAGAUCUCAAGGAUCAUA	495
AD-76720	CCAGGUACCCGGGAGACCA	228	4262-4280	UGGUCUCCCGGUACCCUGG	496
AD-76922	CAGGAUGCCACUAUGUCUA	229	4279-4297	UAGACAUAGUGGCAUCCUG	497
AD-76836	CUUUGCUCACAGACACAGAU	230	4323-4341	AUCUGUGUCUGGAGCAAAG	498
AD-76842	AUGACCUGAAGCAGCUGGA	231	4340-4358	UCCAGCUGCUUCAGGUCAU	499
AD-76699	UUGACAGAUACAUCUCCAA	232	4367-4385	UUGGAGAUGUAUCUGUCAA	500
AD-76855	AAGUAUGAGCUGGACAAAG	233	4384-4402	CUUUGUCCAGCUCUAUCUU	501
AD-76559	UAUGAGCUGGACAAAGCCU	234	4387-4405	AGGCCUUUGUCCAGCUCUAU	502
AD-76650	CUUCUCCGAUAGGAACACA	235	4404-4422	UGUGUUCUUAUCGGAGAAG	503
AD-76601	CCCUCAUCAUCUACCCUGGA	236	4421-4439	UCCAGGUAGAUGAUGAGGG	504
AD-76734	GACAAGGUCUCACACUCUA	237	4438-4456	UAGAGUGUGAGACCUCUGUC	505
AD-76844	UCACACUCUGAGGAUGACU	238	4447-4465	AGUCAUCCUCAGAGUGUGA	506
AD-76622	GAGGAUGACUGUCUAGCUU	239	4456-4474	AAGCUAGACAGUCAUCCUC	507
AD-76880	UUCAAAAGUUCACCAAUACU	240	4474-4492	AGUAUUGGUGAACUUUGAA	508
AD-76683	CUUUAAUGUAGAGCUUAUA	241	4491-4509	UAUAAGCUCUACAUAUAAAG	509
AD-76690	GUCAAGGUCUACGCCUAUU	242	4522-4540	AAUAGGCGUAGACCUUGAC	510
AD-76717	UACAACCUGGAGGAAAAGCU	243	4540-4558	AGCUUUCCUCCAGGUUGUA	511
AD-76661	CUGUACCCGGUUCUACCAU	244	4557-4575	AUGGUAGAACCAGGUACAG	512
AD-76885	AUCCGGAAAAGGAGGAUGA	245	4574-4592	UCAUCCUCCUUUCCGGAU	513
AD-76887	AAAAGGAGGAUGGAAAAGCU	246	4580-4598	AGCUUUCCAUCUCCUUUU	514
AD-76645	CUGAACAAAGCUCUGCCGUA	247	4597-4615	UACGGCAGAGCUUGUUCAG	515
AD-76615	UGAUGAACUCUGCCGCUGU	248	4614-4632	ACAGCGGCACAGUUCAUCA	516
AD-76579	GUGCUGAGGAGAAUUGCUU	249	4631-4649	AAGCAAUUCUCCUCAGCAC	517
AD-76604	AAUUGCUUCAUACAAAAGU	250	4642-4660	ACUUUUGUAUGAAGCAAUU	518
AD-76682	GUCGGAUGACAAGGUCACA	251	4659-4677	UGUGACCUUGUCAUCCGAC	519

TABLE 3-continued

Complement Component C3 unmodified sequences					
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000064.3	Antisense Sequence 5' to 3'	SEQ ID NO:
AD-76903	CCCUGGAAGAACGCGUGGA	252	4676-4694	UCCAGCCGUUCUCCAGGG	520
AD-76630	GACAAGGCCUGUGAGCCAA	253	4693-4711	UUGGCUCACAGGCCUUGUC	521
AD-76575	AGGCCUGUGAGCCAGGAGU	254	4697-4715	ACUCCUGGCUCACAGGCCU	522
AD-76917	CCAGGAGUGGACUAUGUGU	255	4708-4726	ACACAUAGUCCACUCCUGG	523
AD-76886	UACAAGACCCGACUGGUGA	256	4726-4744	UGACCAGUCGGGUCUUGUA	524
AD-76905	UUCAGCUGUCCAUAUGACUU	257	4748-4766	AAGUCAUUGGACAGCUGAA	525
AD-76856	UUUGACGAGUAUCAUGA	258	4765-4783	UCAUGAUGUACUCGUCAAA	526
AD-76737	UACAUCAUGGCCAUUGAGA	259	4774-4792	UCUCAUUGGCCAUGAUGUA	527
AD-76659	CAGACCAUCAAGUCAGGCU	260	4792-4810	AGCCUGACUUGAUGGUCUG	528
AD-76854	CUCGGAUGAGGUGCAGGUU	261	4809-4827	AACCUGCACCUCUCCGAG	529
AD-76711	UUCAUCAGCCCCAUCAAGU	262	4843-4861	ACUUGAUGGGGUGAUGAA	530
AD-76675	UCAAGUCGAGAGAAGCCCU	263	4856-4874	AGGGCUUCUCUGCACUUGA	531
AD-76703	CUGAAGCUGGAGGAGAAGA	264	4873-4891	UCUUCUCCUCCAGCUUCAG	532
AD-76707	AAGCUGGAGGAGAAGAAAC	265	4876-4894	GUUUCUUCUCCUCCAGCUU	533
AD-76649	ACACUACCUCAUGUGGGGU	266	4893-4911	ACCCCAUCAGAGGUAGUGU	534
AD-76631	UUCUGGGGAGAGAAGCCCA	267	4924-4942	UGGGCUUCUCUCCAGAA	535
AD-76598	UACAUAUCGGGAAGGACA	268	4951-4969	UGUCCUUCUCCGAUGAUGUA	536
AD-76658	CACUUGGGUGGAGCACUGA	269	4968-4986	UCAGUGCUCACCCCAAGUG	537
AD-76865	AGGACGAAUGCCAAGACGA	270	4994-5012	UCGUCUUGGCAUUCGUCCU	538
AD-76697	AAGAGAACCAGAAACAUA	271	5012-5030	UAUUGUUUCUGGUUCUUU	539
AD-76611	UGCCAGGACCUCGGCGCCU	272	5029-5047	AGGGCCGAGGUCCUGGCA	540
AD-76837	CUUCACCGAGAGCAUGGUU	273	5046-5064	AACCAUGCUCUCGGUGAAG	541
AD-76704	CACACCCCAUUCUCCCAA	274	5087-5105	UUGGGGAAUGGGGUGUG	542
AD-76910	CUCCAGAUAAAGCUUCAGU	275	5105-5123	ACUGAAGCUUUAUCUGGAG	543
AD-76870	UUUAUUCUCAAAAAAAAAA	276	5123-5141	UUUUUUUUUGAGAUUAAA	544

TABLE 4

Complement Component C3 modified sequences						
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO
AD-76619	UCACUCCUCCCAUCCUCUdTdT	545	AGAGGAUGGGGAGGAGUGAdTdT	813	UCACUCCUCCCAUCCUCU	1081
AD-76864	UCUGUCCUCUGUCCUCUdTdT	546	AGAGGGACAGAGGGACAGAdTdT	814	UCUGUCCUCUGUCCUCU	1082
AD-76867	CAGCACCAUGGGACCCACAdTdT	547	UGUGGGUCCCAUGGUCUGdTdT	815	CAGCACCAUGGGACCCACC	1083
AD-76667	CCUCAGGUCCAGCCUGCUdTdT	548	AGCAGGCUGGGACCUAGGdTdT	816	CCUCAGGUCCAGCCUGCU	1084
AD-76602	CCAGCCUGCUGUCCUGCUdTdT	549	AGCAGGAGCAGCAGGUCGdTdT	817	CCAGCCUGCUGUCCUGCU	1085
AD-76892	UACUAACCCACCUCCUdTdT	550	AGGGGGAGGUGGUUAGUAdTdT	818	UACUAACCCACCUCCU	1086
AD-76665	UACUCUAUCAUACCCCCAdTdT	551	UGGGGGUGAUGAUAGAGUAdTdT	819	UACUCUAUCAUACCCCCA	1087
AD-76900	CAACAUCUUGCGGCGUGGAAdTdT	552	UUCAGCCGCAAGAUGUUGdTdT	820	CAACAUCUUGCGGCGUGGAG	1088
AD-76868	AGAGCGAGGAGACCAUGGUdTdT	553	ACCAUGGUUCCUCCGUCUdTdT	821	AGAGCGAGGAGACCAUGGU	1089
AD-76671	UUCAGUCACUGUUACUGUdTdT	554	ACAGUAACAGUGACUGGAAdTdT	822	UUCAGUCACUGUUACUGU	1090
AD-76564	UUCCCAGGCAAAAAACUAdTdT	555	UUAGUUUUUUGCCUGGGAAdTdT	823	UUCCCAGGCAAAAAACUAG	1091
AD-76600	AGUGCUGUCCAGUGAGAAAdTdT	556	UUUCUCACUGGACAGCACUdTdT	824	AGUGCUGUCCAGUGAGAAAG	1092
AD-76676	AGACUGUGCUGACCCUGAdTdT	557	UCAGGGGUCAGCACAGUCUdTdT	825	AGACUGUGCUGACCCUUGC	1093

TABLE 4-continued

Complement Component C3 modified sequences						
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO
AD-76915	CAGGGAGUUAAGUCAGAAAdTdT	558	UUCUGACUUGAACUCCUGdTdT	826	CAGGGAGUUAAGUCAGAA	1094
AD-76577	UUCAAGUCAGAAAAGGGGAdTdT	559	UCCCUUUUCUGACUUGAAdTdT	827	UUCAAGUCAGAAAAGGGGC	1095
AD-76560	UUCUGACCCGUGCAGGCCAdTdT	560	UGCCUGCAGCCGUGCACGAAdTdT	828	UUCUGACCCGUGCAGGCCA	1096
AD-76664	CAGGCCACCUCCGGGACAdTdT	561	UGGUCCGAAAGGUGGCCUGdTdT	829	CAGGCCACCUCCGGGACCC	1097
AD-76889	CAAGUGGUGGAGAAGGUGAdTdT	562	UCACCUUCCACCACUUGdTdT	830	CAAGUGGUGGAGAAGGUGG	1098
AD-76718	GUGCUGGUCAGCCUGCAGAdTdT	563	UCUGCAGGUCAGCCAGCAdTdT	831	GUGCUGGUCAGCCUGCAGA	1099
AD-76606	AGCGGUUACCUAGAUCAAdTdT	564	UGAUGAAGAGGUAACCGCUdTdT	832	AGCGGUUACCUUUAACUCC	1100
AD-76685	CAGACAGACAAGACCAUCdTdT	565	AGAUGGUCUUGUCUGUCdTdT	833	CAGACAGACAAGACCAUCU	1101
AD-76599	CUACACCCUGGCUCCAdTdT	566	UGUGGAGCCAGGGUGUAGdTdT	834	CUACACCCUGGCUCCACA	1102
AD-76678	ACAGUUCUCUAUCGGAUCdTdT	567	AGAUCGGAUAGAGAACUGdTdT	835	ACAGUUCUCUAUCGGAUCU	1103
AD-76670	UUCACCGUCAACCACAAGAdTdT	568	UCUUGGUGUUGACGGUAAAdTdT	836	UUCACCGUCAACCACAAGC	1104
AD-76609	GGACGGUCAUGGUACAACAAdTdT	569	AUGUUGACCAUGACCGUCCdTdT	837	GGACGGUCAUGGUACAACA	1105
AD-76616	UUGAGAACCUCGGAAGGCAAdTdT	570	AUUCUUUCCGGGUUCUAdTdT	838	UUGAGAACCUCGGAAGGCAU	1106
AD-76629	AUCCCGGUCAGCAGGACUdTdT	571	AGUCCUGCUUGACCGGUAAdTdT	839	AUCCCGGUCAGCAGGACU	1107
AD-76850	CUCCUUGUCUUCUCAGAAAdTdT	572	UUUCUGAGAAGACAAGGAdTdT	840	CUCCUUGUCUUCUCAGAAC	1108
AD-76847	UUGUCUUCUAGCAACCAAdTdT	573	UUGGUUCUGAGAAGCAAdTdT	841	UUGUCUUCUAGCAACCAU	1109
AD-76668	GCUUGGCGUCUUGCCUAdTdT	574	UAAGGGCAAGACGCCAAGCdTdT	842	GCUUGGCGUCUUGCCUUG	1110
AD-76920	UGCCUUGUCUUGGGACAAdTdT	575	AUGUCCCAAGACAAGGGCAdTdT	843	UGCCUUGUCUUGGGACA	1111
AD-76637	AUGGGCCAGUGGAAGAUAdTdT	576	UGAUCUCCACUGGCCAUdTdT	844	AUGGGCCAGUGGAAGAUCC	1112
AD-76627	GCCAGUGGAAGAUCCGAGAdTdT	577	UCUCGGAUCUUCACUGGCdTdT	845	GCCAGUGGAAGAUCCGAGC	1113
AD-76708	CCUACUAUGAAAACUCAdTdT	578	UGUGAGUUUCAUAGUAGGdTdT	846	CCUACUAUGAAAACUCACC	1114
AD-76594	CACUGAGUUUGAGGUGAAAdTdT	579	UUUCACUCCAAACUCAGUdTdT	847	CACUGAGUUUGAGGUGAAG	1115
AD-76858	UUUGAGGUGAAGGAGUACAdTdT	580	UGUACUCCUUCACCUCAAAdTdT	848	UUUGAGGUGAAGGAGUACG	1116
AD-76731	CGUGCUGCCAGUUUCGAAdTdT	581	UUCGAAACUGGGCAGCAGdTdT	849	CGUGCUGCCAGUUUCGAG	1117
AD-76660	AGGUCAUAGUGGAGCCUAdTdT	582	UUAGGCUCCACUAUGACCUdTdT	850	AGGUCAUAGUGGAGCCUAC	1118
AD-76729	CAGAGAAAUUCUACUAdTdT	583	AUGUAGUAGAAUUCUCUdTdT	851	CAGAGAAAUUCUACUACU	1119
AD-76657	UCUAUAACGAGAAGGGCUdTdT	584	AGGCCUUCUCGUUAUAGAdTdT	852	UCUAUAACGAGAAGGGCCU	1120
AD-76882	CUGGAGGUCACCAUCACAdTdT	585	UGUGAUGGUGACUCCAGdTdT	853	CUGGAGGUCACCAUCACCC	1121
AD-76674	GCCAGGUUCCUACGGGAdTdT	586	UCCCGUAGAGGAACCGGAdTdT	854	GCCAGGUUCCUACGGGA	1122
AD-76581	AAGAAAGUGGAGGGAACUAdTdT	587	UAGUUCUCCACUUCUAdTdT	855	AAGAAAGUGGAGGGAACUC	1123
AD-76684	GGAAACUGCCUUGUACUAdTdT	588	AGAUAUCGAGGAGUUCAdTdT	856	GGAAACUGCCUUGUACUUC	1124
AD-76633	UUCGGGAUCCAGGAUGGCAdTdT	589	UGCAACUCCGUAUCCGAAAdTdT	857	UUCGGGAUCCAGGAUGGCG	1125
AD-76902	CGAACAGAGGAUUUCCUAdTdT	590	UAGGGAAUCCUCUUGUAdTdT	858	CGAACAGAGGAUUUCCUC	1126
AD-76921	UUUCCUUGGCUAGAUCCUAdTdT	591	AGGUAUUCAGCGAGGAAAdTdT	859	UUUCCUUGGCUAGAUCCU	1127
AD-76691	CUCAAGCGCAUUCGGAUAdTdT	592	UAAUCGGAUUGCGUUGAdTdT	860	CUCAAGCGCAUUCGGAUG	1128
AD-76592	UGAGGAUGGUCUGGGGAAdTdT	593	UUCCCGGAGCCAUCUAdTdT	861	UGAGGAUGGUCUGGGGAG	1129
AD-76642	AGGUUGUGGUCAGCCGAAdTdT	594	UUGGUAUGGUGACCAACUdTdT	862	AGGUUGUGGUCAGCCGAA	1130
AD-76869	UGCUGAGCCGGAAGGUAdTdT	595	AGUACCUUCCGGUCAGCAdTdT	863	UGCUGAGCCGGAAGGUACU	1131
AD-76716	GGUACUGCUGGACGGGUAAdTdT	596	UACCCGUCGCGAGUACAdTdT	864	GGUACUGCUGGACGGGUG	1132
AD-76689	GCAGAACCCCGAGCAGAdTdT	597	UUUCUGCUGGGGUUCUGCdTdT	865	GCAGAACCCCGAGCAGAA	1133
AD-76679	UACGUGUCUGCCACCGUCAdTdT	598	UGACGGUGGACAGACGUAdTdT	866	UACGUGUCUGCCACCGUCA	1134
AD-76563	UUGCACUCAGGCAGUGAdTdT	599	UGUCACUGCCAGUGCAAdTdT	867	UUGCACUCAGGCAGUGACA	1135
AD-76568	AGGCAGAGCGCAGCGGUAAdTdT	600	AUCCCGUCGCGUCUGCAAdTdT	868	AGGCAGAGCGCAGCGGUA	1136
AD-76574	UUCACCAAGACACCCAAGUdTdT	601	ACUUGGGUUCUUGGUGAAAdTdT	869	UUCACCAAGACACCCAAGU	1137
AD-76875	ACCAAGACACCCAAGUAdTdT	602	AGUACUUGGUGUCUUGGUdTdT	870	ACCAAGACACCCAAGUACU	1138
AD-76677	UUCAAACAGGAUUUCCUAdTdT	603	AGGGCAUUCUGGUUUAGAdTdT	871	UUCAAACAGGAUUUCCUUC	1139
AD-76861	UCGUGACGAACCCUGAUGAdTdT	604	UCAUCAGGGUUCGUCAGAdTdT	872	UCGUGACGAACCCUGAUGG	1140
AD-76672	UCCAGGGCGAGGACACUGdTdT	605	ACAGUGUCCUGCCUGGAdTdT	873	UCCAGGGCGAGGACACUGU	1141
AD-76908	GAGGACACUGGACAGUCUAdTdT	606	UAGACUGCAGUCUUCUAdTdT	874	GAGGACACUGGACAGUCUCU	1142
AD-76625	CUAACCAGGGAGAUGGCAdTdT	607	UGCAUCUCCUGGUAUGdTdT	875	CUAACCAGGGAGAUGGCG	1143
AD-76571	CGUGGCCAAACUCAGCAUAdTdT	608	UAUGCUGAUUUGGCCACGdTdT	876	CGUGGCCAAACUCAGCAUC	1144
AD-76580	UCAACACACACCCAGCCAdTdT	609	UGGCUUGGUGUUGUUGAdTdT	877	UCAACACACACCCAGCCA	1145
AD-76673	CAGAAGCCUUGAGCAUCAdTdT	610	UGAUGCUCAAAGGGUUCUGdTdT	878	CAGAAGCCUUGAGCAUCA	1146
AD-76641	UUGAGCAUCACGGUGGCAAdTdT	611	UCCGCACCCUGAUGCUCAAdTdT	879	UUGAGCAUCACGGUGGCA	1147
AD-76695	GAGCUUCGGAAGGACAGAdTdT	612	UUCUGUCCUGGAGAGUCdTdT	880	GAGCUUCGGAAGGACAGCA	1148
AD-76666	CAGGCUACAGGACCAUGAdTdT	613	UCAUGGUUCUGGAGCCUAdTdT	881	CAGGCUACAGGACCAAUGC	1149
AD-76853	GCAGGCUUCGCCUACAGAdTdT	614	UCUGUAGGCGAGCCUGCdTdT	882	GCAGGCUUCGCCUACAGC	1150
AD-76639	UACAGCACCGUGGCAACUdTdT	615	AGUUGGCCACGGUCUGUAdTdT	883	UACAGCACCGUGGCAACU	1151
AD-76872	CUCCAACAUAUACCGUAdTdT	616	AUGCAGGUAUUGUUGGAdTdT	884	CUCCAACAUAUACCGUAU	1152
AD-76680	CAGUCUACGUACAGAGCUdTdT	617	AGCUCUAGCUAGCAGUAdTdT	885	CAGUCUACGUACAGAGCU	1153
AD-76709	UCAGACCCGGGAGACCUdTdT	618	AGGGUCUCCCGGUCUGAdTdT	886	UCAGACCCGGGAGACCCU	1154
AD-76860	GCGCCACGAGGCAAGAUdTdT	619	AUCUUGGCUUGGUGGCGdTdT	887	GCGCCACGAGGCAAGAU	1155
AD-76610	UCCGUACUACACCUACCUdTdT	620	AGGUAGGUGUAGUAGCGAdTdT	888	UCCGUACUACACCUACCU	1156
AD-76608	CUGAUCUAGAACAAGGCAAdTdT	621	UGCCUUGUUAUGAUCAGdTdT	889	CUGAUCUAGAACAAGGCA	1157
AD-76662	CAGGCUUGUUAAGGCGGAdTdT	622	UCCCGCCUUAACAGCCUAdTdT	890	CAGGCUUGUUAAGGCGGA	1158
AD-76899	GACGCCAGGUCGAGAGCAdTdT	623	UGCUCUGCACCUGGCGUdTdT	891	GACGCCAGGUCGAGAGCC	1159
AD-76640	CCCGGCCAGGACCUUGGAdTdT	624	UACACAGCCUCCUGGCGGdTdT	892	CCCGGCCAGGACCUUGGUG	1160
AD-76705	UUCCGCCUGGUGGCUAdTdT	625	AGUACGCCACCAGCGGAAdTdT	893	UUCCGCCUGGUGGCUACU	1161
AD-76866	CUACACGCUAUCGGUGCAdTdT	626	UGCACCGAUCAGCGUGUAdTdT	894	CUACACGCUAUCGGUGCC	1162
AD-76652	UGGGUGGACUACAAGGACUdTdT	627	AGUCCUUGACUCCACCAAdTdT	895	UGGGUGGACUACAAGGACU	1163
AD-76838	CCUGCGUGGGUCGUGUAdTdT	628	ACCAAGCAGCCACGCAGdTdT	896	CCUGCGUGGGUCGUGGU	1164
AD-76636	UGGUAAAAAGCCGCCAGUAdTdT	629	UACUGGCGCCUUUUACAdTdT	897	UGGUAAAAAGCCGCCAGU	1165
AD-76848	CAGAAGACCCGCAAGCUAdTdT	630	ACAGGCUCCGGUUCUUCdTdT	898	CAGAAGACCCGCAAGCCU	1166
AD-76655	CCUGGGCAGCAGAUACAdTdT	631	UGGUCAUCUGCUGCCAGdTdT	899	CCUGGGCAGCAGAUACCC	1167

TABLE 4-continued

Complement Component C3 modified sequences						
Duplex Name	Sense Sequence 5' to 3'	SEQ ID	Antisense Sequence 5' to 3'	SEQ ID	SEQ NO: mRNA target sequence	
		NO:		NO:		
AD-76700	CUGAAGAUAGAGGGUGACAdTdT	632	UGUCACCCUCUAUCUUCAGdTdT	900	CUGAAGAUAGAGGGUGACC	1168
AD-76583	CCACGGGGCCCGGGUGGUAdTdT	633	UACCACCCGGGCCCGUGGdTdT	901	CCACGGGGCCCGGGUGGUA	1169
AD-76919	ACUGGUGGCCGUGGACAAAdTdT	634	UUUGUCCACGGCCACCAGUdTdT	902	ACUGGUGGCCGUGGACAAG	1170
AD-76681	UAAGAAGAACAACACUGACAdTdT	635	UGUCAGUUUGUUCUUCUAdTdT	903	UAAGAAGAACAACUGAGC	1171
AD-76724	CGCAGAGUAAGAUUCUGGGAdTdT	636	UCCAGAUUUACUCUGCGdTdT	904	CGCAGAGUAAGAUUCUGGGA	1172
AD-76696	GACGUGGUGGAGAAGGCAAdTdT	637	UUGCCUUCUCCACCACGUCdTdT	905	GACGUGGUGGAGAAGGCAG	1173
AD-76890	ACCCCGGGCAGUGGGAAGAdTdT	638	UGUUGUACUUGCCCGGGUdTdT	906	ACCCCGGGCAGUGGGAAGG	1174
AD-76638	GAUUACGCCGUGUCUUCUdTdT	639	AGAAGACACCGGCGUAAUCdTdT	907	GAUUACGCCGUGUCUUCU	1175
AD-76883	UCCGACGCAGGGCUGACCUdTdT	640	AGGUCAGCCUCGUCGCGAdTdT	908	UCCGACGCAGGGCUGACCU	1176
AD-76839	UUCACGAGCAGUCAGUGGCAdTdT	641	UGCCACUGCUCGUGAAdTdT	909	UUCACGAGCAGCAGUGGCC	1177
AD-76590	CCAGCAGACCCGCCAGAGAdTdT	642	UCUCUGGGCGGUCUGCUGdTdT	910	CCAGCAGACCCGCCAGAGG	1178
AD-76721	CCGCCAGAGGGCAGAUCdTdT	643	AGUUCUGCCUCUGGGCGdTdT	911	CCGCCAGAGGGCAGAUCU	1179
AD-76646	UUCAGUCGCCGAGCCAGCAdTdT	644	AGUUCUGCCUCUGGGCGdTdT	912	UUCAGUCGCCGAGCCAGC	1180
AD-76605	GCCGCCCGCCAGCCGUUdTdT	645	AACGGCGUCGGCGGGCGdTdT	913	GCCGCCCGCCAGCCGUU	1181
AD-76719	ACGGAGAAGCGAAUGGACAdTdT	646	UGUCCAUUCGCUUCUCCGUdTdT	914	ACGGAGAAGCGAAUGGACA	1182
AD-76624	AAAGUCGGCAAGUACCCAdTdT	647	UUGGGUACUUGCCCGGUdTdT	915	AAAGUCGGCAAGUACCCCA	1183
AD-76626	CAAGGAGCUGCGCAAGUGAdTdT	648	UCACUUGCGCAGCUUCUdTdT	916	CAAGGAGCUGCGCAAGUGC	1184
AD-76603	GCUGCGAGGACGGCAUGCAdTdT	649	UGCAUGCCGUCCGCGCAdTdT	917	GCUGCGAGGACGGCAUGC	1185
AD-76846	CCAUGAGGUUCUGGCGAdTdT	650	UUGCAUGCAACCUAUGGdTdT	918	CCAUGAGGUUCUGGCGC	1186
AD-76635	UUCUCUGGCCAGCGCCGAdTdT	651	UCCGGCGUGGCACGAGAAdTdT	919	UUCUCUGGCCAGCGCCGGA	1187
AD-76893	CGUUUCAUCUCCUGGGCAdTdT	652	UGCCAGGGAGAUGAAACGdTdT	920	CGUUUCAUCUCCUGGGCG	1188
AD-76923	ACGCGCGGGCCAGCCACCUdTdT	653	AGACUUCUUGACGCCUCdTdT	921	ACGCGCGGGCCAGCCACCU	1189
AD-76736	UUCUGGACUGCUGCAACUdTdT	654	AGUUGCAGCAGUCCAGGAAdTdT	922	UUCUGGACUGCUGCAACU	1190
AD-76566	CUACAUCACAGAGCUGCGAdTdT	655	UCCGAGCUCUGUAGUAGdTdT	923	CUACAUCACAGAGCUGCG	1191
AD-76656	ACGCGCGGGCCAGCCACCUdTdT	656	AGGUGGCGGGCCGCGUdTdT	924	ACGCGCGGGCCAGCCACCU	1192
AD-76596	UGGGCCUGGCCAGGAGUAdTdT	657	UUACUCCUGGCCAGGCCAdTdT	925	UGGGCCUGGCCAGGAGUAA	1193
AD-76562	ACCUGGAUGAGGACAUCAdTdT	658	AUGAUGUCCUACUCCAGGUdTdT	926	ACCUGGAUGAGGACAUCAU	1194
AD-76607	UUGCAGAAAGAGCAUCGUdTdT	659	ACGAUUCUUCUUCUGCAAdTdT	927	UUGCAGAAAGAGCAUCGU	1195
AD-76702	GUUUCGCCAAGUGAGUUCAdTdT	660	UGAACUCACUUCGGGAACdTdT	928	GUUUCGCCAAGUGAGUUC	1196
AD-76879	UUCCCAGAGGUCUGGCUUdTdT	661	ACAGCCAGCUCUCUGGGAAdTdT	929	UUCCCAGAGGUCUGGCUU	1197
AD-76663	GUGGAACGUUAGGACUAdTdT	662	UAGGUCUUCAGGCUUCCAdTdT	930	GUGGAACGUUAGGACUAG	1198
AD-76913	UUGAGGACUUGAAAGAGCAdTdT	663	UGCUCUUCAAGUCUCAAdTdT	931	UUGAGGACUUGAAAGAGCC	1199
AD-76909	AGGACUUGAAAGGCCAdTdT	664	UGUGGCUUUCAAGUCCUdTdT	932	AGGACUUGAAAGAGGCC	1200
AD-76843	CGAAAAUUGGAUUCUAAAdTdT	665	UUAGAGAUUCCAUUUUCGdTdT	933	CGAAAAUUGGAUUCUAA	1201
AD-76597	CUCUACGAAGCUCUAGAUdTdT	666	AUUCAGAGCUCUAGAGdTdT	934	CUCUACGAAGCUCUAGAAU	1202
AD-76732	UAUAUUUUUGAAAGACUAdTdT	667	UGAGUCUUUCAAUAUAdTdT	935	UAUAUUUUUGAAAGACUCC	1203
AD-76871	UAUCAGAAAGGAGAGUdTdT	668	AUUCCCAAGGUCUAGGdTdT	936	UAUCAGAAAGGAGAGUCC	1204
AD-76643	AUUCUGGUGAGGAGUAdTdT	669	ACAUGCUCACAGCCAGAAUdTdT	937	AUUCUGGUGAGGAGAU	1205
AD-76621	UCGGACAAGAAAGGGAUCUdTdT	670	AGAUCUUUCUUGUCCGAdTdT	938	UCGGACAAGAAAGGGAUCU	1206
AD-76884	GCAGACCCUUCGAGGUCAdTdT	671	UAGCCUUCUUCGUCUCdTdT	939	GCAGACCCUUCGAGGUCU	1207
AD-76570	UUCAUCGACCCUGCGCUAdTdT	672	UUAGCCGCGAGGUCGAUAdTdT	940	UUCAUCGACCCUGCGCUAC	1208
AD-76612	ACCCUACUCUGUUGUUCGAdTdT	673	UCGAACAACAGAUAGGGUdTdT	941	ACCCUACUCUGUUGUUCGA	1209
AD-76582	CUGUUGUUCGAAACGAGCAdTdT	674	UGUCUGUUUCGAACAACAGdTdT	942	CUGUUGUUCGAAACGAGCA	1210
AD-76841	CAGGUGGAAAUCCGAGCCAdTdT	675	UGGUCGGAUUUCCACCUdTdT	943	CAGGUGGAAAUCCGAGCCG	1211
AD-76620	CGUUCUCUCAAUUACCGAdTdT	676	UCGGUAAUUUGAAGAAACGdTdT	944	CGUUCUCUCAAUUACCGG	1212
AD-76694	AAGAGUCUAAAGGAGGUGUdTdT	677	ACCCUACCUUGAGCUCUdTdT	945	AAGAGUCUAAAGGAGGUGU	1213
AD-76632	GUGGAACUACUCCACAAdTdT	678	UAUUGUGGAGUAGUCCAdTdT	946	GUGGAACUACUCCACAUC	1214
AD-76591	UACUCCACAUCAGCCUAdTdT	679	AAGCGUGAUGUGGAGUAdTdT	947	UACUCCACAUCAGCCUUC	1215
AD-76593	UUCUGCAACUUGGCCACAdTdT	680	UGGUGGCCAGGUCAGAAAdTdT	948	UUCUGCAACUUGGCCACCA	1216
AD-76713	CACCAAGAGGCGUCACCAAdTdT	681	UUGGUGACGCCUCUUGGUdTdT	949	CACCAAGAGGCGUCACCC	1217
AD-76726	AGCAGACCGUAACCAUCCAdTdT	682	UGGAUGGUACGGUCUGCUdTdT	950	AGCAGACCGUAACCAUCC	1218
AD-76693	UCCUCGUUGUCCGUUCCAdTdT	683	UUGAAGCCGACACGAGGAdTdT	951	UCCUCGUUGUCCGUUCCAU	1219
AD-76876	UAUGUCAUCGUGCCGCUAdTdT	684	UUAGCGGCACGAUGACAUAdTdT	952	UAUGUCAUCGUGCCGCUAA	1220
AD-76589	AGACCGCCUGCAGGAAGUdTdT	685	ACUUCUUCGAGCCGGUCUdTdT	953	AGACCGCCUGCAGGAAGU	1221
AD-76891	GUGGAAGUCAAGGCGUCUAdTdT	686	UAGCAGCCUUGGCUUCCAdTdT	954	GUGGAAGUCAAGGCGUCGU	1222
AD-76907	UUCAUCAGUGACGGUGUCAdTdT	687	UGACACCCGACUGAUGAAdTdT	955	UUCAUCAGUGACGGUGUCA	1223
AD-76613	CAGGAAGUCCUGAAGGUAdTdT	688	UACCUACAGGACUUCUdTdT	956	CAGGAAGUCCUGAAGGU	1224
AD-76572	AAUCAGAAUGAACAAAUCUdTdT	689	AGUUUUUGUUAUCUGAUdTdT	957	AAUCAGAAUGAACAAAACU	1225
AD-76873	CUGUGGCUUGUCACCCUdTdT	690	AGGUGGCAACAGCCACAGdTdT	958	CUGUGGCUUGUCACCCU	1226
AD-76654	CUGGAUCCAGAACCCUGAdTdT	691	UCAGGCUUUCUGAUAUCCAdTdT	959	CUGGAUCCAGAACCCUGG	1227
AD-76578	AAGGAGUGCAGAAAAGAGAdTdT	692	UCCUUCUUCGACUCCUdTdT	960	AAGGAGUGCAGAAAAGAGGA	1228
AD-76894	GUGACCAAGUCCCGGACAAdTdT	693	UUGUCGGGACUUGGUCAdTdT	961	GUGACCAAGUCCCGGACAC	1229
AD-76897	CCGAGUCUGAGACCAGAAUdTdT	694	AUUCUGGUCACAGACUCCGdTdT	962	CCGAGUCUGAGACCAGAAU	1230
AD-76628	UUCUCCUGAACGGACCCAdTdT	695	UGGGUCCUUCAGGAGAAAdTdT	963	UUCUCCUGAACGGACCCC	1231
AD-76688	CCAGUGGCCAGAUAGACAAdTdT	696	UUGUCAUCUGGGCCACUGdTdT	964	CCAGUGGCCAGAUAGACAG	1232
AD-76651	CGUCACGCGGAACCGCUAdTdT	697	UAGCCGUUCCGCGUCGAdTdT	965	CGUCACGCGGAACCGCUC	1233
AD-76692	CGGGGAACAGAACAUAGAUAdTdT	698	UAUCAUGUUUCUUCGCCdTdT	966	CGGGGAACAGAACAUAGAU	1234
AD-76588	UCGGCAUGACGCCACGGUdTdT	699	ACCGUGGGGCUAUGCCGAdTdT	967	UCGGCAUGACGCCACGGU	1235
AD-76916	UACCUUGAUGAACGGAGAdTdT	700	UCUCUUCUUAUCCAGGUAdTdT	968	UACCUUGAUGAACGGAGC	1236
AD-76918	UGGAUGAAACGGAGCAGUAdTdT	701	UACUGUCUCCGUUACUAdTdT	969	UGGAUGAAACGGAGCAGUG	1237
AD-76698	GGGAGAAGUUCGGCCUAGAdTdT	702	UCUAGGCCGAACUUCUCCdTdT	970	GGGAGAAGUUCGGCCUAGA	1238
AD-76723	AGAAGCGCAGGGGGCUUdTdT	703	AAGGCCUUCGCGCUUCUdTdT	971	AGAAGCGCAGGGGGCCU	1239
AD-76735	UUGGAGCUCUAAGAAGAdTdT	704	UCUUCUUGAUGAGCUCAAdTdT	972	UUGGAGCUCUAAGAAGG	1240
AD-76710	GAGCUCAUCAAGAAGGGUdTdT	705	ACCCUUCUUGAUGAGCUCdTdT	973	GAGCUCAUCAAGAAGGGU	1241

TABLE 4-continued

Complement Component C3 modified sequences						
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO
AD-76852	UACACCCAGCAGCUGGCCUdTdT	706	AGGCCAGCUGCUGGGUGUAdTdT	974	UACACCCAGCAGCUGGCCU	1242
AD-76840	CUUCAGACAACCCAGCUCUdTdT	707	AGAGCUGGGUUGUCUGAAGdTdT	975	CUUCAGACAACCCAGCUCU	1243
AD-76727	AGCUCUGCCUUUGCGGCCUdTdT	708	AGGCCGCAAAGGCAGAGCUdTdT	976	AGCUCUGCCUUUGCGGCCU	1244
AD-76573	UUCGUGAACGAGGACCCAdTdT	709	UGGGUCCCGUUCACGAAAdTdT	977	UUCGUGAACGAGGACCC	1245
AD-76878	CAGCACCUUGGUCUACCCAdTdT	710	UGCGGUCAGCCAGGUGCUdTdT	978	CAGCACCUUGGUCUACCC	1246
AD-76618	UACGUGGUCAAGGUCUUCUdTdT	711	AGAAGACCUUGACCACGUAdTdT	979	UACGUGGUCAAGGUCUUCU	1247
AD-76730	CUCUCAGGCUGCAACCUAdTdT	712	UAGGUUAGCAGCCAGAGAdTdT	980	CUCUCAGGCUGCAACCU	1248
AD-76715	UGCGUGAUCCUGGAGAAGAdTdT	713	UCUUCUCCAGGAUCAGCCAdTdT	981	UGCGUGAUCCUGGAGAAGC	1249
AD-76733	CAGAAGCCCGACGGGUCUdTdT	714	AGACCCCGUCGGGUCUCUdTdT	982	CAGAAGCCCGACGGGUCU	1250
AD-76706	GUCUUCAGGAGGAUCCGAdTdT	715	UCGCAUCCUCUGGAAGACdTdT	983	GUCUUCAGGAGGAUCCG	1251
AD-76561	CCCGUGAUACACCAAGAAAdTdT	716	UUUCUUGGUGUAUCACGGGdTdT	984	CCCGUGAUACACCAAGAAA	1252
AD-76669	AUGAUUGGUGGAUACGGAdTdT	717	UCCGUAAUCCACCAUCAUdTdT	985	AUGAUUGGUGGAUACGG	1253
AD-76845	AACAACAACGAGAAAGAdTdT	718	UUGCUUUCUCGUUGUUGUdTdT	986	AACAACAACGAGAAAGCA	1254
AD-76859	UUUGUUCUCAUCUCGCUAdTdT	719	UCAGCGAGAUGAGAACAAAdTdT	987	UUUGUUCUCAUCUCGCU	1255
AD-76888	CAGGAGGCUAAAGAUUUdTdT	720	AAUAUCUUUAGCCUCCUdTdT	988	CAGGAGGCUAAAGAUUUU	1256
AD-76701	UUGCGAGGCAGGUCAAdTdT	721	UUUGAUCCGUCUCCGCAAdTdT	989	UUGCGAGGCAGGUCAAd	1257
AD-76725	ACAGCCUCCAGGCAGCAUdTdT	722	AUGCUGCCUGGCAGGCUdTdT	990	ACAGCCUCCAGGCAGCAU	1258
AD-76728	GCCUGCCAGGCAGCAUAdTdT	723	UUAGUCUGCCUGGCAGGCdTdT	991	GCCUGCCAGGCAGCAUAd	1259
AD-76653	CUAAAGCAGGAGACUUCUdTdT	724	AGGAAGUCUCCUGCUUUAdTdT	992	CUAAAGCAGGAGACUUCU	1260
AD-76687	CUUGAAGCAACUACAUGAdTdT	725	UCAUGUAGUUGGUUCAAGdTdT	993	CUUGAAGCAACUACAUG	1261
AD-76595	UACAUGAACCUACAGAGAUdTdT	726	AUCUCUGUAGGUUCAUGUAdTdT	994	UACAUGAACCUACAGAGAU	1262
AD-76617	AUCCUACACUGUGGCCAUdTdT	727	AUUGGCCACAGUUGAGGAUdTdT	995	AUCCUACACUGUGGCCAU	1263
AD-76874	UUGCUGGCUAUGCUCUGGAdTdT	728	UCCAGAGCAUAGCCAGCAAdTdT	996	UUGCUGGCUAUGCUCUGG	1264
AD-76565	GAAGGGGCCUUCUUAAAdTdT	729	UUUAAGAAGAGGCCUUCdTdT	997	GAAGGGGCCUUCUUAAAC	1265
AD-76849	CAAAUUUCUGACCACAGCAdTdT	730	UCUGUGGUCAGAAUUUGdTdT	998	CAAAUUUCUGACCACAGCC	1266
AD-76587	CAAAGAUAAAGACCGCUAdTdT	731	UCAGCGGUUCUUAUCUUUGdTdT	999	CAAAGAUAAAGACCGCUG	1267
AD-76567	GGGAGGACCCUGUAAGCAdTdT	732	UGCUIACCAGGGUCCUCCdTdT	1000	GGGAGGACCCUGGUAAGC	1268
AD-76686	GACCCUGUAAGCAGCUCUdTdT	733	AGUGUGGUCAGAAUUUGdTdT	1001	GACCCUGUAAGCAGCUCU	1269
AD-76911	UACAACGUGGAGGCCACAUdTdT	734	AUGUGGCCUCCACGUUGUAdTdT	1002	UACAACGUGGAGGCCACAU	1270
AD-76895	CCUAUGCCUCUUGGCCUdTdT	735	AGGGCCAAGAGGGCAUAGGdTdT	1003	CCUAUGCCUCUUGGCCU	1271
AD-76712	AUCUACACUAAAAGACUdTdT	736	AAGUCUUUAGCUCAGUAdTdT	1004	AUCUACACUAAAAGACU	1272
AD-76634	CGUCGUGCGUUGGCUCAdTdT	737	AUUGAGCCAACGACGAdTdT	1005	CGUCGUGCGUUGGCUCAd	1273
AD-76558	CAAUGAACAGAGUAUCUAdTdT	738	UUAGUAUCUCUGUUCUAdTdT	1006	CAAUGAACAGAGUAUCUAd	1274
AD-76863	ACGGUGGUGGCUAUGGCUAdTdT	739	UAGCAUAGCCACCAACCGUdTdT	1007	ACGGUGGUGGCUAUGGCU	1275
AD-76901	UCUACCCAGGCCACCUUCAdTdT	740	UGAAGGUGGCCUGGUAAGAdTdT	1008	UCUACCCAGGCCACCUUC	1276
AD-76898	UUUAUGGUGUCCAAGCCUdTdT	741	AGGCUUGGAACACCAUGAdTdT	1009	UUUAUGGUGUCCAAGCCU	1277
AD-76647	CUUUGGCUAAUACCAAAAdTdT	742	CUUUUGUCUUAGCCAAAdTdT	1010	CUUUGGCUAAUACCAAAAG	1278
AD-76722	AGGACGCCUCCAGCCACAdTdT	743	UGGUGGUCAGGGCGUCCUdTdT	1011	AGGACGCCUCCAGCCACCA	1279
AD-76585	CAGGAACUGAACCUUGAUdTdT	744	UAUCAAGGUUCAGUCCUdTdT	1012	CAGGAACUGAACCUUGAUG	1280
AD-76586	UUGAUGUUGCCUCCACCUdTdT	745	AGUUGGUCUUAGCCAAAdTdT	1013	UUGAUGUUGCCUCCACCU	1281
AD-76857	CUGCCCAGCCGACGUCUAdTdT	746	UGGAGCUGCGGCGUGGCAAdTdT	1014	CUGCCCAGCCGACGUCUAd	1282
AD-76862	CGCAGCUCCAAGAUACCCAdTdT	747	UGGUGAUUCUUGGAGCUGGdTdT	1015	CGCAGCUCCAAGAUACCC	1283
AD-76614	CCACCGUUAUCCACUGGGAdTdT	748	UUNCCAGUAGGUAACGGUGGdTdT	1016	CCACCGUUAUCCACUGGG	1284
AD-76851	CCGUAUCCACUGGGAUUCUdTdT	749	AGAUUCCAGUGGAUACGGdTdT	1017	CCGUAUCCACUGGGAUUCU	1285
AD-76569	UGCCAGCCUCCUGCGAUAdTdT	750	UGAUCGACGGAGGCCUGGAdTdT	1018	UGCCAGCCUCCUGCGAUAd	1286
AD-76644	AGAAGAGCUCACAGGAAAAdTdT	751	AUUUUUCUUGGUCUUCUdTdT	1019	AGAAGAGCUCACAGGAAA	1287
AD-76914	AUGAGGGUUUCACAGUCAAdTdT	752	UUAGUCUGGAAACCCUCAUdTdT	1020	AUGAGGGUUUCACAGUCA	1288
AD-76877	ACAGCUGAAGGAAAAGCCAdTdT	753	UGCCUUUUCCUAGCUGUdTdT	1021	ACAGCUGAAGGAAAAGCC	1289
AD-76881	CCAAGGCACUUUGCGGUAdTdT	754	UACCGACAAAGGUCUUGGdTdT	1022	CCAAGGCACUUUGCGGUAd	1290
AD-76912	UUUGCGGUGGUGACAUAUGUdTdT	755	ACAUUGUCACCACGACAAdTdT	1023	UUUGCGGUGGUGACAUAUGU	1291
AD-76896	GUACCAUGCUAAGGCCAAAdTdT	756	UUUGGCCUUAGCAUUGUAdTdT	1024	GUACCAUGCUAAGGCCAA	1292
AD-76576	CUAAGGCUAAAGAUCAACUdTdT	757	AGUUGAUUCUUUGCCUUAdTdT	1025	CUAAGGCUAAAGAUCAACU	1293
AD-76584	CAACUCACCUGUAAUAAUdTdT	758	AUUUAUACAGGUGAGUUGdTdT	1026	CAACUCACCUGUAAUAAU	1294
AD-76714	UUCGACCUCUAGGUCACCAAdTdT	759	UGGUGACCUCUAGGUCGAAdTdT	1027	UUCGACCUCUAGGUCACCA	1295
AD-76904	UCACCAUAAAACAGCACAdTdT	760	UGUGGUCUUUUUAGGUGAdTdT	1028	UCACCAUAAAACAGCACAd	1296
AD-76648	CGGAAACAGAAAAGAGCCAdTdT	761	UGCCUCUUUUCUGUUCCGdTdT	1029	CGGAAACAGAAAAGAGGCC	1297
AD-76906	UCAGGAUCCGCAAGAACUdTdT	762	AGUGUUCUUGGCAUCCUGAdTdT	1030	UCAGGAUCCGCAAGAACU	1298
AD-76623	UAUGAUCCUUGAGAUUCGUdTdT	763	ACAGAUUCUACAGGAUCAUAdTdT	1031	UAUGAUCCUUGAGAUUCGU	1299
AD-76720	CCAGGUACCGGGGAGACCAAdTdT	764	UGGUCUCCCGGUACCUGGdTdT	1032	CCAGGUACCGGGGAGACCA	1300
AD-76922	CAGGAUGCCACUAUGUCUAdTdT	765	UAGACUAUGUGGCAUCCUdTdT	1033	CAGGAUGCCACUAUGUCUAd	1301
AD-76836	CUUUGCCAGACACAGUAdTdT	766	AUCUGUGUCUGGAGCAAAGdTdT	1034	CUUUGCCAGACACAGUAd	1302
AD-76842	AUGACCUGAAGCAGCUGGAdTdT	767	UCCAGCUGUUCAGGUCUAdTdT	1035	AUGACCUGAAGCAGCUGGC	1303
AD-76699	UUGACAGAUACAUCUCCAdTdT	768	UUUGAGUUAUCUGUCAAdTdT	1036	UUGACAGAUACAUCUCCAd	1304
AD-76855	AAGUAUGAGCUGGACAAAGdTdT	769	CUUUGUCCAGUACUUCUdTdT	1037	AAGUAUGAGCUGGACAAAG	1305
AD-76559	UAUGAGCUGGACAAAGCCUdTdT	770	AGGCUUUUCAGCCUCAUAdTdT	1038	UAUGAGCUGGACAAAGCCU	1306
AD-76650	CUUCUCCGAUAGGAACACAdTdT	771	UGUGUCCUAUCGGAGAAGdTdT	1039	CUUCUCCGAUAGGAACACC	1307
AD-76601	CCUCUACUUAUCCUGGAdTdT	772	UCCAGGUAGAUUGAGGGdTdT	1040	CCUCUACUUAUCCUGGAd	1308
AD-76734	GACAAGGUCUCACACUCUAdTdT	773	UAGAGUGUGAGACCUUGUCdTdT	1041	GACAAGGUCUCACACUCUAd	1309
AD-76844	UCACACUCUGAGGAUGACUdTdT	774	AGUCAUCCACAGAGUGUGAdTdT	1042	UCACACUCUGAGGAUGACU	1310
AD-76622	GAGGAUAGCUGUCUAGCUdTdT	775	AAGCUAGACAGUACUCCUdTdT	1043	GAGGAUAGCUGUCUAGCU	1311
AD-76880	UUCAAAGUUCACCAAUAdTdT	776	AGUAUUGGUGAACUUUGAdTdT	1044	UUCAAAGUUCACCAAUAd	1312
AD-76683	CUUUAAUGUAGAGCUUAdTdT	777	UAUAAGCUCUACAUAAGdTdT	1045	CUUUAAUGUAGAGCUUAd	1313
AD-76690	GUCAAGGUCUACGCCAUdTdT	778	AAUAAGGCGUAGACCUUGAdTdT	1046	GUCAAGGUCUACGCCAUAd	1314
AD-76717	UACAACCUUGGAGGAAAGCUdTdT	779	AGCUUUCCUCCAGGUUGUAdTdT	1047	UACAACCUUGGAGGAAAGCU	1315

TABLE 4-continued

Complement Component C3 modified sequences						
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	
AD-76661	CUGUACCCGGUUCUACCAUdTdT	780	AUGGUAGAACCGGGUACAGdTdT	1048	CUGUACCCGGUUCUACCAU	1316
AD-76885	AUCCGGAAAAGGAGGAUGdTdT	781	UCAUCCUCCUUUCCGGAUdTdT	1049	AUCCGGAAAAGGAGGAUGG	1317
AD-76887	AAAAGGAGGAUGGAAAAGCUdTdT	782	AGCUUUCCAUCCUCCUUUdTdT	1050	AAAAGGAGGAUGGAAAAGCU	1318
AD-76645	CUGAACAAAGCUCUGCCGUdTdT	783	UACGGCAGAGCUUGUUCAGdTdT	1051	CUGAACAAAGCUCUGCCGUG	1319
AD-76615	UGAUGAACUGUGCCGUGdTdT	784	ACAGCGGCACAGUUCACAdTdT	1052	UGAUGAACUGUGCCGUGU	1320
AD-76579	GUGCUGAGGAGAAUUGCUdTdT	785	AAGCAAUUCUCCUCAGCAdTdT	1053	GUGCUGAGGAGAAUUGCUU	1321
AD-76604	AAUUGCUUCAUACAAAAGUdTdT	786	ACUUUUGUAUGAAGCAAUdTdT	1054	AAUUGCUUCAUACAAAAGU	1322
AD-76682	GUCGGAUGACAAGGUCAdTdT	787	UGUGACCUUGUCAUCCGAdTdT	1055	GUCGGAUGACAAGGUCACC	1323
AD-76903	CCCUGGAAGAACGGCUGAdTdT	788	UCCAGCCUUCUCCAGGGdTdT	1056	CCCUGGAAGAACGGCUGGA	1324
AD-76630	GACAAGGCCUGAGCCAAdTdT	789	UUGGCUCACAGCCUUGUCdTdT	1057	GACAAGGCCUGAGCCAG	1325
AD-76575	AGGCCUGUGAGCCAGGAGUdTdT	790	ACUCCUGGCUCACAGGCCUdTdT	1058	AGGCCUGUGAGCCAGGAGU	1326
AD-76917	CCAGGAGUGGACUAUGUGUdTdT	791	ACACAUAGUCCACUCCUGGdTdT	1059	CCAGGAGUGGACUAUGUGU	1327
AD-76886	UACAAGACCCGACUGGUCAdTdT	792	UGACCAGUCGGGUCUUGUAdTdT	1060	UACAAGACCCGACUGGUA	1328
AD-76905	UUCAGCUGUCCAAUGACUUdTdT	793	AAGUCAUUGGACAGCUGAAdTdT	1061	UUCAGCUGUCCAAUGACUU	1329
AD-76856	UUUGACGAGUACAUCAUGAdTdT	794	UCAUGAUUGACUCGUCAAAdTdT	1062	UUUGACGAGUACAUCAUGG	1330
AD-76737	UACAUCUAGGCCAUUGAGAdTdT	795	UCUCAUUGGCCAUGAUGUAdTdT	1063	UACAUCUAGGCCAUUGAGC	1331
AD-76659	CAGACCAUCAAGUCAGGCUdTdT	796	AGCCUGACUUGAUGGUCUGdTdT	1064	CAGACCAUCAAGUCAGGCU	1332
AD-76854	CUCGGAUGAGGUGCAGGUdTdT	797	AACCUGCACCUCUCCGAGdTdT	1065	CUCGGAUGAGGUGCAGGU	1333
AD-76711	UUCAUCAGCCCAUCAAGUdTdT	798	ACUUGAUGGGCUGAUGAAdTdT	1066	UUCAUCAGCCCAUCAAGU	1334
AD-76675	UCAAGUGCAGAGAAGCCUdTdT	799	AGGGCUUCUCGACUUGAdTdT	1067	UCAAGUGCAGAGAAGCCU	1335
AD-76703	CUGAAGCUGGAGGAGAAGdTdT	800	UCUUCUCCUCCAGCUCAGdTdT	1068	CUGAAGCUGGAGGAGAAGA	1336
AD-76707	AAGCUGGAGGAGAGAAGAACTdTdT	801	GUUUUUCUCCUCCAGUdTdT	1069	AAGCUGGAGGAGAGAAGAA	1337
AD-76649	ACACUACCUCUUGGGGUdTdT	802	ACCCACAUUGAGGUAGUGUdTdT	1070	ACACUACCUCUUGGGGU	1338
AD-76631	UUUCUGGGGAGAGAAGCCAdTdT	803	UGGGCUUCUCCCCAGAAdTdT	1071	UUUCUGGGGAGAGAAGCCCA	1339
AD-76598	UACAUCUAGCCGAAGGACAdTdT	804	UGUCCUCCGGAUGAUGUAdTdT	1072	UACAUCUAGCCGAAGGACA	1340
AD-76658	CACUUGGGUGGAGCAGUAdTdT	805	UCAGUGCUCACCCAAGUGdTdT	1073	CACUUGGGUGGAGCAGUGG	1341
AD-76865	AGGACGAAUGCCAAGACGAdTdT	806	UCGUCUUGGCAUUCGUCCUdTdT	1074	AGGACGAAUGCCAAGACGA	1342
AD-76697	AAGAGAACCAGAAACAAdTdT	807	AUUUGUUCUGGUUCUUCUdTdT	1075	AAGAGAACCAGAAACAAG	1343
AD-76611	UGCCAGGACCUCGGCCUdTdT	808	AGGGCCGAGGUCCUGGCAdTdT	1076	UGCCAGGACCUCGGCCU	1344
AD-76837	CUUCACCCGAGCAGUUGUdTdT	809	AACCAUGCUCUCGGUGAAGdTdT	1077	CUUCACCCGAGCAGUUGGU	1345
AD-76704	CACACCCCAUUCGCCAAdTdT	810	UUGGGGAAUGGGGGUGUGdTdT	1078	CACACCCCAUUCGCCCA	1346
AD-76910	CUCCAGAUAAAGCUUCAGUdTdT	811	ACUGAAGCUUUUUCUGGAGdTdT	1079	CUCCAGAUAAAGCUUCAGU	1347
AD-76870	UUUAUUCUCAAAAAAAAAAdTdT	812	UUUUUUUUUGAGAUUAAdTdT	1080	UUUAUUCUCAAAAAAAAA	1348

35

TABLE 5

C3 single dose screen in Hep3B Cells	
Duplex Name	10 nM_AVG
AD-76619	81.34
AD-76864	67.41
AD-76867	27.87
AD-76667	48.22
AD-76602	24.86
AD-76892	63.99
AD-76665	36.80
AD-76900	55.29
AD-76868	33.73
AD-76671	40.23
AD-76564	36.81
AD-76600	16.61
AD-76676	39.51
AD-76915	18.67
AD-76577	68.52
AD-76560	75.89
AD-76664	35.74
AD-76889	62.28
AD-76718	42.42
AD-76606	21.79
AD-76685	52.44
AD-76599	17.98
AD-76678	32.71
AD-76670	75.69
AD-76609	19.35
AD-76616	24.23
AD-76629	91.18
AD-76850	14.52
AD-76847	20.03
AD-76668	55.13
AD-76920	31.43

TABLE 5-continued

C3 single dose screen in Hep3B Cells	
Duplex Name	10 nM_AVG
AD-76637	15.16
AD-76627	27.57
AD-76708	19.49
AD-76594	27.17
AD-76858	80.97
AD-76731	22.06
AD-76660	20.21
AD-76729	45.79
AD-76657	79.53
AD-76882	76.50
AD-76674	37.79
AD-76581	16.70
AD-76684	26.49
AD-76633	70.67
AD-76902	25.01
AD-76921	125.85
AD-76691	86.09
AD-76592	42.69
AD-76642	14.96
AD-76869	33.52
AD-76716	40.09
AD-76689	75.31
AD-76679	59.40
AD-76563	72.41
AD-76568	17.15
AD-76574	91.25
AD-76875	19.37
AD-76677	95.01
AD-76861	32.98
AD-76672	60.88
AD-76908	31.11

TABLE 5-continued

C3 single dose screen in Hep3B Cells		
Duplex Name	10 nM_AVG	
AD-76625	37.69	5
AD-76571	21.83	
AD-76580	47.71	
AD-76673	39.40	
AD-76641	111.17	
AD-76695	80.59	10
AD-76666	40.92	
AD-76853	80.21	
AD-76639	93.17	
AD-76872	31.84	
AD-76680	31.17	
AD-76709	99.88	15
AD-76860	36.14	
AD-76610	73.21	
AD-76608	33.75	
AD-76662	41.33	
AD-76899	41.36	
AD-76640	108.20	20
AD-76705	102.72	
AD-76866	19.58	
AD-76652	86.39	
AD-76838	40.48	
AD-76636	55.70	
AD-76848	23.54	
AD-76655	39.58	25
AD-76700	56.96	
AD-76583	34.13	
AD-76919	53.18	
AD-76681	34.21	
AD-76724	37.68	
AD-76696	37.46	30
AD-76890	79.00	
AD-76638	28.31	
AD-76883	95.52	
AD-76839	27.13	
AD-76590	26.38	
AD-76721	49.09	35
AD-76646	55.65	
AD-76605	56.71	
AD-76719	36.27	
AD-76624	35.67	
AD-76626	13.23	
AD-76603	25.01	40
AD-76846	13.95	
AD-76635	71.91	
AD-76893	70.30	
AD-76923	14.76	
AD-76736	76.07	
AD-76566	16.47	
AD-76656	91.79	45
AD-76596	31.65	
AD-76562	32.29	
AD-76607	24.46	
AD-76702	41.34	
AD-76879	37.36	
AD-76663	40.58	50
AD-76913	18.98	
AD-76909	12.99	
AD-76843	20.73	
AD-76597	18.30	
AD-76732	59.81	
AD-76871	31.99	55
AD-76643	55.95	
AD-76621	101.39	
AD-76884	64.51	
AD-76570	19.64	
AD-76612	9.79	
AD-76582	15.82	
AD-76841	43.31	60
AD-76620	24.98	
AD-76694	62.42	
AD-76632	95.95	
AD-76591	80.65	
AD-76593	76.64	
AD-76713	41.37	65
AD-76726	43.50	

TABLE 5-continued

C3 single dose screen in Hep3B Cells		
Duplex Name	10 nM_AVG	
AD-76693	25.77	
AD-76876	37.92	
AD-76589	18.53	
AD-76891	44.75	
AD-76907	51.95	
AD-76613	18.94	10
AD-76572	38.55	
AD-76873	32.94	
AD-76654	59.32	
AD-76578	25.23	
AD-76894	60.08	
AD-76897	22.16	15
AD-76628	96.02	
AD-76688	40.23	
AD-76651	43.03	
AD-76692	39.71	
AD-76588	38.94	
AD-76916	41.62	20
AD-76918	26.98	
AD-76698	22.86	
AD-76723	50.03	
AD-76735	82.32	
AD-76710	41.56	
AD-76852	88.88	
AD-76840	29.70	25
AD-76727	37.73	
AD-76573	67.80	
AD-76878	93.21	
AD-76618	72.24	
AD-76730	24.79	
AD-76715	36.05	30
AD-76733	69.47	
AD-76706	42.66	
AD-76561	62.17	
AD-76669	32.82	
AD-76845	17.88	35
AD-76859	59.92	
AD-76888	22.05	
AD-76701	42.50	
AD-76725	37.85	
AD-76728	37.43	
AD-76653	29.14	
AD-76687	33.86	40
AD-76595	16.28	
AD-76617	34.00	
AD-76874	109.06	
AD-76565	34.94	
AD-76849	17.38	
AD-76587	25.22	
AD-76567	10.51	45
AD-76686	36.45	
AD-76911	100.13	
AD-76895	29.67	
AD-76712	32.13	
AD-76634	24.10	
AD-76558	10.63	50
AD-76863	71.47	
AD-76901	70.63	
AD-76898	74.44	
AD-76647	83.55	
AD-76722	32.39	
AD-76585	20.68	55
AD-76586	84.87	
AD-76857	12.21	
AD-76862	28.25	
AD-76614	18.38	
AD-76851	22.39	
AD-76569	31.93	60
AD-76644	15.98	
AD-76914	12.42	
AD-76877	25.10	
AD-76881	24.21	
AD-76912	87.11	
AD-76896	11.00	65
AD-76576	12.13	
AD-76584	13.33	

TABLE 5-continued

C3 single dose screen in Hep3B Cells	
Duplex Name	10 nM_AVG
AD-76714	106.13
AD-76904	28.26
AD-76648	40.30
AD-76906	42.29
AD-76623	59.80
AD-76720	33.09
AD-76922	15.26
AD-76836	35.31
AD-76842	36.07
AD-76699	28.16
AD-76855	58.60
AD-76559	145.64
AD-76650	48.94
AD-76601	18.08
AD-76734	30.89
AD-76844	67.03
AD-76622	15.09
AD-76880	42.08
AD-76683	34.59
AD-76690	35.14
AD-76717	67.15
AD-76661	75.24
AD-76885	37.96
AD-76887	58.41
AD-76645	20.75
AD-76615	33.03
AD-76579	33.22
AD-76604	40.15

TABLE 5-continued

C3 single dose screen in Hep3B Cells	
Duplex Name	10 nM_AVG
AD-76682	57.20
AD-76903	24.85
AD-76630	23.33
AD-76575	27.72
AD-76917	24.56
AD-76886	21.18
AD-76905	47.24
AD-76856	48.22
AD-76737	90.72
AD-76659	38.68
AD-76854	31.17
AD-76711	76.77
AD-76675	75.87
AD-76703	45.76
AD-76707	49.43
AD-76649	98.60
AD-76631	49.95
AD-76598	21.87
AD-76658	57.29
AD-76865	13.17
AD-76697	29.14
AD-76611	94.88
AD-76837	27.39
AD-76704	107.56
AD-76910	19.44
AD-76870	69.16

TABLE 6

Additional Complement Component C3 unmodified sense and antisense strand sequences

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range	Antisense Sequence 5' to 3'	SEQ ID NO:	Transcript
AD-60165.1	GCUGAGGAGAAUUGCUUCAUA	1353	4602-4622	UAUGAAGCAAUUCUCCUCAGCAC	1410	NM_000064.2
AD-60153.1	GGAGAAUUGCUUCAUACAAAA	1354	4607-4627	UUUUGUAUGAAGCAAUUCUCCUC	1411	NM_000064.2
AD-60175.1	GCAGCUAAAAGACUUUGACUU	1355	3785-3805	AAGUCAAAAGUCUUUUAGCUGCAG	1412	NM_000064.2
AD-60169.1	AUCCAGACAGACAAGACCAUU	1356	456-476	AAUGGUCUUGUCUGUCUGGAUGA	1413	NM_000064.2
AD-60187.1	GACAGACAAGACCAUCUACAA	1357	461-481	UGUAGAUGGUCUUGUCUGUCUG	1414	NM_000064.2
AD-60149.1	CGUGGUCAAGGUCUUCUCUCU	1358	3305-3325	AGAGAGAAGACCUUGACCACGUA	1415	NM_000064.2
AD-60171.1	UCCAGACAGACAAGACCAUCU	1359	457-477	AGAUGGUCUUGUCUGUCUGGAUG	1416	NM_000064.2
AD-60160.1	AUGAACAAAACUGUGGUCGUU	1360	2874-2894	AACAGCCACAGUUUUGUUAUUC	1417	NM_000064.2
AD-60206.1	CUUCAUGGUAAUCCAAGCCUU	1361	3917-3937	AAGGCUUGGAAUACCAUGAAGGU	1418	NM_009778.2
AD-60176.1	CAUCCAGACAGACAAGACCAU	1362	455-475	AUGGUCUUGUCUGUCUGGAUGAA	1419	NM_000064.2
AD-60156.1	CCAGACAGACAAGACCAUCUA	1363	458-478	UAGAUUGUCUUGUCUGUCUGGAU	1420	NM_000064.2
AD-60155.1	GACAGACAAGACCAUCUACAC	1364	461-481	GUGUAGAUGGUCUUGUCUGUCUG	1421	NM_000064.2
AD-60183.1	UACGUGGUCAAGGUCUUCUCU	1365	3303-3323	AGAGAAGACCUUGACCACGUAGG	1422	NM_000064.2
AD-60161.1	AGACAGACAAGACCAUCUACA	1366	460-480	UGUAGAUGGUCUUGUCUGUCUGG	1423	NM_000064.2
AD-60166.1	ACGUGGUCAAGGUCUUCUCUC	1367	3304-3324	GAGAGAAGACCUUGACCACGUAG	1424	NM_000064.2
AD-60201.1	AGAAAAUUGGAAUCUCUACGA	1368	2446-2466	UCGUAGAGAUUCCAUUUUUCUCU	1425	NM_009778.2
AD-60179.1	UUUGACCUCUUGGUCUUCGUU	1369	1170-1190	AACGAACACCAUGAGGUCAAAGG	1426	NM_000064.2
AD-60164.1	GACAAGACCAUCUACACCCCU	1370	465-485	AGGGGUGUAGAUGGUCUUGUCUG	1427	NM_000064.2
AD-60186.1	GAAAGGGAUCUGUGGGCAGA	1371	2498-2518	UCUGCCACACAGAUCCCUUUCU	1428	NM_000064.2
AD-60162.1	CCAGAUCCACUUCACCAAGAC	1372	1121-1141	GUCUUGGUGAAGUGGAUCUGGUA	1429	NM_000064.2
AD-60189.1	UGUAAUAAAUCGACCUCAAG	1373	4134-4154	CUUGAGGUCGAAUUUAUUACAGG	1430	NM_000064.2
AD-60178.1	AUCCAGACAGACAAGACCAUC	1374	456-476	GAUGGUCUUGUCUGUCUGGAUGA	1431	NM_000064.2
AD-60194.1	AACAAGAAGAACAACACACA	1375	1914-1934	UGUGAGUUUUGUUCUUCUUGUUA	1432	NM_009778.2
AD-60199.1	CUUCACCAAGACACCCAAAUU	1376	1172-1192	AAUUUGGGUGUCUUGGUGAAGUG	1433	NM_009778.2
AD-60174.1	UGACCUCAUGGUGUUCGUGAU	1377	1172-1192	AUCACGAACACCAUGAGGUCAAA	1434	NM_000064.2
AD-60159.1	CCCCUUCGAGGUCACAGUAAU	1378	2519-2539	AUUACUGGACCCUGAAGGGGUC	1435	NM_000064.2
AD-60168.1	ACAGACAAGACCAUCUACACA	1379	462-482	UGUGUAGAUGGUCUUGUCUGUCU	1436	NM_000064.2
AD-60198.1	AUUCACCAAGAAUGAUUGGU	1380	3474-3494	ACCAAUCAUUUCUUGGUGAAUCA	1437	NM_009778.2
AD-60180.1	GGAUGCCAAGAACACUAUGAU	1381	4196-4216	AUCAUAGUGUUCUUGGCAUCCUG	1438	NM_000064.2
AD-60190.1	AACUACAUGAACCUACAGAGA	1382	3597-3617	UCUCUGUAGGUUCAUGUAGUUGG	1439	NM_000064.2
AD-60188.1	UGACCUCUUGGUGUUCGUGAC	1383	1172-1192	GUCACGAACACCAUGAGGUCAAA	1440	NM_000064.2
AD-60205.1	CGUGACAGUGGUGGCAACUU	1384	419-439	AAGUUUGCCACCACUGUCACGUA	1441	NM_009778.2
AD-60184.1	CAGUUUCGAGGUCAUAGUGGA	1385	752-772	UCCACUAGACCUAGAAACUGGG	1442	NM_000064.2
AD-60163.1	AGGGAUCUGUGGGCAGACCA	1386	2501-2521	UGGUCUGCCACACAGAUCUUUU	1443	NM_000064.2
AD-60172.1	AGGGAUCUGUGGGCAGACCC	1387	2501-2521	GGGUCUGCCACACAGAUCUUUU	1444	NM_000064.2
AD-60151.1	ACGUGGUCAAGGUCUUCUCUA	1388	3304-3324	UAGAGAAGACCUUGACCACGUAG	1445	NM_000064.2
AD-60196.1	UUGCCUUUGUCUUGGAACAAU	1389	669-689	AAUGUCCAAGACAAGGCAAGA	1446	NM_009778.2
AD-60204.1	CAUAGAAGAGUUGAAAGAAC	1390	2423-2443	GGUUCUUUAACUCUUCUAUGGU	1447	NM_009778.2

TABLE 6-continued

Additional Complement Component C3 unmodified sense and antisense strand sequences						
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range	Antisense Sequence 5' to 3'	SEQ ID NO:	Transcript
AD-60185.1	CGUGCCGGAAGGAAUCAGAAU	1391	2855-2875	AUUCUGAUUCCUCCGGCAGCAG	1448	NM_000064.2
AD-60170.1	CUCCGUGUGGGUGGACGUCAA	1392	1709-1729	UUGACGUCCACCCACACGGAGUC	1449	NM_000064.2
AD-60158.1	UUGACCUCUAUGGUGUUCGUGA	1393	1171-1191	UCACGAACACCAUGAGGUCAAAAG	1450	NM_000064.2
AD-60200.1	CUGCUGAAAAGACUUUGACUCU	1394	3825-3845	AGAGUCAAAAGUCUUUACAGCAGCA	1451	NM_009778.2
AD-60173.1	CAAGAAAGGGAUCUGUGUGGA	1395	2495-2515	UCCACACAGAUCCUUUCUUGUC	1452	NM_000064.2
AD-60181.1	AAGAAAGGGAUCUGUGUGGCA	1396	2496-2516	UGCCACACAGAUCCUUUCUUGU	1453	NM_000064.2
AD-60152.1	UUUGACCUCUAGGUGUUCGUG	1397	1170-1190	CACGAACCAUGAGGUCAAAAGG	1454	NM_000064.2
AD-60197.1	CACUGUGCAAGACUCCUAAA	1398	278-298	UUUAGGAAGUCUUGCACAGUGAC	1455	NM_009778.2
AD-60191.1	CUGGUUGUGGACCAUAGAAGA	1399	2411-2431	UCUUCUAUGGUCCACAACCAGCU	1456	NM_009778.2
AD-60154.1	UGUUAAAUGGCUGAUCCUGGA	1400	3371-3391	UCCAGGAUCAGCCAUUUAACAGC	1457	NM_000064.2
AD-60167.1	GGAUCUGUGGGCAGACCCCU	1401	2503-2523	AGGGGUCUGCCACACAGAUCUCCU	1458	NM_000064.2
AD-60177.1	ACAGACAAGACCAUCUACACC	1402	462-482	GGUGUAGAUGGUCUUGUCUGUCU	1459	NM_000064.2
AD-60195.1	GUACGUGACAGUGGUGGCAAA	1403	416-436	UUUGCCACCACUGUCACGUACUU	1460	NM_009778.2
AD-60193.1	GGUCAUGAACAUUUUCUCAA	1404	2468-2488	UUGAGAAAGAUGUUAUGACCUU	1461	NM_009778.2
AD-60202.1	GUCGUGCCAGAAGGAAUGAGA	1405	2892-2912	UCUCAUCCUUCUGGCACGACCU	1462	NM_009778.2
AD-60182.1	CAAGAAAGGGAUCUGUGUGGC	1406	2495-2515	GCCACACAGAUCCUUUCUUGUC	1463	NM_000064.2
AD-60203.1	CUGUGCAAGACUCCUAAAAGA	1407	280-300	UCUUUAGGAAGUCUUGCACAGUG	1464	NM_009778.2
AD-60157.1	CCAGAUCACUUCACCAAGAA	1408	1121-1141	UUUCUUGGUGAAGUGGAUCUGGUA	1465	NM_000064.2
AD-60192.1	GCUGUGAAAAGACUUGACUC	1409	3824-3844	GAGUCAAGUCUUCAGCAGCAG	1466	NM_009778.2

TABLE 7

Additional Complement Component C3 modified sense and antisense strand sequences						
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-60165.1	GfscsUfgAfgGfaGfAfAfuUfgCfuUfcAfuAfl96	1467	usAfsuGfaAfgCfaAfmucUfcCfuCfaGfcsasC	1524	GUGCUGAGGAGAAUUGCUUCAUA	1581
AD-60153.1	GfsgsAfgAfaUfuGfCfUfuCfaUfaCfaAfaAfl96	1468	usUfsuUfgUfaUfgAfagcAfaUfuCfuCfcsusC	1525	GAGGAGAAUUGCUUCAUAACAAAA	1582
AD-60175.1	GfscsAfgCfuAfaAfaAfgfaCfuUfuGfaCfuUfl96	1469	asAfsuUfgAfaAfgUfcuuUfuAfgCfuGfcsasg	1526	CUGCAGCUAAAAGACUUUGACUUC	1583
AD-60169.1	AfsusCfcAfgAfcAfgAfcAfaGfaCfcAfuUfl96	1470	asAfsuGfgUfcUfuGfucUgfuCfuGfgAfsusga	1527	UCAUCCAGACAGACAAGACC AUC	1584
AD-60187.1	GfsasCfaGfaCfaAfgAfcCfaUfcUfaCfaAfl96	1471	usUfsuUfgUfaUfgGfucUfgUfcUfgUfcsusg	1528	CAGACAGACAAGACCAUCUACAC	1585
AD-60149.1	CfsgsUfgGfuCfaAfgGfufuUfcUfcUfcUfl96	1472	asGfsaGfaGfaAfgAfcuUfgAfcCfaCfsgsus a	1529	UACGUGGUC AAGGUCUUCUCUUC	1586
AD-60171.1	UfscsCfaGfaCfaGfaAfcfaAfgAfcCfaUfcUfl96	1473	asGfsaUfgGfuCfuUfgucUfgUfcUfgGfususg	1530	CAUCCAGACAGACAAGACCAUCU	1587
AD-60160.1	AfsusGfaAfaAfaAfaAfcfuGfuGfuCfuUfl96	1474	asAfsuAfgCfcAfcAfguuUfuGfuUfcAfsus c	1531	GAAUGAACAAAACUGUGGCUGUU	1588
AD-60206.1	CfsusUfcAfuGfgUfaUfuCfaAfaGfcCfuUfl96	1475	asAfsuGfcUfuGfgAfaaCfaAfuGfaAfgsgsu	1532	ACCUUCAUGGUUAUCCAAGCCUUCU	1589
AD-60176.1	CfsasCfaGfaCfaAfgAfcfaCfaAfgAfcCfaUfl96	1476	asUfsuGfuCfuUfgUfcuUfcUfgGfaUfsgsas a	1533	UUCAUCCAGACAGACAAGACC AU	1590
AD-60156.1	CfscsAfgAfcAfgAfcAfaGfaCfaCfaAfuAfl96	1477	usAfsuAfuGfgUfcUfucUfuCfuGfcsas u	1534	AUCCAGACAGACAAGACCAUCUA	1591
AD-60155.1	GfsasCfaGfaCfaAfgAfcCfaUfcUfaCfaCfl96	1478	gsUfsuUfaGfaUfgGfucUfgUfcUfgUfcsusg	1535	CAGACAGACAAGACCAUCUACAC	1592
AD-60183.1	UfscsCfuUfgGfuCfaAfaGfuCfuUfcUfcUfl96	1479	asGfsaGfaAfgAfcCfuugAfcCfaCfuUfsgsgs g	1536	CCUACGUGGUCAAGGUCUUCUCU	1593
AD-60161.1	AfsgsAfcAfgAfcAfaAfgfaCfaCfuAfuAfl96	1480	usGfsuAfgAfuGfgUfcuuGfuCfuGfuCfususg	1537	CCAGACAGACAAGACCAUCUACA	1594
AD-60166.1	AfscsGfuGfgUfcAfaAfgfgUfcUfuCfuCfl96	1481	gsAfsuAfgAfaGfaCfcuuGfaCfcAfcGfususg	1538	CUACGUGGUCAAGGUCUUCUCUC	1595
AD-60201.1	AfsgsAfaAfaAfuGfgAfaUfcUfcUfaCfaAfl96	1482	usCfsuUfaGfaGfaUfuccAfuUfuUfcUfcsus u	1539	AGAGAAAAUUGGAAUCUCUACGA	1596
AD-60179.1	UfsusUfgAfcCfuCfaUfgGfuGfuUfcUfl96	1483	asAfsuGfaAfcAfcCfaugAfgGfuCfaAfasgsg	1540	CCUUUGACCUC AUGGUGUUCGUG	1597
AD-60164.1	GfsasCfaAfgAfcCfaUfcUfaCfaCfcCfuUfl96	1484	asGfsuGfgUfgUfaGfaugGfuCfuUfgUfcsusg	1541	CAGACAAGACCAUCUACACC CCU	1598
AD-60186.1	GfsasAfaGfgGfaUfcUfgUfgUfgGfcAfgAfl96	1485	usCfsuGfcCfaCfaCfagaUfcCfuUfuUfcsusu	1542	AAGAAAGGGAUCUGUGUGGCAGA	1599
AD-60162.1	CfscsAfgAfuCfcAfcUfuCfaCfcAfaGfaCfl96	1486	gsUfscUfuGfgUfgAfguGfgAfuCfuGfsgusa	1543	UACCAGAUCACUUCACCAA GAC	1600
AD-60189.1	UfsgsUfaAfaAfaAfuUfcGfaCfcUfcAfaGfl96	1487	csUfsuGfaGfgUfcGfaaUfuAfuUfaCfsgsgs g	1544	CCUGUAAUAAAUCGACCUC AAG	1601
AD-60178.1	AfsusCfaAfgAfcAfgAfcAfaGfaCfaCfaCfl96	1488	gsAfsuGfgUfcUfuGfucUfuCfuGfgAfsusg sa	1545	UCAUCCAGACAGACAAGACC AUC	1602

TABLE 7-continued

Additional Complement Component C3 modified sense and antisense strand sequences						
Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-60194.1	AfsasCfaAfgAfaGfAfaFcAfaAfcUfcAfcAfL96	1489	usGfsuGfaGfuUfuGfuccUfuCfuUfgUfusesa	1546	UGAACAAAGAAGAACAAACUCACA	1603
AD-60199.1	CfsusUfcAfcCfaAfgGfAfcAfcCfcAfaAfuUfL96	1490	asAfsuUfuGfgGfuGfuccUfgGfuGfaAfgsusg	1547	CACUUCACCAAGACACCCAAAUU	1604
AD-60174.1	UfsgsAfcCfuCfaUfGfGfuGfuUfcGfuGfaUfL96	1491	asUfscAfcGfaAfcAfccuUfgAfgGfuCfasasa	1548	UUUGACCUC AUGGUGUUCGUGAC	1605
AD-60159.1	CfscsCfcUfuCfGfAfgGfuCfaCfaGfuAfaUfL96	1492	asUfsuAfcUfgUfgAfcuUfgAfaGfgGfsgsusC	1549	GACCCUUCGAGGUCACAGUAUU	1606
AD-60168.1	AfscsAfgAfaAfaGfAfcfcAfuCfuAfcAfcAfL96	1493	usGfsuGfuAfgAfuGfgucUfuGfuCfuGfusesu	1550	AGACAGACAAGACCAUCUACACC	1607
AD-60198.1	AfsusUfcAfcCfaAfgGfAfaAfuGfaUfuGfuUfL96	1494	asCfscAfaUfcAfuUfuccUfgGfuGfaAfusesa	1551	UGAUUCACCAAGAAUUGAUUGGU	1608
AD-60180.1	GfsgsAfuGfcCfaAfgGfaCfaCfuAfuGfaUfL96	1495	asUfscAfuAfgUfgUfuccUfgGfcAfuCfcsusg	1552	CAGGAUGCCAAGAACACUAUGAU	1609
AD-60190.1	AfsasCfuAfcAfuGfAfaFcCfuAfcAfgAfgAfL96	1496	usCfsuCfuGfuAfgGfuccAfuGfuAfgUfusesg	1553	CCAACUACAUGAACCUACAGAGA	1610
AD-60188.1	UfsgsAfcCfuCfaUfGfGfuGfuUfcGfuGfaCfL96	1497	gsUfscAfcGfaAfcAfccuUfgAfgGfuCfasasa	1554	UUUGACCUC AUGGUGUUCGUGAC	1611
AD-60205.1	CfsgsUfgAfcAfgUfgGfuGfgCfaAfaCfuUfL96	1498	asAfsuUfuUfgCfcAfccuUfgGfuCfaCfsgsusg	1555	UACGUGACAGUGGUGGCAACUU	1612
AD-60184.1	CfsasGfuUfuCfGfAfgGfuCfaUfaGfuGfgAfL96	1499	usCfscAfcUfaUfgAfcuUfgAfaAfcUfsgsgs	1556	CCCAGUUUCGAGGUCAUAGUGGA	1613
AD-60163.1	AfsgsGfgAfuCfuGfuUfgfuGfgCfaGfaCfcAfL96	1500	usGfsgUfcUfgCfcAfcacAfgAfuCfcCfususu	1557	AAAGGGAUCUGUGGCGAGACCC	1614
AD-60172.1	AfsgsGfgAfuCfuGfuUfgfuGfgCfaGfaCfcAfL96	1501	gsGfsgUfcUfgCfcAfcacAfgAfuCfcCfususu	1558	AAAGGGAUCUGUGGCGAGACCC	1615
AD-60151.1	AfscsGfuGfuUfcAfaGfgUfcUfuCfuAfuUfL96	1502	usAfsuAfgAfaGfaCfcuUfgAfcAfcGfusasa	1559	CUACGUGUCAAGGUCUUCUCUC	1616
AD-60196.1	UfsusGfcCfuUfuGfuUfcfuUfgGfaAfcAfuUfL96	1503	asAfsuGfuUfcCfaAfgacAfaAfgGfcAfasgsa	1560	UCUUGCCUUGUCUUGGAACAUU	1617
AD-60204.1	CfsasUfaGfaAfgAfGfuUfaAfaGfaAfcCfL96	1504	gsGfsuUfcUfuUfcAfcuUfuUfaUfsgsgsu	1561	ACCAUAGAAGAGUUGAAAGAAC	1618
AD-60185.1	CfsgsUfgCfcGfgAfaGfgAfaUfcAfgAfaUfL96	1505	asUfsuUfuGfaUfuCfcuUfgGfgCfaCfsgsasC	1562	GUCGUGCCGGAAGGAAUCAAAU	1619
AD-60170.1	CfsusCfcGfuGfuGfgGfuGfgAfcGfuCfaAfL96	1506	usUfsgAfcGfuCfcAfcuUfgAfcGfgAfgsusC	1563	GACUCCGUGGGUGGACGUCAA	1620
AD-60158.1	UfsusGfaCfcUfcAfuUfgfgUfuCfuGfgAfL96	1507	usCfsaCfGfaCfaCfcuUfgGfgUfcAfasasg	1564	CUUUGACCUC AUGGUGUUCGUGA	1621
AD-60200.1	CfsusGfgUfuGfuAfaGfgAfcUfuUfgAfcUfcUfL96	1508	asGfsaGfuCfaAfaGfuccUfuCfaGfcAfgscsa	1565	UGCUGUGAAAGACUUUGACUCU	1622
AD-60173.1	CfsasAfgAfaAfgGfgAfuCfuGfuGfuGfgAfL96	1509	usCfscAfcAfcAfgAfcuUfuCfuUfsgsusC	1566	GACAAGAAAGGGAUCUGUGUGGC	1623
AD-60181.1	AfsasGfaAfaGfgGfAfuUfcUfgUfgGfcAfL96	1510	usGfscCfaCfaCfaGfaucUfuUfuUfsgsgsu	1567	ACAAGAAAGGGAUCUGUGUGGCA	1624
AD-60152.1	UfsusUfgAfcCfuCfaUfgfgGfuGfuUfcGfuGfL96	1511	csAfsuGfaAfcAfcfaugAfgGfuCfaAfasgs	1568	CCUUGACCUC AUGGUGUUCGUG	1625
AD-60197.1	CfsasCfuGfuGfcAfaGfgfaCfuUfcCfuAfaAfL96	1512	usUfsuAfgGfaAfgUfcuUfgGfcAfcAfgUfsgsac	1569	GUCACUGUGCAAGACUUCUAAA	1626
AD-60191.1	CfsusGfgUfuGfuGfgGfAfcCfaUfaGfaAfgAfL96	1513	usCfsuUfcUfaUfgGfuccAfcAfaCfcAfgscsu	1570	AGCUGGUUGGACCAUAGAAGA	1627
AD-60154.1	UfsgsUfuAfaAfuGfgGfuGfaUfcCfuGfgAfL96	1514	usCfscAfgGfaUfcAfgccAfuUfuAfaCfasgs	1571	GCUGUAAAUGGCGAUCCUGGA	1628
AD-60167.1	GfsgsAfuCfuGfuGfgGfgCfaGfaCfcUfL96	1515	asGfsgGfgUfcUfgCfcacAfcAfgAfuCfscsu	1572	AGGGAUCUGUGGACAGACCCU	1629
AD-60177.1	AfscsAfgAfaAfaGfAfcfcAfuCfuAfcAfcAfL96	1516	gsGfsuGfuAfgAfuGfgucUfuGfuCfuGfusesu	1573	AGACAGACAAGACCAUCUACACC	1630
AD-60195.1	GfsusAfcGfuGfaCfaGfgfuGfgUfgGfcAfaAfL96	1517	usUfsuGfcCfaCfcAfcuUfgAfcGfuAfcusuu	1574	AAGUACGUGACAGUGGUGGCAAA	1631
AD-60193.1	GfsgsUfcAfuGfaAfcfaCfuUfuCfuCfaAfL96	1518	usUfsgAfgAfaAfgAfguUfcAfuGfaCfcsusu	1575	AAGGUCAUGAACUUCUUCUCAA	1632
AD-60202.1	GfsusCfuGfgCfcAfgGfaGfgAfaUfgAfgAfL96	1519	usCfsuCfaUfuCfcUfuccUfgGfgCfaCfGfasescu	1576	AGGUGUGCCAGAAGGAAUGAGA	1633
AD-60182.1	CfsasAfgAfaAfgGfgAfuCfuGfuGfuGfgCfL96	1520	gsCfscAfcAfcAfgAfcuUfuCfuUfsgsusC	1577	GACAAGAAAGGGAUCUGUGUGGC	1634
AD-60203.1	CfsusGfuGfcAfaGfAfcfuUfcCfuAfaAfgAfL96	1521	usCfsuUfuAfgGfaAfgucUfuGfcAfcAfgsus	1578	CACUGUGCAAGACUUCUCAAAGA	1635
AD-60157.1	CfscsAfgAfuCfcAfcUfuCfaCfcAfaGfaAfL96	1522	usUfscUfuGfgUfgAfguGfgAfuCfuGfsgsu	1579	UACCAGAUCACUUCACCAAAGAC	1636
AD-60192.1	GfscsUfgCfuGfaAfaGfgfaCfuUfuGfaCfuCfL96	1523	gsAfsuUfcAfaAfgUfcuUfcAfgCfaGfscs	1580	CUGCUGUGAAAGACUUCACUC	1637

TABLE 8

C3 single dose screen in Hep3B Cells				
Duplex name	10 nM		0.1 nM	
	Avg	SD	Avg	SD
AD-60165.1	5.54	0.43	13.05	2.69
AD-60153.1	20.13	22.87	22.40	3.76
AD-60175.1	24.94	4.74	42.14	7.68
AD-60169.1	10.66	6.66	49.63	17.36
AD-60187.1	9.49	3.28	51.15	11.65
AD-60149.1	7.49	7.75	55.90	4.41
AD-60171.1	23.77	7.94	60.35	7.27
AD-60160.1	11.49	4.68	60.48	11.60
AD-60206.1	45.64	41.01	63.62	9.90
AD-60176.1	17.30	8.83	66.19	13.81
AD-60156.1	18.22	0.80	66.39	1.67
AD-60155.1	20.48	2.10	68.97	1.73
AD-60183.1	13.82	8.64	69.24	3.35
AD-60161.1	27.49	10.88	80.57	16.13
AD-60166.1	35.21	21.31	81.66	14.48
AD-60201.1	24.60	10.68	83.80	4.87
AD-60179.1	15.21	3.55	85.30	23.07
AD-60164.1	33.93	4.07	85.93	1.00
AD-60186.1	72.24	6.37	88.39	51.31
AD-60162.1	49.58	3.76	89.22	6.06
AD-60189.1	52.59	4.25	89.41	20.79
AD-60178.1	22.65	5.82	90.84	15.23
AD-60194.1	37.79	4.71	91.29	7.32
AD-60199.1	21.84	3.46	91.78	7.69
AD-60174.1	16.68	0.45	92.68	45.25
AD-60159.1	27.58	4.77	92.91	2.22
AD-60168.1	26.91	2.50	92.99	5.86
AD-60198.1	84.91	36.10	93.04	7.23
AD-60180.1	45.91	16.19	93.35	28.54
AD-60190.1	16.67	1.22	95.38	11.83
AD-60188.1	55.44	7.05	95.66	30.36
AD-60205.1	80.10	32.44	96.81	19.27
AD-60184.1	26.47	9.64	97.94	9.88
AD-60163.1	91.18	5.14	99.19	21.40
AD-60172.1	143.57	8.09	99.22	11.58
AD-60151.1	24.05	14.22	101.65	8.27
AD-60196.1	67.05	1.31	102.53	11.85
AD-60204.1	78.88	5.99	103.20	7.01
AD-60185.1	41.42	7.77	103.45	2.47
AD-60170.1	52.73	2.71	104.43	22.03
AD-60158.1	81.03	14.03	105.18	14.20
AD-60200.1	119.02	22.61	106.55	15.55
AD-60173.1	100.25	12.25	108.80	44.49
AD-60181.1	63.50	10.07	109.82	14.56
AD-60152.1	16.58	10.66	112.51	19.82
AD-60197.1	96.07	21.94	112.70	27.14
AD-60191.1	101.85	48.90	112.76	8.49
AD-60154.1	24.21	8.93	112.90	25.58
AD-60167.1	106.64	8.09	115.02	39.17
AD-60177.1	50.71	20.19	116.18	1.49
AD-60195.1	128.54	29.35	116.68	11.92
AD-60193.1	84.93	4.99	117.62	3.53
AD-60202.1	90.00	20.98	119.83	16.90
AD-60182.1	110.82	1.09	121.62	6.78
AD-60203.1	75.57	6.66	125.15	34.15
AD-60157.1	29.07	5.80	125.72	8.08
AD-60192.1	83.74	7.99	141.55	1.82

Example 3. In Vivo Activity

In order to determine the in vivo efficacy of the agents, wild-type (C57BL/6) mice (n=3) were subcutaneously administered a single 1 mg/kg, 3 mg/kg, 5 mg/kg, or 10 mg/kg dose of AD-80806; or a single 1 mg/kg, 3 mg/kg, 5 mg/kg, 10 mg/kg, or 20 mg/kg dose of AD-80807; or PBS as a control. At day 7 post-dose, animals were sacrificed, liver samples were collected and the liver level of C3 mRNA was determined by QRT-PCR. The modified sense and antisense strand nucleotide sequences of AD-80806 and AD-80807 are shown in Table 9 below:

TABLE 9

Duplex ID	Sense (5' to 3')	SEQ ID	Antisense	SEQ ID
		NO: (5' to 3')	NO: (5' to 3')	NO:
AD-80806	asascaagAfaGf AfAfcaaacucac aL96	1638 usGfsugaGfuuuug	uucUfuCfuuguuus csa	1640
AD-80807	csgsugacAfgUf GFgfuggcaaacu uL96	1639 asAfsquUfgcca	ccaCfuGfucacgs usa	1641

As shown in Table 10A, which provides the relative levels of C3 mRNA in mouse livers, with respect to the level of C3 in the livers of PBS-injected mice, both AD-80806 and AD-80807 effectively knock down C3 mRNA at all doses tested.

The in vivo efficacy of AD-80806 and AD-80807 was also assessed in Sprague-Dawley (wild-type) rats. In particular, rats (n=3) were subcutaneously administered a single 1 mg/kg, 3 mg/kg, 5 mg/kg, or 10 mg/kg dose of AD-80806; or a single 1 mg/kg, 3 mg/kg, 5 mg/kg, or 10 mg/kg dose of AD-80807; or PBS as a control. At day 7 post-dose, animals were sacrificed and the liver level of C3 mRNA was determined by QRT-PCR. As shown in Table 10B, which provides the levels of C3 in rat livers, relative to the level of C3 in the livers of PBS-injected rats, a single 10 mg/kg dose of AD-80806 effectively knocks down C3 mRNA.

In separate studies, the in vivo efficacy of AD-80806 was also assessed in Sprague-Dawley (wild-type) rats. In particular, rats (n=5) were subcutaneously administered a single 15 mg/kg dose of AD-80806; or PBS as a control. At day 11 post-dose, animals were sacrificed and the liver level of C3 mRNA was determined by QRT-PCR. As shown in Table 10C, a single 15 mg/kg dose of AD-80806 effectively knocks down C3 mRNA levels.

TABLE 10A

In vivo C3 single dose screen in wild-type mice								
Species	Strain	Dosing regimen	Duplex ID	Dose		C3 mRNA remaining		C3 Knockdown
				Conc	N	Avg	Stdev	
Mouse	WT (C57Bl/6)	Single	AD-80806	1 mg/kg	3	20.1%	4.1%	79.9%
				3 mg/kg	3	13.7%	8.3%	86.3%
				5 mg/kg	3	3.9%	3.0%	96.1%
				10 mg/kg	3	2.1%	0.4%	97.9%

TABLE 10A-continued

In vivo C3 single dose screen in wild-type mice								
Species	Strain	Dosing regimen	Duplex ID	Dose		C3 mRNA remaining		C3
				Conc	N	Avg	Stdev	Knockdown
			AD-80807	1 mg/kg	3	37.7%	22.5%	62.3%
				3 mg/kg	3	17.3%	12.1%	82.7%
				5 mg/kg	3	5.1%	2.4%	94.9%
				10 mg/kg	3	11.6%	14.4%	88.4%
				20 mg/kg	3	2.3%	2.1%	97.7%
				PBS	Control	3	100.0%	14.0%

TABLE 10B

In vivo C3 single dose screen in wild-type rats								
Species	Strain	Dosing regimen	Duplex ID	Dose		C3 mRNA remaining		C3
				Conc	N	Avg	Stdev	Knockdown
Rat	WT (Sprague-Dawley)	Single	AD-80806	1 mg/kg	3	84.16%	17.8%	15.8%
				3 mg/kg	3	79.58%	22.9%	20.4%
				5 mg/kg	3	66.02%	3.7%	34.0%
				10 mg/kg	3	38.14%	1.4%	61.9%
				AD-80807	1 mg/kg	3	98.56%	9.8%
			3 mg/kg	3	73.62%	2.0%	26.4%	
			5 mg/kg	3	62.78%	14.7%	37.2%	
			10 mg/kg	3	55.28%	7.4%	44.7%	
			PBS	Control	3	100.00%	18.7%	

TABLE 10C

In vivo C3 single dose screen in wild-type rats								
Species	Strain	Dosing regimen	Duplex ID	Dose		C3 mRNA remaining		C3
				Conc	N	Avg	Stdev	Knockdown
Rat	WT (Sprague-Dawley)	Single	AD-80806	15 mg/kg	5	11.00%	6.0%	89.0%
			PBS	Control	5	100.00%	5.0%	
Rat	WT (Sprague-Dawley)	Single	AD-80806	15 mg/kg	5	11.00%	3.0%	89.0%
			PBS	Control	5	100.00%	10.0%	
Mouse	Lupus model (MRL/lpr)	Multiple q3W	AD-80806	10 mg/kg	10	2.00%	0.3%	98.0%
			PBS	Control	10	100.00%	27.0%	

Example 4. Efficacy of Multiple Doses of AD-80806 in an Animal Model of Systemic Lupus Erythematosus

The efficacy of multiple doses of AD-80806 was assessed in an art-recognized animal model of systemic lupus erythematosus, MRL/lpr mice. Specifically, at day 0, 8-week old animals were subcutaneously administered a 10 mg/kg dose of AD-80806 (n=10); or PBS (n=16) as a control, and subsequently administered additional 10 mg/kg doses of AD-80806 every three weeks (q3w) thereafter for a total of 17 weeks. At day 119, animals were sacrificed and the serum level of C3 mRNA was determined by QRT-PCR. Serum samples were also collected retroorbitally at 8 weeks (pre-first dose), 11 weeks, 14 weeks, 17 weeks, and 25 weeks of age and the level of serum C3 protein was determined using an ELISA assay. In addition, spot urine was collected at 7 weeks (pre-dose), 11 weeks, 16 weeks, 20 weeks, and 25 weeks of age and the levels of urine albumin and urine creatinine were determined. Seventeen weeks after the initiation of treatment (at 25 weeks of age) or if the animal reached a humane endpoint prior to the end of the study, animals were sacrificed and tissues and serum were harvested for qPCR and complement activity assessment.

Tables 11A and 11B demonstrate that a multi-dose 10 mg/kg regimen of AD-80806 efficaciously knocks down C3

mRNA in the liver and C3 protein in serum; at the end of the study, an average of only 2% of the C3 transcript remained in the liver of the treated animals, when compared to C3 levels in PBS-injected mice, whereas 5.8% of protein remained in circulation in the serum compared to PBS treated animals. Consistent with the reduction in C3 protein levels a significant reduction in the complement alternative pathway activity as measured by a rabbit erythrocyte hemolysis assay was observed with AD-80806 treatment relative to PBS treated animals (Table 11F). Tables 11C and 11D demonstrate that a multi-dose 10 mg/kg administration of AD-80806 at three-week intervals reduced the levels of urinary albumin (Table 11C) and urinary creatinine (Table 11D).

Furthermore, although there was no significant reduction in the urine albumin to urine creatine ratio (indicative of microalbuminuria in these animals) (Table 11E), there was a significant decrease in the alternative pathway hemolytic activity (AH50) in the serum as determined by a rabbit erythrocyte assay (Table 11F) in animals receiving a multi-dose 10 mg/kg regimen of AD-80806 for 17 weeks. Furthermore, there was significant increase in survival of the animals (Table 11G). Indeed, at day 105, 90% of the animals that were administered AD-80806 at three-week intervals had survived, while only 56.3% of the control animals had survived.

In addition, histopathological analyses of the kidneys of these animals demonstrate that there were significantly fewer pathological changes in the glomeruli and tubules of the MRL/lpr mice receiving the q3w regimen of AD-80806 as compared to controls (Tables 11H and 11I).

TABLE 11A

In vivo C3 Multi-Dose Treatment in MRL/lpr Mice									
Species	Strain	Dosing regimen	Duplex ID	Dose		C3 mRNA remaining		C3	
				Conc	N	Avg	Stdev	Knockdown	
Mouse	Lupus model (MRL/lpr)	Multiple q3W	AD-80806	10 mg/kg	10	2.00%	0.3%	98.0%	
			PBS	Control	16	100.00%	27.0%		

TABLE 11B

C3 Protein Levels in MRL/lpr Mice							
Duplex ID	Dose Conc	N	C3 protein remaining (normalized to Day 0 values)				
			Day 0 Average	Day 21 Average	Day 42 Average	Day 63 Average	Day 111 Average
AD-80806	10 mg/kg	10	100.0%	21.8%	13.9%	9.9%	5.8%
PBS	Control	16	100.0%	108.2%	71.4%	66.9%	66.4%

TABLE 11C

Urine Albumin Levels in MRL/lpr Mice							
Duplex ID	Dose Conc	N	Urine Albumin levels (ug/mL)				
			Day 0 Average	Day 21 Average	Day 42 Average	Day 63 Average	Day 111 Average
AD-80806	10 mg/kg	10	3.95	33.50	173.65	160.04	208.61
PBS	Control	16	2.01	136.33	273.14	263.60	293.66

TABLE 11D

Urine Creatinine Levels in MRL/lpr Mice							
Duplex ID	Dose Conc	N	Urine Creatinine levels (ug/mL)				
			Day 0 Average	Day 21 Average	Day 42 Average	Day 63 Average	Day 111 Average
AD-80806	10 mg/kg	10	202.25	239.80	214.20	120.17	129.05
PBS	Control	16	237.17	221.46	275.17	137.42	170.37

TABLE 11E

Urine Albumin to Creatinine Ratio in MRL/lpr Mice							
Duplex ID	Dose Conc	N	Urine Albumin to Creatinine ratio				
			Day 0 Average	Day 21 Average	Day 42 Average	Day 63 Average	Day 111 Average
AD-80806	10 mg/kg	10	0.02	0.14	0.89	1.51	3.11
PBS	Control	16	0.01	0.51	0.98	2.00	3.11

TABLE 11F

Alternative Complement Hemolytic Activity					
Duplex ID	Dose Conc	N	% Hemolysis (Normalized to controls)		
			Average	Stdev	
AD-80806	10 mg/kg	10	19.3%	12.9%	
PBS	Control	16	52.4%	15.4%	

TABLE 11G

Survival							
Duplex ID	Dose Conc	N	Survival Curve				
			Day 0	Day 42	Day 49	Day 70	Day 105
AD-80806	10 mg/kg	10	100.0%	100.0%	90.0%	90.0%	90.0%
PBS	Control	16	100.0%	87.5%	75.0%	62.5%	56.3%

TABLE 11H

Kidney Histology-Glomerulonephritis Score							
Duplex ID	Dose Conc	N	Glomerulopathy Score (0 = Normal 4 = Severe, % in each grade)				
			Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
AD-80806	10 mg/kg	10	0.0%	0.0%	50.0%	40.0%	10.0%
PBS	Control	16	0.0%	0.0%	25.0%	18.8%	56.3%

TABLE 11I

Kidney Histology-Tubular Change Score							
Duplex ID	Dose Conc	N	Tubular Score (0 = Normal 4 = Severe, % in each grade)				
			Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
AD-80806	10 mg/kg	10	30.0%	30.0%	30.0%	0.0%	10.0%
PBS	Control	16	0.0%	25.0%	18.8%	12.5%	43.8%

EQUIVALENTS

As may be recognized by those of ordinary skill in the pertinent art based on the teachings herein, numerous changes and modifications may be made to the above-described and other embodiments of the present disclosure without departing from the spirit of the invention as defined

in the appended claims. Accordingly, this detailed description of embodiments is to be taken in an illustrative, as opposed to a limiting, sense. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the described herein. Such equivalents are intended to be encompassed by the following claims.

```

>gi|726965399|ref|NM_000064.3| Homo sapiens complement component 3 (C3), mRNA
SEQ ID NO: 1
AGATAAAAAGCCAGCTCCAGCAGGCGTGCTCACTCCTCCCCATCCTCTCCCTCTGTCCCTCTGTCCCTC
TGACCCCTGCACTGTCCCAGCACCATGGGACCCACCTCAGGTCCCAGCCTGCTGCTCTGTACTAACC
CCTCCCCCTGGCTCTGGGGAGTCCCATGTACTCTATCATCACCCCAACATCTTGGCGCTGGAGAGCGAG
GAGACCATGGTGTCTGGAGGCCACGACGCGCAAGGGGATGTTCCAGTCACTGTACTGTCCACGACTTCC
CAGGCAAAAAACTAGTGTCTCCAGTGAGAAGACTGTGCTGACCCCTGCCACCAACCACATGGGCAACGT
CACCTTCACGATCCAGCCAACAGGGAGTTCAGTCAGAAAAGGGGCGCAACAAGTTCGTGACCGTGCAG
GCCACCTTCGGGACCCAAGTGGTGGAGAAGTGGTGTGGTTCAGCCTGCAGAGCGGGTACCTCTTCATCC
AGACAGACAAGACCATCTACACCCCTGGCTCCACAGTTCTCTATCGGATCTTCCAGTCAACCACAAGCT
GCTACCCGTGGGCCGGACGGTCAATGGTCAACATTGAGAACC CGGAAGGCATCCCGGTCAAGCAGGACTCC
TTGTCTTCTCAGAACCAGCTTGGCGTCTTGCCCTTGTCTTGGGACATTCGGAACTCGTCAACATGGGCC
AGTGAAGATCCGAGCTACTATGAAAACACACAGCAGGTCTTCTCCACTGAGTTTGAGGTGAAGGA
GTACGTGCTGCCAGTTTCGAGGT CATAGTGGAGCCTACAGAGAAATCTACTACATCTATAACGAGAAG
GGCCTGGAGGTCACCATCACCGCCAGGTTCTCTACGGGAAGAAAGTGGAGGGAAC TGCCTTTGTCATCT
TCGGGATCCAGGATGGCGAACAGAGGATTTCCCTGCCTGAATCCCTCAAGCGCATCCGATTGAGGATGG
CTCGGGGGAGGTTGTGCTGAGCCGGAAGGTACTGCTGGACGGGGTG CAGAACC CCGGAGCAGAAGACCTG
GTGGGGAAGTCTTTGTACGTGTCTGCCACCGTCACTTGC ACTCAGGCAGTGACATGGTGCAGGCAGAGC
GCAGCGGGATCCCCATCGTGACCTCTCCCTACCAGATCCACTTCACCAAGACACCAAGTACTTCAAACC
AGGAATGCCCTTTGACCTCATGGTGTTCGTGACGAACCTGATGGCTCTCCAGCCTACCGAGTCCCGTG
GCAGTCCAGGCGAGGACACTGTGCAGTCTCTAACCCAGGGAGATGGCGTGGC CAAACTCAGCATCAACA
CACACCCAGCAGAAAGCCCTTGAGCATCACGGTGCACGACGAGAAGCAGGAGCTCTCGGAGGCAGAGCA
GGCTACCAGGACCATGCAGGCTCTGCCCTACAGCACCGTGGGCAACTCCAACAATTACCTGCATCTCTCA
GTGCTACGTACAGAGCTCAGACCCGGGAGACCCTCAACGTCAACTTCCTCCTGCGAATGGACCGCGCCC
ACGAGGCCAAGATCCGCTACTACACTACCTGATCATGAACAAGGGCAGGCTGTTGAAGGCGGGACGCCA
GGTGCAGAGCCCGGCCAGGACCTGGTGGTGTGCCCTGTCCATCACCACCGACTTCATCCCTTCCTTC
CGCCTGGTGGCGTACTACACGCTGATCGGTGCCAGCGGCCAGAGGGAGGTGGTGGCCGACTCCGTGTGGG
TGGACGTCAAGGACTCTGCGTGGGCTCGCTGGTGGTAAAAAGCGGCCAGTCAGAAGACCGGCAGCCTGT
ACCTGGGCAGCAGATGACCCTGAAGATAGAGGGTGACCACGGGGCCCGGTGGTACTGGTGGCCGTGGAC
AAGGGCGTGTTCGTGCTGAATAAGAAGAACAAACTGACGCAGAGTAAGATCTGGGACGTGGTGGAGAAGG
CAGACATCGGTGCACCCCGGGCAGTGGGAAGGATTACGCCGGTGTCTTCTCCGACGCAGGGCTGACCTT
CACGAGCAGCAGTGGCCAGCAGACCGCCAGAGGGCAGAACTTCAGTGCCCGCAGCCAGCCGCCGCCGA
CGCCGTTCCGTGCAGCTCACGGAGAAGCGAATGGACAAAGTCGGCAAGTACCCCAAGGAGCTGCGCAAGT
GCTGCGAGGACGGCATGCGGAGAACCCATGAGGTTCTCGTGCCAGCGCGGACCCGTTTCATCTCCCT
GGGCGAGGCGTGCAAGAAGGTTCTTCTGGACTGTGCAACTACATCACAGAGCTGCGGGCGGCAGCACGG
CGGGCCAGCCACCTGGGCTGGCCAGGAGTAACCTGGATGAGGACATCATTGCAGAAGAGAACATCGTTT

```


- continued

CCCCAAGTGAGTTCACAGAGAGCTGGCTGTGGAACTTGAGGACTTGAAAGAGCCACCAGAAAAATGGAAT
CTCTACGAAGCTCATGAATATATTTTTGAAAGACTCCATCACCACGTGGGAGATTCTGGCTGTGAGCATG
TCGGACAAGAAAGGGATCTGTGTGGCAGACCCCTTCGAGGTCACAGTAATGCAGGACTTCTTCATCGACC
TGCGGCTACCCCTACTCTGTGTTCGAAACGAGCAGGTGGAAATCCGAGCCGTTCTCTACAATTACCGGCA
GAACCAAGAGCTCAAGGTGAGGGTGAAGTACTCCACAATCCAGCCTTCTGCAGCCTGGCCACCACCAAG
AGGCGTACCAGCAGACCGTAACCATCCCCCAAGTCTCGTTGTCCGTTCCATATGTCATCGTGCCGC
TAAAGACCGGCTGCAGGAAGTGAAGTCAAGGCTGCTGTCTACCATCATTTTCATCAGTGACGGTGTGAG
GAAGTCCCTGAAGTCTGTCGCGGAAGGAATCAGAATGAACAAAAGTGTGGCTGTTTCGCACCTGGATCCA
GAACGCCTGGGCGTGAAGGAGTGCAGAAAGAGGACATCCACCTGCAGACCTCAGTGACCAAGTCCCGG
ACACCGAGTCTGAGACCAGAATTCTCTGCAAGGGACCCAGTGGCCAGATGACAGAGGATGCCGTGCA
CGCGAACGGCTGAAGCACCTCATTGTGACCCCTCGGGCTGCGGGGAACAGAACATGATCGGCATGACG
CCCACGGTCATCGCTGTGCATTACCTGGATGAAACGAGCAGTGGGAGAAGTTCGGCCTAGAGAAGCGGC
AGGGGGCTTGGAGCTCATCAAGAAGGGGTACACCCAGCAGCTGGCCTCAGACAACCAGCTCTGCCTT
TGCGGCTTCGTGAACGGGCACCCAGCACCTGGCTGACCGCCTACGTGGTCAAGGTCTTCTCTCTGGCT
GTCAACCTCATCGCCATCGACTCCAAGTCTCTCGGGGCTGTTAAATGGCTGATCCTGGAGAAGCAGA
AGCCCGACGGGTCTTCCAGGAGGATGCGCCCGTGATACCAAGAAATGATTGGTGGATTACGGAACAA
CAACGAGAAGACATGGCCCTCACGGCCTTTGTTCTCATCTCGCTGCAGGAGGCTAAAGATATTTGCGAG
GAGCAGGTCAACAGCCTGCCAGGCAGCATCACTAAAGCAGGAGACTTCTTGAAGCCAACACATGAACC
TACAGAGATCTACACTGTGGCCATGTCTGGCTATGCTCTGGCCAGATGGGCAGGCTGAAGGGGCTCT
TCTTAACAAATTTCTGACCACGCCAAAGATAAGAACCCTGGGAGGACCCCTGGTAAGCAGCTCTACAAC
GTGGAGGCCACATCTATGCCCTCTTGCCCTACTGCAGCTAAAAGACTTTGACTTTGTGCCTCCCGTCG
TGCGTTGGCTCAATGAACAGAGATACTACGGTGGTGGCTATGGCTCTACCCAGGCCACCTTCATGGTGT
CCAAGCCTTGCTCAATACCAAAAAGGACGCCCTGACCACCAGGAACGAACCTTGATGTGTCCCTCCAA
CTGCCAGCCGAGCTCCAAGATCACCCACCGTATCCACTGGGAATCTGCCAGCCTCCTGCGATCAGAAG
AGACCAAGGAAAATGAGGGTTTCACAGTCAACAGCTGAAGGAAAAGGCCAAGGCACCTTGTGCGTGGTAC
AATGTACCATGCTAAGGCCAAAGATCAACTCACCTGTAATAAATTCGACCTCAAGGTACCATAAAACCA
GCACCGAAAACAGAAAAGAGCCCTCAGGATGCCAAGAACACTATGATCCTTGAGATCTGTACCAGGTACC
GGGAGACAGGATGCCACTATGTCTATATTTGGACATATCCATGATGACTGGCTTTGTCCAGACACAGA
TGACCTGAAGCAGCTGGCCAATGGTGTGACAGATACATCTCCAAGTATGAGCTGGACAAGCCCTTCTCC
GATAGGAACACCCCTCATCATCTACCTGGACAAGGTCTCACACTCTGAGGATGACTGTCTAGCTTTCAAAG
TTCACCAATACTTTAATGTAGAGCTTATCCAGCCTGGAGCAGTCAAGGTCTACGCCATTACAACTGGA
GGAAAGCTGTACCCGGTCTACCATCCGAAAAGGAGGATGGAAAGCTGAACAAGCTTGCCGTGATGAA
CTGTGCCGCTGTCTGAGGAGAATTGCTTACATAAAAAGTCGGATGACAAGGTCAACCTGGAAGAACGGC
TGGACAAGGCCTGTGAGCCAGGAGTGGACTATGTGTACAAGACCCGACTGGTCAAGGTTGAGCTGTCCAA
TGACTTTGACGAGTACATCATGGCCATTGAGCAGACCATCAAGTCAGGCTCGGATGAGGTGCAGGTGGA
CAGCAGCGCACGTTTATCAGCCCCATCAAGTGCAGAGAAGCCCTGAAGCTGGAGGAGAAGAAACACTACC
TCATGTGGGTCTCTCCCTCCGATTTCTGGGAGAGAAGCCCAACCTCAGCTACATCATCGGGAAGGACAC
TTGGTGGAGCACTGGCCCGAGGAGGACGAATGCCAAGACGAAGAGAACCAGAAAACATGCCAGGACCTC
GGCCCTTACCAGAGCATGGTTGTCTTTGGGTGCCCAACTGACCACACCCCATCCCCACTCCAG
ATAAAGCTTCAGTTATATCTCAAAAAAAAAAAAAAAAAA

Reverse Complement of SEQ ID NO: 1

>gi|726965399|ref|NM_000064.3| *Homo sapiens* complement component 3 (C3), mRNA

SEQ ID NO: 2

AGATAAAAAGCCAGCTCCAGCAGGCGCTGCTCACTCCTCCCCATCCTCCTCTGTCCCTCTGTCCCTC
 TGACCCCTGCACCTGTCCCAGCACCATGGGACCCACCTCAGGTCCCAGCCTGTGCTCCTGCTACTAACCCA
 CCTCCCCCTGGCTCTGGGGAGTCCCATGTACTCTATCATCACCCCCAACATCTTGGCGTGGAGAGCGAG
 GAGACCATGGTGTGGAGGCCACGACGCGCAAGGGATGTTCCAGTCACTGTTACTGTCCAGCACTTCC
 CAGGCAAAAACTAGTGTGTCCAGTGAGAAGACTGTGCTGACCCCTGCCACCAACCACATGGGCAACGT
 CACCTTACAGATCCAGCCACAGGGAGTTCAAGTCAGAAAAGGGCGCAACAAGTTCGTGACCGTGCAG
 GCCACCTTCGGGACCCAAGTGGTGGAGAAGGTGGTGTGGTCCAGCTGCAGAGCGGGTACCTCTTCATCC
 AGACAGACAAGACCATCTACACCCCTGGCTCCACAGTTCTCTATCGGATCTTCACCGTCAACCACAAGCT
 GCTACCCGTGGGCCGAGCGGTATGGTCAACATTGAGAACC CGAAGGCATCCCGGTCAAGCAGGACTCC
 TTGTCTTCTCAGAACCAGCTTGGCGCTTTCGCCCTGTCTTGGGACATTCGGAACTCGTCAACATGGGCC
 AGTGGAAGATCCGAGCCTACTATGAAACTCACCACAGCAGGTCTTCTCCACTGAGTTTGAGGTGAAGGA
 GTACGTGCTGCCAGTTTTCAGGTCATAGTGGAGCCTACAGAGAAATTTACTACATCTATAACGAGAAG
 GGCTTGGAGGTACCATCACCGCCAGGTTCCTACGGGAAGAAAGTGGAGGAACTGCCTTTGTCTATCT
 TCGGGATCCAGGATGGCGAACAGAGGATTTCCCTGCCTGAATCCCTCAAGCGCATTCGATTGAGGATGG
 CTCGGGGGAGGTTGTGTGAGCCGGAAGTACTGTGTGGACGGGTGCAGAACCCCGAGCAGAAGACCTG
 GTGGGAAGTCTTTGTACGTGTCTGCCACCGTCACTTGCACCTCAGGCACTGACATGGTGCAGGCAGAGC
 GCAGCGGATCCCCATCGTGACCTCTCCCTACCAGATCCACTTCACCAAGACACCCAAGTACTTCAAACC
 AGGAATGCCCTTTGACCTCATGGTGTTCGTGACGAACCTGATGGCTCTCCAGCCTACCGAGTCCCGTG
 GCAGTCCAGGGCGAGGACACTGTGCAGTCTCTAACCCAGGGAGATGGCGTGGCCAAACTCAGCATCAACA
 CACACCCAGCCAGAAGCCCTTGAGCATCACGGTCCGCACGAAGAAGCAGGAGCTCTCGGAGGCAGAGCA
 GGCTACCAGGACCATGCAGGCTCTGCCCTACAGCACCGTGGGCAACTCCAACAATTACCTGCATCTCTCA
 GTGTACGTACAGAGCTCAGACCCGGGAGACCTCAACGTCAACTTCTCCTGCGAATGGACCGCGCCC
 ACGAGGCCAAGATCCGCTACTACACCTACCTGATCATGAACAAGGGCAGGCTGTTGAAGGCGGGACGCCA
 GGTGCGAGAGCCCGGCCAGGACCTGGTGGTGTGCCCTGTCCATCACACCGACTTCATCCCTTCCCTTC
 CGCTGGTGGCGTACTACACGTGATCGGTGCCAGCGCCAGAGGGAGGTGGTGGCCGACTCCGTGTGGG
 TGGACGTCAAGGACTCCTGCGTGGGCTCGCTGGTGGTAAAAAGCGGCCAGT CAGAAGACCGGCAGCCTGT
 ACCTGGGCAGCAGATGACCTGAAGATAGAGGGTGACCAGGGGCCCGGGTGGTACTGGTGGCCGTGGAC
 AAGGGCGTGTTCGTCTGAATAAGAAGAACAACCTGACGCAGAGTAAGATCTGGGACGTGGTGGAGAAGG
 CAGACATCGGCTGCACCCCGGCAGTGGGAAGGATTACGCCGGTGTCTTCTCCGACGCAGGGCTGACCTT
 CACGAGCAGCAGTGGCCAGCAGACCGCCAGAGGGCAGAATTTCAGTCCCCGAGCCAGCCGCCCGCCGA
 CGCCGTTCCGTGCAGCTCACGGAGAAGCGAATGGACAAAAGTCGGCAAGTACCCCAAGGAGCTGCACAAGT
 GCTGCGAGGACGGCATGCGGGAGAACCCATGAGGTTCTCGTGCCAGCGCCGACCCGTTTCATCTCCCT
 GGGCGAGGCGTGAAGAAGGTTCTCCTGGACTGCTGCAACTACATCACAGAGCTGCGGCGGCAGCACGCG
 CGGGCCAGCCACTGGGCTGCCAGGAGTAACCTGGATGAGGACATCATTGAGAAGAGAACAATCGTTT
 CCCGAAGTGAGTTCAGAGAGCTGGCTGTGGAACTGAGGACTGAAAAGAGCCACCGAAAAATGGAAT
 CTCTACGAAGCTCATGAATATATTTTGAAGACTCCATCACACCGTGGGAGATCTGGCTGTGAGCATG
 TCGGACAAGAAGGATCTGTGTGGCAGACCCCTTCGAGGTCACAGTAATGCAGGACTTCTTCATCGACC
 TGGCGCTACCTACTCTGTGTTCGAAACGAGCAGGTGGAAATCCGAGCCGTTCTCTACAATTACCGGCA
 GAACCAAGAGCTCAAGGTGAGGGTGGAACTACTCCACAATCCAGCCTTCTGCAGCCTGGCCACCACCAAG

- continued

AGGCGTACCAGCAGACCGTAACCATCCCCCAAGTCTCGTTGTCGGTCCATATGTCATCGTGCCG
 TAAAGACCGGCTGCAGGAAGTGAAGTCAAGGCTGCTGTCTACCATCATTTTCATCAGTGACGGTGTG
 GAAGTCCCTGAAGTCTGCCGGAAGGAATCAGAATGAACAAAACGTGGCTGTTTCGCACCTGGATCCA
 GAACGCCTGGGCGTGAAGGAGTGCAGAAAGAGGACATCCCACCTGCAGACCTCAGTGACCAAGTCCCG
 ACACCGAGTCTGAGACCAGAATTCTCTGCAAGGGACCCAGTGGCCAGATGACAGAGGATGCCGTGCA
 CGCGAACGGCTGAAGCACCTCATTGTGACCCCCGCGGCTGCGGGGAACAGAACATGATCGGCATGACG
 CCCACGGTTCATCGTGTGATTAACCTGGATGAAACGAGCAGTGGGAGAAGTTCGGCCTAGAGAAGCGGC
 AGGGGGCCTTGGAGCTCATCAAGAAGGGGTACACCCAGCAGCTGGCCTCAGACAACCCAGCTCTGCCTT
 TGCGGCTTCGTGAACGGGCACCCAGCACCTGGCTGACCGCTACGTGGTCAAGTCTTCTCTCTGGCT
 GTCAACCTCATCGCCATCGACTCCAAGTCTCTGCGGGCTGTTAAATGGCTGATCCTGGAGAAGCAGA
 AGCCCGACGGGCTTCCAGGAGGATGCGCCCGTGATACCAAGAAATGATTGGTGGATTACGGAAACA
 CAACGAGAAAGACATGGCCCTCACGGCCTTTGTTCTCATCTCGCTGCAGGAGGCTAAAGATATTTGCGAG
 GAGCAGGTCAACAGCCTGCCAGGCAGCATCACTAAAGCAGGAGACTTCCCTGAAGCCAACACATGAACC
 TACAGAGATCCTACACTGTGGCCATGCTGGCTATGCTCTGGCCAGATGGGAGGCTGAAGGGGCTCT
 TCTTAACAAATTTCTGACCACGCCAAAGATAAGAACCGCTGGGAGGACCCCTGGTAAGCAGCTCTACAAC
 GTGGAGGCCACATCCTATGCCCTCTTGCCCTACTGCAGCTAAAAGACTTTGACTTTGTGCCTCCCGTCG
 TCGTTGGCTCAATGAACAGAGATACTACGGTGGTGGCTATGGCTCTACCCAGGCCACCTTCATGGTGT
 CCAAGCCTTGGCTCAATACCAAAAGGACGCCCTGACCACCAGGAACGAACCTTGATGTGCTCCCA
 CTGCCAGCCGAGCTCCAAGATCACCCACCGTATCCACTGGGAATCTGCCAGCCTCCTGCGATCAGAAG
 AGACCAAGGAAATGAGGGTTTACAGTACAGCTGAAGGAAAAGGCCAAGGCACCTTGTGGTGGTGGAC
 AATGTACCATGCTAAGGCCAAAGATCAACTCACCTGTAATAAATTCGACCTCAAGGTACCAATAAACCA
 GCACCGAAAACAGAAAAGAGGCTCAGGATGCCAAGAACACTATGATCCTTGAGATCTGTACAGGTACC
 GGGGAGACCAGGATGCCACTATGCTATATTGGACATATCCATGATGACTGGCTTTGCTCCAGACACAGA
 TGACCTGAAGCAGCTGGCCAATGGTGTGACAGATACATCTCCAAGTATGAGCTGGACAAGCCTTCTCC
 GATAGGAACACCTCATCATCTACCTGGACAAGGTCTCACACTCTGAGGATGACTGTCTAGCTTTCAAAG
 TTCACCAATACTTTAATGTAGAGCTTATCCAGCCTGGAGCAGTCAAGGTCTACGCCATTACAACTGGA
 GGAAAGCTGTACCCGGTTCTACCATCCGGAAAAGGAGGATGGAAAGCTGAACAAGCTCTGCCGTGATGAA
 CTGTGCCGCTGTGCTGAGGAGAATTGCTTCATACAAAAGTCGGATGACAAGGTCAACCTGGAAGAACGGC
 TGGACAAGGCCTGTGAGCCAGGAGTGGACTATGTGTACAAGACCCGACTGGTCAAGGTTACAGCTGTCAA
 TGACTTTGACGAGTACATCATGGCCATTGAGCAGACCATCAAGTCAGGCTCGGATGAGGTGCAGGTTGGA
 CAGCAGCGCACGTTTCATCAGCCCATCAAGTGCAGAGAAGCCCTGAAGCTGGAGGAGAAGAAACTACC
 TCATGTGGGCTCTCTCCCGATTTCTGGGAGAGAAGCCCAACCTCAGCTACATCATCGGAAGGACAC
 TTGGGTGGAGCACTGGCCCGAGGAGGACGAATGCAAGACGAAGAGAACCAGAAACAATGCCAGGACCTC
 GGCGCCTTACCGAGAGCATGTTGTCTTTGGGTGCCCAACTGACCACACCCCAATCCCCACTCCAG
 ATAAAGCTTCAGTTATATCTCAAAAAAAAAAAAAAAAAA

>gi|773669943|ref|NM_009778.3| *Mus musculus* complement component 3 (C3), mRNA

SEQ ID NO: 3

AGAGAGGAGAGCCATATAAAGAGCCAGCGGTACAGCCCAGCTCGCCTCTGCCACCCCTGCCCTTAC
 CCCTTCATTCCTCCACCTTTTCTTCACTATGGGACCAGCTTCAGGGTCCAGCTACTAGTGTACTG

- continued

CTGCTGTTGGCCAGCTCCCCATTAGCTCTGGGGATCCCCATGTATTCCATCATTACTCCCAATGTCTCTAC
 GGCTGGAGAGCGAAGAGACCATCGTACTGGAGGCCACAGATGCTCAGGGTGACATCCAGTCACAGTCAC
 TGTGCAAGACTTCCATAAGAGGCAAGTGTGACCAGTGAGAAGACAGTGTGACAGGAGCCAGTGGACAT
 CTGAGAAGCGTCTCCATCAAGATTCAGCCAGTAAGGAATCAACTCAGATAAGGAGGGGCACAAGTACG
 TGACAGTGGTGGCAAACTTCGGGGAACCGTGGTGGAGAAAGCAGTGTGGTAAGCTTCCAGAGTGGGTA
 CCTCTTCATCCAGACAGACAAGACCATCTACACCCTGGCTCCACTGTCTTATATCGGATCTTCACTGTG
 GACAACAACCTACTGCCGTGGGCAAGACAGTCGTCACTCATTGAGACCCCCGATGGCATTCCTGTCA
 AGAGAGACATTTCTGCTTCCAACAACCAACACGGCATCTTGCCTTTGTCTTGGAACTTCTGAACTGGT
 CAACATGGGGCAGTGAAGATCCGAGCCTTTTACGAACATGCGCCGAAGCAGATCTTCTCCGAGAGTTT
 GAGGTGAAGGAATACGTGCTGCCAGTTTGTAGGTCCGGGTGGAGCCACAGAGACATTTTATTACATCG
 ATGACCCAAATGGCCTGGAAGTTTCCATCATAGCCAAGTTCTGTACGGGAAAAACGTGGACGGGACAGC
 CTTCTGTGATTTTGGGGTCCAGGATGGCGATAAGAAGATTTCTTGGCCCACTCCCTCACGCGCTAGTG
 ATTGAGGATGGTGTGGGGATGCACTGTGACCCGGAAGGTGCTGATGGAGGGGTACGGCTTCCAACG
 CCGACGCCCTGGTGGGGAAGTCCCTGTATGTCTCCGTCACTGTCACTCCTGCACTCAGGTAGTGACATGGT
 AGAGGCAGAGCGCAGTGGGATCCCGATTGTCACTTCCCGTACCAGATCCACTTACCAAGACCCAAA
 TTCTTCAAGCCAGCCATGCCCTTTGACCTCATGGTGTTCGTGACCAACCCGATGGCTCTCCGGCCAGCA
 AAGTGTGGTGGTCACTCAGGGATCTAATGCAAGGCTCTCACCAAGATGATGGCGTGGCCAGCTAAG
 CATCAACACACCCAACAGCCGCAACCCCTGACCATCACAGTCCGCACCAAGAAGGACACTTCCAGAA
 TCACGGCAGGCCACCAAGACAATGGAGGCCATCCCTACAGCACTATGCACAACCTCAACAACCTACTAC
 ACTTGTCACTGTCAAGATGGAGCTCAAGCCGGGGACAACCTCAATGTCAACTTCCACTGCGCACAGA
 CCCAGGCCATGAGGCCAAGATCCGATACTACACTACCTGTTATGAACAAGGGGAAGCTCTGAAGGCA
 GGCCGCCAGGTTCCGGAGCCTGGCCAGGACCTGGTGGTCTTGTCCCTGCCATCACTCCAGAGTTTATTC
 CTTCAATTTGCGCTGGTGGCTTACTACCCCTGATTGGAGCTAGTGGCCAGAGGGAGGTGGTGGCTGACTC
 TGTGTGGTGGATGTAAGGATTCCTGTATTGGCACGCTGGTGGTGAAGGGTGACCAAGAGATAACCAT
 CTCGCACCTGGGCAACAAACGACACTCAGGATTGAAGGAAACAGGGGGCCGAGTGGGGCTAGTGGCTG
 TGGACAAGGGAGTGTTTGTGCTGAACAAGAACAACCTCACACAGAGCAAGATCTGGGATGTGGTAGA
 GAAGGCAGACATTTGGTGCACCCAGGCAAGTGGGAAGAACTATGCTGGTGTCTTCAATGGATGCAGGCCTG
 GCCTTCAAGACAAGCCAAGGACTGCAGACTGAACAGAGAGCAGATCTTGTAGTGCACCAAGCCAGCAGCCC
 GCCCGCTCGCTCAGTACAGTTGATGGAAAGAAGGATGGACAAGCTGGTCACTGACAGAGGTCT
 TCGGAAGTGTGTGAGGATGGTATGCGGGATATCCCTATGAGATACAGCTGCCAGCGCCGGGCACGCCTC
 ATCACCCAGGGCGAGAAGTGCATAAAGGCTTTCATAGACTGCTGCAACCACATCACCAAGCTGCGTGAAC
 AACACAGAAGAGACCACGTGCTGGGCCCTGGCCAGGAGTGAATTGGAGGAAGACATAATTCAGAAGAAGA
 TATTATCTCTAGAAGCCACTTCCACAGAGCTGGTTGTGACCATAGAAGAGTTGAAAGAACCAGAGAAA
 AATGGAATCTCTACGAAGGTATGAACATCTTCTCAAAGATTCCATCACCACTGGGAGATTCTGGCAG
 TGAGCTTGTACAGCAAGAAAGGATCTGTGTGGCAGACCCCTATGAGATCAGAGTGTGCAGGACTTCTT
 CATTGACCTGCGGCTGCCCTACTCTGTAGTGCACGAACAGGTGGAGATCAGAGCTGTGCTTCAAC
 TACCGTGAACAGGAGGAACTTAAGGTGAGGGTGGAACTGTTGCATAATCCAGCCTTCTGCAGCATGGCCA
 CCGCAAGAATCGTACTTCCAGACCATCAAAATCCCTCCCAAGTCTCGGTGGCTGTACCGTATGTCTAT
 TGTCCCTTGAAGATCGGCCAACAAGAGGTGGAGGTCAAGGCTGTGCTTCAATCACTTATCAGTGTAT
 GGTGTCAAGAAGACACTGAAGTGTGTCAGAGGAATGAGAATCAACAAAACCTGTGGCCATCCATACAC
 TGACCCAGAGAAAGCTCGGTCAAGGGGAGTGCAGAAGGTGGATGTGCTGCCGAGACCTTAGCGACCA

- continued

AGTGCCAGACACAGACTCTGAGACCAGAATTATCCTGCAAGGGAGCCCGGTGGTTCAGATGGCTGAAGAT
GCTGTGGACGGGGAGCGGCTGAAACACCTGATCGTGACCCCCGCAGGCTGTGGGGAACAGAACATGATTG
GCATGACACCAACAGTCATTGCGGTACACTACCTGGACCAGACCGAACAGTGGGAGAAGTTCGGCATAGA
GAAGAGGCAAGAGGCCCTGGAGCTCATCAAGAAAGGGTACACCCAGCAGCTGGCCTTCAAACAGCCCAGC
TCTGCCTATGCTGCCTTCAACAACCGGCCCCCGCAGCCTGGCTGACAGCCTACGTGGTCAAGGTCTTCT
CTCTAGCTGCCAACCTCATCGCCATCGACTCTCACGTCCTGTGTGGGCTGTTAAATGGTTGATTCTGGA
GAAACAGAAGCCGGATGGTGTCTTTTCAGGAGGATGGGCCCGTGATTACCAAGAAATGATTGGTGGCTTC
CGGAACGCCAAGGAGGCAGATGTGTCACTCACAGCCTTCGTCTCATCGCACTGCAGGAAGCCAGGGACA
TCTGTGAGGGGAGGTCAATAGCCTTCTGGGAGCATCAACAAGGCAGGGGAGTATATTGAAGCCAGTTA
CATGAACCTGCAGAGACCATAACAGTGGCCATTGCTGGGTATGCCCTGGCCCTGATGAACAACTGGAG
GAACCTTACCTCGGCAAGTTTCTGAACACAGCCAAAGATCGGAACCGCTGGGAGGAGCCTGACCAGCAGC
TCTACAACGTAGAGCCACATCCTACGCCCTCCTGGCCCTGCTGTGCTGAAAGACTTTGACTCTGTGCC
CCCTGTAGTGCCTGGCTCAATGAGCAAAGATACTACGGAGGCGGCTATGGCTCCACCCAGGCTACCTTC
ATGGTATTCCAAGCCTTGGCCCAATATCAAACAGATGTCCCTGACCATAAGGACTTGAACATGGATGTGT
CCTTCCACCTCCCCAGCCGTAGCTCTGCAACCACGTTTTCGCCCTGCTCTGGGAAAATGGCAACCTCCTGCC
ATCGGAAGAGACCAAGCAAATGAGGCCCTTCTCTAACAGCCAAAGGAAAAGGCCGAGGCACATTGTGC
GTGGTGGCAGTGTATCATGCCAACTCAAAGCAAAGTCACTGCAAGAAGTTTGACCTCAGGGTCAGCA
TAAGACCAGCCCTGAGACAGCCAAGAAGCCCGAGGAAGCCAAGAATACCATGTTCTTGAATCTGCAC
CAAGTACTTGGGAGATGTGGAGCCACTATGTCCATCCTGGACATCTCCATGATGACTGGCTTTGCTCCA
GACACAAAGGACCTGGAAGTCTGGCCCTGGAGTAGATAGATACATCTCCAAGTACGAGATGAACAAAG
CCTTCTCCAACAAGAACACCCTCATCATCTACCTAGAAAAGATTTACACACCCGAAGAAGACTGCCTGAC
CTTCAAAGTTCACCAAGTACTTTAATGTGGGACTTATCCAGCCCGGGTGGTCAAGGTCTACTCCTATTAC
AACCTCGAGGAATCATGCACCCGGTTCATCATCCAGAGAAGGACGATGGGATGCTCAGCAAGCTGTGCC
ACAGTGAATGTGCCGTGTGCTGAAGAGAAGTCTTATGCAACAGTACAGGAGAAGATCAACCTGAA
TGTCCGGCTAGACAAGGCTTGTGAGCCCGAGTGCAGTATGTGTACAAGACCGAGCTAACCAACATAGAG
CTGTTGGATGATTTTGTGAGTACACCATGACCATCCAGCAGGTCAAGTCAAGGCTCAGATGAGGTGC
AGGCAGGGCAGCAACGCAAGTTCATCAGCCACATCAAGTGCAGAAAACGCCCTGAAGTGCAGAAAAGGAA
GAAGTACCTCATGTGGGGCTCTCTCTGACCTCTGGGGAGAAAAGCCCAACACCAGCTACATCATTGGG
AAGGACACGTGGGTGGAGCACTGGCCGTGAGGCAGAAGAATGCCAGGATCAGAAGTACCAGAAAACAGTGGC
AAGAAGTGGGGCATTACAGAATCTATGGTGGTTTATGGTTGTCCCAACTGACTACAGCCAGCCCTCT
AATAAAGCTTCAGTTGTATTTCAACCATC

Reverse Complement of SEQ ID NO: 3

SEQ ID NO: 4

GATGGGTGAAATACAACGAAGCTTTATTAGAGGGCTGGGCTGTAGTCAGTTGGGACAACCATAAACCACCATAG
ATTCTGTGAATGCCCAAGTCTTCGCACTGTTTCTGGTACTTCTGATCCTGGCATTCTTCTGCCTCAGGCCAGT
GCTCCACCCACGTGTCCCTTCCAATGATGTAGCTGGTGTGGGCTTTTCTCCAGAGGTGAGGAGAGGGCCC
ACATGAGTACTTCTCCCTTTCTGCAGCTTCAGGGCGTTTCTGCACCTGATGTGGCTGATGAACTTGCCTGTGCT
GCCCTGCCTGCACCTCATCTGAGCCTGACTTGATGACCTGCTGGATGGTCAAGTGTACTCATAAAATCATCCA
ACAGCTCTATGTTGGTTAGCTCGGTCTTGTACACATAGTCGACTCCGGGCTCACAAAGCCTTGTCTAGCCGGACAT
TCAGGTTGATCTTCTCTGTGACTGTTGCATGAAGCAGTTCTCTTACGACACCCGGCACATTTCACTGTGGCACA

-continued

GCTTGCTGAGCATCCCATCGTCTTCTCTGGATGATAGAACCGGGTGCATGATTCTCGAGGTTGTAATAGGAGT
 AGACCTTGACCGACCCGGGCTGGATAAGTCCACATTAAGTACTGGTGAACCTTGAAGGTCAGGCAGTCTTCTT
 CGGTGTGTGAAATCTTTCTAGGTAGATGATGAGGGTGTCTTGTGTGGAGAAGGCTTTGTTTCATCTCGTACTTGG
 AGATGTATCTATCTACTCCAGAGGCCAGCAGTTCAGGTCTTTGTGTCTGGAGCAAAGCCAGTCATCATGGAGA
 TGTCAGGATGGACATAGTGGCGTCCACATCTCCCAAGTACTTGGTGCAGATTTCAAGGAACATGGTATTCTTGG
 CTTCCTCGGGCTTCTTGGCTGTCTCAGGGCTGGTCTTATGTGACCCTGAGGTCAAACCTTTGCAGGTGACTT
 TGCTTTGAGTTTGGCATGATACACTGCCACCACCGACAATGTGCCTCGGCCTTTTCTTTGGCTGTTAGAGAGA
 AGGCCTCATTTTGGCTTGGTCTCTCCGATCGCAGGAGGTTGCCATTTTCCAGAGCAGGCAGAAACGTGGTTGCAG
 AGTACCGCTGGGGAGGTGAAGGACACATCCATGTTCAAGTCTTATGGTCAGGGACATCTGTTTGATATTGGG
 CCAAGGCTTGAATACCATGAAGGTAGCCTGGGTGGAGCCATAGCCGCTCCGTAGTATCTTTGCTCATTGAGCC
 AGCGCACTACAGGGGGCACAGAGTCAAAGTCTTTCAGCAGCAGCAGGGCCAGGAGGGCGTAGGATGTGGCCTCTA
 CGTTGTAGAGCTGTGGTCAAGGCTCTCCAGCGGTTCCGATCTTTGGCTGTGTTCAAGAACTTGCCGAGGTAAG
 GTTCTCCAGTTTGTTCATCAGGGCCAGGGCATACCAGCAATGGCCACTGTGTATGGTCTCTGCAGGTTTCATGT
 AACTGGCTTCAATATACTCCCTGCCTTGTGTGATGCTCCAGGAAGGCTATTGACCTGCCCTCACAGATGTCCC
 TGGCTTCTGCAGTGCATGAGGACGAAGGCTGTGAGTGACACATCTGCCTCCTTGGCGTCCGGAAGCCACCAA
 TCATTTCTTGGTGAATCAGGGCCCATCTCTGAAAGACACCATCCGGCTTCTGTTTCTCCAGAATCAACCATT
 TAACAGCCCCACACAGGACGTGAGAGTCGATGGCGATGAGGTTGGCAGCTAGAGAGAAGACCTTGACCACGTAGG
 CTGTACGCAGGTGTGGGGGGCCGGTGTGTAAGGCAGCATAGGCAGAGCTGGGCTGTTGAAGGCCAGCTGCT
 GGGTGTACCTTTCTTGTAGAGCTCCAGGGCCTTTCGCTCTTCTCTATGCCGAACCTTCTCCACTGTTGCGTCT
 GGTCAGGTAGTGTACCGCAATGACTGTTGGTGTATGCCAATCATGTTCTGTTCCCCACAGCCTGCGGGGTCA
 CGATCAGGTGTTTACGCGCTCCCGTCCACAGCATCTTCAGCCATCTGAACCACCGGGCTCCCTTGCAAGATAA
 TTCTGGTCTCAGAGTCTGTGTCTGGCACTTGGTCTGTAAGTCTGCGGCAGGCACATCCACCTTCTGCACTCCC
 CTTGACCAGCTTCTCTGGGTCCAGTGTATGGATGGCCACAGTTTTGTTGATTCTCATTCCTTCTGGCACGACCT
 TCAGTGTCTTCTTGACCCATCACTGATGAAGTGATTGAAGACAGCAGCCTGACCTCCACCTCTTGTGGCCGA
 TCTTCAAGGGGACAATGACATACGGTACAGCCACCGAGGACTTGGGAGGGATTTTGTATGGTCTGGAAGTAGCGAT
 TCTTGGCGGTGGCCATGCTGCAGAAGGCTGGATTATGCAACAGTTCACCCCTCACCTTAAGTCTCTCTGTTTAC
 GGTAGTTGAAGACACAGCTCTGATCTCCACCTGTTGTTGCGCACTACAGAGTAGGGCAGCCGAGGTCAATGA
 AGAAGTCTGCATCACTCTGATCTCATAGGGTCTGCCACACAGATCCCTTCTTGTCTGACAAGCTCACTGCCA
 GAATCTCCAGGTGGTGTGGAATCTTTGAGAAAGATGTTTCATGACCTTCGTAGAGATTCATTTTCTCTGGTT
 CTTTCAACTCTTCTATGGTCCACAACAGCTCTGTGGGAAGTGGCTTCTAGAGATAATATCTTCTTCTGGAATTA
 TGTCTTCTCCAATCACTCTGCGCAGGCCAGCAGTGGTCTTCTGTGTTGTTACGCAGCTTGGTGTATGT
 GGTTGCAGCAGTCTATGAAGGCTTTATGCAGTCTCGCCCTGGGTGATGAGGCGTGCCGGCGCTGGCAGCTGT
 ATCTCATAGGATAATCCCGCATAACCATCTCACAACTTCCGAAGACCTTGTGAGTACTGACCAGCTTTGT
 CCATCTTCTTCCATCAACTGACTGAGCGACGGCGGGCTGCTGGCTTGGTGCCTCAAGATCTGCTCTCT
 GTTCACTCTGCAGTCTTGGCTTGTCTTGAAGGCCAGGCTGCATCCATGAAGACACCAGCATAGTCTTCCCAC
 TGCTTGGGGTGCAGCAATGTCTGCTTCTCTACCACATCCAGATCTTGCTCTGTGTGAGTTTGTCTTCTTGT
 TCAGCACAACACTCCCTTGTCCACAGCCACTAGCCCACTCGGGCCCCCTGGTTTCTTCAATCTGAGTGTG
 TTTGTTGCCAGGTGCGAGATGGTTATCTTGGGTCAACCCTTACCACCAGCGTCCAATACAGGAATCCTTCA
 CATCCACCACACAGAGTCAGCCACCACCTCCCTCTGGCCACTAGCTCCAATCAGGGTGTAGTAAGCCACCAGGC
 GAAATGAAGGAATAAACTCTGGAGTGTGGGCAGGACAAGACCACCAGGTCCTGGCCAGGCTCCCGAACCTGGC

- continued

GGCCCTGCCTTCAGGAGCTCCCTTGTTCATAAC CAGGTAGGTGTAGTATCGGATCTTGGCCTCATGGCCTGGGT
 CTGTGCGCAGGTGGAAGTTGACATTGAGGTTGCCCCCGGCTTGAGCTCCATTTCGTGACACTGACAAGTGTAGGT
 AGTTGTGGAGTTGTGCATAGTGTGTAGGGATGGGCCTCCATTGTCTTGGTGGCCTGCCGTGATTCTGGGAGAG
 TGTCTTCTTGGTGCAGGACTGTGATGGTCAGGGGTTGGCGGCTGTTGGGTGTGTTGATGCTTAGCTTGGCCACGC
 CATCATCTTGGGTGAGAGCCTTTGCATTAGATCCCTGAGTGACCACCAGCACTTTGCTGGCCGGAGAGCCATCGG
 GGTGGTACGAAACACCATGAGGTCAAAGGCATGGCTGGCTTGAAGAATTGGGTGTCTTGGTGAAGTGGATCT
 GGTACGGGGAAGTGACAATCGGGATCCCACTGCGCTCTGCCTTACCATGTCACTACCTGAGTGACAGGATGACAG
 TGACGGAGACATACAGGACTTCCCAACAGGGCGTCGGCGTTGGAAGCCGTACCCCTCCATCAGCACCTTCC
 GGGTCAGCACTGCATCCCCACACCATCCTCAATCACTACGCGCTGAGGGAGTGGGCCAGAGAAATCTTCTTAT
 CGCCATCCTGGACCCAAAAATCACGAAGGCTGTCCCGTCCACGTTTTTCCCGTACAGGAACTTGGCTATGATGG
 AACTTCCAGGCCATTTGGGTTCATCGATGTAATAAAATGTCTCTGTGGCTCCACCCGGACCTCAAACTGGGCA
 GCACGTATTCCTTACCTCAAACCTGCGGAGAAGATCTGCTTCGGCGCATGTTTCGTAAGGCTCGGATCTTCC
 ACTGCCCCATGTTGACCAGTTGAGAAATGTTCAAGACAAAGGCAAGATGCCGTGTTGGTTGTTGGAAGACAGAA
 TGTCTCTCTTGACAGGAATGCCATCGGGGTTCTCAATGAGGATGACGACTGTCTTGCCACGGGCAGTAGGTTGT
 TGTCCACAGTGAAGATCCGATATAAGACAGTGGAGCCAGGGGTGTAGATGGTCTTGTCTGTCTGGATGAAGAGGT
 ACCCACTCTGGAAGCTTACCATCACTGCTTTCTCCACCACGTTTCCCGAAGTTTGCACCACCTGTACAGTACT
 TGTGCCCTCCTTATCTGAGTTGAATTCCTTACTGGCTGGAATCTTGATGGAGACGCTTCTCAGATGTCCACTGG
 CTCTGTCAACACTGTCTTCTCACTGGTCAGCACTTGCCTTTTAGGAAGTCTTGACAGTGACTGTGACTGGGA
 TGTACCCCTGAGCATCGTGGGCTCCAGTACGATGGTCTTTCGCTCTCCAGCCGTAGGACATTGGGAGTAATGA
 TGAATAACATGGGATCCCCAGAGCTAATGGGAGCTGGCCAACAGCAGCAGTAGCACTAGTAGCTGGGACCTG
 AAGCTGGTCCCATAGTGAAGGAAAAAGGTGGAAGGAATGAAGGGGTAAGGGGCAGGGGTGGGCAGAGGCGAGCTG
 GGGCTGTAGCCGCTGGCTCTTTATATGGCTCTCCTCTCT

>gi|158138560|ref|NM_016994.2| *Rattus norvegicus* complement component 3 (C3), mRNA

SEQ ID NO: 5

CTACCCCTTACCCCTCACTCCTTCCACCTTTGTCCTTTACCATGGGACCCACGTCAGGGTCCAGCTACT
 AGTGCTACTGTGCTGTTGGCCAGCTCCCTGCTAGCTCTGGGGAGCCCATGTAATCCATCATTACTCCC
 AATGTCCTGCGGCTGGAGAGTGAAGAGACTTTCATACTAGAGGCCATGATGCTCAGGGTGTATGCCAG
 TCACGTCACTGTGCAAGACTTCTAAAGAAGCAAGTGTGACCAGTGAGAAGACAGTGTGACAGGAGC
 CACTGGACATCTGAACAGGGTCTCCATCAAGATTCCAGCCAGTAAGGAATCAATGCAGATAAGGGGCAC
 AAGTACGTGACAGTGGTGGCAAACCTTCGGGGCAACAGTGGTGGAGAAAGCGGTGCTAGTAAGCTTTCAGA
 GTGGTTACCTCTTCATCCAGACAGACAAGCCATCTACACCCAGGCTCCACTGTTTTCTATCGGATCTT
 CACTGTGGACAACAACCTATTGCCTGTGGGCAAGACAGTCTCATCGTCATTGAGACCCCGGACGGCGTT
 CCCATCAAGAGAGACATCTATCTTCCACAACCAATATGGCATCTTGCCTTTGTCTTGGAACTTCCAG
 AACTGGTCAACATGGGGCAGTGAAGATCCGAGCCTTCTATGAACATGCACAAAGCAGACCTTCTCTGC
 AGAGTTTGAGGTGAAGGAATACGTGCTGCCAGTTTTCGAAGTCTGGTGGAGCCTACAGAGAAATTTTAT
 TACATCGATGACCCAAAGGCTCGGAAGTTTCCATCACAGCCAGATTCTGTATGGGAAGAACGTGGACG
 GGACAGCTTTCGTGATCTTGGGGTCCAGGATGAGGATAAGAAGATTTCTCTGGCCAGTCCCTCACCCG
 CGTGCTGATCGAGGATGGTTCAGGGGAGGCAGTGTCTCAGCCGAAAAGTCTGATGGACGGGGTACGGCC
 TCCAGCCCAGAAGCCCTAGTGGGGAAGTCCCTGTACGCTCTGTCACTGTTATCTGCACTCAGGTAGCG
 ACATGGTAGAGGCAGAGCGCAGTGGGATCCCAATGTCACTTCCCGTACCAGATCCACTTCCACCAAGAC

- continued

ACCCAAATTCCTCAAGCCAGCCATGCCCTTCGACCTCATGGTGTGGTGTGACCAACCCTGATGGCTCTCCA
GCCCCGAGAGTGCCAGTAGTCACTCAGGGATCCGACGCGCAGGCTCTCACCAGGATGATGGTGTGGCCA
AGCTGAGCGTCAACACACCCAAACACCGCCAAACCCTGACTATCACGGTCCGCACCAAGAGGAGGTAT
CCCGACGCGCGCCAGGCCACCAGGACGATGCAGGCCAGCCCTACAGCACTATGCACAATTCACCAAC
TACCTGCACTTGTCACTGTCTCGGGTGGAGCTCAAGCCTGGGGACAACCTCAATGTCAACTCCACCTGC
GCACGGACGCTGGCCAAGAGCCAAAGATCCGATACTACACCTATCTGGTTATGAACAAGGGGAAGTTACT
GAAGGCAGGCCGTCAGGTTCCGGAGCCTGGCCAGGACCTGGTGGTCTTGTCACTGCCATCACTCCAGAA
TTTATACCTTCTCCGCTGGTGGCTTACTACACCTGATTGGAGCTAATGGCCAAAGGGAGGTGGTGG
CCGACTCAGTGTGGTGGATGTGAAGGACTCCTGTGTAGGCACGCTGGTGGTGAAGGTGACCCAAAGAGA
TAACCGACAGCCCGCCTGGGCATCAAACGACACTAAGGATCGAGGGGAACAGGGGGCCCGAGTGGGG
CTAGTGGCTGTGGACAAGGGGTGTGTGTGCTGAACAAGAAGAACTCACACAGAGCAAGATCTGGG
ATGTAGTAGAGAAGGCAGACATTGGCTGCACCCAGGCAGTGGGAAGAACTATGCGGGTGTCTTCATGGA
TGCTGGCCTGACCTTCAAGACAACCAAGGCCCTGCAGACTGATCAGAGAGAAGATCCTGAGTGCGCCAAG
CCAGCTGCCCGCCCGCTCGCTCAGTGCAGTTGATGGAAAGGAGGATGGACAAGCTGGTCACTACACCG
ACAAGGCTCTGCGGAAGTGTGTGAGGATGGCATGCGTGATATCCCTATGAAGTACAGTGCAGCGCCG
GGCTCGCTCATCACCCAGGGCGAGAGCTGCCTGAAGCCTTCATGGACTGCTGCAACTATATCACCAAG
CTTCGTGAGCAGCAGACAAGAGACCATGTGCTGGGCTGGCCAGGAGTGTGGATGAAGACATAATCC
CAGAAGAAGATATTATCTCTAGAAGCCACTTCCCAGAGAGCTGGTTGTGGACCATAGAAGAGTTGAAAGA
ACCAGAGAAAAATGGAATCTCTACGAAGGTATGAACATCTTTCTCAAAGATTCCATCACCACTGGGAG
ATTCTGGCAGTGAGCTTGTCCGACAAGAAAGGGATCTGTGTGGCAGACCCCTATGAGATCACAGTGATGC
AGGACTTCTTCACTGACCTGCAGCTGCCCTACTCTGTGGTGCAGCAATGAACAGGTGGAGATCAGAGCTGT
GCTCTTCAATTACCGTGAACAGGAGAACTTAAGGTAAGGGTGGAACTGTTGCATAACCCAGCCTTCTGC
AGCATGGCCACTGCCAAGAAGCGGTACTACCAGACCATCGAAATCCCTCCAAGTCTCTGTGGCTGTGC
CTTATGTCAATGTCCCTTGAAGATCGGCTCCAGGAGTGGAGGTCAAGGCCGCGCTTTCACCACTT
CATCAGTGATGGTGTCAAGAAGATACTGAAGTCTGTCCAGAAGGAATGAGAGTCAACAAAACCTGTGGCT
GTCCGTACACTGGATCCAGAACACCTCGGTCAAGGGGAGTGCAGAGGGAGGATGTACCTGCAGCAGACC
TCAGTGACCAAGTGCCAGACACAGATTCTGAGACCAGAATCTCCTGCAAGGACCCCGGTGGCTCAGAT
GGCCGAGGACGCTGTGGACGGGAGCGCTGAAACACCTGATCGTGACCCCTCTGGCTGTGGGAGCAG
AACATGATTGGCATGACACCCAGGTCATTGCAGTACACTATCTGGATCAGACCGAACAGTGGGAGAAAT
TCGGCCTAGAGAAGAGGCAAGAAGCTCTGGAGCTCATCAAGAAAGGTACACCCAGCAGCTGGCTTTCAA
ACAGCCAGCTCTGCCTATGCTGCCTTCAACAACCGGCTCCAGCACCTGGCTGACAGCCTATGTGGTC
AAGGTCTTCTCTGGCTGCCAACCTCATCGCCATCGACTCTCAGGTCTGTGTGGGCTGTCAAATGGC
TGATTCTGGAGAAACAGAAGCCAGATGGTGTCTTTCAGGAGGACGGACAGTGATTACCAAGAAATGAT
TGGTGGCTTCCGGAACCAAGGAGGAGATGTGTGCTTACAGCCTTGTCTCATCGCACTGCAGGAA
GCCAGAGATATCTGTGAGGGGAGGTCACAGCCTTCCCGGAGCATCAACAAGGCAGGGGAGTATCTTG
AAGCCAGTTACCTGAACCTGCAGAGACCATACACAGTAGCCATTGCTGGGTATGCCCTGGCCCTGATGAA
CAAATGGAGGAACCTTACCTCACCAAGTTTCTGAACACAGCCAAAGATCGGAACCGCTGGGAGGAGCCT
GGCCAGCAGCTCTACAATGTGGAGGCCACCTCTACGCCCTCCTGGCCCTGCTGTGCTGAAAGACTTTG
ACTCTGTGCCCTCTGTGGTGCCTGGCTCAACGAGCAAGATACTACGGAGGTGGCTATGGCTCCACGCA
GGCTACCTTCATGGTATTCCAAGCCTTGGCTCAATACCAACAGATGTCCCTGACCACAAGGACTTGAAC

- continued

ATGGATGTGTCCTCCACCTCCCCAGCCGACGCTCCCAACTGTGTTTCGCCTGCTATGGGAAAGTGGCA
 GTCTCCTGAGATCAGAAGAGACCAAGCAGAATGAGGGCTTTTCTCTGACAGCCAAGGAAAAAGCCAAGG
 CACACTGTCGGTGGTGACAGTGTATCACGCCAAAGTCAAAGGCAAAGCCACCTGCAAGAAGTTTGACCTC
 AGGGTCACCATAAAACCAGCCCTGAGACAGCCAAGAAGCCCAGGATGCCAAGAGTTCTATGATCCTTG
 ACATCTGCACCAGTACTTGGGAGACGTGGATGCTACTATGTCCATCCTGGACATCTCCATGATGACTGG
 CTTTATTCAGACACAAACGACCTGGAAGTCTGAGCTCTGGAGTAGACAGATACATTCCAAGTATGAG
 ATGGACAAAGCCTTCTCCAACAAGAACACCCTCATCATCTACCTAGAAAAGATCTCACACTCCGAAGAAG
 ACTGCCTGTCTTCAAAGTCCACCGTCTTTAACGTGGGACTTATCCAGCCGGGGTGGTCAAGGTCTA
 CTCTACTACAATCTAGAGGAGTCTATGCACCCGGTCTATCATCCGGAGAAGGACGATGGAATGTGAGC
 AAGCTGTGCCACAATGAAATGTGCCGTGTGCAGAGGAGAACTGCTTCATGCATCAGTACAGGATCAGG
 TCAGCCTGAATGAACGACTAGACAAGGCTTGTGAGCCTGGAGTGGACTACGTGTACAAGACCAAGCTAAC
 GACGATAGAGCTGTGGATGATTTTGTAGTACATCATGACCATCGAGCAGGTATCAAGTCAGGCTCA
 GATGAGGTGCAGGCAGGTGAGAACGAAGTTTCATCAGCCACGTCAAGTGCAGAAACGCCCTAAAGCTGC
 AGAAAGGGAAGCAGTACCTCATGTGGGGCTCTCCTCCGACCTCTGGGGAGAAAAGCCCAATACCAGCTA
 CATCATTTGGGAAGGACAGTGGGTGGAGCACTGGCCGAGGCAGAGGAATGTCAGGATCAGAAGAACCAG
 AAACAGTGCGAAGACCTCGGGCATTCACAGAAACAATGGTGGTTTTTCGGCTGCCCAACTGACCACAAC
 CTCCAATAAAGCTTCAGTTGATTTTACCCATCAAAAAAAAAAAAAAAAAA

Reverse Complement of SEQ ID NO : 5

SEQ ID NO : 6

TTTTTTTTTTTTTTTTTGATGGGTAAATAACAACCTGAAGCTTTATTGGAGTTGTGGTCAGTTGGGGCAGCCGA
 AAACCACCATGTTTCTGTGAATGCCCCGAGGCTTCGCACGTCTTCTGGTCTTCTGATCCTGCATTCTCTG
 CCTCGGGCCAGTGCTCCACCCACGTGCTTCCCAATGATGTAGCTGGTATGGGCTTTTCTCCCAGAGGTCGG
 AGGAGAGGCCCCACATGAGGTACTGCTTCCCTTTCTGCAGCTTTAGGGCGTTTCTGCACTTGACGTGGCTGATGA
 ACCTTCGTTCTGACCTGCCTGCACCTCATCTGACCTGACTTGATGACCTGCTCGATGGTCATGATGACTCAT
 CAAAATCATCCGACAGCTCTATCGTCGTAGCTTGGTCTTGTACACGTAGTCCACTCCAGGCTCACAAGCCTTGT
 CTAGTCGTTTCAATTCAGGCTGACCTGATCCTGTGACTGATGCATGAAGCAGTTCTCCTCTGCACAGCGGCACATTT
 CATTGTGGCACAGCTTGTCTCAGCATTCCATCGTCTTCTCCGGATGATAGAACCGGGTGCATGACTCCTCTAGAT
 TGATAGTAGGAGTAGACCTTGACCGACCCCGCTGGATAAGTCCCACGTTAAGAAGTGGTGGACTTTGAAGGACA
 GGCAGTCTTCTCGAGTGTGAGATCTTTCTAGGTAGATGATGAGGGTGTCTTGTGGAGAAGGCTTTGTCCA
 TCTCATACTTGGAAATGTATCTGTCTACTCCAGAGCTCAGCAGTTCAGGTCGTTTGTGTCTGGAATAAAGCCAG
 TCATCATGGAGATGTCAGGATGGACATAGTAGCATCCACGTCTCCAAGTACCTGGTGCAGATGTCAAGGATCA
 TAGAACTCTTGGCATCCTGGGGCTTCTGGCTGTCTCAGGGGCTGGTTTTATGGTGACCTGAGGTCAAACCTCT
 TGCAGGTGGCTTTGCCCTTGTACTTGGCGTGATACACTGTCAACCCGACAGTGTGCCTTGGCTTTTCTTTGG
 CTGTGAGAAAAGCCCTCATCTGCTTGGTCTCTCTGATCTCAGGAGACTGCCACTTCCCATAGCAGGCGAA
 ACACAGTTGGGGAGCTGCGCTGGGGAGGTGGAGGGACACATCCATGTTCAAGTCTTGTGGTCAGGGACATCTG
 TTTGGTATTGAGCCAAGGCTTGAATAACCATGAAGTAGCCTGCGTGGAGCCATAGCCACCTCCGTAGTATCTTT
 GCTCGTTGAGCCAGCGCACCAAGGAGGACAGAGTCAAAGTCTTTCAGCAGCAGCAGGGCCAGGAGGGCTAGG
 AGGTGGCCTCCACATTGTAGAGCTGCTGCCAGGCTCCTCCAGCGGTTCCGATCTTTGGCTGTGTTTCAGAACT
 TGGTGAAGTAAGTTCTCCAGTTTGTTCATCAGGGCCAGGCATACCCAGCAATGGCTACTGTGTATGGTCTCT
 GCAGGTTCAAGTAACTGGCTTCAAGATACTCCCTGCCTTGTGATGCTCCGGGAAGGCTGTTGACCTGCCCT

- continued

CACAGATATCTCTGGCTTCCCTGCAGTGCAGATGAGGACAAAGGCTGTAAGCGACACATCTGCCTCCTTGGTGTCC
GGAAGCCACCAATCATTCTTGGTGAATCACTGGTCCGTCTCTGAAAGACACCATCTGGCTTCTGTTCTCCA
GAATCAGCCATTTGACAGCCCCACACAGGACCTGAGAGTCGATGGCGATGAGGTTGGCAGCCAGAGAGAAGACCT
TGACCACATAGGCTGTCAGCCAGGTGCTGGGAGGCCGGTGTGTAAGGCAGCATAGGCAGAGCTGGGCTGTTGA
AAGCCAGCTGCTGGGTGTACCTTTCTTGTAGAGCTCCAGAGCTTCTTGCCCTTCTCTAGGCCGAATTTCTCCC
ACTGTTGGTCTGATCCAGATAGTGTACTGCAATGACCGTGGGTGTATGCCAATCATGTTCTGCTCCCCACAGC
CAGAGGGGGTACGATCAGGTGTTTCCAGCCGCTCCCCGTCCACAGCGTCTCGGCCATCTGAGCCACCGGGGTCC
CTTGACAGGAGAATTTGGTCTCAGAATCTGTGCTGGCACTTGGTCACTGAGGTCTGCTGCAGGTACATCCTCCC
TCTGCACTCCCCCTTGACCGAGGTGTTCTGGATCCAGTGTACGGACAGCCACAGTTTTGTTGACTCTCATTCTT
CTGGCAGACCTTCAGTATCTTCTTGACACCATCACTGATGAAGTGGTTGAAGACGGCGGCCCTTGACCTCCACCT
CCTGGAGGCCGATCTTCAAGGGGACAATGACATAAGGCACAGCCACAGAGGACTTGGGAGGGATTTTCATGGTCT
GGTAGTACCGTTCTTGGCAGTGGCCATGCTGCAGAAGGCTGGGTTATGCAACAGTTCACCCCTTACCTTAAGTT
TCTCTGTTACGGTAATTGAAGAGCACAGCTCTGATCTCCACCTGTTTTCATGCGCACACAGAGTAGGGCAGTC
GCAGGTCAATGAAGAAGTCTGCATCACTGTGATCTCATAGGGGTCTGCCACACAGATCCCTTTCTTGTTCGGACA
AGCTCACTGCCAGAATCTCCAGGTGGTGTGGAATCTTGAAGAAAGATGTTTCATGACCTTCGTAGAGATTCAT
TTTTCTCTGGTTCTTTCAACTCTTCTATGGTCCACAACCAGCTCTCTGGGAAGTGGCTTCTAGAGATAATATCTT
CTTCTGGGATATAGTCTTCATCCACATCACTCTGGCCAGGCCAGCACATGGTCTCTTCTGTGTGCTCACGAA
GCTTGGTGATATAGTTGCAGCAGTCCATGAAGGCCCTTCAGGCAGCTCTCGCCCTGGGTGATGAGGCGAGCCCGC
GCTGGCAGCTGTACTTCATAGGATATCACGCATGCCATCCTCACAACACTTCCGACAGCCCTTGTCCGGTGTACT
GACCAGCTTTGTCCATCTCTTTCCATCAACTGCACTGAGCGACGGCGGGCGGAGCTGGCTTGGCGCACTCAG
GATCTTCTCTGATCAGTCTGCAGGCCTGGTTTGTCTTGAAGGTGAGGCCAGCATCCATGAAGACACCCGCAT
AGTCTTCCCACTGCCTGGGTGCAGCCAATGCTGCTCTCTACTACATCCCAGATCTTGCTCTGTGTGAGTT
TGTTCTTCTTGTTCAGCACAAACACCCCTTGTCACAGCCACTAGCCCACTCGGGCCCCCTGGTTCCCTCGA
TCCTTAGTGTGTTGATGCCAGGCGCGGGCTGTGCGTTATCTCTTGGGTACCTTTCACCACCAGCGTGCCTA
CACAGGAGTCTTCACATCCACCCACACTGAGTCCGCCACCACCTCCCTTTGGCCATTAGCTCCAATCAGGGTGT
AGTAAGCCACCAGGCGGAAGGAAGGTATAAATCTGAGTGTGAGGCGAGTACAAGACCACCAGGTCTGGCCAG
GCTCCCGAACCTGACGGCTGCCTTCACTAAGTCTCCCTTGTTCATAACAGATAGGTGTAGTATCGGATCTTGG
CCTCTTGGCCAGCGTCCGTCGCGAGGTGGAAGTTGACATTGAGGTGTCCCAGGCTTGAGCTCCACCCGAGACA
CTGACAAGTGCAGGTAGTTGTGGAATGTGATAGTGTGAGGGCTGGGCTGCATCGTCTGTTGGTGGCTGCC
GCGCGTCCGGGATACCCCTCTTGGTGCAGGACCTGATAGTACAGGGTTGGCGGTTGTTGGGTGTGTGACGC
TCAGCTTGGCCACACCATCATCTGGGTGAGAGCCTGCGCGTCGGATCCCTGAGTGACTACTGGCACTCTGCGGG
CTGGAGAGCCATCAGGGTGGTTCACAAACACCATGAGGTGCAAGGCATGGCTGGCTTGAAGAATTTGGGTGTCT
TGGTGAAGTGGATCTGGTACGGGGAAGTGAACAATGGGATCCCACTGCGCTCTGCTTACCATGTGCTACCTG
AGTGCAGGATAACAGTGACAGAGACGTACAGGGACTTCCCACTAGGGCTTCTGGGCTGGAGGGCCGTACCCCGT
CCATCAGCACTTTTTCGGCTGAGCACTGCCTCCCTGAACCATCTCGATCAGCACGCGGGTGGGGACTGGGCCA
GAGAAATCTTCTTATCTCATCTGGACCCCAAGATCACGAAAGCTGTCCCGTCCAGTCTTCTCCCATACAGGA
ATCTGGCTGTGATGGAACTTCCAGGCCCTTTGGGTCACTGATGTAATAAAATTTCTCTGTAGGCTCCACCAGGA
CTTCAAAGTGGGAGCAGTATCTTCTCACCTCAAACCTGTCAGAGAAGGTCTGCTTGGTGCATGTTTATAGA
AGGCTCGGATCTTCCACTGCCCATGTTGACAGGTTCTGGAATGTTCCAAGACAAAGGCAAGATGCCATATGGT
TGTGGGAAGATAGAATGTCTCTTGTATGGGAACGCCGTCCGGGTCTCAATGACGATGACGACTGTCTTGCCCA
CAGGCAATAGGTTGTTGTCCACAGTGAAGATCCGATAGAAAACAGTGGAGCCTGGGGTGTAGATGGTCTTGTCTG

-continued

TCTGGATGAAGAGGTAACCACTCTGAAAGCTTACTAGCACCGCTTCTCCACCACTGTGCCCCGAAGTTTGCCA
 CCACTGTACAGTACTTGTGCCCTTATCTGCATTGAATTCCTTACTGGCTGGAATCTTGATGGAGACCTGTTC
 GATGTCCAGTGGCTCCTGTCAACACTGTCTTCTCACTGGTCAGCACTTGCTTCTTTAGGAAGTCTTGACAGTGA
 CAGTGACTGGGACATCACCTGAGCATCATGGGCTCTAGTATGAAAGTCTCTTCACTCTCCAGCCGAGGACAT
 TGGGAGTAATGATGGAGTACATGGGGCTCCCCAGAGCTAGCAGGGAGCTGGCCAACAGCAGCAGTAGCACTAGTA
 GCTGGGACCTGACGTGGGTCCCATGGTAAAGGACAAAGGTGGAAGGAGTGAGGGTAAGGGGTAG

>gi|982312947|ref|XM_005587719.2| PREDICTED: *Macaca fascicularis*
 complement component 3 (C3), mRNA

SEQ ID NO: 7

AAAGCCTCCAGCAGTCACTGCTCACTCCTCCCATCCTCTCCCTGTCCCTGTCCCTGTCCCTGTGACCC
 TGCCTGTCCAGCACCATGGGACTCACCTCAGGTCAGGCTCCAGCTGCTGCTCCTGCTACTAATCCACCTCCC
 CCTGGCTCTGGGACTCCCATGTACTCTATGATCACCCAAACGCTTGTGGCTGGAGAGTGAGGAGACC
 GTGGTGTGGAGGCCATGACGCGAATGGGATGTTCCGGTCACTGTCACTGTCCACGACTTCCAGGCA
 AAAAAGTGGTGTCTCAGTGAAGAAGCCGTGCTGACCCCTGCCACCAGCCACATGGGCAGCGTCAACAT
 CAGGATCCAGCCAACAAGGAGTCAAGTCAGAAAAGGGCACAACAAGTTCGTGACTGTGCAGGCCACC
 TTCGGGGCCCAAGTGGTGGAGAAGGTGGTACTGGTCAAGCTTCAGAGCGGGTACCTCTCATCCAGACAG
 ACAAGACCATCTACACCCCTGGCTCCACAGTTCTCTGTCCGATCTTCCCGTCAACCACAAGCTGCTACC
 CGTGGGCCGAGCGTGTGTTCAACATTGAGAACCAGGACCGGATCCCGGTCAAGCAGGACTCCTTGTCT
 TCTCAGAACCAATTTGGCATCTTGGCCTTGTCTTGGACATTCGGAACCTCGTCAACATGGGCCAGTGA
 AGATCCGAGCCTACTATGAAAATTCGCCGCAACAGGTCTTCTCCACTGAGTTTGAGGTGAAGGAGTACGT
 GCTGCCAGTTCAGAGTCAAGTGGAGCCTACAGAGAAATCTACTACATCTATAACAGAAGGGCCTG
 GAGGTCAACATCACCGCCAGGTTCTCTATGGAAAGAAAGTGGAGGGAACGCTTGTGATCTTCCGGA
 TCCAGGATGGCGAGCAGAGGATTTCCCTGCCTGAATCCCTCAAGCGCATCCAGATTGAGGATGGCTCAGG
 AGACGCCGTGCTGAGCCGAAGTACTGCTGGACGGGTGCAGAATCCCGACCGGAAGACCTAGTGGGG
 AAGTCTTGTATGTCTGTACCGTTATCTGCACTCAGGCAGTGACATGGTGCAGGCGGAGCGCAGCG
 GGATCCCATCGTGACCTCTCCCTACCAGATCCACTTCCCAAGACGCCAAGTACTTCAAACCAGGAAT
 GCCCTTTGACCTCATGGTGTCTGTGACGAACCCGATGGCTCTCCAGCCTACCGAGTCCCGTGGCAGTC
 CAGGCGGAGGACGCTGTGCAGTCTTAACCCAGGAGACGGCGTGGCCAACTCAGCATCAACACACACC
 CCAGCCAGAAGCCCTTGAAGATCACGGTGCACGAGGAGCGGAGCTCTCGGAGCGGAGCAGGCTAC
 CAGGACCATGGAGGCTCAGCCCTACAGCACCGTGGGCACTCAACAATTACCTGCATCTCTCAGTGCCA
 CGTGCAGAGCTCAGACCTGGGAGACCCCTCAACGTCAACTTCTCTGCGAATGGACCGCACCCAGGAGG
 CCAAGATCCGCTACTACACCTACTGATTATGAACAAAGGCAAGCTGTTGAAGGTGGGACGCCAGGTGCG
 AGAGCCTGGCCAGGACCTGGTGGTGTGCCCCCTGTCCATCACACCGACTTATCCCTTCTTCCGCCTG
 GTGGCTACTACACGCTGATCGGCGCAACCGCCAGAGGGAAGTGGTGGCCGACTCCGTGTGGTGGACG
 TCAAGGACTCTTGCCTGGGCTCGCTGGTGGTAAAAGCGGCCAGTCAAGAGACAGGCAGCCTTTACCCGG
 GCAGCAGATGACCTGAAGATAGAGGGTGACACGGGGCCCGGGTGGGACTGGTGGCTGTGGACAAGGGC
 GTGTTTGTGCTGAATAAGAAGAACAAGCTGACGCAGAGTAAGATCTGGGACGTGGTGGAGAAGGCAGACA
 TCGGCTGCACCCAGGCAGTGGGAAGGATTACGCTGGTGTCTTCTCGGATGCAGGCCTGACCTTTGCGAG
 CAGCAGTGGCCAGCAGCGCCAGAGGGCAGAACTTCAAGTCCACAGCCAGCCGCCCGCCGACCGCT
 TCCGTGCAGCTCGCGGAGAAGAGAATGGACAAGTTGGTCACTACCCCAAGGAGCTGCGCAAGTGTGCG

- continued

AGCACGGTATGCGGGAGAAACCCCATGAGGTTCATGCCAGCGCCGGACCCGTTACATCACCCCTGGACGA
 GGCGTGCAAGAAGGCCCTTCTGGACTGTGCAACTACATCACCGAGCTGCGGGCGCAGCACGCGCGGGCC
 AGTCACCTGGGCTGGCCAGGAGTAACCTGGATGAGGACATCATCGCAGAAGAGAACATCGTTTTCCGAA
 GTGAGTCCCAGAGAGTTGGCTGTGGAAGATTGAAGAGTTGAAAGAGGCACCGAAAAACGGAATCTCCAC
 GAAGCTCATGAATATATTTTTGAAAGACTCCATCACACGTTGGGAGATTCTGGCCGTGAGCTTGTGAGAC
 AAGAAAGGGATCTGTGTGGCAGACCCCTTCGAGGTCACAGTAATGCAGGACTTCTTCATCGACCTGCGGC
 TACCCTACTCTGTTGTTGAAACGAGCAGGTGGAATCCGAGCTGTTCTCTACAATTACCGGCAGAACCA
 AGAGCTCAAGGTGAGGGTGAAGTACTCCACAATCCAGCCTTCTGCAGCCTGGCCACCGCCAAGAGGCGT
 CACCAGCAGACCGTAACCATCCCCCAAGTCTCGTGTCCGTTCTTATGTCATCGTGCCCTAAAGA
 CCGGCCAGCAGGAAGTGAAGTCAAGGCTGCCGTCTACCATTTTTTCATCAGTGACGGTGTGAGGAAGT
 CCTGAAGTCTGTCGCGAAGGAATCAGAATGAACAAAAGTGTGGTGTTCGCACGCTGGATCCAGAAGC
 CTGGGCCAGGAAGGAGTGCAGAGAGGAGACTCCACCTGCAGACCTCAGTGACCAAGTCCCGGACACCG
 AGTCTGAGACCAGAATTCTCTGCAAGGGACCCCGGTGGCCAGATGACAGAGGATGCCATCGATGCGGA
 ACGGCTGAAGCACCTCATCGTGACCCCTCGGGCTGCGGAGAACAGAACATGATCACCATGACGCCACA
 GTCATCGTGTGCATTACTGGATGAAACGGAACAGTGGGAGAAGTTCGGCCCGGAGAAGCGGCAGGGG
 CCTTGGAGCTCATCAAGAAGGGGTACACCCAGCAGCTGGCCTTCCAGACAACCCAGCTTGCCCTTTCGGC
 CTTCTGAACCGGGACCCAGCACCTGGCTGACCGCTACGTGGTCAAGGCTTCTCTCTGGCTGTCAAC
 CTCATTGCCATCGACTCCAGGTCTCTGCGGGCTGTTAAATGGCTGATCCTGGAGAAGCAGAAGCCCG
 ACGGGTCTTCCAGGAGGATGCGCCCGTGATACATCAAGAAATGACTGGTGGATTCCGGAACACCAACGA
 GAAAGACATGGCCCTCACGGCTTTGTTCTCATCTCGCTGCAAGAGGCTAAAGAGATTGCGAGGAGCAG
 GTCAACAGCCTGCCCGGCAGCATCACTAAAGCAGGAGACTTCCTTGAAGCCAACTACATGAACCTACAGA
 GATCCTACACTGTGGCCATCGCTGCCTATGCCCTGGCCAGATGGGCAGGCTGAAGGGACCTTCTCTCAA
 CAAATTTCTGACCACAGCCAAAGATAAGAACCGCTGGGAGGAGCCTGGTCAAGCTCTACAATGTGGAG
 GCCACATCCTATGCCCTTGGCCCTACTGCAGCTAAAAGACTTTGACTTTGTGCTCCCGTCGTGCGTT
 GGCTCAATGAACAGAGATACTACGGTGGTGGCTATGGCTCTACCCAGGCCACCTTCATGGTGTCCAAGC
 CTTGGCTCAATACAAAAGGATGTCCCTGATCACAAGGAACCTGAACCTGGATGTGTCCCTCCAACCTGCCC
 AGTCGCAGCTCCAAGATCATCCACCGTATCCACTGGGAATCTGCCAGCCTCCTGCGATCAGAAGAGCCA
 AGGAAAATGAGGGTTTACAGTACAGCTGAAGGAAAAGGCCAAGGCACCTTGTGCGTAGTGACAATGTA
 CCATGCTAAGGCCAAAGGTCAACTCACCTGTAATAAATTCGACCTCAAGGTACCATAAAACAGCACCG
 GAAACAGAAAAGAGCCTCAGGATGCCAAGAACTATGATCCTTGAGATCTGTACCAGGTACCGGGGAG
 ACCAGGATGCCACTATGTCTATACTGGACATATCCATGATGACTGGCTTCGTTCCAGACACAGATGACCT
 CAAGCAGCTGGCAAACGGCGTTGACAGATACATCTCCAAGTATGAGCTGGACAAAGCCTTCTCCGATAGG
 AACACCCTCATCATCTACTGGACAAGGTCTCACACTCTGAGGATGACTGTATAGCTTTCAAAGTTCACC
 AATATTTAATGTAGAGCTTATCCAGCCTGGTGCAGTCAAGGCTACGCCTATTACAACCTGGCGGAAAG
 CTGTACCCGGTTTACCACCCAGAAAAGGAGGATGGAAGCTGAACAAGCTCTGTCGTGATGAGCTGTGC
 CGCTGTGCTGAGGAGAATTGCTTCATACAAAAGTTGGATGACAAAAGTCAACCTGGAAGAACGGCTGGACA
 AGGCCTGTGAGCCAGGAGTGGACTATGTGTACAAGACCCGACTGGTCAAGGCCAGCTGTCCAATGACTT
 TGACGAGTACATCATGGCCATGAGCAGATCATCAAGTCAGGCTCGGATGAGGTGCAGGTGGACAACAG
 CGCACGTTTCATCAGCCCATCAAGTGCAGGGAAGCCCTGAAGCTGGAGGAGAGGAAACTACCTCATGT
 GGGTCTCTCTCCGATTTCTGGGAGAGAAAACCAATCTCAGCTACATCATCGGAAGGACACCTGGGT
 GGAGCACTGGCCCGAGGAGGACGAATGCCAAGATGAAGAGAACCAGAAACAATGCCAGGACCTCGGCACC

-continued

TTCACCTGAGAACATGGTTGTCTTTGGGTGCCCAACTGACCACACCCCATTCCTCCACTCCCAATAAAG
CTTCAGTTATATTTCA

Reverse Complement of SEQ ID NO: 7

SEQ ID NO: 8

TGAAATATAACTGAAGCTTTATTGGGAGTGGGGGAATGGGGGTGGTTCAGTTGGGGCACCCAAAGACAACCATG
TTCTCAGTGAAGGTGCCGAGGTCTCGCATTGTTTCTGGTTCTTTCATCTTGGCATTCTCTCTCGGGCCAG
TGCTCCACCCAGGTGCTCTCCCGATGATGTAGCTGAGATTGGGTTTCTCTCCCAGAAATCGGAGGAGAGACCC
CACATGAGGTAGTGTTCCTCTCCTCCAGCTTCAGGGCTTCCCTGCACTTGATGGGGCTGATGAACGTGCGCTGT
TGTCCAACCTGCACCTCATCCGAGCCTGACTTGATGATCTGCTCAATGGCCATGATGTACTCGTCAAAGTCATTG
GACAGCTGGGCCTTGACCAGTCCGGTCTTGTACACATAGTCCACTCCTGGCTCACAGGCTTGTCCAGCCGTTCT
TCCAGGGTGACTTTGTCATCCAACCTTTTGTATGAAGCAATTCTCCTCAGCACAGCGGCACAGCTCATCACGACAG
AGCTTGTTCAGCTTTCCATCCTCTTTTCTGGGTGGTAGAACCGGGTACAGCTTTCGCGCAGGTTGTAATAGGCG
TAGACCTTGACTGCACCAGGCTGGATAAGCTCTACATTAATAATTTGGTGAACCTTGAAGCTATACAGTCATCC
TCAGAGTGTGAGACCTTGTCCAGGTAGATGATGAGGGTGTTCCTATCGGAGAAGGCTTGTCCAGCTCATACTTG
GAGATGTATCTGTCAACGCCGTTTCCAGCTGCTTGAGGTCATCTGTGTCTGGAACGAAGCCAGTCATCATGGAT
ATGTCCAGTATAGACATAGTGGCATCCTGGTCTCCCGGTACCTGGTACAGATCTCAAGGATCATAGTGTCTTG
GCATCCTGAGGCCTTTTTCTGTTTCCGGTGTGGTTTATGGTGACCTTGAGGTCGAATTTATTACAGGTGAGT
TGACCTTTGGCCTTAGCATGGTACATTGTCACTACCGACAAGGTGCCCTGGCCTTTTCTTCAGCTGTGACTGTG
AAACCTCATTTTCTCTGGTCTCTTCTGATCGCAGGAGGCTGGCAGATTCCAGTGGATACGGTGGATGATCTTG
GAGCTGCGACTGGGAGTGGAGGGACACATCCAGGTTGAGTTCCTTGTGATCAGGGACATCCTTTTGGTATTGA
GCCAAGGCTTGGAAACCATGAAGTGGCCTGGGTAGAGCCATAGCCACCACCGTAGTATCTCTGTTCAATTGAGC
CAACGCACGACGGGAGGCACAAAGTCAAAGTCTTTAGCTGCAGTAGGGCCAAAGGGCATAGGATGTGGCCTCC
ACATTGTAGAGCTGTGACCAGGCTCCTCCAGCGGTTCTTATCTTTGGCTGTGGTCAAGAAATTTGTTGAGAAGA
GGTCCCTTCAGCCTGCCATCTGGGCCAGGCATAGGCAGCGATGGCCACAGTGTAGGATCTCTGTAGGTTTCATG
TAGTTGGCTTCAAGGAAGTCTCCTGCTTTAGTGATGCTGCCGGGACAGGCTGTGACCTGCTCCTCGCAAATCTCT
TTAGCCTCTTGACGAGATGAGAACAAGGCGGTGAGGGCCATGTCTTCTCGTTGGTGTCCGGAATCCACCA
GTCATTTCTTGATGTATCACGGGCGATCCTCCTGGAAGACCCGTCGGGCTTCTGCTTCTCCAGGATCAGCCAT
TTAACAGCCCCGAGAGGACCTGGGAGTCGATGGCAATGAGGTTGACAGCCAGAGAGAAGACCTTGACCACGTAG
GCGGTCAGCCAGGTGCTGGGTGCCCGTTCAGGAAGGCCGCAAGGCAGAGCTGGGTTGTCTGAAGGCCAGCTGC
TGGGTGTACCCCTTCTTGATGAGCTCCAAGGCCCTGCCGCTTCTCCGGGCCGAACTTCTCCACTGTTCGGTT
TCATCCAGGTAATGCACAGCGATGACTGTGGGCGTCATGGTGATCATGTTCTGTCTCCGCAGCCGAGGGGTC
ACGATGAGGTGCTTCCAGCGTTCGCATCGATGGCATCCTCTGTCTATCTGGGCCACCGGGTCCCTTGCAGGAGA
ATTCTGGTCTCAGACTCGGTGTCGGGACTTGGTCACTGAGGCTGTCAGGTGGGACGTCCTCTCTGCACTCCT
TCCTGGCCAGGCTTCTGGATCCAGCGTGCAGACAGCCACAGTTTGTTCATTCTGATTCCTTCCGGCACGACC
TTCAGGGACTTCTGACACCGTCACTGATGAAAAATGGTAGACGGCAGCCTTGACTTCCACTTCTCTGCTGGCCG
GTCTTTAGGGGCACGATGACATAAGGAACGGACAGCGAGGACTTGGGGGGATGGTTACGGTCTGCTGGTGACGC
CTCTTGGCGGTGGCCAGGCTGCAGAAGGCTGGATTGTGGAGTAGTTCCACCCTCACCTTGAGCTCTGGTTCTGC
CGGTAATTGTAGAGAACAGCTCGGATTTCCACCTGCTCGTTTCGAACAACAGAGTAGGGTAGCCGAGGTCGATG
AAGAAGTCTGCATTACTGTGACCTCGAAGGGGCTGCCACACAGATCCCTTTCTGTCTGACAAGCTCACGGCC
AGAATCTCCACGTTGGTATGGAGTCTTCAAAAATATATTCATGAGCTTCGTGGAGATTCCGTTTTTCCGGTGCC

-continued

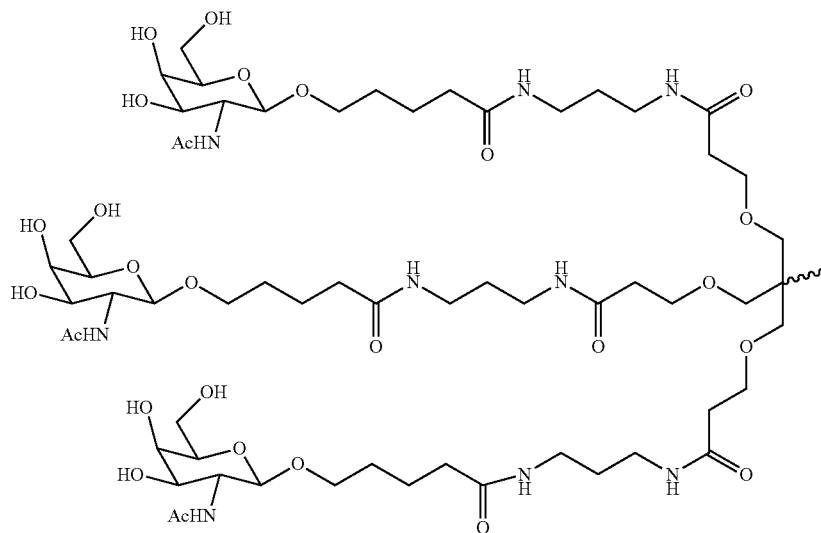
TCTTTCAACTCTTCAATCTTCCACAGCCAACCTCTCTGGGAACTCACTTCGGGAAACGATGTTCTTCTTTCGCGATG
ATGTCTCATCCAGGTTACTCTCGGCCAGGCCAGGTGACTGGCCCGCGCTGCTGCCGCCAGCTCGGTGATG
TAGTTGCAGCAGTCCAGGAAGGCCTTCTTGACCGCTCGTCCAGGGTATGTAACGGGTCCGGCGCTGGCATGAG
AACCTCATGGGGTTCTCCCGCATAACCGTCTCGCAGCACTTGCGCAGCTCCTTGGGGTACTGACCAACTTTGTCC
ATTCTTCTTCCCGCAGCTGCACGGAACGGCGTGGCGGGCGGTGGCTGTGGGCACTGAAGTTCTGCCCTCTGG
GCCGTCTGCTGGCCACTGCTGCTCGCAAGGTGAGCCTGCATCCGAGAAGACACCAGCGTAATCCTTCCCACTG
CCTGGGGTGCAGCCGATGTCTGCCTTCTCCACCACGTCACAGATCTTACTCTGCGTCAGCTTGTCTTCTTATTC
AGCACAAACACGCCCTTGTCCACAGCCACCAGTCCACCCGGGCCCCGTGGTCACCCCTCTATCTTCAGGGTCATC
TGCTGCCCGGGTAAAGGCTGCCTGTCTTCTGACTGGCCGCTTTTTACCACCAGCGAGCCACGCAAGAGTCCTTG
ACGTCCACCCACACGGAGTCGGCCACCCTTCCCTCTGGCCGTGGCGCCGATCAGCGTGTAGTAGGCCACCAGG
CGGAAGGAAGGGATGAAGTCGGTGGTATGGACAGGGGCAGCACCACCAGGTCCTGGCCAGGCTCTCGCACCTGG
CGTCCACCTTCAACAGCTTGCCCTTGTTCATAATCAGGTAGGTGTAGTAGCGATCTTGGCCTCCTGGGTGCGG
TCCATTGCGAGAGGAAGTTGACGTTGAGGGTCTCCCAAGTCTGAGCTCTGCACGTGGCACTGAGAGATGCAGG
TAATTGTTGGAGTTGCCACGGTGTGTAGGGCTGAGCCTCCATGGTCTGGTAGCCTGCTCCGCTCCGAGAGC
TCCCCTTCTTCTGTCGCACCGTGTATGCTCAAGGGCTTCTGGCTGGGGTGTGTGTTGATGCTGAGTTTGGCCACG
CCGTCTCCCTGGGTTAGAGACTGCACAGCGTCTCGCCCTGACTGCCACGGGGACTCGGTAGGCTGGAGAGCCA
TCGGGGTTCGTACGAACACCATGAGGTCAAAGGCATTCCTGGTTTGAAGTACTTGGGCGTCTTGGTGAAGTGG
ATCTGGTAGGAGAGGTCACGATGGGGATCCGCTGCGCTCCGCTGCACCATGCTACTGCTGAGTGCAGGATA
ACGGTGACAGACACATAAAGGACTTCCCACTAGGTCTTCCGGTCGGGGATTCTGCACCCCGTCCAGCAGTACC
TCCCGCTCAGCACGGCGTCTCCTGAGCCATCCTCAATCTGGATGCGCTTGAGGGATT CAGGCAGGAAATCCTC
TGCTCGCATCCTGGATCCGAAGATGACAAAGGCAGTTCCTCCACTTCTTTCCATAGAGGAACCTGGCGGTG
ATGGTGACCTCCAGGCCCTTCTGGTTATAGATGTAGTAGAATTTCTCTGTAGGCTCCACTATGACCTCGAAACTG
GGCAGCACGTACTCCTTCACTCAAACCTCAGTGGAGAAGACCTGTTGCGGCGAATTTTCATAGTAGGCTCGGATC
TTCCACTGGCCATGTTGACGAGTTCGGAAATGTCCCAAGACAAGGGCAAGATGCCAAATGGTTCTGAGAAGAC
AAGGAGTCTGCTTGACCGGGATGCCGTCGGGTCTCAATGTTGACCACGACCGTCCGGCCACGGGTAGCAGC
TTGTGGTTGACGGTGAAGATCCGACAGAGAACTGTGGAGCCAGGGGTGTAGATGGTCTGTCTGTCTGGATGAAG
AGGTACCCGCTCTGAAGCTGACCAGTACCCTTCTCCACCCTTGGGCCCGAAGGTGGCCTGCACAGTCACG
AACTGTTGTGCCCTTTTCTGACTTGAACCTCTGTTGGCTGGGATCCTGATGGTGACGCTGCCATGTGGCTG
GTGGCAGGGTTCAGCACGGTCTTCTCACTGGACAGCACCAGTTTTTTGCCTGGGAAGTCGTGGACAGTGACAGT
ACCGGAACATCCCATTCGCGTCATGGCCCTCCAGCACCAGGTCTCCTCACTCTCCAGCCGAAGACGTTTGGG
GTGATCATAGAGTACATGGGAGTCCCAGAGCCAGGGGGAGGTGGATTAGTAGCAGGAGCAGCAGGCTGGGACCT
GAGGTGAGTCCCATGGTGTGGGACAGTGCAGGGTCAGAGGGACAGAGGGACAGAGGGAGAGGATGGGGAGGAGT
GAGCAGTGACTGCTGGAGTTGGCTTT

SEQUENCE LISTING

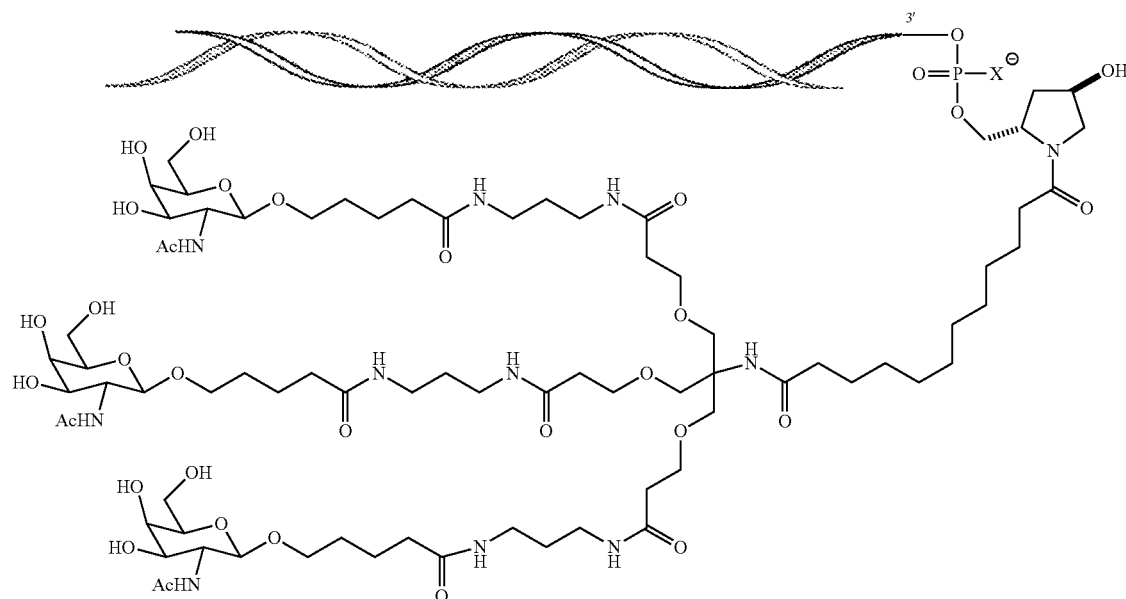
The patent contains a lengthy sequence listing. A copy of the sequence listing is available in electronic form from the USPTO web site (<https://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US11866701B2>). An electronic copy of the sequence listing will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of complement component C3 in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein each strand is independently 15-30 nucleotides in length, wherein the antisense strand comprises at least 15 contiguous nucleotides from the complement of nucleotides 4614-4632 of SEQ ID NO:1, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a nucleotide modification, and
 - 15
 - 20
 - 25
2. The dsRNA agent of claim 1, further comprising at least one phosphorothioate or methylphosphonate internucleotide linkage.
3. The dsRNA agent of claim 1, wherein at least one strand comprises a 3' overhang of at least one nucleotide.
4. The dsRNA agent of claim 1, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.
5. The dsRNA agent of claim 1, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.
6. The dsRNA agent of claim 5, wherein the ligand is
 - wherein at least one strand is conjugated to a ligand.



7. The dsRNA agent of claim 5, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

8. The dsRNA agent of claim 7, wherein the X is O.

9. An isolated cell comprising the dsRNA agent of claim 1.

10. A pharmaceutical composition for inhibiting expression of a complement component C3 gene comprising the dsRNA agent of claim 1.

11. The dsRNA agent of claim 1, wherein the antisense strand comprises at least 17 contiguous nucleotides from the complement of nucleotides 4614-4632 of SEQ ID NO:1.

12. The dsRNA agent of claim 1, wherein the antisense strand comprises at least 19 contiguous nucleotides from the complement of nucleotides 4614-4632 of SEQ ID NO:1.

13. The dsRNA agent of claim 1, wherein the sense strand comprises the nucleotide sequence 5'-UGAUGAACUGUGCCGUGU-3' (SEQ ID NO:248) and the antisense strand comprises the nucleotide sequence 5'-ACAGCGGCACAGUUCAUCA-3' (SEQ ID NO:516).

14. The dsRNA agent of claim 1, wherein each strand is independently 19-25 nucleotides in length.

15. The dsRNA agent of claim 1, wherein at least one of the nucleotide modifications is selected from the group consisting of a 2'-O-methyl nucleotide modification, a nucleotide comprising a 5'-phosphorothioate nucleotide group modification, a deoxy-nucleotide modification, a 3'-terminal deoxy-thymine (dT) nucleotide modification, a 2'-fluoro nucleotide modification, a 2'-deoxy-nucleotide modification, a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group nucleotide modification, a 2'-deoxy-2'-fluoro nucleotide modification, a locked nucleotide modification, an unlocked nucleotide modification, a conformationally restricted nucleotide modification, a constrained ethyl nucleotide modification, an abasic nucleotide modification, a 2'-amino-nucleotide modification, a 2'-O-allyl-nucleotide modification, a 2'-C-alkyl-nucleotide modification, a 2'-hydroxyl-nucleotide modification, a 2'-methoxyethyl nucleotide modification, a 2'-O-

30

alkyl-nucleotide modification, a morpholino nucleotide modification, a phosphoramidate modification, a non-natural base nucleotide modification, a tetrahydropyran nucleotide modification, a 1,5-anhydrohexitol nucleotide modification, a cyclohexenyl nucleotide modification, a 5'-phosphate nucleotide modification, and a 5'-phosphate mimic nucleotide modification.

35

16. The dsRNA agent of claim 2, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

40

17. The dsRNA agent of claim 16, wherein the strand is the antisense strand.

18. The dsRNA agent of claim 16, wherein the strand is the sense strand.

45

19. The dsRNA agent of claim 2, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

50

20. The dsRNA agent of claim 19, wherein the strand is the antisense strand.

55

21. The dsRNA agent of claim 19, wherein the strand is the sense strand.

22. The dsRNA agent of claim 2, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at both the 5'- and 3'-terminus of one strand.

60

23. The dsRNA agent of claim 22, wherein the strand is the antisense strand.

65

24. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of complement component C3 in a cell,

wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein each strand is independently 15-30 nucleotides in length,

wherein the antisense strand comprises at least 15 contiguous nucleotides from the complement of nucleotides 4614-4632 of SEQ ID NO:1,

171

172

wherein all of the nucleotide of the sense strand and all of
the nucleotides of the antisense strand comprise a
nucleotide modification,
wherein the dsRNA agent comprises 6-8 phosphorothio-
ate internucleotide linkages, and
wherein at least one strand is conjugated to an N-acetyl-
galactosamine ligand.

5

* * * * *