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(54) Title: FACTOR XII (HAGEMAN FACTOR) (F12), KALLIKREIN B, PLASMA (FLETCHER FACTOR) 1 (KLKB1), AND KININOGEN 1 (KNG1) IRNA COMPOSITIONS AND METHODS OF USE THEREOF

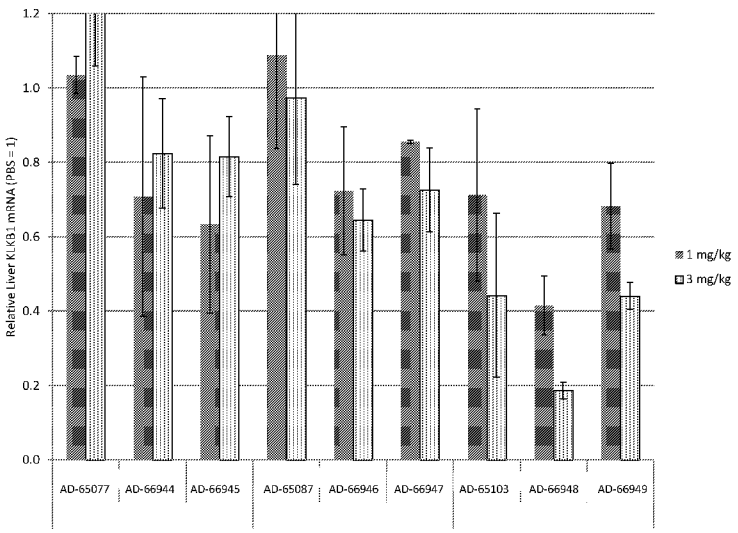

Figure 1

(57) Abstract: The present invention relates to RNAi agents, *e.g.*, double stranded RNAi agents, targeting the Kallikrein B, Plasma (Fletcher Factor) 1 (KLKB1) gene, the Factor XII (Hageman Factor (F12) gene, or the Kininogen 1 (KNG1) gene, and methods of using such RNAi agents to inhibit expression of a KLKB1 gene, an F12 gene, and/or a KNG1 gene, and methods of treating subjects having an hereditary angioedema (HAE) and/or a contact activation pathway-associated disorder.

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FACTOR XII (HAGEMAN FACTOR) (F12), KALLIKREIN B, PLASMA (FLETCHER FACTOR) 1 (KLKB1), AND KININOGEN 1 (KNG1) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

Related Applications

This application claims the benefit of priority to U.S. Provisional application No. 62/157,890, filed on May 6, 2015, to U.S. Provisional Patent Application No. 62/260,887, filed on November 30, 2015, and to U.S. Provisional Patent Application No. 62/266,958, filed on December 14, 2015. The entire contents of 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 and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 3, 2016, is named 121301-03120_SL.txt and is 721,827 bytes in size.

Background of the Invention

The blood coagulation system is essential for hemostasis, responding to vascular injury with local production of a clot formed of fibrin mesh and activated platelets. Blood coagulation, thrombin generation, and fibrin formation can be initiated by two distinct pathways, referred to as the extrinsic and intrinsic pathways.

The extrinsic pathway involves binding of plasma factor VIIa (FVIIa) to extravascular tissue factor (TF) at a site of vessel injury.

The intrinsic pathway is initiated by the surface-dependent activation of plasma factor XII (F12) to F12a in a process called contact activation. Contact activation involves two other proteins, prekallikrein and high molecular weight kininogen which circulate as a bi-molecular complex. Collectively, these three proteins, FXII, prekallikrein and HK, comprise the "contact activation pathway," also referred to as the "Kallikrein-Kinin System." When the contact activation pathway is initiated by binding of F12 to negatively charged surfaces (or macromolecules), a conformational change in F12 is induced resulting in formation of active F12 (F12a). F12a cleaves prekallikrein to generate active kallikrein (α-kallikrein), which in turn reciprocally activates F12 to generate additional F12a. The active kallikrein then digests high-molecular-weight kininogen to liberate bradykinin. F12a generated by contact activation also activates factor XI (F11) to F11a, triggering a series of proteolytic cleavage events that culminates in thrombin generation and fibrin clot formation.

Interestingly, it has been shown that the contact system is not required for hemostasis. Humans and other animals deficient in a contact activation protein are largely asymptomatic and homozygous F12 deficiency is not associated with any disease or disorder. However, the contact system has been shown to play an important role in thrombotic disease, as pharmacologic inhibition of F12a or ablation of the F12 or high molecular weight kininogen genes can protect mice from experimentally induced thrombosis in a variety of models.

In healthy subjects, a homeostatic balance between procoagulant forces and anticoagulant and fibrinolytic forces exists. However, numerous genetic, acquired, and environmental factors can dysregulate this balance in favor of coagulation, leading to thrombosis, the pathologic formation of thrombi, triggering life-threatening events For example, formation of thrombi in a vein may result in, *e.g.,* deep venous thrombosis (DVT), and formation of thrombi in an artery or a cardiac chamber may result in, *e.g.,* myocardial infarction or stroke. Thrombi may obstruct blood flow at the site of formation or detach and embolize to block a distant blood vessel (*e.g.,*a pulmonary embolism or embolic stroke).

Acquired/enviornomental factors that can lead to pathological contact activation and contact pathway-mediated thrombosis include various dental, surgical and medical settings, such as atrial fibrillation, cancer treatment, immobilization, central venous catheters, implants, and extracorporeal oxygenation. As a result of such medical and surgical settings, tissue damage releases tissue factor and exposes various triggers of the contact pathway, such as DNA, RNA, phosphate, collagen, and laminin) which activate the contact pathway leading to thrombosis.

A genetic disorder that dysregulates the homeostatic balance between procoagulant forces and anticoagulant and fibrinolytic forces is Hereditary Angioedema (HAE). HAE is a rare autosomal dominant disorder that causes recurrent edema and swelling of the extremities, face, larynx, upper respiratory tract, abdomen, trunk, and genetials and a nonpruritic rash in one-third of patients. Untreated HAE patients experience an average of one-to-two angioedema attacks per month, but the frequency and severity of episodes can vary significantly. Edema swelling is often disfiguring and disabling, results in frequent hospitalization, and patients sometimes require psychiatric care to treat disease-associated anxiety. Abdominal attacks can cause severe pain, nausea and vomiting, and sometimes lead to inappropriate surgeries. Furthermore, over half of HAE patients also experience life-threatening laryngeal edema during their lifetime that may require emergency tracheostomy to prevent asphyxiation. HAE affects an estimated 6,000 to 10,000 individuals of varying ethnic groups in the United States and causes significant economic harm to patients, accounting for 15,000 to 30,000 hospital visits and 20 to 100 sick days per year.

HAE results from a mutation of the C1 inhibitor (C1INH, SERPING1) gene that results in a deficiency of C1INH protein. Over 250 different C1INH mutations have been demonstrated to cause an HAE clinical presentation. These C1INH mutations are typically

inherited genetically, however, up to 25% of HAE cases result from *de novo* mutation of C1INH. HAE type I is caused by C1INH mutations that result in lower levels of truncated or misfolded proteins that are inefficiently secreted, and accounts for approximately 85% of HAE cases. HAE type II constitutes about 15% of cases and is caused by mutations near the C1INH's active site that result in normal levels of dysfunctional C1INH protein. In addition, HAE type III, a rare third form the disease, occurs because of a gain-of-function mutation in coagulation factor XII (F12) (Hageman Factor).

C1 inhibitor is a serine protease inhibitor of the serpin family and a major inhibitor of proteases in the complement and contact activation pathways, as well as a minor inhibitor of fibrinolytic protease plasmin. These plasma proteolytic cascades are activated during an HAE attack, generating substances that increase vascular permeability, *e.g.,* bradykinin. Studies have shown that the bradykinin peptide, which activates proinflammatory signaling pathways that dilate vessels and induces chemotaxis of neutrophils, is the primary substance that enhances vascular permeability in an HAE attack by binding to the bradykinin receptor on vascular endothelial cells.

Typically, C1INH inhibits the autoactivation of F12 the ability of F12a to activate prekallilrein, the activation of high molecular weight kininogen by kallikrein, and the feedback activation of F12 by kallikrein. Consequently, mutations causing C1INH deficiency or F12 gain-of-function result in excess production of bradykinin and onset of HAE angioedema.

Currently, HAE may be treated with 17α-alkylated androgens prophylactically to reduce to probability of recurrent episodes, or with disease-specific therapeutics to treat acute attacks. About 70% of individuals with HAE are treated with androgens or remain untreated, and about 30% receive therapeutics. Androgens are unsuitable for short-term treatment of acute attacks because they take several days to become effective, and they can have significant side effects and may affect growth and development adversely. As a result, androgens are used only for long-term prophylaxis and are typically not administered to pregnant women or children. Furthermore, current therapeutics used to treat acute attacks must be administered intravenously numerous times per week or may cause side-effects that require drug administration and subsequent patient monitoring in a hospital, thereby limiting their regular prophylactic use to manage the disease long-term. Therefore, in the absence of regimens which be administered safely, effectively and by more convenient routes and regimens to treat acute angioedema attacks and prophylactically manage recurrent attacks in a large proportion of patients, including pregnant women and children, there is a need for alternative therapies for subjects suffering from HAE.

Accordingly, there is a need in the art for compositions and methods to inhibit thrombosis in a subject at risk of forming a thrombus, such as a subject having a genetic, an acquired, or an environmental risk of forming a thrombus.

Summary of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a Kallikrein B, Plasma (Fletcher Factor) 1 (KLKB1) gene, RNA transcripts of a Factor XII (F12) gene, or RNA transcripts of a kininogen (KNG1) gene. For simplicity, and unless otherwise specified, the term "contact activation pathway gene" as used herein refers to a KLKB1 gene, an F12 gene, or a KNG1 gene. The contact activation pathway gene may be within a cell, *e.g.,* a cell within a subject, such as a human.

Accordingly, in one aspect, the present invention provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of Factor XII (Hageman Factor) (F12), wherein the double stranded RNAi agent 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 the nucleotide sequence of SEQ ID NO:9 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:10.

In another aspect, the present invention provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of a Factor XII (Hageman Factor) (F12), wherein the double stranded RNAi agent 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 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27.

In another aspect, the present invention provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of a Factor XII (Hageman Factor) (F12), wherein the double stranded RNAi agent 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 nucleotides 2000-2060 of SEQ ID NO:9. In some embodiments, the antisense strand comprises a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 2000-2030 of SEQ ID NO:9. In other embodiments, the antisense strand comprises a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 2030-2060 of SEQ ID NO:9. In one embodiment, the antisense strand comprises a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 2010-2040 of SEQ ID NO:9. In one embodiment, the antisense strand comprises a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 2010-2035 of SEQ ID NO:9. In another embodiment, the antisense strand comprises a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 2015-2040 of SEQ ID NO:9. In another embodiment, the antisense strand comprises a

region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 2015-2045 of SEQ ID NO:9. In another embodiment, the antisense strand comprises a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 2020-2050 of SEQ ID NO:9. In another embodiment, the antisense strand comprises a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 2020-2045 of SEQ ID NO:9. In still other embodiments, the antisense strand comprises a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the ranges of SEQ ID NO:9 provided in Table 24. In one embodiment, the antisense strand comprises a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 2018-2040 of SEQ ID NO:9. In one embodiment, the antisense strand comprises a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of the antisense strand of AD-67244 (5' – UUCAAAGCACUUUAUUGAGUUUC – 3') (SEQ ID NO: 25). In one embodiment, the sense strand comprises the sense strand nucleotide sequence of AD-67244. In some embodiments, the region of complementarity comprises 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides differing by no more than 3 nucleotides from nucleotides 2015-2040 of SEQ ID NO:9. In some embodiments, the region of complementarity comprises 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides differing by no more than 3 nucleotides from nucleotides 2015-2045 of SEQ ID NO:9. In some embodiments, the region of complementarity comprises 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides differing by no more than 3 nucleotides from nucleotides 2018-2040 of SEQ ID NO:9. In some embodiments, the region of complementarity comprises 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides differing by no more than 3 nucleotides from nucleotides 2018-2045 of SEQ ID NO:9. In one embodiment, the agent comprises at least one modified nucleotide. In another embodiment, all of the nucleotides of theagent are modified nucleotides. In one embodiment, the agent further comprises a ligand, *e.g.,* a ligand attached to the 3'-end of the sense strand. In one embodiment, the sense strand and the antisense strand are each independently 15-30 nucleotides in length. In another embodiment, the sense strand and the antisense strand are each independently 19-25 nucleotides in length.

In one aspect, the present invention provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of Kallikrein B, Plasma (Fletcher Factor) 1 (KLKB1), wherein the double stranded RNAi agent 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 the nucleotide sequence of 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 acid (RNAi) agents for inhibiting expression of a Kallikrein B, Plasma (Fletcher Factor) 1 (KLKB1), wherein the double stranded RNAi agent 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, 19A, or 19B.

In one aspect, the present invention provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of Kininogen 1 (KNG1), wherein the double stranded RNAi agent 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 the nucleotide sequence of SEQ ID NO:17 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:18.

In another aspect, the present inventionprovides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of a Kininogen 1 (KNG1), wherein the double stranded RNAi agent 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 15, 16, 19E, or 19F.

In one embodiment, the antisense strand comprises 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, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27.

In one embodiment, the double stranded RNAi agents provided herein comprise at least one modified nucleotide.

In one aspect, the present invention provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of Kallikrein B, Plasma (Fletcher Factor) 1 (KLKB1), wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of 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, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand is conjugated to a ligand attached at the 3'-terminus.

In another aspect, the present invention provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of Factor XII (Hageman Factor) (F12), wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides

differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:9 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:10, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand is conjugated to a ligand attached at the 3'-terminus.

In a further aspect, the present invention provides double stranded ribonucleic acid (RNAi) agents for inhibiting expression of Kininogen 1 (KNG1), wherein the double stranded RNAi agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:17 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:18, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand is conjugated to a ligand attached at the 3'-terminus.

In certain embodiments, the dsRNA comprises at least one modified nucleotide. In certain embodiments, the dsRNA comprises no more than 4 (*i.e.*, 4, 3, 2, 1, or 0) unmodified nucleotides in the sense strand. In certain embodiments, the dsRNA comprises no more than 4 (*i.e.*, 4, 3, 2, 1, or 0) unmodified nucleotides in the antisense strand. In certain embodiments, the dsRNA comprises no more than 4 (*i.e.*, 4, 3, 2, 1, or 0) unmodified nucleotides in both the sense strand and the antisense strand. In certain embodiments, all of the nucleotides in the sense strand of the dsRNA are modified nucleotides. In certain embodiments,all of the nucleotides in the antisense strand of the dsRNA are modified nucleotides. In certain embodiments, all of the nucleotides in the sense strand of the dsRNA and all of the nucleotides of the antisense strand are modified nucleotides.

In certain embodiments, the at least one of the modified nucleotides is selected from the group consisting of 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 locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, and a nucleotide comprising a 5'-phosphate mimic, *e.g.,* a vinyl phosphate.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of 2'-O-methyl and 2'fluoro modifications.

In certain embodiments, the antisesense strand of the double stranded RNAi agents of any of the invention comprise no more than 8 2′-fluoro modifications, no more than 7 2′-fluoro modifications, no more than 6 2′-fluoro modifications, no more than 5 2′-fluoro modifications, no more than 4 2′-fluoro modifications, no more than 3 2′-fluoro modifications, no more than 2 2′-fluoro modifications, no more than 1 2′-fluoro modifications, or no more than 1 2′-fluoro modifications. In other embodiments, the sesense strand of the double stranded RNAi agents of any of the invention comprise no more than 6 2′-fluoro modifications, no more than 5 2′-fluoro modifications, no more than 4 2′-fluoro modifications, no more than 3 2′-fluoro modifications, no more than 2 2′-fluoro modifications, no more than 1 2′-fluoro modifications, or no more than 1 2′-fluoro modifications.

In one embodiment, the double stranded RNAi agent further comprises at least one phosphorothioate internucleotide linkage. In one embodiment, the double stranded RNAi agent comprises 6-8 phosphorothioate internucleotide linkages.

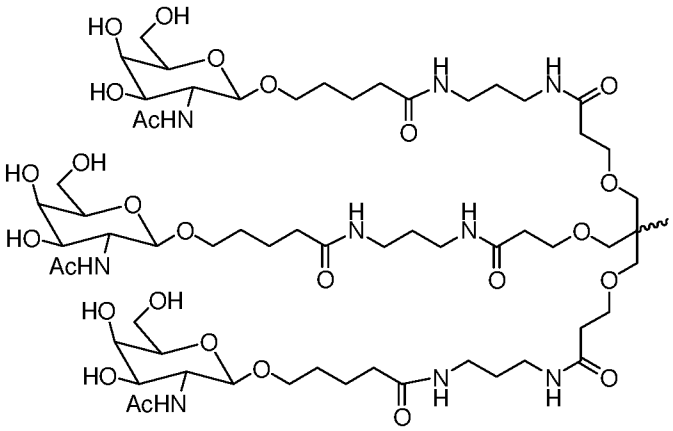
The region of complementarity may be at least 17 nucleotides in length, 18 nucleotides in length, 19 nucleotides in length, 20 nucleotides in length, or 21 nucleotides in length.

In certain embodiment, the region of complementarity may be 19 to 21 nucleotides in length or 21 to 23 nucleotides in length.

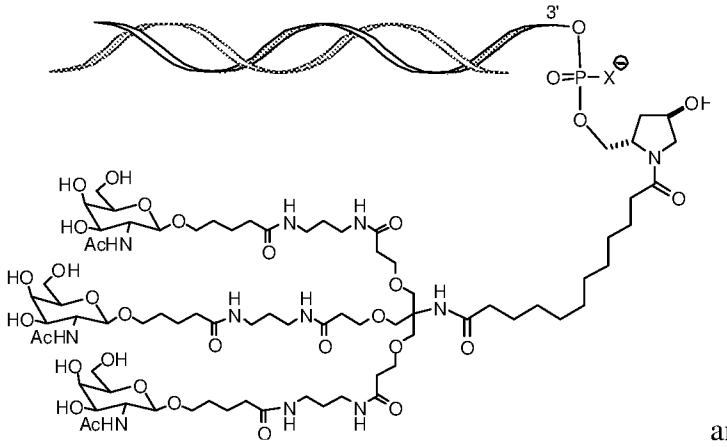
In certain embodiments, each strand of the double stranded RNAi agent is no more than 30 nucleotides in length. In certain embodiments, the double stranded RNAi agent is at least 15 nucleotides in length.

In certain embodiments, at least one strand of the double stranded RNAi agent comprises a 3' overhang of at least 1 nucleotide. In certain embodiments, the at least one strand comprises a 3' overhang of at least 2 nucleotides.

In certain embodiments, the double stranded RNAi agent further comprises a ligand. In certain embodiments, the ligand is conjugated to the 3' end of the sense strand of the dsRNA. In certain embodiments, the ligand is an N-acetylgalactosamine (GalNAc) derivative. In certain embodiments, the ligand is one or more GalNAc derivatives attached through a monovalent, a bivalent, or a trivalent branched linker. In certain embodiments, the ligand is



In certain embodiments, the dsRNA is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

In one embodiment, the X is O.

In one embodiment, the sense and antisense sequences are selected from any one of those sequences listed in any one of Tables 3, 4, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27.

In one embodiment, the region of complementarity consists of any one of the antisense sequences listed in any one of Tables 3, 4, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27.

In one embodiment, the dsRNA agent that inhibits the expression of F12 is selected from the group consisting of AD-66170, AD-66173, AD-66176, AD-66125, AD-66172 , AD-66167 , AD-66165, AD-66168, AD-66163, AD-66116, AD-66126, and AD-67244. In another embodiment, the dsRNA agent that inhibits the expression of F12 is AD-67244.

In one embodiment, the dsRNA agent that inhibits the expression of KLKB1 is selected from the group consisting of AD-65077, AD-65170, AD-65103, AD-65083, AD-65087, AD-65149, AD-64652, AD-65162, AD-65153, AD-65084, AD-65099, and AD-66948. In another embodiment, the dsRNA agent that inhibits the expression of KLKB1 is AD-66948.

In one embodiment, the dsRNA agent that inhibits the expression of KNG1 is selected from the group consisting of AD-66259, AD-66261, AD-66262, AD-66263, AD-6634, and AD-67344. In another embodiment, the dsRNA agent that inhibits the expression of KNG1 is AD-67344.

In one aspect, the present invention provides cells comprising a double stranded RNAi agent of the invention targeting KLKB1. In one aspect, the present invention provides cells comprising a double stranded RNAi agent of the invention targeting F12. In a further aspect, the present invention provides cells comprising a double stranded RNAi agent of the invention targeting KNG1.

In one aspect, the present invention provides vectors encoding at least one strand of of a double stranded RNAi agent of the invention targeting KLKB1. In another aspect, the present inventionprovides vectors encoding at least one strand of of a double stranded RNAi agent of the invention targeting F12. In a further aspect, the present invention provides vectors encoding at least one strand of of a double stranded RNAi agent of the invention targeting KNG1.

In one aspect, the present invention provides pharmaceutical compositions for inhibiting expression of a KLKB1 gene comprising a double stranded RNAi agent or vector of the invention. In another aspect, the present invention provides pharmaceutical compositions for inhibiting expression of a F12 gene comprising a double stranded RNAi agent or vector of the invention. In a further aspect, the present invention provides pharmaceutical compositions for inhibiting expression of a KNG1 gene comprising a double stranded RNAi agent or vector of the invention.

The pharmaceutical compositions provided herein may be administered in an unbuffered solution, *e.g.,* saline or water, or administered with a buffer solution, *e.g.,* a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS).

In one embodiment, the pharmaceutical compositions of the invention comprise a double stranded RNAi agent as described herein, and a lipid formulation.

In one aspect, the present invention provides methods of inhibiting KLKB1 expression in a cell. The methods include contacting the cell with a double stranded RNAi agent or a pharmaceutical composition of the invention; and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of a KLKB1 gene, thereby inhibiting expression of the KLKB1 gene in the cell.

In another aspect, the present invention provides methods F12 expression in a cell. The methods include contacting the cell with a double stranded RNAi agent or a pharmaceutical composition of the invention; and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of a F12 gene, thereby inhibiting expression of the F12 gene in the cell.

In a further aspect, the present invention provides methods KNG1 expression in a cell. The methods include contacting the cell with a double stranded RNAi agent or a pharmaceutical composition of the invention; and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of a KNG1 gene, thereby inhibiting expression of the KNG1 gene in the cell.

In one embodiment, the cell is within a subject, such as a human subject.

In one embodiment, the KLKB1 expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

In one embodiment, the F12 expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

In one embodiment, the KNG1 expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

In one aspect, the present invention provides methods of treating a subject having a disease or disorder that would benefit from reduction in expression of a contact activation pathway gene. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent or pharmaceutical composition of invention, thereby treating the subject.

In one embodiment, the contact activation pathway gene is KLKB1. In another embodiment, the contact activation pathway gene is F12. In yet another embodiment, the contact activation pathway gene is KNG1.

In another aspect, the present invention provides methods of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in expression of a contact activation pathway gene. The methods include administering to the subject a prophylactically effective amount of a double stranded RNAi agent or pharmaceutical composition of invention, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in expression of a contact activation pathway gene.

In one embodiment, the contact activation pathway gene is KLKB1. In another embodiment, the contact activation pathway gene is F12. In yet another embodiment, the contact activation pathway gene is KNG1. In one embodiment, the contact activation pathway gene is F12 and the methods further comprise administering to the subject a double stranded RNAi agent of the invention targeting KLKB1. In another embodiment, the contact activation pathway gene is F12 and the methods further comprise administering to the subject a double stranded RNAi agent of the invention targeting KNG1.

In one embodiment, the administration of the double stranded RNAi to the subject causes a decrease in bradykinin levels or a decrease in coagulation factor XII activity.

In one embodiment, the disorder is a contact activation pathway-associated disease, such as a thrombophilia, hereditary angioedema (HAE), Flectcher Factor Deficiency, or essential hypertension.

In certain embodiment, the at least one symptom is an angioedema attack or a thrombus formation.

In one embodiment, the subject is human.

In one embodiment, the methods further comprise administering an anti-KLKB1 antibody, or antigen-binding fragment thereof, to the subject.

In one embodiment, the methods further comprise measuring bradykinin and/or coagulation factor XII levels in the subject.

In another aspect, the present invention provides methods of inhibiting the expression of F12 in a subject. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent of the invention targeting F12, thereby inhibiting the expression of F12 in the subject.

In one aspect, the present invention provides methods of inhibiting the expression of KLKB1 in a subject. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent of the invention targeting KLKB1, thereby inhibiting the expression of KLKB1 in the subject.

In one aspect, the present invention provides methods of inhibiting the expression of KNG1 in a subject. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent of the invention targeting KNG1, thereby inhibiting the expression of KNG1 in the subject.

In one aspect, the present invention provides methods of treating a subject having a thrombophilia. The methods include administering to the subject a therapeutically effective amount of a double stranded RNAi agent of the invention targeting F12, or a pharmaceutical composition comprising a double stranded RNAi agent of the invention targeting F12, thereby treating the subject.

In another aspect, the present invention provides methods of preventing at least one symptom in a subject having a thrombophilia. The methods include administering to the subject a prophylactically effective amount of of a double stranded RNAi agent of the invention targeting F12, or a pharmaceutical composition comprising a double stranded RNAi agent of the invention targeting F12, thereby preventing at least one symptom in the subject.

In one embodiment, the methods further comprise administering to the subject a double stranded RNAi agent of the invention targeting KLKB1. In another embodiment,the methods further comprise administering to the subject a double stranded RNAi agent of the invention targeting KNG1.

In one aspect, the present invention provides methods of treating a subject having hereditary angioedema (HAE). The methods include administering to the subject a therapeutically effective amount of of a double stranded RNAi agent of the invention targeting F12, or a pharmaceutical composition comprising a double stranded RNAi agent of the invention targeting F12, thereby treating the subject.

In another aspect, the present invention provides methods of preventing at least one symptom in a subject having hereditary angioedema (HAE). The methods include administering to the subject a prophylactically effective amount of a double stranded RNAi agent of the invention targeting F12, or a pharmaceutical composition comprising a double stranded RNAi agent of the invention targeting F12, thereby preventing at least one symptom in the subject.

In one embodiment, the methods further comprise administering to the subject a double stranded RNAi agent of the invention targeting KLKB1. In another embodiment,the methods further comprise administering to the subject a double stranded RNAi agent of the invention targeting KNG1.

In another aspect, the present invention provides methods of preventing the formation of a thrombus in a subject at risk of forming a thrombus. The methods includea dministering to the subject a prophylactically effective amount of of a double stranded RNAi agent of the invention targeting F12, or a pharmaceutical composition comprising a double stranded RNAi agent of the invention targeting F12, thereby inhibiting formation of a thrombus in the subject at risk of forming a thrombus.

In one embodiment, the subject at risk of forming a thrombus has a contact activation pathway-associated disease or disorder.

In one embodiment, the contact activation pathway-associated disease is thrombophilia. In another embodiment, the contact activation pathway-associated disease is hereditary angioedema (HAE).

In other embodments, the contact activation pathway-associated disease is Flectcher Factor Deficiency or essential hypertension.

In one embodiment, the subject at risk of forming a thrombus is selected from the group consisting of a surgical patient; a medical patient; a pregnant subject; a postpartum subject; a subject that has previously had a thrombus; a subject undergoing hormone replacement therapy; a subject sitting for long periods; and an obese subject.

In one embodiment, the methods further comprise administering to the subject a double stranded RNAi agent of the invention targeting KLKB1. In another embodiment,the methods further comprise administering to the subject a double stranded RNAi agent of the invention targeting KNG1.

In another aspect, the present invention provides methods of preventing an angioedema attack in a subject having heriditary angioedema (HAE). The methods include administering to the subject a prophylactically effective amount of a double stranded RNAi

agent of the invention targeting F12, or a pharmaceutical composition comprising a double stranded RNAi agent of the invention targeting F12, thereby preventing an angioedema attack.

In one embodiment, the methods further comprise administering to the subject a double stranded RNAi agent of the invention targeting KLKB1. In another embodiment,the methods further comprise administering to the subject a double stranded RNAi agent of the invention targeting KNG1.

Brief Description of the Drawings

Figure 1 is a graph depicting KLKB1 mRNA suppression following a single subcutaneous 1 mg/kg or 3 mg/kg dose of the indicated agents at 7-10 days post-dose in wild-type mice.

Figure 2 is a graph depicting F12 mRNA suppression following a single subcutaneous 1 mg/kg dose or a single 3 mg/kg dose, or a single 1 mg/kg dose or a single 10 mg/kg dose of the of the indicated agents at 7-10 days post-dose wild-type mice.

Figure 3 is a graph depicting KNG1 mRNA suppression following a single subcutaneous 1 mg/kg or 3 mg/kg dose of the indicated agents at 7-10 days post-dose in wild-type mice.

Figure 4A is a graph depicting the amount of Evans blue dye in the blood of mice administered a single 0 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg dose of AD-66948 and captopril at day 7 post-dose.

Figure 4B is a graph depicting the amount of Evans blue dye in the intestines of mice administered a single 0 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg dose of AD-66948 and captopril at day 7 post-dose.

Figure 4C is a graph depicting KLKB1 mRNA suppression in the liver of mice administered a single 0 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg dose of AD-66948 and captopril at day 7 post-dose.

Figure 4D is a graph depicting the relative permeability of the intestine in mice administered a single 0 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg dose of AD-66948 and captopril at day 7 post-dose.

Figure 5A is a graph depicting the amount of Evans blue dye in the blood of mice administered a single 0 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg dose of AD-67244 and captopril at day 7 post-dose.

Figure 5B is a graph depicting the amount of Evans blue dye in the intestines of mice administered a single 0 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg dose of AD-67244 and captopril at day 7 post-dose.

Figure 5C is a graph depicting F12 mRNA suppression in the liver of mice administered a single 0 mg/kg, 0.1 mg/kg , 0.3 mg/kg, 1 mg/kg, or 3 mg/kg dose of AD-67244 and captopril at day 7 post-dose.

Figure 5D is a graph depicting the relative permeability of the intestine in mice administered a single 0 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg dose of AD-67244 and captopril at day 7 post-dose.

Figure 6A is a graph depicting the amount of Evans blue dye in the blood of mice administered a single 0 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg dose of AD-67344 and captopril at day 7 post-dose.

Figure 6B is a graph depicting the amount of Evans blue dye in the intestines of mice administered a single 0 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg dose of AD-67344 and captopril at day 7 post-dose.

Figure 6C is a graph depicting KNG1 mRNA suppression in the liver of mice administered a single 0 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg dose of AD-67344 and captopril at day 7 post-dose.

Figure 6D is a graph depicting the relative permeability of the intestine in mice administered a single 0 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg dose of AD-67344 and captopril at day 7 post-dose.

Figure 7 depicts the modified nucleotide sequences of the indicated double stranded RNAi agents targeting a KLKB1 gene. F is a 2′-fluoro nucleotide modification; OMe is a 2′-O-methyl (2′-OMe) nucleotide modification; and s is a phosphorothioate linkage Figure discloses SEQ ID NOS 2285-2302, respectively, in order of appearance.

Figure 8 depicts the modified nucleotide sequences of the indicated double stranded RNAi agents targeting an F12 gene. F is a 2′-fluoro nucleotide modification; OMe is a 2′-O-methyl (2′-OMe) nucleotide modification; and s is a phosphorothioate linkage. Figure discloses SEQ ID NOS 2303-2320, respectively, in order of appearance.

Figure 9 depicts the modified nucleotide sequences of the indicated double stranded RNAi agents targeting a KNG1 gene. F is a 2′-fluoro nucleotide modification; OMe is a 2′-O-methyl (2′-OMe) nucleotide modification; and s is a phosphorothioate linkage. Figure discloses SEQ ID NOS 2321-2332, respectively, in order of appearance.

Figure 10A is a graph depicting the amount of Evans blue dye in the ears of mice administered a single 0.1 mg/kg, 0.5 mg/kg , or 3 mg/kg dose of AD-67244 in combination with a single 10 mg/kg dose of a dsRNA agent targeting C1-INH at day 7 post-dose. Error bars = standard deviation.

Figure 10B is a graph depicting dose-dependent F12 mRNA suppression following a single subcutaneous 0.1 mg/kg, 0.5 mg/kg , or 3 mg/kg dose of AD-67244 in combination with a single 10 mg/kg dose of a dsRNA agent targeting C1-INH at day 7 post-dose.

Figure 11 is a graph depicting F12 protein suppression in the plasma of female *Cynomolgus* monkeys subcutaeoulsy administered a single 3 mg/kg, 1 mg/kg, 0.3 mg/kg, or 0.1 mg/kg dose of AD-67244. The plasma F12 levels shown are the relative F12 protein levels which were normalized to the average pre-dose baseline F12 protein level. Error bars = standard deviation.

Figure 12 is a graph depicting F12 protein suppression in the plasma of wild-type mice administered a single 0.5 mg/kg dose of either AD-67244 or AD-74841.

Figure 13 is a graph depicting the effect of 5'-end modifications on the *in vivo* efficacy of the indicated agents.

Detailed Description of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a contact activation pathway gene (*i.e.,* Kallikrein B, Plasma (Fletcher Factor) 1 (KLKB1) gene, a "Factor XII (Hageman Factor) (F12) gene, or a Kininogen 1 (KNG1) gene). The gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the correponding gene (the KLKB1 gene, the F12 gene, or the KNG1 gene) in mammals.

The RNAi agents of the invention have been designed to target protein-coding and 3' UTR regions in the human KLKB1 gene, including portions of the gene that are conserved in the KLKB1 othologs of other mammalian species. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing properties and the specific target sites 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 contact activation pathway gene, *i.e.,* the KLKB1 gene, the F12 gene, or the KNG1 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 contact activation pathway gene, *i.e.,* the KLKB1 gene, the F12 gene, or the KNG1 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* and *in vivo* assays, the present inventors have demonstrated that iRNAs targeting a contact activation pathway gene can potently mediate RNAi, resulting in significant inhibition of expression the contact activation pathway gene, *i.e.,* the KLKB1

gene, the F12 gene, or the KNG1 gene. The present inventors have also demonstrated that the RNAi agents of the invention are exceptionally stable in the cytoplasm and lysosme. Thus, methods and compositions including these iRNAs are useful for treating a subject having a contact activation pathway-associated disease or disorder, *e.g.,* a thrombophilia, HAE, and for preventing at least one symptom in a subject having a contact activation pathway-associated disease or disorder or a subject at risk of developing a contact activation pathway-associated disease or disorder.

Accordingly, the present invention also provides methods for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a contact activation pathway gene, *e.g.*, a contact activation pathway-associated disease, such as a thrombophilia or hereditary angioedema (HAE), using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a contact activation pathway gene.

Very low dosages of the iRNAs of the invention, in particular, can specifically and efficiently mediate RNA interference (RNAi), resulting in significant inhibition of expression of the correponding gene (contact activation pathway gene).

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a contact activation pathway gene (*i.e.,* a KLKB1 gene, an F12 gene, or a KNG1 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 contact activation pathway gene (*i.e.,* a KLKB1 gene, an F12 gene, or a KNG1 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 "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, ranges include both the upper and lower limit.

As used herein, "Kallikrein B, Plasma (Fletcher Factor) 1," used interchangeably with the terms "Prekallikrein" and "KLKB1," refers to the naturally occurring gene that encodes the zymogen form of kallikrein, prekallikrein. Plasma prekallikrein is converted to plasma kallikrein (also referred to as active kallikrein) by F12a and proteolytically releases bradykinin from high-molecular weight kininogen and activates F12. Bradykinin is a peptide that enhances vascular permeability and is present in elevated levels in HAE patients. The amino acid and complete coding sequences of the reference sequence of the KLKB1 gene may be found in, for example, GenBank Accession No. GI:78191797 (RefSeq Accession No. NM_000892.3; SEQ ID NO:1; SEQ ID NO:2). Mammalian orthologs of the human KLKB1 gene may be found in, for example, GenBank Accession Nos. GI:544436072 (RefSeq Accession No. XM_005556482, cynomolgus monkey; SEQ ID NO:7 and SEQ ID NO:8); GI:380802470 (RefSeq Accession No. JU329355, rhesus monkey); GI:236465804 (RefSeq Accession No. NM_008455, mouse; SEQ ID NO:3 and SEQ ID NO:4); GI:162138904 (RefSeq Accession No. NM_012725, rat; SEQ ID NO:5 and SEQ ID NO:6).

Additional examples of KLKB1 mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, and OMIM.

As used herein, "Factor XII (Hageman Factor)," used interchangeably with the terms "coagulation factor XII," "FXII," "F12," active F12," and "F12a," refers to the naturally occurring gene that encodes the zymogen form of F12a. F12 a is an enzyme (EC 3.4.21.38) of the serine protease (or serine endopeptidase) class that cleaves prekallikrein to form kallikrein, which subsequently releases bradykinin from high-molecular weight kininogen and activates F12. The amino acid and complete coding sequences of the reference sequence of the F12 gene may be found in, for example, GenBank Accession No. GI:145275212 (RefSeq Accession No. NM_000505; SEQ ID NO:9; SEQ ID NO:10). Mammalian orthologs of the human F12 gene may be found in, for example, GenBank Accession Nos. GI:544441267 (RefSeq Accession No. XM_005558647, cynomolgus monkey; SEQ ID NO:11 and SEQ ID NO:12); GI:805299477 (RefSeq Accession No. NM_021489, mouse; SEQ ID NO:13 and SEQ ID NO:14); GI:62078740 (RefSeq Accession No. NM_001014006, rat; SEQ ID NO:15 and SEQ ID NO:16).

Additional examples of F12 mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, and OMIM.

As used herein, "Kininogen 1," used interchangeably with the terms "Fitzgerald Factor," "Williams-Fitzgerald-Flaujeac Factor," "high-molecular weight kininogen" ("HMWK" or "HK"), "low-molecular weight kininogen" ("LMWK)", and"KNG1," refers to

the naturally occurring gene that is alternatively spliced to generate HMWK and LMWK. Cleavage of HMWK by active kallikrein releases bradykinin. The amino acid and complete coding sequences of the reference sequence of the KNG1 gene may be found in, for example, GenBank Accession No. GI:262050545 (RefSeq Accession No. NM_001166451; SEQ ID NO:17; SEQ ID NO:18). Mammalian orthologs of the human KNG1 gene may be found in, for example, GenBank Accession Nos. GI:544410550 (RefSeq Accession No. XM_005545463, cynomolgus monkey; SEQ ID NO:19 and SEQ ID NO:20); GI:156231028 (RefSeq Accession No. NM_001102409, mouse; SEQ ID NO:21 and SEQ ID NO:22); GI:80861400 (RefSeq Accession No. NM_012696, rat; SEQ ID NO:23 and SEQ ID NO:23).

Additional examples of KNG1 mRNA sequences are readily available using publicly available databases, *e.g.*, GenBank, UniProt, and OMIM.

For simplicity, as used herein, unless otherwise specified, a "contact activation pathway gene" refers to a KLKB1 gene, an F12 gene, or a KNG1 gene.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a contact activation pathway 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 contact activation pathway gene. In one embodiment, the target sequence is within the protein coding region of the contact activation pathway gene. In another embodiment, the target sequence is within the 3' UTR of the contact activation pathway 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 KLKB1 gene in a cell, *e.g.,* a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that interacts with a target RNA sequence, *e.g.,* a contact activation pathway gene, *i.e.,* a KLKB1 target mRNA sequence, an F12 target mRNA sequence, or a KNG1 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). Thus, in one aspect the invention relates to a single stranded RNA (siRNA) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.,* a contact activation pathway gene. Accordingly, the term "siRNA" is also used herein to refer to an RNAi as described above.

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

In another embodiment, an "iRNA" for use in the compositions, uses, and methods of the invention is a double stranded RNA and is referred to herein as a "double stranded RNAi agent," "double stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA", refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.,* a contact activation pathway gene, *i.e.,* a KLKB1 gene, an F12 gene, or a KNG1 gene. In some embodiments of the invention, a double stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.,* an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

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

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

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

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

Where the two substantially complementary strands of a dsRNA are comprised by 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, an RNAi agent of the invention is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, *e.g.*, a contact activation pathway gene, *i.e.,* a KLKB1 target mRNA sequence, an F12 target mRNA sequence, or a KNG1 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).

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

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

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

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 antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the 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 KLKB1 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 contact activation pathway gene 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 includea a nucleotide mismatch in the antisense strand. In another embodiment, a double stranded RNAi agent of the invention includea 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 Hoogstein base pairing.

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

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding a contact activation pathway gene). For example, a polynucleotide is complementary to at least a part of a KLKB1 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding a KLKB1 gene.

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

In one embodiment, the antisense polynucleotides disclosed herein are fully complementary to the target KLKB1 sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target KLKB1 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 Nos:1 and 2, or a fragment of any one of SEQ ID Nos:1 and 2, 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 KLKB1 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, 19A, or 19B, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 3, 4, 19A, or 19B, 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 KLKB1 sequence and comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in any one of Tables 3, 4, 19A, or 19B, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 3, 4, 19A, or 19B, 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, the antisense polynucleotides disclosed herein are fully complementary to the target F12 sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target F12 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 SEQ ID Nos:9 or 10, or a fragment of SEQ ID Nos:9 or 10, 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 embodiment, the antisense strand polynucleotides are substantially complementary to the target F12 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 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27, 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 F12 sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of antisense strand nucleotide sequences in any one ofTables 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27, 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, the sense strand polynucleotides and the antisense polynucleotides disclosed herein are fully complementary to the target KNG1 sequence. In other embodiments, the sense strand polynucleotides and/or the antisense polynucleotides

disclosed herein are substantially complementary to the target KNG1 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 SEQ ID Nos:17 or 18, or a fragment of SEQ ID Nos:17 or 18, 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 embodiment, the antisense strand polynucleotides are substantially complementary to the target KNG 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 15 or 16, 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 KNG1 sequence and comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in Table 15 or 16, or a fragment of any one of the antisense strand nucleotide sequences in Table 15 or 16, 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 general, the majority of nucleotides of each strand are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, an "iRNA" may include ribonucleotides with chemical modifications. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modifications, as used in an iRNA molecule, are encompassed by "iRNA" for the purposes of this specification and claims.

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

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 contact activation pathway gene expression (*i.e.,* KLKB1 gene expression, F12 gene expression, and/or KNG1 gene expression) and/or replication; a human at risk for a disease, disorder or condition that would benefit from reduction in contact activation pathway gene expression; a human having a disease, disorder or condition that would benefit from reduction in contact activation pathway gene expression; and/or human being treated for a disease, disorder or condition that would benefit from reduction in contact activation pathway gene expression, as described herein.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more symptoms associated with contact activation pathway gene expression (*i.e.,* KLKB1 gene expression, F12 gene expression, and/or KNG1 gene expression) and/or contact activation pathway protein production (*i.e.,* KLKB1 protein production, F12 protein production, and/or KNG1 protein production) , *e.g.,* a thrombophilia, *e.g.,* the formation of a thrombus, the presence of elevated bradykinin, heredity angioedema (HAE), such as hereditary angioedema type I; hereditary angioedema type II; hereditary angioedema type III; or any other hereditary angioedema caused by elevated levels of bradykinin, an angioedema attack, edema swelling of the extremities, face, larynx, upper respiratory tract, abdomen, trunk, and genetials, prodrome; laryngeal swelling; nonpruritic rash; nausea; vomiting; abdominal pain. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

The term "lower" in the context of the level of contact activation pathway gene expression and/or contact activation pathway protein production in a subject or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or more and is preferably down to a level accepted as within the range of normal for an individual without such disorder.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder or condition thereof, that would benefit from a reduction in expression of a contact activation pathway gene and/or production of a contact activation pathway protein, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, or a reduction in the frequency and/or duration of a symptom associated with such a disease, disorder, or condition, *e.g.,* a symptom of contact activation

pathway gene expression, such as the formation of a venous thrombus, an arterial thrombus, a cardiac chamber thrombus, a thromboembolism, the presence of elevated bradykinin, an angioedema attack, hereditary angioedema type I; hereditary angioedema type II; hereditary angioedema type III; any other hereditary angioedema caused by elevated levels of bradykinin; edema swelling of the extremities, face, larynx, upper respiratory tract, abdomen, trunk, and genetials, prodrome; laryngeal swelling; nonpruritic rash; nausea; vomiting; abdominal pain. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom associated with such a disease, disorder or condition (*e.g.*, by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms delayed (*e.g.*, by days, weeks, months or years) is considered effective prevention.

As used herein, the term "contact activation pathway-associated disease," is a disease or disorder that is caused by, or associated with contact activation pathway gene expression (*i.e.,* KLKB1 gene expression, F12 gene expression, and/or KNG1 gene expression) or contact activation pathway protein production (*i.e.,* KLKB1 protein production, F12 protein production, and/or KNG1 protein production). The term "contact activation pathway-associated disease" includes a disease, disorder or condition that would benefit from reduction in contact activation pathway gene expression and/or contact activation pathway protein activity. A contact activation pathway-associated disease may be a genetic disorder or an acquired disorder.

Non-limiting examples of contact activation pathway-associated diseases include, for example, thrombophilia, heredity angioedema (HAE) (such as hereditary angioedema type I; hereditary angioedema type II; hereditary angioedema type III; or any other hereditary angioedema caused by elevated levels of bradykinin), prekallikrein deficiency (inherited or acquired), also known as Fletcher Factor Deficiency, malignant essential hypertension, hypertension, end stage renal disease.

In one embodiment, the contact activation pathway-associated disease is a thrombophilia. As used herein, the term "thrombophilia," also referred to as "hypercoagulability" or "a prothrombotic state", is any disease or disorder associated with an abnormality of blood coagulation that increases the risk of thrombosis and the development of a thrombus. As used herein, the term "thrombosis" refers to the process of local coagulation or clotting of the blood (formation of a "thrombus" or "clot") in a part of the circulatory system. A thrombophilia may be inherited, acquired, or the result on an environmental condition. Exemplary inherited thrombophilias include inherited antithrombin deficiency, inherited Protein C deficiency, inherited Protein S deficiency, inherited Factor V Leiden thrombophilia, and Prothrombin (Factor II) G20210A. An exemplary acquired thrombophilia includes Antiphospholipid syndrome. Acquired/environmentally acquired thrombophilias may be the result of, for example trauma, fracture, surgery, *e.g.,* orthopedic surgery, oncological surgery, oral contraceptive use, hormone replacement therapy,

pregnancy, puerperium, hypercoaguability, previous thrombus, age, immobilization (*e.g.,* more than three days of bed rest), prolonged travel, metabolic syndrome, and air pollution (see, *e.g.,* Previtali, *et al.* (2011) *Blood Transfus* 9:120). Accordingly, "subjects at risk of forming a thrombus" include surgical patients (*e.g.,* subjects having general surgery, dental surgery, orthopedic surgery (*e.g.,* knee or hip replacement surgery), trauma surgery, oncological sugery); medical patients (*e.g.,* subjects having an immobilizing disease, *e.g.,*subjects having more than three days of bed rest and/or subjects having long-term use of an intravenous catheter; subjects having atrial fibrillation; elderly subjects; subjects having renal impairment; subjects having a prosthetic heart valve; subjects having heart failure; subjects having cancer); pregnant subjects; postpartum subjects; subjects that have previously had a thrombus; subjects undergoing hormone replacement therapy; subjects sitting for long periods of time, such as in a plane or car; and obese subjects.

In one embodiment, the contact activation pathway-associated disease is hereditary angioedema (HAE). As used herein, "hereditary angioedema," used interchangeably with the term "HAE," refers to an autosomal dominant disorder caused by mutation of the C1 inhibitor (C1INH), SERPING1) gene or the coagulation factor XII (F12) gene that causes recurrent edema swelling in patients. Typical symptoms of HAE include severe swelling of the arms, legs, hands, feet, face, tongue and larynx, abdomen, trunk, genitals, nausea, vomiting, abdominal pain, and nonpriuric rash. Elevanted levels of bradykinin peptide are observed during HAE attacks or episodes..

In another embodiment, the contact activation pathway-associated disease is prekallikrein deficiency.

In another embodiment, the contact activation pathway-associated disease is malignant essential hypertension.

In another embodiment, the contact activation pathway-associated disease is hypertension.

In another embodiment, the contact activation pathway-associated disease is end stage renal disease.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a patient for treating a subject having HAE and/or contact activation pathway-associated disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, stage of pathological processes mediated by contact activation pathway gene expression, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject who does not yet experience or display symptoms of a contact activation pathway-associated disease, but who may be predisposed or at risk, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylacticaly 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. RNAi agents employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

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

II. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of a contact activation pathway gene (*i.e.*, a KLKB1 gene, an F12 gene, or a KNG1 gene). In one embodiment, the iRNA agent includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a contact activation pathway gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human having a contact activation pathway-associated disease, *e.g.*, a thrombophilia or hereditary angioedema, or at risk of developing a contact activation pathway-associated disease, *e.g.*, a thrombophilia, or an angioedema attack.

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 contact activation pathway 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 contact activation pathway gene, the iRNA inhibits the expression of the contact activation pathway gene (*e.g.,* a human, a primate, a non-primate, or a bird contact activation pathway gene) by at least about 10% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, Western Blotting or flowcytometric techniques.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of a contact activation pathway gene (*i.e.,* a KLKB1 gene, an F12 gene, or a KNG1 gene). The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

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

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

In some embodiments, the dsRNA is about 15 to about 20 nucleotides in length, or about 25 to about 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well-known in the art that dsRNAs

longer than about 21-23 nucleotides in length may serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 9 to 36 base pairs, *e.g.*, about 10-36, 11-36, 12-36, 13-36, 14-36, 15-36, 9-35, 10-35, 11-35, 12-35, 13-35, 14-35, 15-35, 9-34, 10-34, 11-34, 12-34, 13-34, 14-34, 15-34, 9-33, 10-33, 11-33, 12-33, 13-33, 14-33, 15-33, 9-32, 10-32, 11-32, 12-32, 13-32, 14-32, 15-32, 9-31, 10-31, 11-31, 12-31, 13-32, 14-31, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target contact activation pathway gene expression is not generated in the target cell by cleavage of a larger dsRNA.

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

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

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

nucleotides can be easily prepared. Single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In one aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences provided in any one of Tables 3, 4, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 3, 4, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27.

In one embodiment, the sense strand is selected from the group of sequences provided in any one of Tables 3, 4, 19A, and 19B and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 3, 4, 19A, and 19B. 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 KLKB1 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, 19A, and 19B and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 3, 4, 19A, and 19B. In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

In one embodiment, the sense strand is selected from the group of sequences provided in any one of any one of Tables 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27. 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 an F12 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 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27. In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

In one embodiment, the sense strand is selected from the group of sequences provided in any one of Tables 15, 16, 19E, and 19F, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 15, 16, 19E, and 19F. 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 KNG1 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 15, 16, 19E, and 19F, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 15, 16, 19E, and 19F. In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It will be understood that, although some of the sequences in Tables 3, 4, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27 are described as modified and/or conjugated sequences, the RNA of the iRNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth in Tables 3, 4, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27 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 about 20 to about 23 base pairs, *e.g.,* 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, *EMBO* 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in any one of Tables 3, 4, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27, 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, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27 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, 19A, and 19B and differing in their ability to inhibit the expression of a KLKB1 gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, 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 9, 10,19C, 19D, 20, and 21, and differing in their ability to inhibit the expression of an F12 gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, and 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 15, 16, 19E, and 19F, and differing in their ability to inhibit the expression of a KNG1 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, 19A, and 19B identify a site(s) in a KLKB1 transcript that is susceptible to RISC-mediated cleavage, the RNAs provided in any one of Tables 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27 identify a site(s) in

an F12 transcript that is susceptible to RISC-mediated cleavage, and RNAs provided in any one of Tables 15, 16, 19E, and 19F identify a site(s) in a KNG1 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, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in the contact activation pathway 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, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27 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, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27 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 a contact activation pathway 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 contact activation pathway gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of a contact activation pathway gene is important, especially if the particular region of complementarity in a contact activation pathway 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. In some embodiments, substantially all of the nucleotides of an iRNA of the invention are modified and the iRNA comprises no more than 8 2'-fluoro modifications (*e.g.*, no more than 7 2'-fluoro modifications, no more than 6 2'-fluoro modifications, no more than 5 2'-fluoro modification, no more than 4 2'-fluoro modifications, no more than 3 2'-fluoro modifications, or no more than 2 2'-fluoro modifications) on the sense strand and no more than 6 2'-fluoro modifications (*e.g.*, no more than 5 2'-fluoro modifications, no more than 4 2'-fluoro modifications, no more than 3 2'-fluoro modifications, or no more than 2 2'-fluoro modifications) on the antisense strand. In other embodiments, all of the nucleotides of an iRNA of the invention are modified and the iRNA comprises no more than 8 2'-fluoro modifications (*e.g.*, no more than 7 2'-fluoro

modifications, no more than 6 2'-fluoro modifications, no more than 5 2'-fluoro modification, no more than 4 2'-fluoro modifications, no more than 3 2'-fluoro modifications, or no more than 2 2'-fluoro modifications) on the sense strand and no more than 6 2'-fluoro modifications (*e.g.*, no more than 5 2'-fluoro modifications, no more than 4 2'-fluoro modifications, no more than 3 2'-fluoro modifications, or no more than 2 2'-fluoro modifications) on the antisense strand.

The nucleic acids featured in the invention can be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al.* (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position) or replacement of the sugar; and/or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6, 239,265; 6,277,603; 6,326,199; 6,346,614;

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

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

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

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

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

5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506.

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

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

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

guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Patent Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

The RNA of an iRNA can also be modified to include one or more bicyclic sugar moities. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A "bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4′ -carbon and the 2′ -carbon of the sugar ring. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH2-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural

conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *Et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4′ and the 2′ ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4′ to 2′ bridge. Examples of such 4′ to 2′ bridged bicyclic nucleosides, include but are not limited to 4′ -(CH2)−O-2′ (LNA); 4′ -(CH2)−S-2′ ; 4′ -(CH2)2−O-2′ (ENA); 4′ -CH(CH3)−O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′ -CH(CH2OCH3)−O-2′ (and analogs thereof; see, *e.g.*, U.S. Pat. No. 7,399,845); 4′ -C(CH3)(CH3)−O-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,283); 4′ -CH2−N(OCH3)-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,425); 4′ -CH2−O−N(CH3)-2′ (see, e.g.,U.S. Patent Publication No. 2004/0171570); 4′ -CH2−N(R)−O-2′ , wherein R is H, C1-C12 alkyl, or a protecting group (see, *e.g.*, U.S. Pat. No. 7,427,672); 4′ -CH2−C(H)(CH3)-2′ (see, *e.g.*, Chattopadhyaya *et al., J. Org. Chem.*, 2009, 74, 118-134); and 4′ -CH2—C(=CH2)-2′ (and analogs thereof; see, *e.g.*, US Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

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

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

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

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

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

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

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

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-0-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

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.

A. Modified iRNAs Comprising Motifs of the Invention

In certain aspects of the invention, the double stranded RNAi agents of the invention include agents with chemical modifications as disclosed, for example, in U.S. Provisional Application No. 61/561,710, filed on November 18, 2011, or in PCT/US2012/065691, filed on November 16, 2012, the entire contents of each of which are incorporated herein by reference.

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

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

Accordingly, the invention provides double stranded RNAi agents capable of inhibiting the expression of a target gene (*i.e.*, a contact activation pathway gene, *i.e.,* a KLKB1 gene, an F12 gene, or a KNG1 gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may range from 12-30 nucleotides in length. For example, each strand may be between 14-30 nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

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

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

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

on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

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

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

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

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

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

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

agent is blunt, while the other end comprises a 2 nucleotide overhang. Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand.

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

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

In one embodiment, the RNAi agent comprises sense and antisense strands, wherein the RNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end; wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the

first strand, wherein the duplex region region which is at least 25 nucleotides in length, and the second strand is sufficiently complemenatary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein dicer cleavage of the RNAi agent preferentially results in an siRNA comprising the 3' end of the second strand, thereby reducing expression of the target gene in the mammal. Optionally, the RNAi agent further comprises a ligand.

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

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

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

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

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

one or more nucleotide than the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

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

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

each n_p and n_q independently represent an overhang nucleotide;

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

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

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

In one embodiment, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8, 7, 8, 9, 8, 9, 10, 9, 10, 11, 10, 11,12 or 11, 12, 13) of - the sense strand, the

count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end.

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

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

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

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

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

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

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

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

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

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

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

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

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

wherein:

k and l are each independently 0 or 1;

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(III)

wherein:

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

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

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

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

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

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

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

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

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

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

(IIIa)

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

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

(IIIb)

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

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

(IIIc)

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

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

(IIId)

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

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

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

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

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

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

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

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

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

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

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

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

In one embodiment, the RNAi agent is a multimer containing three, four, five, six or more duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable.

Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

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

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

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

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

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

In certain specific embodiments, the RNAi agent for use in the methods of the invention is an agent selected from the group of agents listed in any one of Tables 3, 4, 9, 10, 15, 16, 19A, 19B, 19C, 19D, 19E, 19F, 20, 21, 23, 24, 26, and 27. In one embodiment, the agent is any one of the agents listed in any one of Tables 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27. These agents may further comprise a ligand.

IV. iRNAs Conjugated to Ligands

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

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

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

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

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

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

cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

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

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

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

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

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

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

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

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

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

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

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

B. Cell Permeation Agents

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

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

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 26). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 27) 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: 28) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 29) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

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

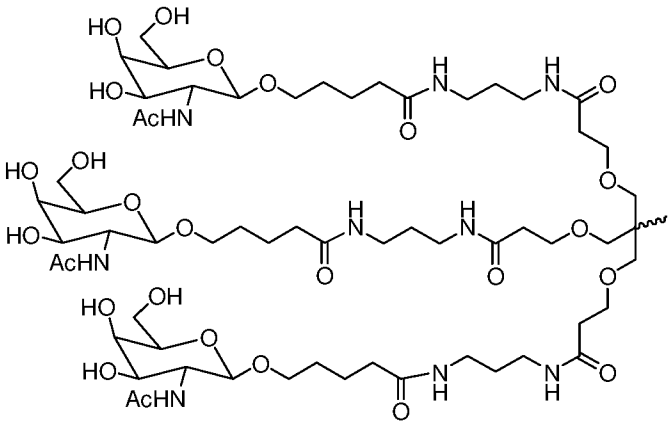
moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.

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

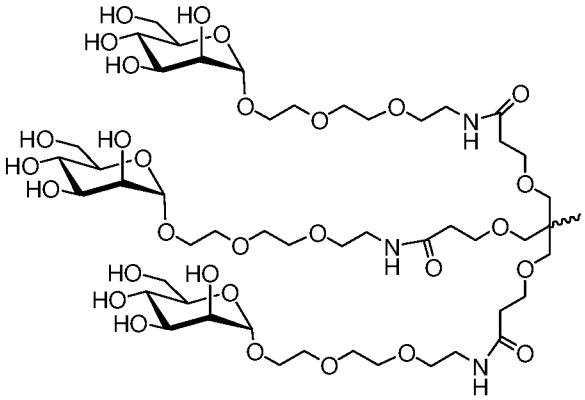
C. Carbohydrate Conjugates

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



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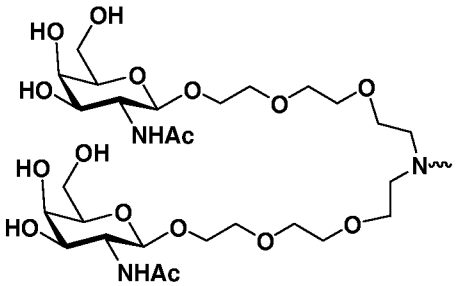

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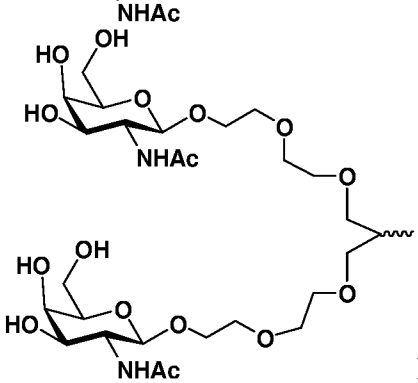

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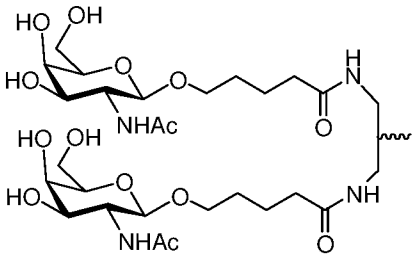

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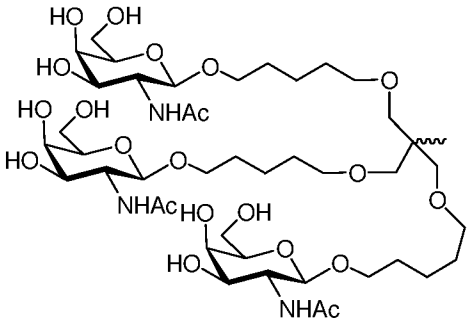

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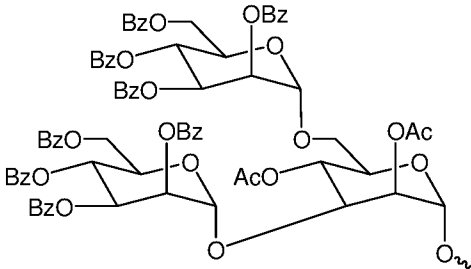

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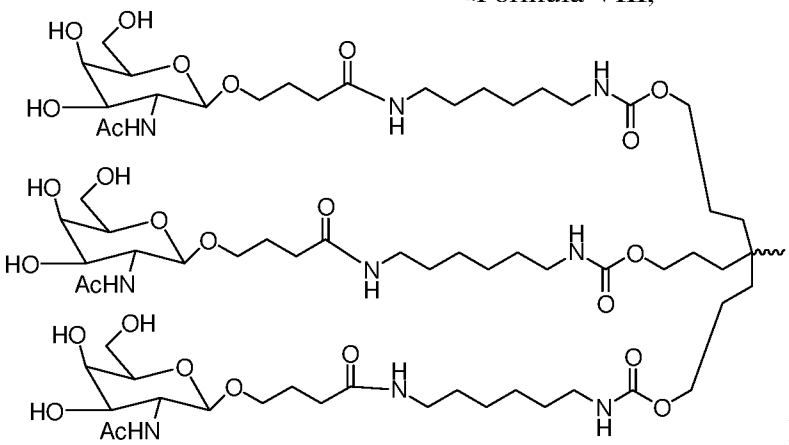

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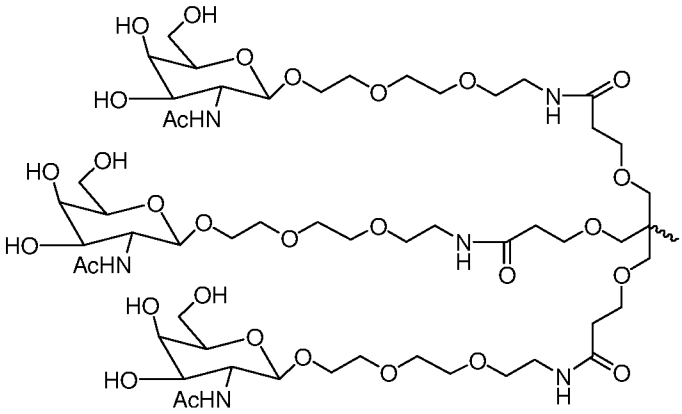

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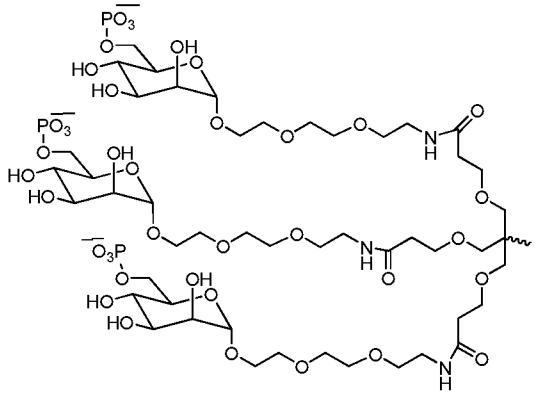

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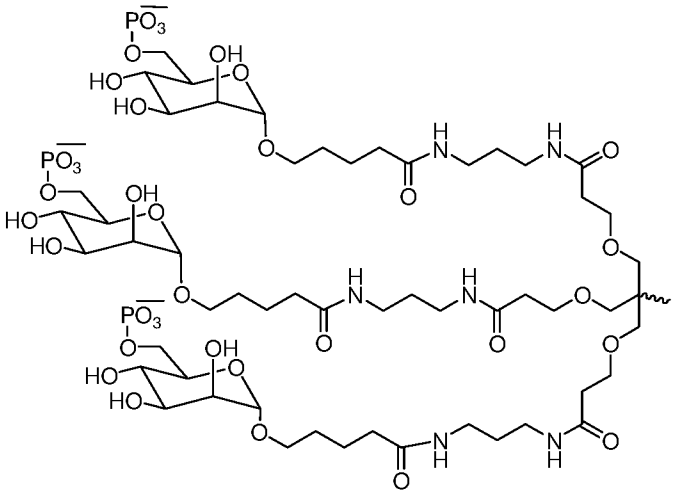

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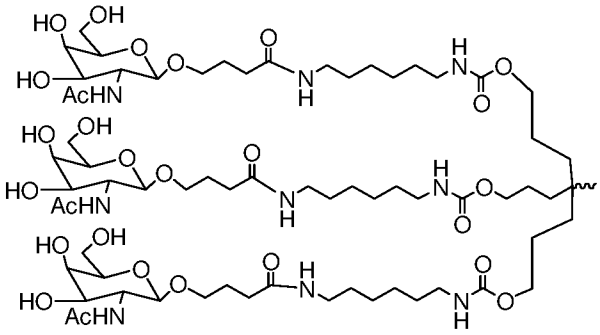

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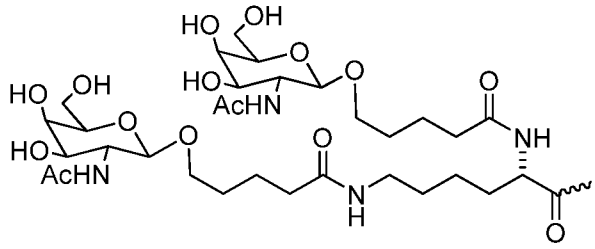

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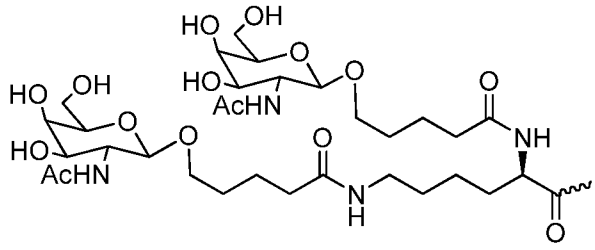

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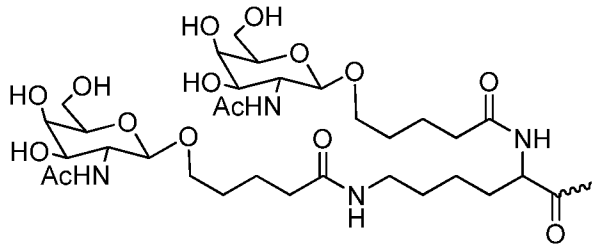

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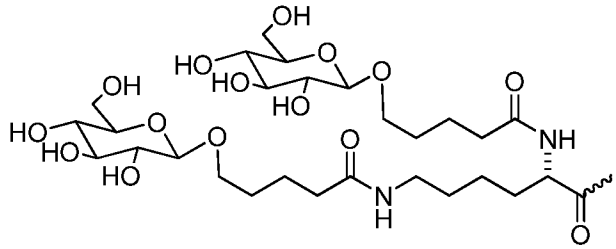
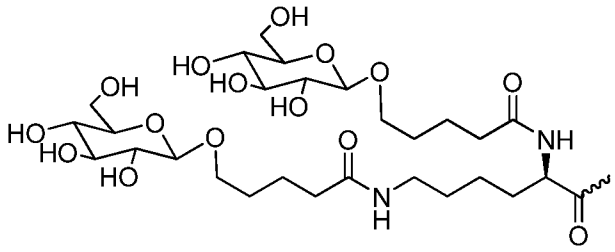
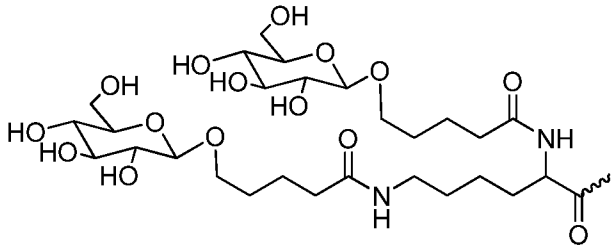


Formula XVII,



Formula XVIII,



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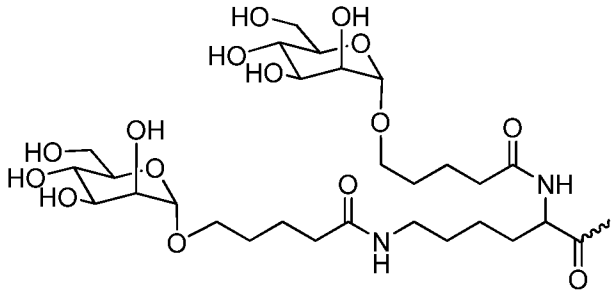
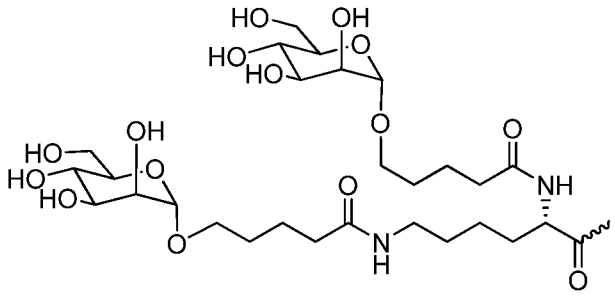
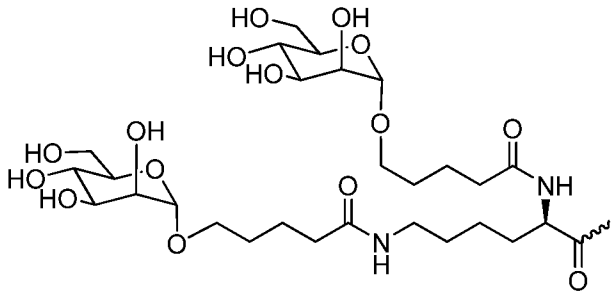


Formula XX,

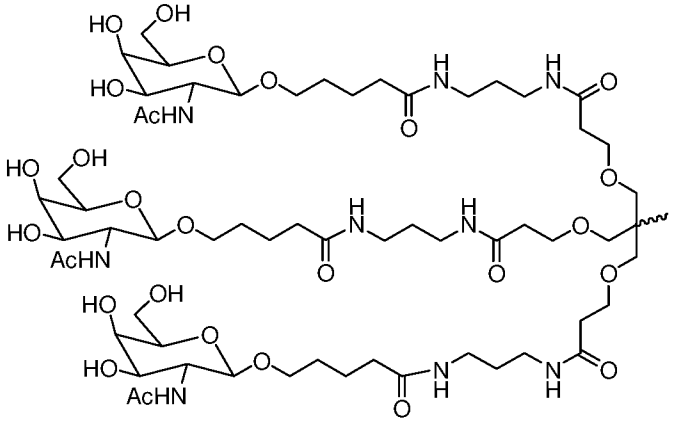


Formula XXI,



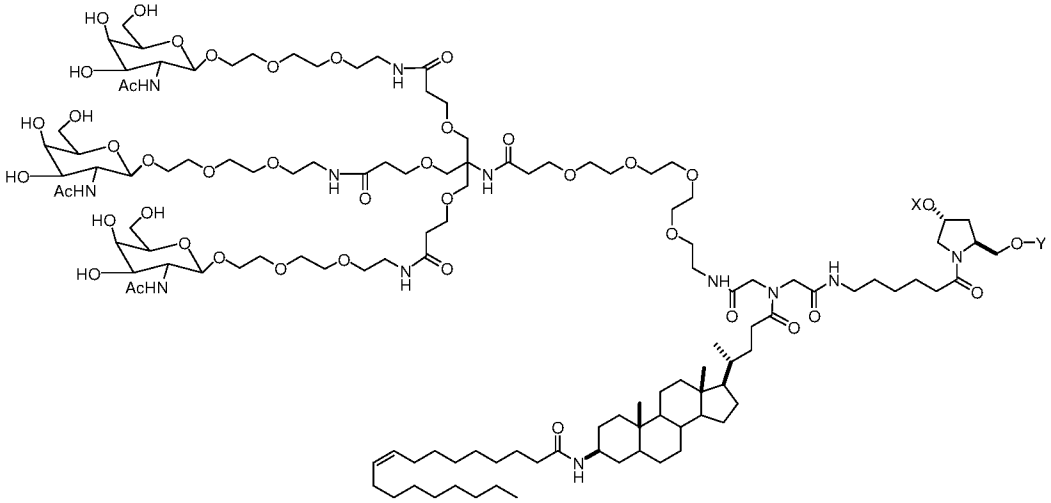
Formula XXII.

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



Formula II.

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



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

In certain embodiments of the invention, 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.

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, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic or substituted aliphatic. In one embodiment, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

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

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

those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

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

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

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

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

i. Redox cleavable linking groups

In one embodiment, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage

which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

ii. Phosphate-based cleavable linking groups

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

iii. Acid cleavable linking groups

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

iv. Ester-based linking groups

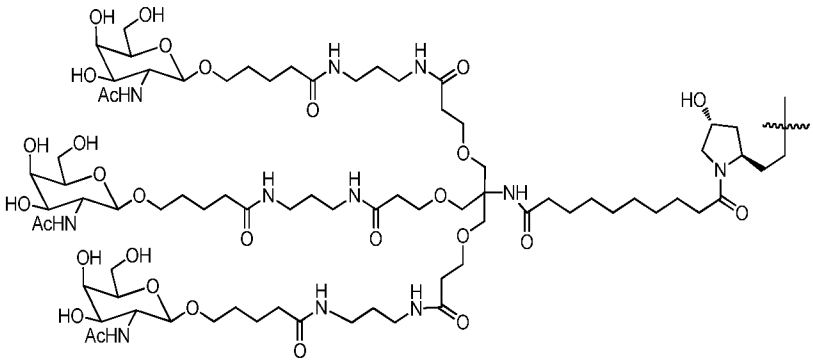
In another embodiment, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not

limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula -C(O)O-, or -OC(O)-. These candidates can be evaluated using methods analogous to those described above.

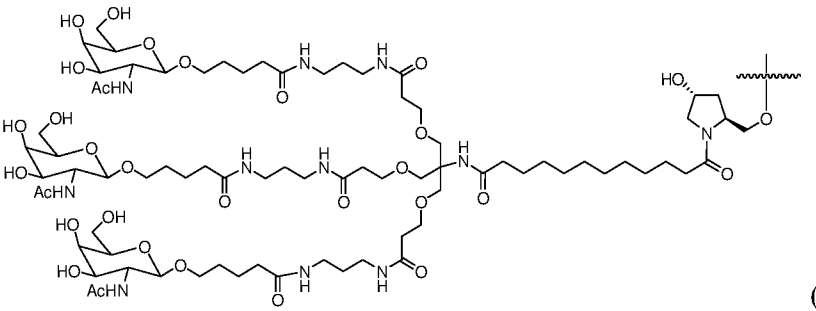
v. Peptide-based cleaving groups

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

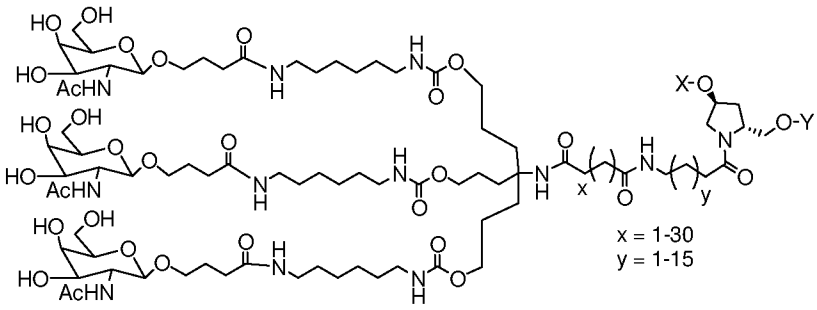
In one embodiment, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



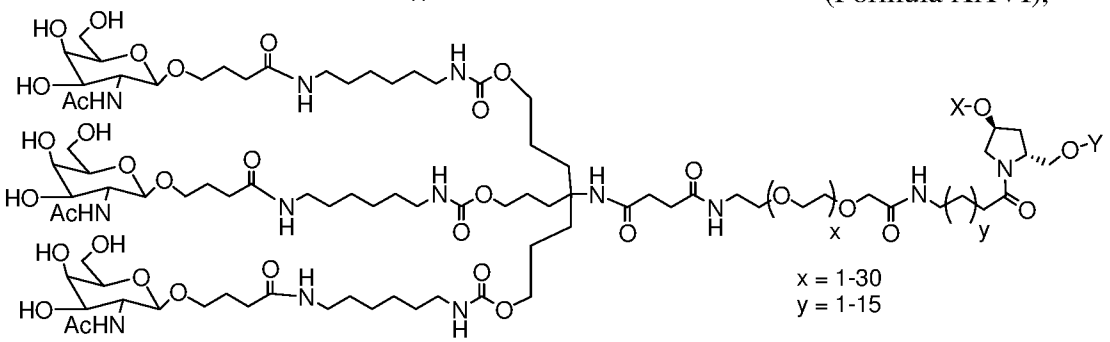
(Formula XXIV),



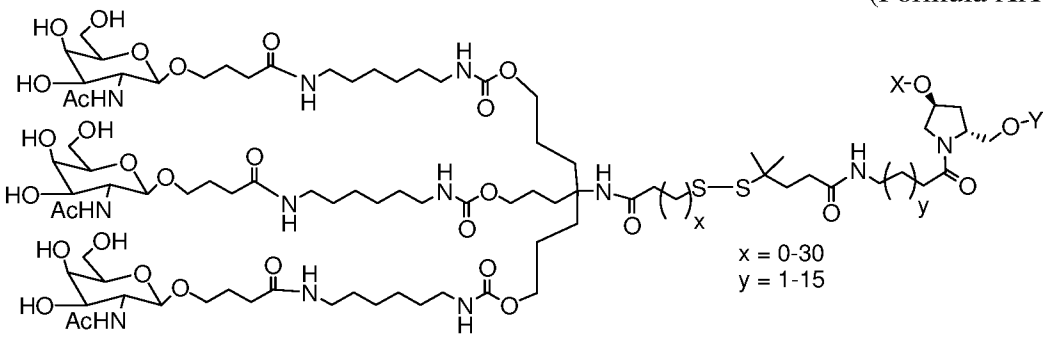
(Formula XXV),



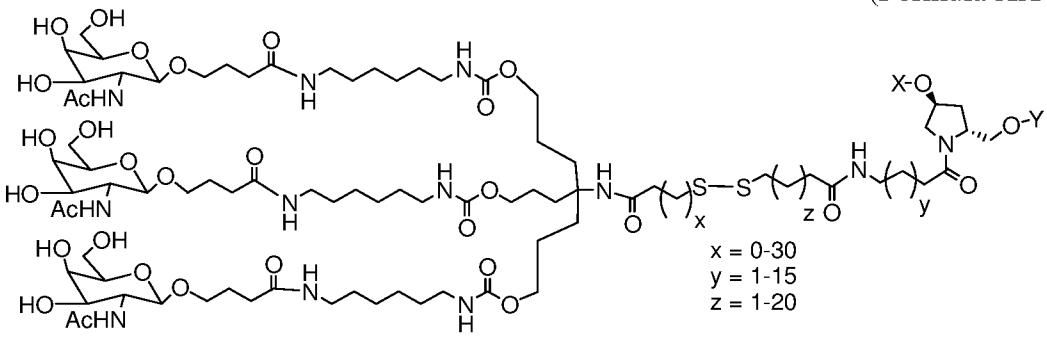
(Formula XXVI),



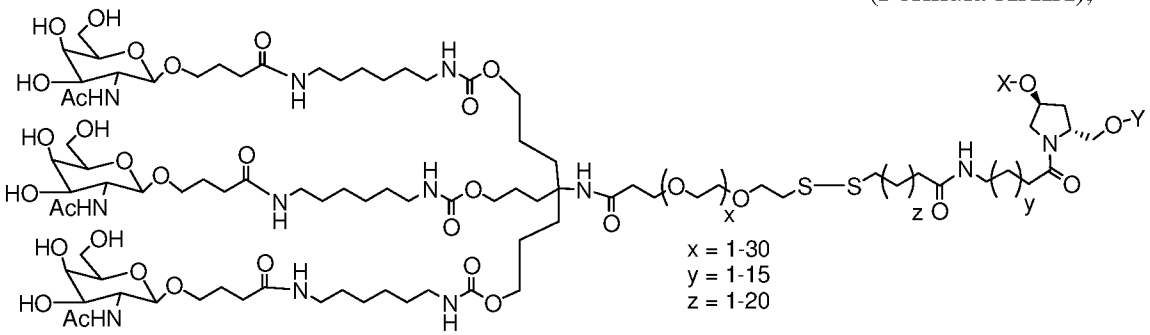
(Formula XXVII),



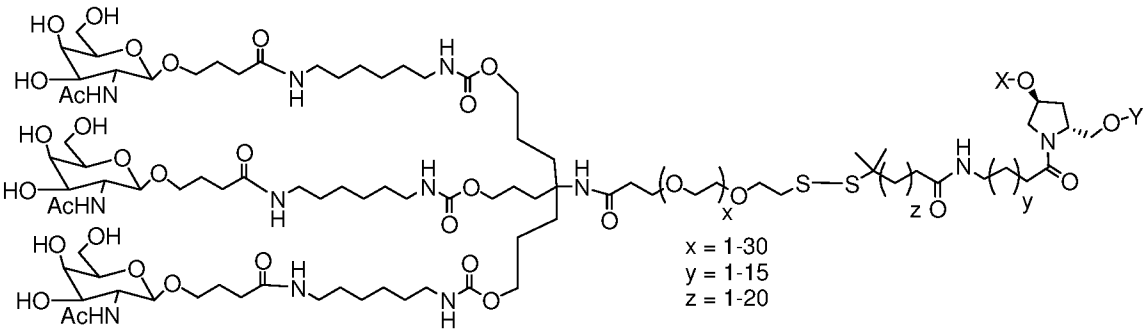
(Formula XXVIII),



(Formula XXIX),



(Formula XXX), and



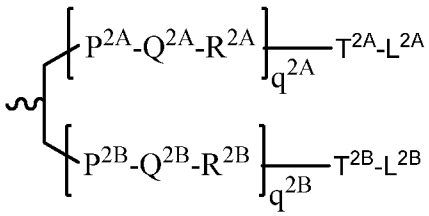
(Formula XXXI),

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

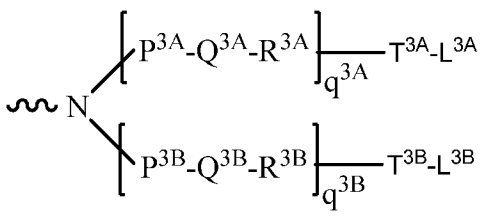
In certain embodiments of the compositions and methods of the invention, a ligand is one or more "GalNAc" (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

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

Formula XXXII



Formula XXXIII



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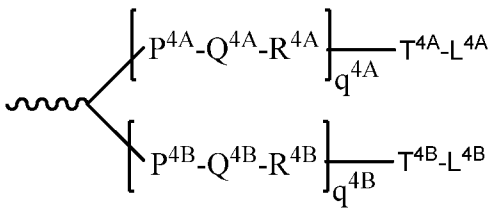
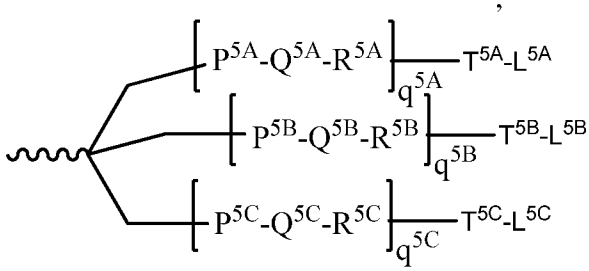


,

Formula XXXIV



;

Formula XXXV

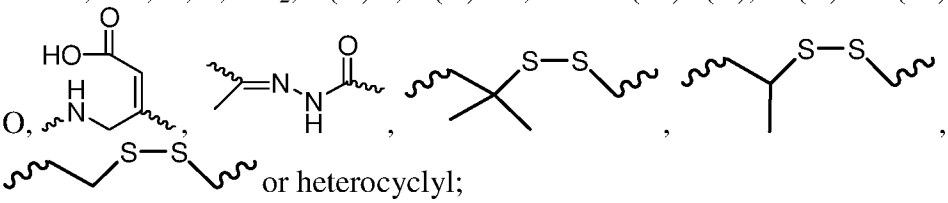
wherein:

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

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

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

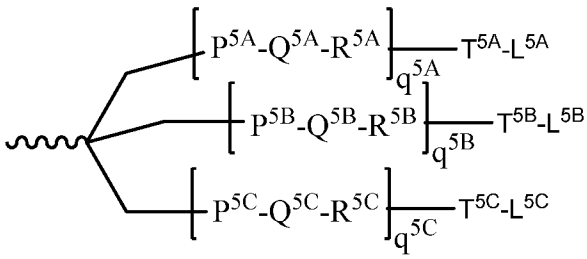
R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, NHCH(R^a)C(O), -C(O)-CH(R^a)-NH-, CO, CH=N-



or heterocyclyl;

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

Formula XXXV



,

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

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

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

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

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

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

then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

V. Delivery of an iRNA of the Invention

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

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

intranasal administration (Howard, KA., *et al* (2006) *Mol. Ther.* 14:476-484; Zhang, X., *et al* (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V., *et al* (2005) *Nat. Med.* 11:50-55). For administering an iRNA systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases *in vivo*. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA composition to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J., *et al* (2004) *Nature* 432:173-178). Conjugation of an iRNA to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO., *et al* (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the iRNA can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim SH., *et al* (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al* (2003) *J. Mol. Biol* 327:761-766; Verma, UN., *et al* (2003) Clin. *Cancer Res.* 9:1291-1300; Arnold, AS *et al* (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, DR., *et al* (2003), supra; Verma, UN., *et al* (2003), *supra*), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS., *et al* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY., *et al* (2005) *Cancer Gene Ther.* 12:321-328; Pal, A., *et al* (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME., *et al* (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA., *et al* (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., *et al* (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in U.S. Patent No. 7,427,605, which is herein incorporated by reference in its entirety.

A. Vector encoded iRNAs of the Invention

iRNA targeting a contact activation pathway gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; Skillern, A., *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Pat. No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

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

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

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

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

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

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

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

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

Adenoviruses are also contemplated for use in delivery of iRNAs of the invention. Adenoviruses are especially attractive vehicles, *e.g.*, for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease.

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

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

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

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

The pharmaceutical preparation of a vector can include the vector in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

VI. Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for treating a disease or disorder associated with the expression or activity of a contact activation pathway gene (*i.e.*,a KLKB1 gene, an F12 gene, and/or a KNG1 gene). 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 a contact activation pathway gene.

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 intravenous (IV) or for subcutaneous delivery. Another example is compositions that are formulated for direct delivery into the liver, *e.g.*, by infusion into the liver, such as by continuous pump infusion.

The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a contact activation pathway gene. In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of an iRNA of the invention will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, preferably about 0.3 mg/kg and about 3.0 mg/kg.A repeat-dose regimine may include administration of a therapeutic amount of iRNA on a regular basis, such as every other day or once a year. In certain embodiments, the iRNA is administered about once per month to about once per quarter (*i.e.*, about once every three months).

After an initial treatment regimen, the treatments can be administered on a less frequent basis.

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

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as disorders that would benefit from reduction in the expression of a contact activation pathway 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).

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

A. iRNA Formulations Comprising Membranous Molecular Assemblies

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

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

A liposome containing an iRNA 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 iRNA agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the iRNA agent and condense around the iRNA agent to form a liposome. After condensation, the detergent is removed, *e.g.*, by dialysis, to yield a liposomal preparation of iRNA agent.

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

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

775:169, 1984). These methods are readily adapted to packaging iRNA agent preparations into liposomes.

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

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

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

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

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

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

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

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

In one embodiment, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are taken up by macrophages *in vivo* and can be used to deliver iRNA 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 iRNA agents in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

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

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

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

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

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

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

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

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

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

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

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

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use

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

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

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

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

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

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

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

A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of the siRNA composition, an alkali metal C_8 to C_{22} alkyl sulphate, and a micelle forming compounds. Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic

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

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

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

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

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

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

B. *Lipid particles*

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

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

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

The cationic lipid can be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I -(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (Dlin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDaP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPz), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane

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

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

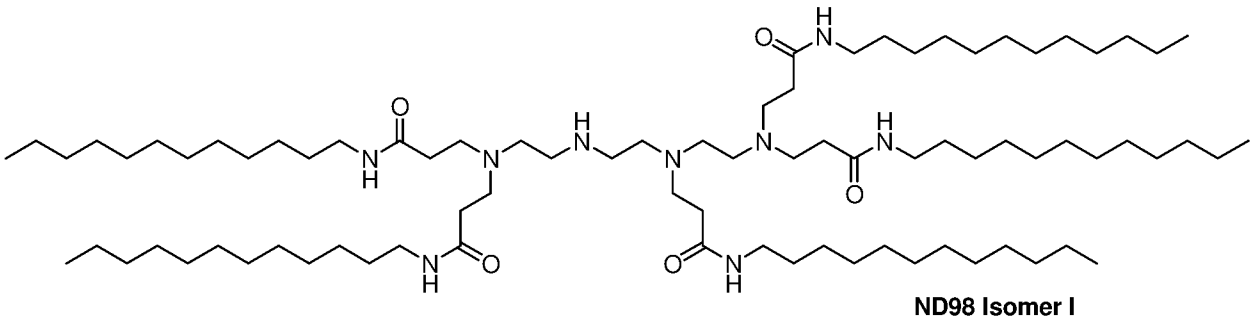
In one embodiment, the lipid-siRNA particle includes 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 siRNA/Lipid Ratio.

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

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

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

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



Formula 1

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

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

Table 1

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

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

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

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

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

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

SNALP (1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed April 15, 2009, which is hereby incorporated by reference.

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

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

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

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

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the invention are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.*, sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric

acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the invention can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.,* p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Patent 6,887,906, US Publn. No. 20030027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

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

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

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

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

including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension can also contain stabilizers.

C. Additional Formulations

i. Emulsions

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

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion can be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants,

naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

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

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

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

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

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

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

ii. Microemulsions

In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically

microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

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

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

polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

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

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

iii. Microparticles

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

iv. Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that

even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

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

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

Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C1-20 alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (see e.g., Touitou, E., et al. Enhancement in Drug Delivery, CRC Press, Danvers, MA, 2006; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri et al., J. Pharm. Pharmacol., 1992, 44, 651-654).

The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (see e.g., Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman et al. Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate),

glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (see e.g., Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto et al., J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita et al., J. Pharm. Sci., 1990, 79, 579-583).

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

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

Agents that enhance uptake of iRNAs at the cellular level can also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al., PCT Application WO 97/30731), are also known to enhance the cellular uptake of dsRNAs. Examples of commercially available transfection reagents include, for example Lipofectamine™ (Invitrogen; Carlsbad, CA),

Lipofectamine 2000™ (Invitrogen; Carlsbad, CA), 293fectin™ (Invitrogen; Carlsbad, CA), Cellfectin™ (Invitrogen; Carlsbad, CA), DMRIE-C™ (Invitrogen; Carlsbad, CA), FreeStyle™ MAX (Invitrogen; Carlsbad, CA), Lipofectamine™ 2000 CD (Invitrogen; Carlsbad, CA), Lipofectamine™ (Invitrogen; Carlsbad, CA), iRNAMAX (Invitrogen; Carlsbad, CA), Oligofectamine™ (Invitrogen; Carlsbad, CA), Optifect™ (Invitrogen; Carlsbad, CA), X-tremeGENE Q2 Transfection Reagent (Roche; Grenzacherstrasse, Switzerland), DOTAP Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), DOSPER Liposomal Transfection Reagent (Grenzacherstrasse, Switzerland), or Fugene (Grenzacherstrasse, Switzerland), Transfectam® Reagent (Promega; Madison, WI), TransFast™ Transfection Reagent (Promega; Madison, WI), Tfx™-20 Reagent (Promega; Madison, WI), Tfx™-50 Reagent (Promega; Madison, WI), DreamFect™ (OZ Biosciences; Marseille, France), EcoTransfect (OZ Biosciences; Marseille, France), TransPass[a] D1 Transfection Reagent (New England Biolabs; Ipswich, MA, USA), LyoVec™/LipoGen™ (Invitrogen; San Diego, CA, USA), PerFectin Transfection Reagent (Genlantis; San Diego, CA, USA), NeuroPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), GenePORTER Transfection reagent (Genlantis; San Diego, CA, USA), GenePORTER 2 Transfection reagent (Genlantis; San Diego, CA, USA), Cytofectin Transfection Reagent (Genlantis; San Diego, CA, USA), BaculoPORTER Transfection Reagent (Genlantis; San Diego, CA, USA), TroganPORTER™ transfection Reagent (Genlantis; San Diego, CA, USA), RiboFect (Bioline; Taunton, MA, USA), PlasFect (Bioline; Taunton, MA, USA), UniFECTOR (B-Bridge International; Mountain View, CA, USA), SureFECTOR (B-Bridge International; Mountain View, CA, USA), or HiFect™ (B-Bridge International, Mountain View, CA, USA), among others.

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

v. Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity per se) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-

2,2'-disulfonic acid (Miyao et al., DsRNA Res. Dev., 1995, 5, 115-121; Takakura et al., DsRNA & Nucl. Acid Drug Dev., 1996, 6, 177-183.

vi. Excipients

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

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

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

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

vii. Other Components

The compositions of the present invention can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and

stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

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

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA compounds and (b) one or more agents which function by a non-iRNA mechanism and which are useful in treating a hemolytic disorder. Examples of such agents include, but are not lmited to an anti-inflammatory agent, anti-steatosis agent, anti-viral, and/or anti-fibrosis agent.

In addition, other substances commonly used to protect the liver, such as silymarin, can also be used in conjunction with the iRNAs described herein. Other agents useful for treating liver diseases include telbivudine, entecavir, and protease inhibitors such as telaprevir and other disclosed, for example, in Tung et al., U.S. Application Publication Nos. 2005/0148548, 2004/0167116, and 2003/0144217; and in Hale et al., U.S. Application Publication No. 2004/0127488.

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

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*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 contact activation pathway gene expression (*i.e.,* KLKB1 gene expression, F12 gene expression, and/or KNG1 gene 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 Contact Activation Pathway Gene Expression

The present invention also provides methods of inhibiting expression of a contact activation pathway gene (*i.e.,* a KLKB1 gene , an F12 gene , and/or a KNG1 gene) in a cell.

In one embodiment, the invention provides methods for inhibiting expression of a KLKB1 gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.,* double stranded RNAi agent, in an amount effective to inhibit expression of KLKB1 in the cell, thereby inhibiting expression of KLKB1 in the cell.

In one embodiment, the invention provides methods for inhibiting expression of an F12 gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNAi agent, in an amount effective to inhibit expression of F12 in the cell, thereby inhibiting expression of F12 in the cell.

In one embodiment, the invention provides methods for inhibiting expression of a KNG1 gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNAi agent, in an amount effective to inhibit expression of KNG1 in the cell, thereby inhibiting expression of KNG1 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 are also possible. Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a $GalNAc_3$ ligand, or any other ligand that directs the RNAi agent to a site of interest.

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

The phrase "inhibiting expression of a contact activation pathway gene" is intended to refer to inhibition of expression of any contact activation pathway gene (such as, *e.g.*, a mouse contact activation pathway gene, a rat contact activation pathway gene, a monkey contact activation pathway gene, or a human contact activation pathway gene) as well as variants or mutants of a contact activation pathway gene.

The phrase "inhibiting expression of a KLKB1" is intended to refer to inhibition of expression of any KLKB1gene (such as, *e.g.*, a mouse KLKB1 gene, a rat KLKB1 gene, a monkey KLKB1 gene, or a human KLKB1 gene) as well as variants or mutants of a KLKB1 gene. Thus, the KLKB1 gene may be a wild-type KLKB1 gene, a mutant KLKB1 gene (such as a mutant KLKB1 gene giving rise to amyloid deposition), or a transgenic KLKB1 gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of a KLKB1 gene" includes any level of inhibition of a KLKB1 gene, *e.g.*, at least partial suppression of the expression of a KLKB1 gene. The expression of the KLKB1 gene may be assessed based on the level, or the change in the level, of any variable associated with KLKB1 gene expression, *e.g.*, KLKB1 mRNA level, KLKB1 protein level, or the number or extent of amyloid deposits. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

The phrase "inhibiting expression of F12" is intended to refer to inhibition of expression of any F12 gene (such as, *e.g.*, a mouse F12 gene, a rat F12 gene, a monkey F12 gene, or a human F12 gene) as well as variants or mutants of an F12 gene. Thus, the F12 gene may be a wild-type F12 gene, a mutant F12 gene (such as a mutant F12 gene), or a transgenic F12 gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of an F12 gene" includes any level of inhibition of an F12 gene, *e.g.*, at least partial suppression of the expression of an F12 gene. The expression of the F12 gene may be assessed based on the level, or the change in the level, of any variable associated with F12 gene expression, *e.g.*, F12 mRNA level, F12 protein level, or the number or extent of amyloid deposits. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

The phrase "inhibiting expression of KNG1" is intended to refer to inhibition of expression of any KNG1 gene (such as, *e.g.*, a mouse KNG1 gene, a rat KNG1 gene, a monkey KNG1 gene, or a human KNG1 gene) as well as variants or mutants of an KNG1 gene. Thus, the KNG1 gene may be a wild-type KNG1 gene, a mutant KNG1 gene (such as a mutant KNG1 gene), or a transgenic KNG1 gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of an KNG1 gene" includes any level of inhibition of an KNG1 gene, *e.g.*, at least partial suppression of the expression of an KNG1 gene. The expression of the KNG1 gene may be assessed based on the level, or the change in the level, of any variable associated with KNG1 gene expression, *e.g.*, KNG1 mRNA level, KNG1 protein level, or the number or extent of amyloid deposits. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with contact activation pathway gene expression compared with a control level. The control level may be any type of control level that is utilized in the

art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In some embodiments of the methods of the invention, expression of a contact activation pathway gene (*i.e.*, a KLKB1 gene, an F12 gene, and/or a KNG1 gene) is inhibited by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%,at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%. at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

Inhibition of the expression of a contact activation pathway 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 contact activation pathway 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 contact activation pathway gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s)). In preferred embodiments, the inhibition is assessed by expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

Alternatively, inhibition of the expression of a contact activation pathway gene may be assessed in terms of a reduction of a parameter that is functionally linked to contact activation pathway gene expression, *e.g.*, KLKB1 protein expression, F12 protein expression, KNG1 protein expression, fibrin deposition, thrombus generation, or bradykinin level. Contact activation pathway gene silencing may be determined in any cell expressing a contact activation pathway gene, either constitutively or by genomic engineering, and by any assay known in the art.

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

A control cell or group of cells that may be used to assess the inhibition of the expression of a contact activation pathway gene includes a cell or group of cells that has not

yet been contacted with an RNAi agent of the invention. For example, the control cell or group of cells may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent.

The level of contact activation pathway mRNA that is expressed by a cell or group of cells, or the level of circulating contact activation pathway mRNA, may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of a contact activation pathway gene in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.,* mRNA of the KLKB1 gene, mRNA of the F12 gene, and/or mRNA of the KNG1 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 KLKB1 mRNA may be detected using methods the described in PCT/US2012/043584, the entire contents of which are hereby incorporated herein by reference.

In one embodiment, the level of expression of a contact activation pathway gene 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 contact activation pathway gene. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to KLKB1 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 contact activation pathway gene mRNA.

An alternative method for determining the level of expression of a contact activation pathway gene 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 a contact activation pathway gene is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System).

The expression levels of a contact activation pathway 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 KLKB1 expression level may also comprise using nucleic acid probes in solution.

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

The level of contact activation pathway 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 contact activation pathway-associated disease, such as reduction in edema swelling of the extremities, face, larynx, upper respiratory tract, abdomen, trunk, and genitals, prodrome; laryngeal swelling; nonpruritic rash; nausea; vomiting; or abdominal pain. These 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 organis. 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 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 or plasma drawn from the subject. In further embodiments, a "sample derived from a subject" refers to liver tissue or retinal tissue derived from the subject.

In some embodiments of the methods of the invention, the RNAi agent is administered to a subject such that the RNAi agent is delivered to a specific site within the subject. The inhibition of expression of a contact activation pathway gene may be assessed using measurements of the level or change in the level of contact activation pathway gene mRNA or contact activation pathway protein in a sample derived from fluid or tissue from the specific site within the subject. In preferred embodiments, the site is selected from the group consisting of liver, choroid plexus, retina, and pancreas. 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 of Treating or Preventing Contact Activation Pathway-Associated Diseases

The present invention provides therapeutic and prophylactic methods which include administering to a subject having a contact activation pathway gene-associated disease, disorder, and/or condition, or prone to developing, a contact activation pathway gene-associated disease, disorder, and/or condition, compositions comprising an iRNA agent (*i.e.,* an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, an iRNA agent targeting a KNG1 gene, or a combination of any of the foregoing, *i.e.,* a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting an F12 gene, or a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting an F12 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, and an iRNA agent targeting a KNG1 gene), or pharmaceutical compositions comprising an iRNA agent (*i.e.,* an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, an iRNA agent targeting a KNG1 gene, or a combination of any of the foregoing), or vectors comprising an iRNA (*i.e.,* an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, an iRNA agent targeting a KNG1 gene, or a combination of any of the foregoing) of the invention. Non-limiting examples of contact activation pathway gene-associated diseases include, for example, a thrombophilia, heredity angioedema (HAE) (such as hereditary angioedema type I; hereditary angioedema type II; hereditary angioedema type III; or any other hereditary

angioedema caused by elevated levels of bradykinin), prekallikrein deficiency, malignant essential hypertension, hypertension, end stage renal disease, Fletcher Factor Deficiency, edema swelling of the extremities, face, larynx, upper respiratory tract, abdomen, trunk, and genitals, prodrome; laryngeal swelling; nonpruritic rash; nausea; vomiting; abdominal pain.

In one embodiment, the contact activation pathway gene-associated disease is a thrombophilia. In another embodiment, the contact activation pathway gene-associated disease is HAE. In another embodiment, the contact activation pathway gene-associated disease is prekallikrein deficiency. In another embodiment, the contact activation pathway gene-associated disease is malignant essential hypertension. In another embodiment, the contact activation pathway gene-associated disease is hypertension. In another embodiment, the contact activation pathway gene-associated disease is end stage renal disease. In another embodiment, the contact activation pathway gene-associated disease is Fletcher Factor Deficiency.

The methods of the invention are useful for treating a subject having a contact activation pathway gene-associated disease, *e.g.,* a subject that would benefit from reduction in contact activation pathway gene expression and/or contact activation pathway protein production. In one aspect, the present invention provides methods of reducing the level of Kallikrein B, Plasma (Fletcher Factor) 1 (KLKB1) gene expression in a subject having hereditary angioedema (HAE). In another aspect, the present invention provides methods of reducing the level of KLKB1 protein in a subject with HAE. In one aspect, the present invention provides methods of reducing the level of Factor XII (Hageman Factor) (F12) gene expression in a subject having hereditary angioedema (HAE). In another aspect, the present invention provides methods of reducing the level of F12 protein in a subject with HAE. In one aspect, the present invention provides methods of reducing the level of Kininogen 1 (KNG1) gene expression in a subject having hereditary angioedema (HAE). In another aspect, the present invention provides methods of reducing the level of KNG1 protein in a subject with HAE.

The present invention also provides methods of reducing the level of bradykinin in a subject with contact activation pathway-associated disease, *e.g.,* a thrombophilia or hereditary angioedema. For example, in one embodiment, the invention provides methods of reducing the level of bradykinin in a subject with hereditary angioedema which include administering to the subject a therapeutically effective amount or a prophylactically effective amount of a dsRNA agent of the invention, (*i.e.,* an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, an iRNA agent targeting a KNG1 gene, or a combination of any of the foregoing, *i.e.,* a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting an F12 gene, or a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting an F12 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, and an iRNA agent targeting

a KNG1 gene), or a pharmaceutical composition or vector comprising such agents, or combinations of such agents.

In one aspect, the present invention provides methods of treating a subject having an contact activation pathway-associated disease, *e.g.,* a thrombophilia, hereditary angioedema type I; hereditary angioedema type II; hereditary angioedema type III; any other hereditary angioedema caused by elevated levels of bradykinin. In one embodiment, 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 of the invention targeting a KLKB1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting a KLKB1 gene or a vector of the invention comprising an iRNA agent targeting an KLKB1 gene. In another embodiment, 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 of the invention targeting an F12 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an F12 gene or a vector of the invention comprising an iRNA agent targeting an F12 gene. In yet another embodiment, 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 of the invention targeting an KNG1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an KNG1 gene or a vector of the invention comprising an iRNA agent targeting an KNG1 gene. In other embodiments, the treatment methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a therapeutically effective amount of a combination of dsRNA agents of the invention, (*i.e.,* a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting an F12 gene, or a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting an F12 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, and an iRNA agent targeting a KNG1 gene), or a pharmaceutical composition or vector comprising such agents, or combinations of such agents.

In another aspect, the present invention provides methods of treating a subject having HAE. In one embodiment, the methods (and uses) of the invention for treating a subject having HAE include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent of the invention targeting a F12 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting a F12 gene or a vector of the invention comprising an iRNA agent targeting an F12 gene. In another embodiment, the methods (and uses) of the invention for treating a subject having HAE include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent of the invention targeting an KLKB1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an KLKB1 gene or a vector of the invention comprising an iRNA agent targeting an KLKB1 gene. In yet another embodiment, the methods (and uses)

of the invention for treating a subject having HAE include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent of the invention targeting an KNG1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an KNG1 gene or a vector of the invention comprising an iRNA agent targeting an KNG1 gene. In other embodiments, the methods (and uses) of the invention for treating a subject having HAE include administering to the subject, *e.g.,* a human, a therapeutically effective amount of a combination of dsRNA agents of the invention, (*i.e.,* a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting an F12 gene, or a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting an F12 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, and an iRNA agent targeting a KNG1 gene), or a pharmaceutical composition or vector comprising such agents, or combinations of such agents.

In another aspect, the present invention provides methods of treating a subject having a thrombophilia. In one embodiment, the methods (and uses) of the invention for treating a subject having thrombophilia include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent of the invention targeting a F12 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting a F12 gene or a vector of the invention comprising an iRNA agent targeting an F12 gene. In another embodiment, the methods (and uses) of the invention for treating a subject having thrombophilia include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent of the invention targeting an KLKB1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an KLKB1 gene or a vector of the invention comprising an iRNA agent targeting an KLKB1 gene. In yet another embodiment, the methods (and uses) of the invention for treating a subject having thrombophilia include administering to the subject, *e.g.,* a human, a therapeutically effective amount of an iRNA agent of the invention targeting an KNG1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an KNG1 gene or a vector of the invention comprising an iRNA agent targeting an KNG1 gene. In other embodiments, the methods (and uses) of the invention for treating a subject having thrombophilia include administering to the subject, *e.g.,* a human, a therapeutically effective amount of a combination of dsRNA agents of the invention, (*i.e.,* a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting an F12 gene, or a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting an F12 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, and an iRNA agent targeting a KNG1 gene), or a pharmaceutical composition or vector comprising such agents, or combinations of such agents.

In one aspect, the invention provides methods of preventing at least one symptom in a subject having a contact activation pathway-associated disease, *e.g.,* a thrombophilia, hereditary angioedema (HAE), *e.g.,* the presence of elevated bradykinin, edema swelling of the extremities, face, larynx, upper respiratory tract, abdomen, trunk, and genitals, prodrome; laryngeal swelling; nonpruritic rash; nausea; vomiting; abdominal pain. The methods include administering to the subject a prohylactically effective amount of the iRNA agent, *e.g.* dsRNA, pharmaceutical compositions, or vectors of the invention, thereby preventing at least one symptom in a subject having a contact activation pathway-associated disease. In one embodiment, the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of an iRNA agent of the invention targeting a KLKB1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting a KLKB1 gene or a vector of the invention comprising an iRNA agent targeting an KLKB1 gene. In another embodiment, the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of an iRNA agent of the invention targeting an F12 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an F12 gene or a vector of the invention comprising an iRNA agent targeting an F12 gene. In yet another embodiment, the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of an iRNA agent of the invention targeting an KNG1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an KNG1 gene or a vector of the invention comprising an iRNA agent targeting an KNG1 gene. In other embodiments,the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of a combination of dsRNA agents of the invention, (*i.e.,* a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting an F12 gene, or a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting an F12 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, and an iRNA agent targeting a KNG1 gene), or a pharmaceutical composition or vector comprising such agents, or combinations of such agents.

In one aspect, the present invention provides methods of preventing the formation of a thrombus in a subject at risk of forming a thrombus. The methods include administering to the subject a prohylactically effective amount of the iRNA agent, *e.g.* dsRNA, pharmaceutical compositions, or vectors of the invention, thereby preventing the formation of a thrombus in the subject at risk of forming a thrombus. In one embodiment, the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of an iRNA agent of the invention targeting a KLKB1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting a

KLKB1 gene or a vector of the invention comprising an iRNA agent targeting an KLKB1 gene. In another embodiment, the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of an iRNA agent of the invention targeting an F12 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an F12 gene or a vector of the invention comprising an iRNA agent targeting an F12 gene. In yet another embodiment, the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of an iRNA agent of the invention targeting an KNG1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an KNG1 gene or a vector of the invention comprising an iRNA agent targeting an KNG1 gene. In other embodiments,the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of a combination of dsRNA agents of the invention, (*i.e.,* a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting an F12 gene, or a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting an F12 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, and an iRNA agent targeting a KNG1 gene), or a pharmaceutical composition or vector comprising such agents, or combinations of such agents.

"Subjects at risk of forming a thrombus" include surgical patients (*e.g.,* subjects having general surgery, dental surgery, orthopedic surgery (*e.g.,* knee or hip replacement surgery), trauma surgery, oncological sugery); medical patients (*e.g.,* subjects having an immobilizing disease, *e.g.,*subjects having more than three days of bed rest and/or subjects having long-term use of an intravenous catheter; subjects having atrial fibrillation; elderly subjects; subjects having renal impairment; subjects having a prosthetic heart valve; subjects having heart failure; subjects having cancer); pregnant subjects; postpartum subjects; subjects that have previously had a thrombus; subjects undergoing hormone replacement therapy; subjects sitting for long periods of time, such as in a plane or car; and obese subjects.

In one aspect, the present invention provides methods of preventing an angioedema attack in a subject having HAE. The methods include administering to the subject a prohylactically effective amount of the iRNA agent, *e.g.* dsRNA, pharmaceutical compositions, or vectors of the invention, thereby preventing the formation of a thrombus in the subject at risk of forming a thrombus. In one embodiment, the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of an iRNA agent of the invention targeting a KLKB1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting a KLKB1 gene or a vector of the invention comprising an iRNA agent targeting an KLKB1 gene. In another embodiment, the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of an iRNA

agent of the invention targeting an F12 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an F12 gene or a vector of the invention comprising an iRNA agent targeting an F12 gene. In yet another embodiment, the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of an iRNA agent of the invention targeting an KNG1 gene or a pharmaceutical composition comprising an iRNA agent of the invention targeting an KNG1 gene or a vector of the invention comprising an iRNA agent targeting an KNG1 gene. In other embodiments,the prophylactic methods (and uses) of the invention include administering to the subject, *e.g.,* a human, a prophylactically effective amount of a combination of dsRNA agents of the invention, (*i.e.,* a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting an F12 gene, or a combination of an iRNA agent targeting a KLKB1 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting an F12 gene and an iRNA agent targeting a KNG1 gene, or a combination of an iRNA agent targeting a KLKB1 gene, an iRNA agent targeting an F12 gene, and an iRNA agent targeting a KNG1 gene), or a pharmaceutical composition or vector comprising such agents, or combinations of such agents.

In one 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 KLKB1 gene expression.

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 F12 gene expression.

In yet another aspect, the present invention provides uses of a therapeutically effective amount of an iRNA agent of the invention for treating a subject, *e.g.*, a subject that would benefit from a reduction and/or inhibition of KNG1 gene expression.

In another aspect, the present invention provides uses of an iRNA agent, *e.g.*, a dsRNA, of the invention targeting an KLKB1 gene or pharmaceutical composition comprising an iRNA agent targeting a KLKB1 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 KLKB1 gene expression and/or KLKB1 protein production, such as a subject having a disorder that would benefit from reduction in KLKB1 gene expression, *e.g.*, a contact activation pathway-associated disease.

In one aspect, the present invention provides uses of an iRNA agent, *e.g.*, a dsRNA, of the invention targeting an F12 gene or pharmaceutical composition comprising an iRNA agent targeting an F12 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 F12 gene expression and/or F12 protein production, such as a subject having a disorder that would benefit from reduction in F12 gene expression, *e.g.*, a contact activation pathway-associated disease.

In another aspect, the present invention provides uses of an iRNA agent, *e.g.*, a dsRNA, of the invention targeting an KNG1 gene or pharmaceutical composition comprising an iRNA agent targeting a KNG1 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 KNG1 gene expression and/or KNG1 protein production, such as a subject having a disorder that would benefit from reduction in KNG1 gene expression, *e.g.*, a contact activation pathway-associated disease.

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 KLKB1 gene expression and/or KLKB1 protein production.

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 F12 gene expression and/or F12 protein production.

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 KNG1 gene expression and/or KNG1 protein production.

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 KLKB1 gene expression and/or KLKB1 protein production, such as a contact activation pathway-associated disease.

In one embodiment, an iRNA agent targeting KLKB1 is administered to a subject having hereditary angioedema (HAE) and/or an KLKB1-associated disease such that the expression of a KLKB1 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 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 KLKB1 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 KLKB1 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.

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 F12 gene expression and/or F12 protein production, such as a contact activation pathway-associated disease.

In one embodiment, an iRNA agent targeting F12 is administered to a subject having hereditary angioedema (HAE) and/or a contact activation pathway-associated disease such that the expression of a F12 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 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 F12 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 F12 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.

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 KNG1 gene expression and/or KNG1 protein production, such as a contact activation pathway-associated disease.

In one embodiment, an iRNA agent targeting KNG1 is administered to a subject having hereditary angioedema (HAE) and/or a contact activation pathway-associated disease such that the expression of a KNG1 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 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 KNG1 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 KNG1 gene is decreased for an extended duration, *e.g.*, at least about two, three, four, five, six, seven days or more, *e.g.*, about one week, two weeks, three weeks, or about four weeks or longer.

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

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of HAE may be assessed, for example, by periodic monitoring of HAE symptoms or bradykinin levels. Comparison of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of an iRNA targeting a contact activation pathway gene or pharmaceutical composition thereof, "effective against" a contact activation pathway-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 HAE and/or a contact activation pathway-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, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.15 mg/kg, 0.2 mg/kg, 0.25 mg/kg, 0.3 mg/kg, 0.35 mg/kg, 0.4 mg/kg, 0.45 mg/kg, 0.5 mg/kg, 0.55 mg/kg, 0.6 mg/kg, 0.65 mg/kg, 0.7 mg/kg, 0.75 mg/kg, 0.8 mg/kg, 0.85 mg/kg, 0.9 mg/kg, 0.95 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, 2.0 mg/kg, 2.1 mg/kg, 2.2 mg/kg, 2.3 mg/kg, 2.4 mg/kg, 2.5 mg/kg dsRNA, 2.6 mg/kg dsRNA, 2.7 mg/kg dsRNA, 2.8 mg/kg dsRNA, 2.9 mg/kg dsRNA, 3.0 mg/kg dsRNA, 3.1 mg/kg dsRNA, 3.2 mg/kg dsRNA, 3.3 mg/kg dsRNA, 3.4 mg/kg dsRNA, 3.5 mg/kg dsRNA, 3.6 mg/kg dsRNA, 3.7 mg/kg dsRNA, 3.8 mg/kg dsRNA, 3.9 mg/kg dsRNA, 4.0 mg/kg dsRNA, 4.1 mg/kg dsRNA, 4.2 mg/kg dsRNA, 4.3 mg/kg dsRNA, 4.4 mg/kg dsRNA, 4.5 mg/kg dsRNA, 4.6 mg/kg dsRNA, 4.7 mg/kg dsRNA, 4.8 mg/kg dsRNA, 4.9 mg/kg dsRNA, 5.0 mg/kg dsRNA, 5.1 mg/kg dsRNA, 5.2 mg/kg dsRNA, 5.3 mg/kg dsRNA, 5.4 mg/kg dsRNA, 5.5 mg/kg dsRNA, 5.6 mg/kg dsRNA, 5.7 mg/kg dsRNA, 5.8 mg/kg dsRNA, 5.9 mg/kg dsRNA, 6.0 mg/kg dsRNA, 6.1 mg/kg dsRNA, 6.2 mg/kg dsRNA, 6.3 mg/kg dsRNA, 6.4 mg/kg dsRNA, 6.5 mg/kg dsRNA, 6.6 mg/kg dsRNA, 6.7 mg/kg dsRNA, 6.8 mg/kg dsRNA, 6.9 mg/kg dsRNA, 7.0 mg/kg dsRNA, 7.1 mg/kg dsRNA, 7.2 mg/kg dsRNA, 7.3 mg/kg dsRNA, 7.4 mg/kg dsRNA, 7.5 mg/kg dsRNA, 7.6 mg/kg dsRNA, 7.7 mg/kg dsRNA, 7.8 mg/kg dsRNA, 7.9 mg/kg dsRNA, 8.0 mg/kg dsRNA, 8.1 mg/kg dsRNA, 8.2 mg/kg dsRNA, 8.3 mg/kg dsRNA, 8.4 mg/kg dsRNA, 8.5 mg/kg dsRNA, 8.6 mg/kg dsRNA, 8.7 mg/kg dsRNA, 8.8 mg/kg dsRNA, 8.9 mg/kg dsRNA, 9.0 mg/kg dsRNA, 9.1 mg/kg dsRNA, 9.2 mg/kg dsRNA, 9.3 mg/kg dsRNA, 9.4 mg/kg dsRNA, 9.5 mg/kg dsRNA, 9.6 mg/kg dsRNA, 9.7 mg/kg dsRNA, 9.8 mg/kg dsRNA, 9.9 mg/kg dsRNA, 9.0 mg/kg dsRNA, 10 mg/kg dsRNA, 15 mg/kg dsRNA, 20 mg/kg dsRNA, 25 mg/kg dsRNA, 30 mg/kg dsRNA, 35 mg/kg dsRNA, 40 mg/kg dsRNA, 45 mg/kg dsRNA, or about 50 mg/kg dsRNA. In one embodiment, subjects can be administered 0.5 mg/kg of the dsRNA. Values and ranges intermediate to the recited values are also intended to be part of this invention.

In certain embodiments, for example, when a composition of the invention comprises a dsRNA as described herein and a lipid, subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 5 mg/kg, about 0.01 mg/kg to about 10 mg/kg, about 0.05 mg/kg to about 5 mg/kg, about 0.05 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 5 mg/kg, about 0.1 mg/kg to about 10 mg/kg, about 0.2 mg/kg to about 5 mg/kg, about 0.2 mg/kg to about 10 mg/kg, about 0.3 mg/kg to about 5 mg/kg, about 0.3 mg/kg to about 10 mg/kg, about 0.4 mg/kg to about 5 mg/kg, about 0.4 mg/kg to about 10 mg/kg, about 0.5 mg/kg to about 5 mg/kg, about 0.5 mg/kg to about 10 mg/kg, about 1 mg/kg to about 5 mg/kg, about 1 mg/kg to about 10 mg/kg, about 1.5 mg/kg to about 5 mg/kg, about 1.5 mg/kg to about 10 mg/kg, about 2 mg/kg to about 2.5 mg/kg, about 2 mg/kg to about 10 mg/kg, about 3 mg/kg to about 5 mg/kg, about 3 mg/kg to about 10 mg/kg, about 3.5 mg/kg to about

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

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

In other embodiments, for example, when a composition of the invention comprises a dsRNA as described herein and an N-acetylgalactosamine, subjects can be administered a therapeutic amount of iRNA, such as a dose of about 0.1 to about 50 mg/kg, about 0.25 to about 50 mg/kg, about 0.5 to about 50 mg/kg, about 0.75 to about 50 mg/kg, about 1 to about 50 mg/kg, about 1.5 to about 50 mg/kg, about 2 to about 50 mg/kg, about 2.5 to about 50 mg/kg, about 3 to about 50 mg/kg, about 3.5 to about 50 mg/kg, about 4 to about 50 mg/kg, about 4.5 to about 50 mg/kg, about 5 to about 50 mg/kg, about 7.5 to about 50 mg/kg, about 10 to about 50 mg/kg, about 15 to about 50 mg/kg, about 20 to about 50 mg/kg, about 20 to about 50 mg/kg, about 25 to about 50 mg/kg, about 25 to about 50 mg/kg, about 30 to about 50 mg/kg, about 35 to about 50 mg/kg, about 40 to about 50 mg/kg, about 45 to about 50 mg/kg, about 0.1 to about 45 mg/kg, about 0.25 to about 45 mg/kg, about 0.5 to about 45 mg/kg, about 0.75 to about 45 mg/kg, about 1 to about 45 mg/kg, about 1.5 to about 45 mg/kg, about 2 to about 45 mg/kg, about 2.5 to about 45 mg/kg, about 3 to about 45 mg/kg, about 3.5 to about 45 mg/kg, about 4 to about 45 mg/kg, about 4.5 to about 45 mg/kg, about 5 to about 45 mg/kg, about 7.5 to about 45 mg/kg, about 10 to about 45 mg/kg, about 15 to about 45 mg/kg, about 20 to about 45 mg/kg, about 20 to about 45 mg/kg, about 25 to about 45 mg/kg, about 25 to about 45 mg/kg, about 30 to about 45 mg/kg, about 35 to about 45 mg/kg, about 40 to about 45 mg/kg, about 0.1 to about 40 mg/kg, about 0.25 to about 40 mg/kg, about 0.5 to about 40 mg/kg, about 0.75 to about 40 mg/kg, about 1 to about 40 mg/kg, about 1.5 to about 40 mg/kg, about 2 to about 40 mg/kg, about 2.5 to about 40 mg/kg, about 3 to about 40 mg/kg, about 3.5 to about 40 mg/kg, about 4 to about 40 mg/kg, about 4.5 to about 40 mg/kg, about 5 to about 40 mg/kg, about 7.5 to about 40 mg/kg, about 10 to about 40 mg/kg, about 15 to about 40 mg/kg, about 20 to about 40 mg/kg, about 20 to about 40 mg/kg, about 25 to about 40 mg/kg, about 25 to about 40 mg/kg, about 30 to about 40 mg/kg, about 35 to about 40 mg/kg, about 0.1 to about 30 mg/kg, about 0.25 to about 30 mg/kg,

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

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

In certain embodiments of the invention, for example, when a double stranded RNAi agent includes a modification (*e.g.,* one or more motifs of three identical modifications on three consecutive nucleotides), including one such motif at or near the cleavage site of the agent, six phosphorothioate linkages, and a ligand, such an agent is administered at a dose of about 0.01 to about 0.5 mg/kg, about 0.01 to about 0.4 mg/kg, about 0.01 to about 0.3 mg/kg, about 0.01 to about 0.2 mg/kg, about 0.01 to about 0.1 mg/kg, about 0.01 mg/kg to about 0.09 mg/kg, about 0.01 mg/kg to about 0.08 mg/kg, about 0.01 mg/kg to about 0.07 mg/kg, about 0.01 mg/kg to about 0.06 mg/kg, about 0.01 mg/kg to about 0.05 mg/kg, about 0.02 to about 0.5 mg/kg, about 0.02 to about 0.4 mg/kg, about 0.02 to about 0.3 mg/kg, about 0.02 to about 0.2 mg/kg, about 0.02 to about 0.1 mg/kg, about 0.02 mg/kg to about 0.09 mg/kg, about 0.02 mg/kg to about 0.08 mg/kg, about 0.02 mg/kg to about 0.07 mg/kg, about 0.02 mg/kg to about 0.06 mg/kg, about 0.02 mg/kg to about 0.05 mg/kg, about 0.03 to about 0.5 mg/kg, about 0.03 to about 0.4 mg/kg, about 0.03 to about 0.3 mg/kg, about 0.03 to about 0.2 mg/kg, about 0.03 to about 0.1 mg/kg, about 0.03 mg/kg to about 0.09 mg/kg, about 0.03 mg/kg to about 0.08 mg/kg, about 0.03 mg/kg to about 0.07 mg/kg, about 0.03 mg/kg to about 0.06

mg/kg, about 0.03 mg/kg to about 0.05 mg/kg, about 0.04 to about 0.5 mg/kg, about 0.04 to about 0.4 mg/kg, about 0.04 to about 0.3 mg/kg, about 0.04 to about 0.2 mg/kg, about 0.04 to about 0.1 mg/kg, about 0.04 mg/kg to about 0.09 mg/kg, about 0.04 mg/kg to about 0.08 mg/kg, about 0.04 mg/kg to about 0.07 mg/kg, about 0.04 mg/kg to about 0.06 mg/kg, about 0.05 to about 0.5 mg/kg, about 0.05 to about 0.4 mg/kg, about 0.05 to about 0.3 mg/kg, about 0.05 to about 0.2 mg/kg, about 0.05 to about 0.1 mg/kg, about 0.05 mg/kg to about 0.09 mg/kg, about 0.05 mg/kg to about 0.08 mg/kg, or about 0.05 mg/kg to about 0.07 mg/kg. Values and ranges intermediate to the foregoing recited values are also intended to be part of this invention, *e.g.*, the RNAi agent may be administered to the subject at a dose of about 0.015 mg/kg to about 0.45 mg/kg.

For example, the RNAi agent, *e.g.*, RNAi agent in a pharmaceutical composition, may be administered at a dose of about 0.01 mg/kg, 0.0125 mg/kg, 0.015 mg/kg, 0.0175 mg/kg, 0.02 mg/kg, 0.0225 mg/kg, 0.025 mg/kg, 0.0275 mg/kg, 0.03 mg/kg, 0.0325 mg/kg, 0.035 mg/kg, 0.0375 mg/kg, 0.04 mg/kg, 0.0425 mg/kg, 0.045 mg/kg, 0.0475 mg/kg, 0.05 mg/kg, 0.0525 mg/kg, 0.055 mg/kg, 0.0575 mg/kg, 0.06 mg/kg, 0.0625 mg/kg, 0.065 mg/kg, 0.0675 mg/kg, 0.07 mg/kg, 0.0725 mg/kg, 0.075 mg/kg, 0.0775 mg/kg, 0.08 mg/kg, 0.0825 mg/kg, 0.085 mg/kg, 0.0875 mg/kg, 0.09 mg/kg, 0.0925 mg/kg, 0.095 mg/kg, 0.0975 mg/kg, 0.1 mg/kg, 0.125 mg/kg, 0.15 mg/kg, 0.175 mg/kg, 0.2 mg/kg, 0.225 mg/kg, 0.25 mg/kg, 0.275 mg/kg, 0.3 mg/kg, 0.325 mg/kg, 0.35 mg/kg, 0.375 mg/kg, 0.4 mg/kg, 0.425 mg/kg, 0.45 mg/kg, 0.475 mg/kg, or about 0.5 mg/kg. Values intermediate to the foregoing recited values are also intended to be part of this invention.

In some embodiments, the RNAi agent is administered as a fixed dose of between about 100 mg to about 900 mg, e.g., between about 100 mg to about 850 mg, between about 100 mg to about 800 mg, between about 100 mg to about 750 mg, between about 100 mg to about 700 mg, between about 100 mg to about 650 mg, between about 100 mg to about 600 mg, between about 100 mg to about 550 mg, between about 100 mg to about 500 mg, between about 200 mg to about 850 mg, between about 200 mg to about 800 mg, between about 200 mg to about 750 mg, between about 200 mg to about 700 mg, between about 200 mg to about 650 mg, between about 200 mg to about 600 mg, between about 200 mg to about 550 mg, between about 200 mg to about 500 mg, between about 300 mg to about 850 mg, between about 300 mg to about 800 mg, between about 300 mg to about 750 mg, between about 300 mg to about 700 mg, between about 300 mg to about 650 mg, between about 300 mg to about 600 mg, between about 300 mg to about 550 mg, between about 300 mg to about 500 mg, between about 400 mg to about 850 mg, between about 400 mg to about 800 mg, between about 400 mg to about 750 mg, between about 400 mg to about 700 mg, between about 400 mg to about 650 mg, between about 400 mg to about 600 mg, between about 400 mg to about 550 mg, or between about 400 mg to about 500 mg.

In some embodiments, the RNAi agent is administered as a fixed dose of about 100 mg, about 125 mg, about 150 mg, about 175 mg, 200 mg, about 225 mg, about 250 mg, about

275 mg, about 300 mg, about 325 mg, about 350 mg, about 375 mg, about 400 mg, about 425 mg, about 450 mg, about 475 mg, about 500 mg, about 525 mg, about 550 mg, about 575 mg, about 600 mg, about 625 mg, about 650 mg, about 675 mg, about 700 mg, about 725 mg, about 750 mg, about 775 mg, about 800 mg, about 825 mg, about 850 mg, about 875 mg, or about 900 mg.

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

Administration of the iRNA can reduce the presence of contact activation pathway protein (*i.e.,* KLKB1 protein, F12 protein, and/or KNG1 protein) and/or bradykinin levels, *e.g.*, in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more.

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

Owing to the inhibitory effects on contact activation pathway 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 contact activation pathway gene expression are those having hereditary angioedema (HAE) and/or a contact activation pathway-associated disease or disorder as described herein.

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

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 contact activation pathway gene expression, *e.g.,* a subject having a contact activation pathway-associated disease, in combination with other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders.

For example, in certain embodiments, an iRNA targeting a contact activation pathway gene is administered in combination with, *e.g.*, an agent useful in treating an contact activation pathway-associated disease as described elsewhere herein. For example, additional therapeutics and therapeutic methods suitable for treating a subject that would benefit from reduction in contact activation pathway gene expression, *e.g.,* a subject having a contact activation pathway-associated disease, include an iRNA agent targeting a different portion of the contact activation pathway gene, an androgen, or a therapeutic agent, *e.g.,* a C1INH replacement protein, a kallikrein inhibitor peptide, a bradkinin B2 receptor antagonist peptide, or other therapeutic agents and/or procedures for treating a contact activation pathway-associated disease or a combination of any of the foregoing. In one embodiment, the additional therapeutic is selected from the group consisting of an androgen, such as danazol or oxandrolone, Berinert®, Cinryze™, Rhuconest®, Ecallantide, Firazyr®, Kalbitor®, and a combination of any of the foregoing.

In certain embodiments, a first iRNA agent targeting a contact activation pathway gene is administered in combination with a second iRNA agent targeting a different portion of the contact activation pathway gene. For example, the first RNAi agent comprises a first sense strand and a first antisense strand forming a double stranded region, wherein substantially all of the nucleotides of said first sense strand and substantially all of the nucleotides of the first antisense strand are modified nucleotides, wherein said first sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; and the second RNAi agent comprises a second sense strand and a second antisense strand forming a double stranded region, wherein substantially all of the nucleotides of the second sense strand and substantially all of the nucleotides of the second antisense strand are modified nucleotides, wherein the second sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

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

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

In certain embodiments, a first iRNA agent targeting a contact activation pathway gene is administered in combination with a second iRNA agent targeting a gene that is different from the contact activation pathway gene. For example, the iRNA agent targeting the KLKB1 gene may be administered in combination with an iRNA agent targeting the coagulation factor XII (F12) gene. The first iRNA agent targeting a KLKB1 gene and the second iRNA agent targeting a gene different from the KLKB1 gene, *e.g.*, the coagulation factor XII (F12) gene, may be administred as parts of the same pharmaceutical composition. Alternatively, the first iRNA agent targeting a KLKB1 gene and the second iRNA agent targeting a gene different from the KLKB1 gene, *e.g.*, the coagulation factor XII (F12) gene, may be administered as parts of different pharmaceutical compositions.

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.

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 contact activation pathway gene expression (*i.e.,* KLKB1 expression, F12 expression, or KNG1 expression) in a cell. In other aspects, the present invention provides an iRNA of the invention and/or a composition comprising an iRNA of the invention for use in reducing and/or inhibiting contact activation pathway gene expression (*i.e.,* KLKB1 expression, F12 expression, or KNG1 expression) in a cell. In yet 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 contact activation pathway gene expression (*i.e.,* KLKB1 expression, F12 expression, or KNG1 expression) in a cell are provided. In still other aspects, the the present invention provides an iRNA of the invention and/or a

composition comprising an iRNA of the invention for use in reducing and/or inhibiting contact activation pathway protein production (*i.e.,* KLKB1 protein production, F12 protein production, or KNG1 protein production) in a cell. In yet 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 contact activation pathway protein production (*i.e.,* KLKB1 protein production, F12 protein production, or KNG1 protein production) in a cell are provided. The methods and uses include contacting the cell with an iRNA, *e.g.,* a dsRNA, of the invention and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of the contact activation pathway gene, thereby inhibiting expression of the contact activation pathway gene or inhibiting contact activation pathway protein production in the cell.

Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of KLKB1 may be determined by determining the mRNA expression level of KLKB1 using methods routine to one of ordinary skill in the art, *e.g.,* Northern blotting, qRT-PCR, by determining the protein level of KLKB1 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 KLKB1.

In the methods and uses of the invention the cell may be contacted *in vitro* or *in vivo*, *i.e.*, the cell may be within a subject.

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

Contact activation pathway 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%.

Contact activation pathway 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%.

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 contact activation pathway 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 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 KLKB1, 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 an KLKB1 gene in a mammal, *e.g.,* a human. The present invention also provides a composition comprising an iRNA, *e.g.,* a dsRNA, that targets an KLKB1 gene in a

cell of a mammal for use in inhibiting expression of the KLKB1 gene in the mammal. In another aspect, the present invention provides use of an iRNA, *e.g.,* a dsRNA, that targets an KLKB1 gene in a cell of a mammal in the manufacture of a medicament for inhibiting expression of the KLKB1 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 an KLKB1 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the KLKB1 gene, thereby inhibiting expression of the KLKB1 gene in the mammal.

In another aspect, the present invention also provides methods for inhibiting the expression of an F12 gene in a mammal, *e.g.,* a human. The present invention also provides a composition comprising an iRNA, *e.g.,* a dsRNA, that targets an F12 gene in a cell of a mammal for use in inhibiting expression of the F12 gene in the mammal. In another aspect, the present invention provides use of an iRNA, *e.g.,* a dsRNA, that targets an F12 gene in a cell of a mammal in the manufacture of a medicament for inhibiting expression of the F12 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 an F12 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the F12 gene, thereby inhibiting expression of the F12 gene in the mammal.

In yet another aspect, the present invention also provides methods for inhibiting the expression of an KNG1 gene in a mammal, *e.g.,* a human. The present invention also provides a composition comprising an iRNA, *e.g.,* a dsRNA, that targets an KNG1 gene in a cell of a mammal for use in inhibiting expression of the KNG1 gene in the mammal. In another aspect, the present invention provides use of an iRNA, *e.g.,* a dsRNA, that targets an KNG1 gene in a cell of a mammal in the manufacture of a medicament for inhibiting expression of the KNG1 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 an KNG1 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the KNG1 gene, thereby inhibiting expression of the KNG1 gene in the mammal.

Reduction in gene expression can be assessed in peripheral blood sample of the iRNA-administered subject by any methods known it the art, *e.g.* qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known it the art and by methods, *e.g.*, ELISA or Western blotting, described herein. In one embodiment, a tissue sample serves as the tissue material for monitoring the reduction in contact activation pathway gene and/or protein expression. In another embodiment, a blood sample serves as

the tissue material for monitoring the reduction in contact activation pathway gene and/or protein expression.

In one embodiment, verification of RISC medicated 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).

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

EXAMPLES

Example 1. KLKB1 iRNA Synthesis

Source of reagents

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

Transcripts

siRNA Design

A set of siRNAs targeting human KLKB1, "kallikrein B, plasma (Fletcher factor) 1" (REFSeq Accession No. NM_000892.3, GI:78191797, GeneID: 3818, SEQ ID NO:1 and SEQ ID NO.2) and KLKB1 orthologs from toxicology species (cynomolgus monkey: RefSeq Accession No. XM_005556482, GI:544436072; rhesus monkey: RefSeq JU329355, GI:380802470; mouse: RefSeq NM_008455, GI:236465804; rat: RefSeq NM_012725, GI:162138904) was designed using custom R and Python scripts. The human KLKB1 RefSeq mRNA has a length of 2252 bases. The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 72 through position 2252 of human KLKB1 mRNA (containing the the coding region and 3' UTR) was determined using a linear model that predicted the direct measure of mRNA knockdown based on the data of more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. Subsets of the KLKB1 siRNAs were designed with perfect or near-perfect matches between human, cynomolgus monkey and rhesus monkey. A further subset was designed with perfect or near-perfect matches to mouse and rat KLKB1 orthologs. For each strand of the siRNA, a custom Python script was used in a brute force search to measure the number and positions of mismatches between the siRNA and all potential

alignments in the target species transcriptome. Extra weight was given to mismatches in the seed region, defined here as positions 2-9 of the antisense oligonucleotide, as well the cleavage site of the siRNA, defined here as positions 10-11 of the antisense oligonucleotide. The relative weights for the mismatches were 2.8 for seed mismatches, 1.2 for cleavage site mismatches, and 1 mismatches in other positions up through antisense position 19. Mismatches in the first position were ignored. A specificity score was calculated for each strand by summing the value of each weighted mismatch. Preference was given to siRNAs whose antisense score in human and cynomolgus monkey was greater than or equal to 3.0 and predicted efficacy was greater than or equal to 70% knockdown of the KLKB1 transcript. One set of siRNAs containing structure-activity modifications, including various 2'-O-methyl and 2'-fluoro substitution patterns, were also designed, synthesized and screened.

A detailed list of the unmodified KLKB1 sense and antisense strand sequences is shown in Table 3. A detailed list of the modified KLKB1 sense and antisense strand sequences is shown in Table 4.

siRNA Synthesis

KLKB1 siRNA sequences were synthesized at 1 μmol scale on a Mermade 192 synthesizer (BioAutomation) using the solid support mediated phosphoramidite chemistry. The solid support was controlled pore glass (500 A) loaded with custom GalNAc ligand or universal solid support (AM biochemical). Ancillary synthesis reagents, 2'-F and 2'-O-Methyl RNA and deoxy phosphoramidites were obtained from Thermo-Fisher (Milwaukee, WI) and Hongene (China). 2'F 2'-O-Methyl, GNA (glycol nucleic acids), 5'phosphate and other modifications were introduced using the corresponding phosphoramidites. Synthesis of 3' GalNAc conjugated single strands was performed on a GalNAc modified CPG support. Custom CPG universal solid support was used for the synthesis of antisense single strands. Coupling time for all phosphoramidites (100 mM in acetonitrile) was 5 min employing 5-Ethylthio-1H-tetrazole (ETT) as activator (0.6 M in acetonitrile). Phosphorothioate linkages were generated using a 50 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (1:1 v/v). Oxidation time was 3 minutes. All sequences were synthesized with final removal of the DMT group ("DMT off").

Upon completion of the solid phase synthesis, oligoribonucleotides were cleaved from the solid support and deprotected in sealed 96 deep well plates using 200 μL Aqueous Methylamine reagents at 60°C for 20 minutes. For sequences containing 2' ribo residues (2'-OH) that are protected with a tert-butyl dimethyl silyl (TBDMS) group, a second step deprotection was performed using TEA.3HF (triethylamine trihydro fluoride) reagent. To the methylamine deprotection solution, 200uL of dimethyl sulfoxide (DMSO) and 300ul TEA.3HF reagent was added and the solution was incubated for additional 20min at 60°C. At the end of cleavage and deprotection step, the synthesis plate was allowed to come to room

temperature and was precipitated by addition of 1mL of acetontile: ethanol mixture (9:1). The plates were cooled at -80 C for 2 hrs, superanatant decanted carefully with the aid of a multi channel pipette. The oligonucleotide pellet was re-suspended in 20mM NaOAc buffer and were desalted using a 5 mL HiTrap size exclusion column (GE Healthcare) on an AKTA Purifier System equipped with an A905 autosampler and a Frac 950 fraction collector. Desalted samples were collected in 96-well plates. Samples from each sequence were analyzed by LC-MS to confirm the identity, UV (260 nm) for quantification and a selected set of samples by IEX chromatography to determine purity.

Annealing of KLKB1 single strands was performed on a Tecan liquid handling robot. Equimolar mixture of sense and antisense single strands were combined and annealed in 96 well plates. After combining the complementary single strands, the 96-well plate was sealed tightly and heated in an oven at 100°C for 10 minutes and allowed to come slowly to room temperature over a period 2-3 hours. The concentration of each duplex was normalized to 10μM in 1X PBS and then submitted for *in vitro* screening assays.

Example 2. *In vitro* screening of KLKB1 siRNA duplexes

Cell culture and transfections

Cos7 cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO2 in DMEM (ATCC) supplemented with 10% FBS, before being released from the plate by trypsinization. Dual-Glo® Luciferase constructs were generated in the psiCHECK2 plasmid containing either approximately 2.2 kb of human KLKB1 genomic sequence or 2.5 kb of orthologous mouse KLKB1 genomic sequence. Each dual-luciferase plasmid was co-transfected with siRNA into approximately $15x10^4$ cells using Lipofectamine 2000 (Invitrogen, Carlsbad CA. cat # 11668-019). For each well of a 96 well plate, 0.2 μl of Lipofectamine was added to 10 ng of plasmid vector and a single siRNA (Tables 3 and 4) in 14.8 μl of Opti-MEM and allowed to complex at room temperature for 15 minutes. The mixture was then added to the cells which were resuspended in 80 μl of fresh complete media. Cells were incubated for 24 hours before luciferase was measured.

Single dose experiments were performed at 10nM and 0.01nM final duplex concentration and dose response experiments were done over a range of doses from 10nM to 36fM final duplex concentration over 8, 6-fold dilutions.

Dual-Glo® Luciferase assay

Forty-eight hours after the siRNAs were transfected, Firefly (transfection control) and Rinella (fused to KLKB1 target sequence) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding 75 μl of Dual-Glo® Luciferase Reagent equal to the culture medium volume to each well and mix. The mixture was incubated at room temperature for 30 minutes before lunimescense (500 nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal.

Renilla luciferase activity was measured by adding 75 µl of room temperature Dual-Glo® Stop & Glo® Reagent to each well and the plates were incubated for 10-15 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent, quenches the firefly luciferase signal and sustaines luminescence for the Renilla luciferase reaction. siRNA activity was determined by normalizing the Renilla (KLKB1) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were transfected with the same vector but were not treated with siRNA or were treated with a non-targeting siRNA. All transfections were done in triplicate.

Table 5 shows the results of a single dose screen in Cos7 cells transfected with the indicated human KLKB1 iRNAs. Table 6 shows the results of a single dose screen in Cos7 cells transfected with the indicated mouse KLKB1 iRNAs. Data are expressed as percent of mRNA remaining relative to negative control.

Table 2. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	**Nucleotide(s)**
A	Adenosine-3’-phosphate
Af	2’-fluoroadenosine-3’-phosphate
Afs	2’-fluoroadenosine-3’-phosphorothioate
As	adenosine-3’-phosphorothioate
C	cytidine-3’-phosphate
Cf	2’-fluorocytidine-3’-phosphate
Cfs	2’-fluorocytidine-3’-phosphorothioate
Cs	cytidine-3’-phosphorothioate
G	guanosine-3’-phosphate
Gf	2’-fluoroguanosine-3’-phosphate
Gfs	2’-fluoroguanosine-3’-phosphorothioate
Gs	guanosine-3’-phosphorothioate
T	5’-methyluridine-3’-phosphate
Tf	2’-fluoro-5-methyluridine-3’-phosphate
Tfs	2’-fluoro-5-methyluridine-3’-phosphorothioate
Ts	5-methyluridine-3’-phosphorothioate
U	Uridine-3’-phosphate
Uf	2’-fluorouridine-3’-phosphate
Ufs	2’-fluorouridine -3’-phosphorothioate
Us	uridine -3’-phosphorothioate

N	any nucleotide (G, A, C, T or U)
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'- phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'- phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'- phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3
(dt)	deoxy-thymine
Y34	2-hydroxymethyl-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose)
Y44	2-hydroxymethyl-tetrahydrofurane-5-phosphate
(Agn)	Adenosine-glycol nucleic acid (GNA)
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
(Cgn)	Cytidine-glycol nucleic acid (GNA)
P	Phosphate
VP	Vinyl-phosphate

Table 3. Unmodified Sense and Antisense Strand Sequences of KLKB1 dsRNAs

Duple Name	Sense Oligo Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Oligo Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Position in NM_000892.3
AD-65077	A-129940	AAUCCAAAAUAUUCUACAAAA	30	A-129941	UUUUGUAGAAUAUUUUGGAUUUC	117	1661-1682
AD-65170	A-130248	CUGGUCAUCAAAUAAGUGCUU	31	A-130249	AAGCACUUAUUUGAUGACCAGAU	118	382-403
AD-65103	A-130010	GUGGUCAUCAAAUAAGUGCUU	32	A-130011	AAGCACUUAUUUGAUGACCACAU	119	382-403
AD-65083	A-129942	CAUGGACUGGAUUUUAGAGAA	33	A-129943	UUCUCUAAAAUCCAGUCCAUGUA	120	1922-1943
AD-65087	A-130004	ACCAAAGUCGCUGAGUACAUA	34	A-130005	UAUGUACUCAGCGACUUUGGUGU	121	1905-1926
AD-65149	A-130178	GAUGGACUGGAUUUUAGAGAA	35	A-130179	UUCUCUAAAAUCCAGUCCAUCUA	122	1922-1943
AD-64652	A-129275	UAUGAGAGGAGUCAAUUUUAA	36	A-129276	UUAAAAUUGACUCCUCUCAUAUC	123	431-452
AD-65162	A-130198	AAUGAGAGGAGUCAAUUUUAA	37	A-130199	UUAAAAUUGACUCCUCUCAUUUC	124	431-452
AD-65153	A-130242	UCCAAAGUCGCUGAGUACAUA	38	A-130243	UAUGUACUCAGCGACUUUGGAGU	125	1905-1926
AD-65084	A-129958	AUUCUACAAAAGGUAAAUAUU	39	A-129959	AAUAUUUACCUUUUGUAGAAUAU	126	1671-1692
AD-65099	A-129948	UUUCUCACAAAUAAAAGAGAU	40	A-129949	AUCUCUUUUAUUUGUGAGAAAGG	127	1457-1478
AD-65100	A-129962	UAUCAAGAUUAUAAAAUAACA	41	A-129963	UGUUAUUUUAUAAUCUUGAUAUC	128	1725-1746
AD-65090	A-129960	CUUCUUGAAAGAUAGUGUUAA	42	A-129961	UUAACACUAUCUUUCAAGAAGCA	129	302-323
AD-65085	A-129972	GAAUGUUUGCCAAGAGACUUA	43	A-129973	UAAGUCUCUUGGCAAACAUUCAC	130	1016-1037
AD-65062	A-129980	AUAUUCCUUUGGUAACAAAUA	44	A-129981	UAUUUGUUACCAAAGGAAUAUUU	131	1687-1708
AD-65164	A-130230	CGUCAUCAAAUAAGUGCUUGA	45	A-130231	UCAAGCACUUAUUUGAUGACGAC	132	384-405
AD-65139	A-130206	UCCUGCAAAAGAACUUUACCU	46	A-130207	AGGUAAAGUUCUUUUGCAGGAUA	133	918-939
AD-65151	A-130210	CAAUGUUUGCCAAGAGACUUA	47	A-130211	UAAGUCUCUUGGCAAACAUUGAC	134	1016-1037
AD-65158	A-130228	AGACACAAGCACAAUUUAUAA	48	A-130229	UUAUAAAUUGUGCUUGUGUCUCC	135	1595-1616

AD-65078	A-129956	CGAGUCACAAAGAAAUGUUUA	49	A-129957	UAAACAUUUCUUUGUGACUCGAU	136	818-839
AD-65161	A-130182	AUCUUGAAAGAUAGUGUUACA	50	A-130183	UGUAACACUAUCUUUCAAGAUGC	137	303-324
AD-65076	A-130016	AAUGUGGUCAUCAAAUAAGUA	51	A-130017	UACUUAUUUGAUGACCACAUUGC	138	379-400
AD-65093	A-130006	GUUACUCUUUGAGAUUGUGUA	52	A-130007	UACACAAUCUCAAAGAGUAACCA	139	1177-1198
AD-65156	A-130196	GUUCUUGAAAGAUAGUGUUAA	53	A-130197	UUAACACUAUCUUUCAAGAACCA	140	302-323
AD-65059	A-129934	GACUUUGGAGGAGAAGAAUUA	54	A-129935	UAAUUCUUCUCCUCCAAAGUCAA	141	972-993
AD-65073	A-129968	ACCUGCAAAAGAACUUUACCU	55	A-129969	AGGUAAAGUUCUUUUGCAGGUUA	142	918-939
AD-65074	A-129984	AUCGAGUCACAAAGAAAUGUU	56	A-129985	AACAUUUCUUUGUGACUCGAUUU	143	816-837
AD-65092	A-129990	UGACACAAGCACAAUUUAUAA	57	A-129991	UUAUAAAUUGUGCUUGUGUCACC	144	1595-1616
AD-65097	A-129992	GGUCAUCAAAUAAGUGCUUGA	58	A-129993	UCAAGCACUUAUUUGAUGACCAC	145	384-405
AD-65101	A-129978	UGGUCAUCAAAUAAGUGCUUA	59	A-129979	UAAGCACUUAUUUGAUGACCACA	146	383-404
AD-65131	A-130172	AGCCAGAAAAGAUAUCAAGAU	60	A-130173	AUCUUGAUAUCUUUUCUGGCUUU	147	1713-1734
AD-65159	A-130244	CUUACUCUUUGAGAUUGUGUA	61	A-130245	UACACAAUCUCAAAGAGUAAGCA	148	1177-1198
AD-65150	A-130194	UUUCUACAAAAGGUAAAUAUU	62	A-130195	AAUAUUUACCUUUUGUAGAAAAU	149	1671-1692
AD-65060	A-129950	CACAAUGGAAUGUGGCGUUUA	63	A-129951	UAAACGCCACAUUCCAUUGUGUU	150	1827-1848
AD-65098	A-130008	UUACUCUUUGAGAUUGUGUAA	64	A-130009	UUACACAAUCUCAAAGAGUAACC	151	1178-1199
AD-65067	A-129966	UACUCUUUGAGAUUGUGUAAA	65	A-129967	UUUACACAAUCUCAAAGAGUAAC	152	1179-1200
AD-65065	A-129936	UGCCAGAAAAGAUAUCAAGAU	66	A-129937	AUCUUGAUAUCUUUUCUGGCAUU	153	1713-1734
AD-65095	A-129946	UUCUUGAAAGAUAGUGUUACA	67	A-129947	UGUAACACUAUCUUUCAAGAAGC	154	303-324
AD-65126	A-130186	GACAAUGGAAUGUGGCGUUUA	68	A-130187	UAAACGCCACAUUCCAUUGUCUU	155	1827-1848
AD-65157	A-130212	AAUAAAAUAACCCAACGGAUA	69	A-130213	UAUCCGUUGGGUUAUUUUAUUAU	156	1734-1755
AD-65086	A-129988	CUUAAAACACAUCUGAAAGUGGA	70	A-129989	UCCACUUUCAGAUGUUUUAAGAA	157	843-864
AD-65167	A-130200	AAUCAAGAUUAUAAAAUAACA	71	A-130201	UGUUAUUUUAUAAUCUUGAUUUC	158	1725-1746
AD-65071	A-129938	AGUAACGUGGAAUCUGGAUUA	72	A-129939	UAAUCCAGAUUCCACGUUACUCA	159	618-639

AD-65066	A-129952	UAUAAAGGAGUUGAUAUGAGA	73	A-129953	UCUCAUAUCAACUCCUUUAUAAA	160	417-438
AD-65165	A-130246	AUACUCUUUGAGAUUGUGUAA	74	A-130247	UUACACAAUCUCAAAGAGUAUCC	161	1178-1199
AD-65132	A-130188	AAUAAAGGAGUUGAUAUGAGA	75	A-130189	UCUCAUAUCAACUCCUUUAUUAA	162	417-438
AD-65125	A-130170	CACUUUGGAGGAGAAGAAUUA	76	A-130171	UAAUUCUUCUCCUCCCAAAGUGAA	163	972-993
AD-65091	A-129974	UAUAAAAUAACCCAACGGAUA	77	A-129975	UAUCCGUUGGGUUAUUUUAUAAU	164	1734-1755
AD-65136	A-130252	GAAUGUGGUCAUCAAAUAAGU	78	A-130253	ACUUAUUUGAUGACCACAUUCCU	165	378-399
AD-65137	A-130174	UGUAACGUGGAAUCUGGAUUA	79	A-130175	UAAUCCAGAUUCCACGUUACACA	166	618-639
AD-65140	A-130222	UUCGAGUCACACAAAGAAAUGUU	80	A-130223	AACAUUUCUUUGUGACUCGAAUU	167	816-837
AD-65128	A-130218	UUAUUCCUUUGGUAACAAAUA	81	A-130219	UAUUUGUUACCAAAGGAAUAAUU	168	1687-1708
AD-65088	A-130020	ACACAAGCACAAUUUAUACCA	82	A-130021	UGGUAUAAAUUGUGCUUGUGUCA	169	1597-1618
AD-65160	A-130260	CUGGAAUCUGGAUUCUCACUA	83	A-130261	UAGUGAGAAUCCAGAUUCCAGGU	170	624-645
AD-65152	A-130226	GUUAAAACAUCUGAAAGUGGA	84	A-130227	UCCACUUUCAGAUGUUUUAACAA	171	843-864
AD-65133	A-130204	AACUCUUUGAGAUUGUGUAAA	85	A-130205	UUUACACAAUCUCAAAGAGUUAC	172	1179-1200
AD-65082	A-130018	GCAAUGUGGUCAUCAAAUAAA	86	A-130019	UUUAUUUGAUGACCACAUUGCUU	173	377-398
AD-65094	A-130022	GUGGAAUCUGGAUUCUCACUA	87	A-130023	UAGUGAGAAUCCAGAUUCCACGU	174	624-645
AD-65155	A-130180	GGCAUUGUUGGAGGAACAAAA	88	A-130181	UUUUGUUCCUCCAACAAUGCCUG	175	1239-1260
AD-65163	A-130214	UUUGUUGGAGGAACAAACUCU	89	A-130215	AGAGUUUGUUCCUCCAACAAAGC	176	1242-1263
AD-65144	A-130192	GGAGUCACAAAGAAAUGUUUA	90	A-130193	UAAACAUUUCUUUGUGACUCCAU	177	818-839
AD-65096	A-129976	AUUGUUGGAGGAACAAACUCU	91	A-129977	AGAGUUUGUUCCUCCAACAAUGC	178	1242-1263
AD-65142	A-130254	UAUGUGGUCAUCAAAUAAGUA	92	A-130255	UACUUAUUUGAUGACCACAUAGC	179	379-400
AD-65141	A-130238	CAAAAGAUGCUUGUAAGGGAA	93	A-130239	UUCCCUUACAAGCAUCUUUUGCC	180	1780-1801
AD-65079	A-129970	GUCUGUGCUGGCUAUAAAGAA	94	A-129971	UUCUUUAUAGCCAGCACAGACCA	181	1755-1776
AD-65102	A-129994	AGCAGUGUUGAAGAAUGCCAA	95	A-129995	UUGGCAUUCUUCAACACUGCUAA	182	465-486
AD-65138	A-130190	GUGUGUGGAGGGUCACUCAUA	96	A-130191	UAUGAGUGACCCUCCACACACGU	183	1323-1344

AD-65075	A-130000	GAAAAGAUGCUUGUAAGGGAA	97	A-130001	UUCCCUUACAAGCAUCUUUUCCC	184	1780-1801
AD-65080	A-129986	ACAUCUGAAAGUGGCACACCA	98	A-129987	UGGUGUGCCACUUUCAGAUGUUU	185	849-870
AD-65145	A-130208	CUCUGUGCUGGCUAUAAAGAA	99	A-130209	UUCUUUAUAGCCAGCACAGAGCA	186	1755-1776
AD-65169	A-130232	UGCAGUGUUGAAGAAUGCCAA	100	A-130233	UUGGCAUUCUUCAACACUGCAAA	187	465-486
AD-65061	A-129964	UUCUUAAAACAUCUGAAAGUA	101	A-129965	UACUUUCAGAUGUUUUAAGAAGA	188	841-862
AD-65135	A-130236	UAGCACAAUUUAUACCAACUA	102	A-130237	UAGUUGGUAUAAAUUGUGCUAGU	189	1601-1622
AD-65068	A-129982	UGGAAUGUGGCGUUUGGUGGA	103	A-129983	UCCACCAAACGCCACAUUCCAUU	190	1832-1853
AD-65148	A-130256	CCAAUGUGGUCAUCAAAUAAA	104	A-130257	UUUAUUUGAUGACCACAUUGGUU	191	377-398
AD-65072	A-129954	CUGUGUGGAGGGUCACUCAUA	105	A-129955	UAUGAGUGACCCUCCACACAGGU	192	1323-1344
AD-65146	A-130224	UCAUCUGAAAGUGGCACACCA	106	A-130225	UGGUGUGCCACUUUCAGAUGAUU	193	849-870
AD-65129	A-130234	CCACAAUUUAUACCAACUGUU	107	A-130235	AACAGUUGGUAUAAAUUGUGGUU	194	1603-1624
AD-65064	A-130012	CACAAGCACACAAUUUAUACCAA	108	A-130013	UUGGUAUAAAUUGUGCUUGUGUC	195	1598-1619
AD-65134	A-130220	AGGAAUGUGGCGUUUGGUGGA	109	A-130221	UCCACCAAACGCCACAUUCCUUU	196	1832-1853
AD-65063	A-129996	GCACAAUUUAUACCAACUGUU	110	A-129997	AACAGUUGGUAUAAAUUGUGCUU	197	1603-1624
AD-65089	A-129944	CGCAUUGUUGGAGGAACAAAA	111	A-129945	UUUUGUUCCUCCAACAAUGCGUG	198	1239-1260
AD-65069	A-129998	AAGCACAAUUUAUACCAACUA	112	A-129999	UAGUUGGUAUAAAUUGUGCUUGU	199	1601-1622
AD-65130	A-130250	GACAAGCACACAAUUUAUACCAA	113	A-130251	UUGGUAUAAAUUGUGCUUGUCUC	200	1598-1619
AD-65147	A-130240	UUUUACCCGGGAGUUGACUUU	114	A-130241	AAAGUCAACUCCCGGGUAAAAUU	201	957-978
AD-65081	A-130002	AUUUACCCGGGAGUUGACUUU	115	A-130003	AAAGUCAACUCCCGGGUAAAUUU	202	957-978
AD-65154	A-130258	UCACAAGCACACAAUUUAUACCA	116	A-130259	UGGUAUAAAUUGUGCUUGUGACA	203	1597-1618

Table 4. Modified Sense and Antisense Strand Sequences of KLKB1 dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisence Oligo Name	Antisense Sequence 5' to 3'	SEQ ID NO:
AD-65077	A-129940	AfsasUfcCfaAfaAfUfAfuUfcUfaCfaAfaAfL96	204	A-129941	usUfsuUfgUfaGfaAfuauUfuUfgGfaUfususc	291
AD-65170	A-130248	CfsusGfgUfcAfuCfAfAfaUfaAfgUfgCfuUfL96	205	A-130249	asAfsgCfaCfuUfaUfuugAfuGfaCfcAfgsasu	292
AD-65103	A-130010	GfsusGfgUfcAfuCfAfAfaUfaAfgUfgCfuUfL96	206	A-130011	asAfsgCfaCfuUfaUfuugAfuGfaCfcAfcsasu	293
AD-65083	A-129942	CfsasUfgGfaCfuGfGfAfuUfuUfaGfaGfaAfL96	207	A-129943	usUfscUfcUfaAfaAfuccAfgUfcCfaUfgsusa	294
AD-65087	A-130004	AfscsCfaAfaGfuCfGfCfuGfaGfuAfcAfuAfL96	208	A-130005	usAfsuGfuAfcUfcAfgcgAfcUfuUfgGfusgsu	295
AD-65149	A-130178	GfsasUfgGfaCfuGfGfAfuUfuUfaGfaGfaAfL96	209	A-130179	usUfscUfcUfaAfaAfuccAfgUfcCfaUfcsusa	296
AD-64652	A-129275	UfsasUfgAfgAfgGfAfGfuCfaAfuUfuUfaAfL96	210	A-129276	usUfsaAfaAfuUfgAfcucCfuCfuCfaUfasusc	297
AD-65162	A-130198	AfsasUfgAfgAfgGfAfGfuCfaAfuUfuUfaAfL96	211	A-130199	usUfsaAfaAfuUfgAfcucCfuCfuCfaUfususc	298
AD-65153	A-130242	UfscsCfaAfaGfuCfGfCfuGfaGfuAfcAfuAfL96	212	A-130243	usAfsuGfuAfcUfcAfgcgAfcUfuUfgGfasgsu	299
AD-65084	A-129958	AfsusUfcUfaCfaAfAfAfgGfuAfaAfuAfuUfL96	213	A-129959	asAfsuAfuUfuAfcCfuuuUfgUfaGfaAfusasu	300
AD-65099	A-129948	UfsusUfcUfcAfcAfAfAfuAfaAfaGfaGfaUfL96	214	A-129949	asUfscUfcUfuUfuAfuuuGfuGfaGfaAfasgsg	301
AD-65100	A-129962	UfsasUfcAfaGfaUfUfAfuAfaAfaUfaAfcAfL96	215	A-129963	usGfsuUfaUfuUfuAfuaaUfcUfuGfaUfasusc	302
AD-65090	A-129960	CfsusUfcUfuGfaAfAfGfaUfaGfuGfuUfaAfL96	216	A-129961	usUfsaAfcAfcUfaUfcuuUfcAfaGfaAfgscsa	303
AD-65085	A-129972	GfsasAfuGfuUfuGfCfCfaAfgAfgAfcUfuAfL96	217	A-129973	usAfsaGfuCfuCfuUfggcAfaAfcAfuUfcsasc	304
AD-65062	A-129980	AfsusAfuUfcCfuUfUfGfgUfaAfcAfaAfuAfL96	218	A-129981	usAfsuUfuGfuUfaCfcaaAfgGfaAfuAfususu	305
AD-65164	A-130230	CfsgsUfcAfuCfaAfAfUfaAfgUfgCfuUfgAfL96	219	A-130231	usCfsaAfgCfaCfuUfauuUfgAfuGfaCfgsasc	306
AD-65139	A-130206	UfscsCfuGfcAfaAfAfGfaAfcUfuUfaCfcUfL96	220	A-130207	asGfsgUfaAfaGfuUfcuuUfuGfcAfgGfasusa	307
AD-65151	A-130210	CfsasAfuGfuUfuGfCfCfaAfgAfgAfcUfuAfL96	221	A-130211	usAfsaGfuCfuCfuUfggcAfaAfcAfuUfgsasc	308
AD-65158	A-130228	AfsgsAfcAfcAfaGfCfAfcAfaUfuUfaUfaAfL96	222	A-130229	usUfsaUfaAfaUfuGfugcUfuGfuGfuCfuscsc	309
AD-65078	A-129956	CfsgsAfgUfcAfcAfAfAfgAfaAfuGfuUfuAfL96	223	A-129957	usAfsaAfcAfuUfuCfuuuGfuGfaCfuCfgsasu	310
AD-65161	A-130182	AfsusCfuUfgAfaAfGfAfuAfgUfgUfuAfcAfL96	224	A-130183	usGfsuAfaCfaCfuAfucuUfuCfaAfgAfusgsc	311

AD-65076	A-130016	AfsasUfgUfgGfuCfAfUfcAfaAfuAfaGfuAfL96	225	A-130017	usAfscUfuAfuUfuGfaugAfcCfaCfaUfusgsc	312
AD-65093	A-130006	GfsusUfaCfuCfuUfUfGfaGfaUfuGfuGfuAfL96	226	A-130007	usAfscAfcAfaUfcUfcaaAfgAfgUfaAfcscsa	313
AD-65156	A-130196	GfsusUfcUfuGfaAfAfGfaUfaGfuGfuUfaAfL96	227	A-130197	usUfsaAfcAfcUfaUfcuuUfcAfaGfaAfcscsa	314
AD-65059	A-129934	GfsasCfuUfuGfgAfGfGfaGfaAfgAfaUfuAfL96	228	A-129935	usAfsaUfuCfuUfcUfccuCfcAfaAfgUfcsasa	315
AD-65073	A-129968	AfscsCfuGfcAfaAfAfGfaAfcUfuUfaCfcUfL96	229	A-129969	asGfsgUfaAfaGfuUfcuuUfuGfcAfgGfususa	316
AD-65074	A-129984	AfsusCfgAfgUfcAfCfAfaAfgAfaAfuGfuUfL96	230	A-129985	asAfscAfuUfuCfuUfuguGfaCfuCfgAfususu	317
AD-65092	A-129990	UfsgsAfcAfcAfaGfCfAfcAfaUfuUfaUfaAfL96	231	A-129991	usUfsaUfaAfaUfuGfugcUfuGfuGfuCfascsc	318
AD-65097	A-129992	GfsgsUfcAfuCfaAfAfUfaAfgUfgCfuUfgAfL96	232	A-129993	usCfsaAfgCfaCfuUfauuUfgAfuGfaCfcsasc	319
AD-65101	A-129978	UfsgsGfuCfaUfcAfAfAfuAfaGfuGfcUfuAfL96	233	A-129979	usAfsaGfcAfcUfuAfuuuGfaUfgAfcCfascsa	320
AD-65131	A-130172	AfsgsCfcAfgAfaAfAfGfaUfaUfcAfaGfaUfL96	234	A-130173	asUfscUfuGfaUfaUfcuuUfuCfuGfgCfususu	321
AD-65159	A-130244	CfsusUfaCfuCfuUfUfGfaGfaUfuGfuGfuAfL96	235	A-130245	usAfscAfcAfaUfcUfcaaAfgAfgUfaAfgscsa	322
AD-65150	A-130194	UfsusUfcUfaCfaAfAfAfgGfuAfaAfuAfuUfL96	236	A-130195	asAfsuAfuUfuAfcCfuuuUfgUfaGfaAfasasu	323
AD-65060	A-129950	CfsasCfaAfuGfgAfAfUfgUfgGfcGfuUfuAfL96	237	A-129951	usAfsaAfcGfcCfaCfauuCfcAfuUfgUfgsusu	324
AD-65098	A-130008	UfsusAfcUfcUfuUfGfAfgAfuUfgUfgUfaAfL96	238	A-130009	usUfsaCfaCfaAfuCfucaAfaGfaGfuAfascsc	325
AD-65067	A-129966	UfsasCfuCfuUfuGfAfGfaUfuGfuGfuAfaAfL96	239	A-129967	usUfsuAfcAfcAfaUfcucAfaAfgAfgUfasasc	326
AD-65065	A-129936	UfsgsCfcAfgAfaAfAfGfaUfaUfcAfaGfaUfL96	240	A-129937	asUfscUfuGfaUfaUfcuuUfuCfuGfgCfasusu	327
AD-65095	A-129946	UfsusCfuUfgAfaAfGfAfuAfgUfgUfuAfcAfL96	241	A-129947	usGfsuAfaCfaCfuAfucuUfuCfaAfgAfasgsc	328
AD-65126	A-130186	GfsasCfaAfuGfgAfAfUfgUfgGfcGfuUfuAfL96	242	A-130187	usAfsaAfcGfcCfaCfauuCfcAfuUfgUfcsusu	329
AD-65157	A-130212	AfsasUfaAfaAfuAfAfCfcCfaAfcGfgAfuAfL96	243	A-130213	usAfsuCfcGfuUfgGfguuAfuUfuUfaUfusasu	330
AD-65086	A-129988	CfsusUfaAfaAfcAfUfCfuGfaAfaGfuGfgAfL96	244	A-129989	usCfscAfcUfuUfcAfgauGfuUfuUfaAfgsasa	331
AD-65167	A-130200	AfsasUfcAfaGfaUfUfAfuAfaAfaUfaAfcAfL96	245	A-130201	usGfsuUfaUfuUfuAfuaaUfcUfuGfaUfususc	332
AD-65071	A-129938	AfsgsUfaAfcGfuGfGfAfaUfcUfgGfaUfuAfL96	246	A-129939	usAfsaUfcCfaGfaUfuccAfcGfuUfaCfuscsa	333
AD-65066	A-129952	UfsasUfaAfaGfgAfGfUfuGfaUfaUfgAfgAfL96	247	A-129953	usCfsuCfaUfaUfcAfacuCfcUfuUfaUfasasa	334
AD-65165	A-130246	AfsusAfcUfcUfuUfGfAfgAfuUfgUfgUfaAfL96	248	A-130247	usUfsaCfaCfaAfuCfucaAfaGfaGfuAfuscsc	335

AD-65132	A-130188	AfsasUfaAfaGfgAfGfUfuGfaUfaUfgAfgAfL96	249	A-130189	usCfsuCfaUfaUfcAfacuCfcUfuUfaUfusasa	336
AD-65125	A-130170	CfsasCfuUfuGfgAfGfGfaGfaAfgAfaUfuAfL96	250	A-130171	usAfsaUfuCfuUfcUfccuCfcAfaAfgUfgsasa	337
AD-65091	A-129974	UfsasUfaAfaAfuAfAfCfcCfaAfcGfgAfuAfL96	251	A-129975	usAfsuCfcGfuUfgGfguuAfuUfuUfaUfasasu	338
AD-65136	A-130252	GfsasAfuGfuGfgUfCfAfuCfaAfaUfaAfgUfL96	252	A-130253	asCfsuUfaUfuUfgAfugaCfcAfcAfuUfcscsu	339
AD-65137	A-130174	UfsgsUfaAfcGfuGfGfAfaUfcUfgGfaUfuAfL96	253	A-130175	usAfsaUfcCfaGfaUfuccAfcGfuUfaCfascsa	340
AD-65140	A-130222	UfsusCfgAfgUfcAfCfAfaAfgAfaAfuGfuUfL96	254	A-130223	asAfscAfuUfuCfuUfuguGfaCfuCfgAfasusu	341
AD-65128	A-130218	UfsusAfuUfcCfuUfUfGfgUfaAfcAfaAfuAfL96	255	A-130219	usAfsuUfuGfuUfaCfcaaAfgGfaAfuAfasusu	342
AD-65088	A-130020	AfscsAfcAfaGfcAfCfAfaUfuUfaUfaCfcAfL96	256	A-130021	usGfsgUfaUfaAfaUfuguGfcUfuGfuGfuscsa	343
AD-65160	A-130260	CfsusGfgAfaUfcUfGfGfaUfuCfuCfaCfuAfL96	257	A-130261	usAfsgUfgAfgAfaUfccaGfaUfuCfcAfgsgsu	344
AD-65152	A-130226	GfsusUfaAfaAfcAfUfCfuGfaAfaGfuGfgAfL96	258	A-130227	usCfscAfcUfuUfcAfgauGfuUfuUfaAfcsasa	345
AD-65133	A-130204	AfsasCfuCfuUfuGfAfGfaUfuGfuGfuAfaAfL96	259	A-130205	usUfsuAfcAfcAfaUfcucAfaAfgAfgUfusasc	346
AD-65082	A-130018	GfscsAfaUfgUfgGfUfCfaUfcAfaAfuAfaAfL96	260	A-130019	usUfsuAfuUfuGfaUfgacCfaCfaUfuGfcsusu	347
AD-65094	A-130022	GfsusGfgAfaUfcUfGfGfaUfuCfuCfaCfuAfL96	261	A-130023	usAfsgUfgAfgAfaUfccaGfaUfuCfcAfcsgsu	348
AD-65155	A-130180	GfsgsCfaUfuGfuUfGfGfaGfgAfaCfaAfaAfL96	262	A-130181	usUfsuUfgUfuCfcUfccaAfcAfaUfgCfcsusg	349
AD-65163	A-130214	UfsusUfgUfuGfgAfGfGfaAfcAfaAfcUfcUfL96	263	A-130215	asGfsaGfuUfuGfuUfccuCfcAfaCfaAfasgsc	350
AD-65144	A-130192	GfsgsAfgUfcAfcAfAfAfgAfaAfuGfuUfuAfL96	264	A-130193	usAfsaAfcAfuUfuCfuuuGfuGfaCfuCfcsasu	351
AD-65096	A-129976	AfsusUfgUfuGfgAfGfGfaAfcAfaAfcUfcUfL96	265	A-129977	asGfsaGfuUfuGfuUfccuCfcAfaCfaAfusgsc	352
AD-65142	A-130254	UfsasUfgUfgGfuCfAfUfcAfaAfuAfaGfuAfL96	266	A-130255	usAfscUfuAfuUfuGfaugAfcCfaCfaUfasgsc	353
AD-65141	A-130238	CfsasAfaAfgAfuGfCfUfuGfuAfaGfgGfaAfL96	267	A-130239	usUfscCfcUfuAfcAfagcAfuCfuUfuUfgscsc	354
AD-65079	A-129970	GfsusCfuGfuGfcUfGfGfcUfaUfaAfaGfaAfL96	268	A-129971	usUfscUfuUfaUfaGfccaGfcAfcAfgAfcscsa	355
AD-65102	A-129994	AfsgsCfaGfuGfuUfGfAfaGfaAfuGfcCfaAfL96	269	A-129995	usUfsgGfcAfuUfcUfucaAfcAfcUfgCfusasa	356
AD-65138	A-130190	GfsusGfuGfuGfgAfGfGfgUfcAfcUfcAfuAfL96	270	A-130191	usAfsuGfaGfuGfaCfccuCfcAfcAfcAfcsgsu	357
AD-65075	A-130000	GfsasAfaAfgAfuGfCfUfuGfuAfaGfgGfaAfL96	271	A-130001	usUfscCfcUfuAfcAfagcAfuCfuUfuUfcscsc	358
AD-65080	A-129986	AfscsAfuCfuGfaAfAfGfuGfgCfaCfaCfcAfL96	272	A-129987	usGfsgUfgUfgCfcAfcuuUfcAfgAfuGfususu	359

AD-65145	A-130208	CfsusCfuGfuGfcUfGfGfcUfuAfuAfaGfaAfL96	273	A-130209	usUfscUfuUfuAfuGfccaGfcAfcAfgAfgscsa	360
AD-65169	A-130232	UfsgsCfaGfuGfuUfGfAfaGfaAfuGfcCfaAfL96	274	A-130233	usUfsgGfcAfuUfcUfucaAfcAfcUfgCfasasa	361
AD-65061	A-129964	UfsusCfuUfaAfaAfCfAfuCfuGfaAfaGfuAfL96	275	A-129965	usAfscUfuUfcAfgAfuguUfuUfaAfgAfasgsa	362
AD-65135	A-130236	UfsasGfcAfcAfaUfUfUfaUfaCfcAfaCfuAfL96	276	A-130237	usAfsgUfuGfgUfaUfaaaUfuGfuGfcUfasgsu	363
AD-65068	A-129982	UfsgsGfaAfuGfuGfGfCfgUfuUfgGfuGfgAfL96	277	A-129983	usCfscAfcCfaAfaCfgccAfcAfuUfcCfasusu	364
AD-65148	A-130256	CfscsAfaUfgUfgGfUfCfaUfcAfaAfuAfaAfL96	278	A-130257	usUfsuAfuUfuGfaUfgacCfaCfaUfuGfgsusu	365
AD-65072	A-129954	CfsusGfuGfuGfgAfGfGfgUfcAfcUfcAfuAfL96	279	A-129955	usAfsuGfaGfuGfaCfccuCfcAfcAfcAfgsgsu	366
AD-65146	A-130224	UfscsAfuCfuGfaAfAfGfuGfgCfaCfaCfcAfL96	280	A-130225	usGfsgUfgUfgCfcAfcuuUfcAfgAfuGfasusu	367
AD-65129	A-130234	CfscsAfcAfaUfuUfAfUfaCfcAfaCfuGfuUfL96	281	A-130235	asAfscAfgUfuGfgUfauaAfaUfuGfuGfgsusu	368
AD-65064	A-130012	CfsasCfaAfgCfaCfAfaUfuAfuAfcCfaAfL96	282	A-130013	usUfsgGfuAfuAfaAfuugUfgCfuUfgUfgsusc	369
AD-65134	A-130220	AfsgsGfaAfuGfuGfGfCfgUfuUfgGfuGfgAfL96	283	A-130221	usCfscAfcCfaAfaCfgccAfcAfuUfcCfususu	370
AD-65063	A-129996	GfscsAfcAfaUfuUfAfUfaCfcAfaCfuGfuUfL96	284	A-129997	asAfscAfgUfuGfgUfauaAfaUfuGfuGfcsusu	371
AD-65089	A-129944	CfsgsCfaUfuGfuUfGfGfaGfgAfaCfaAfaAfL96	285	A-129945	usUfsuUfgUfuCfcUfccaAfcAfaUfgCfgsusg	372
AD-65069	A-129998	AfsasGfcAfcAfaUfUfUfaUfaCfcAfaCfuAfL96	286	A-129999	usAfsgUfuGfgUfaUfaaaUfuGfuGfcUfusgsu	373
AD-65130	A-130250	GfsasCfaAfgCfaCfAfaUfuAfuAfcCfaAfL96	287	A-130251	usUfsgGfuAfuAfaAfuugUfgCfuUfgUfcsusc	374
AD-65147	A-130240	UfsusUfuAfcCfcGfGfGfaGfuUfgAfcUfuUfL96	288	A-130241	asAfsaGfuCfaAfcUfcccGfgGfuAfaAfasusu	375
AD-65081	A-130002	AfsusUfuAfcCfcGfGfGfaGfuUfgAfcUfuUfL96	289	A-130003	asAfsaGfuCfaAfcUfcccGfgGfuAfaAfususu	376
AD-65154	A-130258	UfscsAfcAfaGfcAfCfAfaUfuUfaUfaCfcAfL96	290	A-130259	usGfsgUfaUfaAfaUfuguGfcUfuGfuGfascsa	377

Table 5. Human KLKB1 single dose screen using Dual-Glo Luciferase® Assay

Duplex ID	10nM Avg	0.1nM Avg	10nM SD	0.1nM_SD
AD-65077	15.04	36.85	1.97	0.94
AD-65170	11.72	37.36	1.61	3.43
AD-65103	11.77	40.29	1.72	2.58
AD-65083	14.90	46.32	1.64	3.59
AD-65087	14.83	47.05	0.93	3.15
AD-65149	15.68	47.95	1.10	5.95
AD-64652	17.40	48.15	0.98	2.10
AD-65162	20.26	48.59	0.11	6.03
AD-65153	13.45	49.10	0.80	3.51
AD-65084	16.25	49.14	1.79	4.63
AD-65099	14.44	49.82	2.09	1.40
AD-65100	19.10	50.71	0.37	1.49
AD-65090	18.90	50.81	1.95	7.82
AD-65085	15.98	52.77	0.74	3.97
AD-65062	16.20	54.87	0.06	3.28
AD-65164	14.22	55.83	0.13	5.41
AD-65139	14.30	56.04	0.82	4.47
AD-65151	15.78	56.12	2.34	8.24
AD-65158	22.09	56.30	2.11	4.24
AD-65078	16.43	56.83	2.21	4.52
AD-65161	20.86	56.93	1.98	2.35
AD-65076	15.06	57.79	1.13	3.90
AD-65093	18.51	58.48	1.65	2.72
AD-65156	21.88	58.48	1.23	5.06
AD-65059	23.66	59.20	2.39	9.41
AD-65073	14.96	59.62	0.84	4.37
AD-65074	20.38	59.64	1.70	4.26
AD-65092	25.49	59.65	1.13	5.25
AD-65097	16.10	59.84	1.05	6.04
AD-65101	17.79	60.00	1.09	7.50
AD-65131	26.32	60.83	3.13	4.22
AD-65159	20.30	60.84	1.29	5.93
AD-65150	26.14	60.87	2.74	5.87
AD-65060	21.85	61.24	2.64	8.69
AD-65098	21.82	61.42	1.59	2.06
AD-65067	14.78	61.63	1.49	1.15
AD-65065	30.49	61.91	1.08	3.88
AD-65095	20.31	62.19	0.93	3.55
AD-65126	22.68	62.58	2.00	3.65
AD-65157	37.47	63.14	1.23	3.92

AD-65086	32.28	63.19	2.00	6.01
AD-65167	26.43	63.54	1.61	3.80
AD-65071	26.58	64.16	2.30	2.64
AD-65066	22.13	64.20	1.26	3.77
AD-65165	21.89	64.31	2.14	3.57
AD-65132	21.03	64.52	2.67	2.21
AD-65125	25.73	64.78	3.64	10.30
AD-65091	35.66	65.28	3.85	0.92
AD-65136	19.19	65.74	1.46	2.65
AD-65137	28.04	65.76	1.12	4.54
AD-65140	27.71	65.90	2.52	2.03
AD-65128	24.33	66.14	3.88	6.36
AD-65088	38.37	66.28	0.75	4.58
AD-65160	25.02	66.42	1.10	2.11
AD-65152	48.65	66.46	2.84	2.02
AD-65133	14.03	66.60	1.79	1.76
AD-65082	28.29	66.66	3.48	4.83
AD-65094	26.65	66.78	0.56	1.41
AD-65155	40.50	66.99	2.70	1.23
AD-65163	35.04	67.16	3.15	4.42
AD-65144	22.23	67.27	1.79	0.87
AD-65096	36.47	67.31	2.64	1.97
AD-65142	19.07	67.32	3.01	1.34
AD-65141	15.21	67.58	1.60	3.45
AD-65079	29.27	67.76	2.80	3.80
AD-65102	30.46	68.54	2.45	1.65
AD-65138	41.51	68.68	2.13	5.34
AD-65075	16.72	69.00	1.04	1.14
AD-65080	36.41	69.03	4.21	3.96
AD-65145	34.78	69.34	2.61	2.95
AD-65169	30.06	69.63	2.17	4.29
AD-65061	41.00	70.18	4.34	3.71
AD-65135	67.86	70.58	2.28	5.97
AD-65068	30.14	71.51	3.78	4.08
AD-65148	37.81	71.67	7.51	2.54
AD-65072	43.46	71.73	1.45	6.71
AD-65146	55.99	71.80	5.50	3.07
AD-65129	34.09	71.81	1.22	2.86
AD-65064	37.23	71.84	4.61	2.50
AD-65134	34.90	71.86	3.14	2.91
AD-65063	32.52	72.21	2.92	4.47
AD-65089	34.88	73.21	0.07	0.46
AD-65069	59.34	73.27	4.47	4.89
AD-65130	38.52	73.89	1.69	2.78

AD-65147	69.27	76.69	7.33	4.28
AD-65081	55.75	77.78	4.77	5.96
AD-65154	45.69	79.81	2.01	7.82

Table 6. Mouse KLKB1 single dose screen using Dual-Glo Luciferase® Assay

Duplex ID	10nM Avg	0.1nM Avg	10nM SD	0.1nM_SD
AD-65077	8.67	44.09	0.45	0.12
AD-65103	13.99	52.56	1.01	0.70
AD-65087	11.03	55.15	0.53	0.44
AD-65101	18.50	57.32	1.40	5.05
AD-65151	13.94	58.04	0.90	1.91
AD-65097	17.04	58.72	1.99	3.06
AD-65170	25.69	59.35	1.72	3.72
AD-65062	32.25	60.50	3.49	0.75
AD-65064	34.48	61.67	1.44	0.10
AD-65085	13.68	61.86	1.70	3.87
AD-65063	17.43	61.92	1.12	2.56
AD-65153	23.75	62.67	2.17	2.36
AD-65089	30.93	62.79	0.75	2.15
AD-65074	43.09	63.07	1.73	4.37
AD-65067	18.02	63.49	2.41	2.86
AD-65099	48.62	63.58	2.12	4.44
AD-65091	67.51	64.14	8.48	2.38
AD-65086	41.17	64.14	4.31	2.61
AD-65059	75.31	64.20	2.59	3.27
AD-65158	59.18	64.32	3.51	2.00
AD-65095	65.59	64.35	6.12	5.81
AD-65083	32.77	64.54	1.99	3.38
AD-65169	66.29	64.54	3.45	4.37
AD-65125	71.21	64.58	3.75	2.19
AD-65141	34.27	64.65	2.11	5.10
AD-65073	47.93	64.68	2.48	1.94
AD-65142	49.73	64.74	4.36	5.42
AD-65128	37.04	64.86	3.14	0.92
AD-65075	34.53	65.38	3.98	1.48
AD-65068	75.01	65.42	1.57	3.56
AD-65148	54.43	65.52	3.04	1.57
AD-65065	110.49	65.55	4.29	2.79
AD-65071	54.17	65.62	3.09	2.92
AD-65061	112.26	65.87	2.27	2.26
AD-65060	63.69	65.90	5.04	0.97

AD-65076	27.86	65.98	2.99	3.32
AD-65098	51.86	66.08	3.95	6.10
AD-64652	70.91	66.42	5.35	3.70
AD-65154	65.58	66.44	3.77	1.79
AD-65131	104.49	66.84	3.11	3.42
AD-65152	46.64	66.91	2.11	2.46
AD-65160	18.79	67.16	0.67	4.48
AD-65133	23.73	67.16	1.62	3.56
AD-65096	55.79	67.18	3.11	4.26
AD-65163	62.97	67.20	4.28	0.88
AD-65157	66.01	67.22	4.10	3.60
AD-65092	51.19	67.53	3.56	1.95
AD-65093	52.08	67.55	2.16	1.72
AD-65130	57.52	67.76	6.66	1.12
AD-65100	74.97	67.91	6.18	2.73
AD-65102	63.88	67.94	2.90	2.22
AD-65159	59.98	67.94	6.46	4.35
AD-65165	62.31	67.95	1.86	3.06
AD-65150	81.30	68.12	8.66	0.38
AD-65164	34.95	68.44	0.63	3.58
AD-65136	50.50	68.53	3.84	0.44
AD-65161	71.46	68.64	3.85	1.88
AD-65088	48.15	68.68	3.38	1.80
AD-65134	68.99	68.91	2.75	1.56
AD-65078	63.09	69.05	4.57	3.85
AD-65129	18.28	69.24	1.92	0.72
AD-65155	45.01	69.32	3.93	1.78
AD-65079	47.09	69.38	0.79	3.42
AD-65126	67.47	69.39	3.54	7.01
AD-65144	65.85	69.71	2.58	4.73
AD-65084	81.65	69.74	4.96	3.77
AD-65162	74.13	69.80	5.71	3.24
AD-65140	54.32	69.81	1.87	3.84
AD-65139	46.64	69.97	6.00	2.50
AD-65147	66.48	69.99	4.21	3.72
AD-65069	67.61	70.00	2.85	2.74
AD-65149	45.54	70.01	2.63	2.08
AD-65072	73.76	70.21	3.59	1.93
AD-65146	79.06	70.25	5.25	2.65
AD-65145	41.44	70.43	1.00	3.89
AD-65132	72.39	70.57	2.20	1.67
AD-65090	112.31	70.73	6.67	2.16
AD-65094	35.82	70.81	2.77	0.09
AD-65066	75.47	70.83	3.28	4.93

AD-65137	57.80	71.08	2.54	3.10
AD-65081	62.66	71.12	4.17	6.09
AD-65082	42.77	71.30	1.68	1.58
AD-65138	72.87	71.49	1.82	2.34
AD-65080	68.06	72.17	3.20	0.58
AD-65167	73.30	72.47	5.89	3.39
AD-65135	76.47	72.83	0.50	2.14
AD-65156	104.49	73.62	4.93	2.80
AD-65077	15.04	36.85	1.97	0.94
AD-65170	11.72	37.36	1.61	3.43
AD-65103	11.77	40.29	1.72	2.58

A subset of duplexes were also assayed for dose response for silencing human KLKB1 and mouse KLKB1 mRNA using the Dual-Glo® Luciferase assay, as described above. The results of the human KLKB1 screen in Cos7 cells transfected with the indicated KLKB1 iRNAs are shown in Table 7. The results of the mouse KLKB1 screen in Cos7 cells transfected with the indicated KLKB1 iRNAs are shown in Table 8. Data are expressed as percent of mRNA remaining relative to negative control at 48 hours.

Table 7. Human KLKB1 dose response screen in Cos7 cells using Dual-Glo Luciferase® Assay

Duplex ID	IC50 (nM)
AD-65077	0.0004
AD-65170	0.0084
AD-65103	0.0344
AD-65083	0.0704
AD-65087	0.0593
AD-65149	0.0854
AD-64652	0.123
AD-65162	0.1323
AD-65153	0.0683
AD-65084	0.0987
AD-65099	0.0211

Table 8. Mouse KLKB1 dose response screen in Cos7 cells using Dual-Glo Luciferase® Assay

Duplex ID	IC50 (nM)
AD-65077	0.0083
AD-65170	0.206
AD-65103	0.1216

AD-65083	1.2257
AD-65087	0.1381
AD-65149	36.5482
AD-64652	N/A
AD-65162	N/A
AD-65153	0.4234
AD-65084	N/A
AD-65099	246.7682

Example 3. F12 iRNA Synthesis

Source of reagents

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

Transcripts

siRNA Design

A set of siRNAs targeting the human F12, "coagulation factor XII" (human: NCBI refseqID NM_000505; NCBI GeneID: 2161), as well as toxicology-species F12 orthologs (cynomolgus monkey: XM_005558647; mouse: NM_021489; rat, NM_001014006) were designed using custom R and Python scripts. The human F12 REFSEQ mRNA has a length of 2060 bases. The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 50 through position 2060 (the coding region and 3' UTR) of human F12 mRNA (containing the the coding region and 3' UTR) was determined using a linear model that predicted the direct measure of mRNA knockdown based on the data of more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. Subsets of the F12 siRNAs were designed with perfect or near-perfect matches between human, cynomolgus and rhesus monkey. A further subset was designed with perfect or near-perfect matches to mouse and rat F12 orthologs. For each strand of the siRNA, a custom Python script was used in a brute force search to measure the number and positions of mismatches between the siRNA and all potential alignments in the target species transcriptome. Extra weight was given to mismatches in the seed region, defined here as positions 2-9 of the antisense oligonucleotide, as well the cleavage site of the siRNA, defined here as positions 10-11 of the antisense oligonucleotide. The relative weights for the mismatches were 2.8 for seed mismatches, 1.2 for cleavage site mismatches, and 1 mismatches in other positions up through antisense position 19. Mismatches in the first position were ignored. A specificity score was calculated for each strand by summing the value of each weighted mismatch. Preference was given to siRNAs whose antisense score in

human and cynomolgus monkey was >= 3.0 and predicted efficacy was >= 70% knockdown of the F12 transcript.

A detailed list of the unmodified F12 sense and antisense strand sequences is shown in Table 9. A detailed list of the modified F12 sense and antisense strand sequences is shown in Table 10.

siRNA Synthesis

F12 siRNA sequences were synthesized at 1 μmol scale on a Mermade 192 synthesizer (BioAutomation) using the solid support mediated phosphoramidite chemistry. The solid support was controlled pore glass (500 A) loaded with custom GalNAc ligand or universal solid support (AM biochemical). Ancillary synthesis reagents, 2'-F and 2'-O-Methyl RNA and deoxy phosphoramidites were obtained from Thermo-Fisher (Milwaukee, WI) and Hongene (China). 2'F 2'-O-Methyl, GNA (glycol nucleic acids), 5'phosphate and other modifications were introduced using the corresponding phosphoramidites. Synthesis of 3' GalNAc conjugated single strands was performed on a GalNAc modified CPG support. Custom CPG universal solid support was used for the synthesis of antisense single strands. Coupling time for all phosphoramidites (100 mM in acetonitrile) was 5 min employing 5-Ethylthio-1H-tetrazole (ETT) as activator (0.6 M in acetonitrile). Phosphorothioate linkages were generated using a 50 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (1:1 v/v). Oxidation time was 3 minutes. All sequences were synthesized with final removal of the DMT group ("DMT off").

Upon completion of the solid phase synthesis, oligoribonucleotides were cleaved from the solid support and deprotected in sealed 96 deep well plates using 200 μL Aqueous Methylamine reagents at 60°C for 20 minutes. For sequences containing 2' ribo residues (2'-OH) that are protected with a tert-butyl dimethyl silyl (TBDMS) group, a second step deprotection was performed using TEA.3HF (triethylamine trihydro fluoride) reagent. To the methylamine deprotection solution, 200uL of dimethyl sulfoxide (DMSO) and 300ul TEA. 3HF reagent was added and the solution was incubated for additional 20min at 60°C. At the end of cleavage and deprotection step, the synthesis plate was allowed to come to room temperature and was precipitated by addition of 1mL of acetontile: ethanol mixture (9:1). The plates were cooled at -80 C for 2 hrs, superanatant decanted carefully with the aid of a multi channel pipette. The oligonucleotide pellet was re-suspended in 20mM NaOAc buffer and were desalted using a 5 mL HiTrap size exclusion column (GE Healthcare) on an AKTA Purifier System equipped with an A905 autosampler and a Frac 950 fraction collector. Desalted samples were collected in 96-well plates. Samples from each sequence were

analyzed by LC-MS to confirm the identity, UV (260 nm) for quantification and a selected set of samples by IEX chromatography to determine purity.

Annealing of F12 single strands was performed on a Tecan liquid handling robot. Equimolar mixture of sense and antisense single strands were combined and annealed in 96 well plates. After combining the complementary single strands, the 96-well plate was sealed tightly and heated in an oven at 100°C for 10 minutes and allowed to come slowly to room temperature over a period 2-3 hours. The concentration of each duplex was normalized to 10μM in 1X PBS and then submitted for *in vitro* screening assays.

Table 9. Unmodified F12 Sequences

Duplex name	sense oligo name	Sense Sequence 5' to 3'	SEQ ID NO:	antis oligo name	Antisense Sequence 5' to 3'	Position in NM_000505	SEQ ID NO:
AD-66186	A-132464	GGUGAGCUUGGAGUCAACACU	378	A-132465	AGUGUUGACUCCAAGCUCACCAG	79_102	428
AD-66157	A-132406	GAGCUUGGAGUCAACACUUUA	379	A-132407	UAAAGUGUUGACUCCAAGCUCAC	82_105	429
AD-66118	A-132326	CUUGGAGUCAACACUUUCGAU	380	A-132327	AUCGAAAGUGUUGACUCCAAGCU	85_108	430
AD-66115	A-132320	UUGGAGUCAACACUUUCGAUU	381	A-132321	AAUCGAAAGUGUUGACUCCAAGC	86_109	431
AD-66170	A-132432	AACACUUUCGAUUCCACCUUA	382	A-132433	UAAGGUGGAAUCGAAAGUGUUGA	94_117	432
AD-66166	A-132424	AGGAGCAUAAGUACAAAGCUA	383	A-132425	UAGCUUUGUACUUAUGCUCCUUG	126_149	433
AD-66173	A-132438	GAGCAUAAGUACAAAGCUGAA	384	A-132439	UUCAGCUUUGUACUUAUGCUCCU	128_151	434
AD-66177	A-132446	UAAGUACAAAGCUGAAGAGCA	385	A-132447	UGCUCUUCAGCUUUGUACUUAUG	133_156	435
AD-66161	A-132414	AAGUACAAAGCUGAAGAGCAA	386	A-132415	UUGCUCUUCAGCUUUGUACUUAU	134_157	436
AD-66114	A-132318	UACCACAAAUGUACCCACAAA	387	A-132319	UUUGUGGGUACAUUUGUGGUACA	218_241	437
AD-66179	A-132450	CCACAAAUGUACCCACAAGGA	388	A-132451	UCCUUGUGGGUACAUUUGUGGUA	220_243	438
AD-66160	A-132412	UACUGUUUGGAGCCCAAGAAA	389	A-132413	UUUCUUGGGCUCCAAACAGUAUC	305_328	439
AD-66171	A-132434	ACUGUUUGGAGCCCAAGAAAA	390	A-132435	UUUUCUUGGGCUCCAAACAGUAU	306_329	440
AD-66189	A-132470	CUGUUUGGAGCCCAAGAAAGU	391	A-132471	ACUUUCUUGGGCUCCAAACAGUA	307_330	441
AD-66122	A-132334	GGAGCCCAAGAAAGUGAAAGA	392	A-132335	UCUUUCACUUUCUUGGGCUCCAA	313_336	442
AD-66176	A-132444	GAGCCCAAGAAAGUGAAAGAA	393	A-132445	UUCUUUCACUUUCUUGGGCUCCA	314_337	443
AD-66125	A-132340	AGCCCAAGAAAGUGAAAGACA	394	A-132341	UGUCUUUCACUUUCUUGGGCUCC	315_338	444
AD-66112	A-132314	GCCCAAGAAAGUGAAAGACCA	395	A-132315	UGGUCUUUCACUUUCUUGGGCUC	316_339	445
AD-66172	A-132436	CCCAAGAAAGUGAAAGACCAA	396	A-132437	UUGGUCUUUCACUUUCUUGGGCU	317_340	446
AD-66127	A-132344	CAAGAAAGUGAAAGACCAUUA	397	A-132345	UAAUGGUCUUUCACUUUCUUGGG	319_342	447
AD-66162	A-132416	GAAAGUGAAAGACCACUGCAA	398	A-132417	UUGCAGUGGUCUUUCACUUUCUU	322_345	448

AD-66181	A-132454	AAAGUGAAAGACCACUGCAGA	399	A-132455	UCUGCAGUGGUCUUUCACUUUCU	323_346	449
AD-66184	A-132460	UCACUGGAAACCACUGCCAGA	400	A-132461	UCUGGCAGUGGUUUCCAGUGAGG	420_443	450
AD-66182	A-132456	ACUGCCAGAAAGAGAAGUGCU	401	A-132457	AGCACUUCUCUUUCUGGCAGUGG	432_455	451
AD-66167	A-132426	CUGCCAGAAAGAGAAGUGCUU	402	A-132427	AAGCACUUCUCUUUCUGGCAGUG	433_456	452
AD-66165	A-132422	CAGAAAGAGAAGUGCUUUGAA	403	A-132423	UUCAAAGCACUUCUCUUUCUGGC	437_460	453
AD-66155	A-132402	AGAAAGAGAAGUGCUUUGAGA	404	A-132403	UCUCAAAGCACUUCUCUUUCUGG	438_461	454
AD-66159	A-132410	AGUGCUUUGAGCCUCAGCUUA	405	A-132411	UAAGCUGAGGCUCAAAGCACUUC	447_470	455
AD-66168	A-132428	UUCCACAAGAAUGAGAUAUGA	406	A-132429	UCAUAUCUCAUUCUUGUGGAAAA	476_499	456
AD-66185	A-132462	UCCACAAGAAUGAGAUAUGGU	407	A-132463	ACCAUAUCUCAUUCUUGUGGAAA	477_500	457
AD-66156	A-132404	CCACAAGAAUGAGAUAUGGUA	408	A-132405	UACCAUAUCUCAUUCUUGUGGAA	478_501	458
AD-66113	A-132316	AAGAAUGAGAUAUGGUAUAGA	409	A-132317	UCUAUACCAUAUCUCAUUCUUGU	482_505	459
AD-66188	A-132468	UGGUAUAGAACUGAGCAAGCA	410	A-132469	UGCUUGCUCAGUUCUAUACCAUA	494_517	460
AD-66190	A-132472	GUAUAGAACUGAGCAAGCAGA	411	A-132473	UCUGCUUGCUCAGUUCUAUACCA	496_519	461
AD-66180	A-132452	AUAGAACUGAGCAAGCAGCUA	412	A-132453	UAGCUGCUUGCUCAGUUCUAUAC	498_521	462
AD-66117	A-132324	CCAGAUGCCAGUGCAAGGGUA	413	A-132325	UACCCUUGCACUGGCAUCUGGCC	522_545	463
AD-66169	A-132430	GCCAGUGCAAGGGUCCUGAUA	414	A-132431	UAUCAGGACCCUUGCACUGGCAU	528_551	464
AD-66174	A-132440	CAGUGCAAGGGUCCUGAUGCA	415	A-132441	UGCAUCAGGACCCUUGCACUGGC	530_553	465
AD-66175	A-132442	ACCAAGGCAAGCUGCUAUGAU	416	A-132443	AUCAUAGCAGCUUGCCUUGGUGU	683_706	466
AD-66158	A-132408	CCAAGGCAAGCUGCUAUGAUA	417	A-132409	UAUCAUAGCAGCUUGCCUUGGUG	684_707	467
AD-66119	A-132328	AGGCUUCAUGUCCCCACUCAUA	418	A-132329	UAUGAGUGGGACAUGAAGCCUAG	974_997	468
AD-66187	A-132466	GGCUCCGCAAGAGUCUGUCUU	419	A-132467	AAGACAGACUCUUGCGGAGCCGC	1131_1154	469
AD-66163	A-132418	GCUCCGCAAGAGUCUGUCUUA	420	A-132419	UAAGACAGACUCUUGCGGAGCCG	1132_1155	470
AD-66116	A-132322	CCGCAAGAGUCUGUCUUCGAU	421	A-132323	AUCGAAGACAGACUCUUGCGGAG	1135_1158	471
AD-66137	A-132364	GUUCGAGGGGGCUGAAGAAUA	422	A-132365	UAUUCUUCAGCCCCCUCGAACUG	1570_1593	472
AD-66183	A-132458	GGAAGGCAAGAUUGUGUCCCA	423	A-132459	UGGGACACAAUCUUGCCUUCCAU	1956_1979	473

AD-66164	A-132420	AGGCAAGAUUGUGUCCCAUUA	424	A-132421	UAAUGGGACACAAUCUUGCCUUC	1959_1982	474
AD-66121	A-132332	AACUCAAUAAAGUGCUUUGAA	425	A-132333	UUCAAAGCACUUUAUUGAGUUUC	2017_2040	475
AD-66126	A-132342	AAUAAAGUGCUUUGAAAACGU	426	A-132343	ACGUUUUCAAAGCACUUUAUUGA	2022_2045	476
AD-66178	A-132448	AGUGCUUUGAAAAUGCUGAGA	427	A-132449	UCUCAGCAUUUUCAAAGCACUUU	2027_2050	477

Table 10. Modified F12 Sequences

Duplex name	sense oligo name	Sense Sequence 5' to 3'	SEQ ID NO:	antis oligo name	Antisense Sequence 5' to 3'	SEQ ID NO:
AD-66186	A-132464	GfsgsUfgAfgCfuUfGfGfaGfuCfaAfcAfcUfL96	478	A-132465	asGfsuGfuUfgAfcUfccaAfgCfuCfaCfcsasg	528
AD-66157	A-132406	GfsasGfcUfuGfgAfGfUfcAfaCfaCfuUfuAfL96	479	A-132407	usAfsaAfgUfgUfuGfacuCfcAfaGfcUfcsasc	529
AD-66118	A-132326	CfsusUfgGfaGfuCfAfAfcAfcUfuUfcGfaUfL96	480	A-132327	asUfscGfaAfaGfuGfuugAfcUfcCfaAfgscsu	530
AD-66115	A-132320	UfsusGfgAfgUfcAfAfCfaCfuUfuCfgAfuUfL96	481	A-132321	asAfsuCfgAfaAfgUfguuGfaCfuCfcAfasgsc	531
AD-66170	A-132432	AfsasCfaCfuUfuCfGfAfuUfcCfaCfcUfuAfL96	482	A-132433	usAfsaGfgUfgGfaAfucgAfaAfgUfgUfusgsa	532
AD-66166	A-132424	AfsgsGfaGfcAfuAfAfGfuAfcAfaAfgCfuAfL96	483	A-132425	usAfsgCfuUfuGfuAfcuuAfuGfcUfcCfususg	533
AD-66173	A-132438	GfsasGfcAfuAfaGfUfAfcAfaAfgCfuGfaAfL96	484	A-132439	usUfscAfgCfuUfuGfuacUfuAfuGfcUfcscsu	534
AD-66177	A-132446	UfsasAfgUfaCfaAfAfGfcUfgAfaGfaGfcAfL96	485	A-132447	usGfscUfcUfuCfaGfcuuUfgUfaCfuUfasusg	535
AD-66161	A-132414	AfsasGfuAfcAfaAfGfCfuGfaAfgAfgCfaAfL96	486	A-132415	usUfsgCfuCfuUfcAfgcuUfuGfuAfcUfusasu	536
AD-66114	A-132318	UfsasCfcAfcAfaAfUfGfuAfcCfcAfcAfaAfL96	487	A-132319	usUfsuGfuGfgGfuAfcauUfuGfuGfgUfascsa	537
AD-66179	A-132450	CfscsAfcAfaAfuGfUfAfcCfcAfcAfaGfgAfL96	488	A-132451	usCfscUfuGfuGfgGfuacAfuUfuGfuGfgsusa	538
AD-66160	A-132412	UfsasCfuGfuUfuGfGfAfgCfcCfaAfgAfaAfL96	489	A-132413	usUfsuCfuUfgGfgCfuccAfaAfcAfgUfasusc	539
AD-66171	A-132434	AfscsUfgUfuUfgGfAfGfcCfcAfaGfaAfaAfL96	490	A-132435	usUfsuUfcUfuGfgGfcucCfaAfaCfaGfusasu	540
AD-66189	A-132470	CfsusGfuUfuGfgAfGfCfcCfaAfgAfaAfgUfL96	491	A-132471	asCfsuUfuCfuUfgGfgcuCfcAfaAfcAfgsusa	541

AD-66122	A-132334	GfsgsAfgCfcCfaAfGfAfaAfgUfgAfaAfgAfL96	492	A-132335	usCfsuUfuCfaCfuUfucuUfgGfgCfuCfcsasa	542
AD-66176	A-132444	GfsasGfcCfcAfaGfAfAfaGfuGfaAfaGfaAfL96	493	A-132445	usUfscUfuUfcAfcUfuucUfuGfgGfcUfcscsa	543
AD-66125	A-132340	AfsgsCfcCfaAfgAfAfAfgUfgAfaAfgAfcAfL96	494	A-132341	usGfsuCfuUfuCfaCfuuuCfuUfgGfgCfuscsc	544
AD-66112	A-132314	GfscsCfcAfaGfaAfAfGfuGfaAfaGfaCfcAfL96	495	A-132315	usGfsgUfcUfuUfcAfcuuUfcUfuGfgGfcsusc	545
AD-66172	A-132436	CfscsCfaAfgAfaAfGfUfgAfaAfgAfcCfaAfL96	496	A-132437	usUfsgGfuCfuUfuCfacuUfuCfuUfgGfgscsu	546
AD-66127	A-132344	CfsasAfgAfaAfgUfGfAfaAfgAfcCfaUfuAfL96	497	A-132345	usAfsaUfgGfuCfuUfucaCfuUfuCfuUfgsgsg	547
AD-66162	A-132416	GfsasAfaGfuGfaAfAfGfaCfcAfuUfgCfaAfL96	498	A-132417	usUfsgCfaAfuGfgUfcuuUfcAfcUfuUfcsusu	548
AD-66181	A-132454	AfsasAfgUfgAfaAfGfAfcCfaUfuGfcAfgAfL96	499	A-132455	usCfsuGfcAfaUfgGfucuUfuCfaCfuUfuscsu	549
AD-66184	A-132460	UfscsAfcUfgGfaAfAfCfcAfcUfgCfcAfgAfL96	500	A-132461	usCfsuGfgCfaGfuGfguuUfcCfaGfuGfasgsg	550
AD-66182	A-132456	AfscsUfgCfcAfgAfAfAfgAfgAfaGfuGfcUfL96	501	A-132457	asGfscAfcUfuCfuCfuuuCfuGfgCfaGfusgsg	551
AD-66167	A-132426	CfsusGfcCfaGfaAfAfGfaGfaAfgUfgCfuUfL96	502	A-132427	asAfsgCfaCfuUfcUfcuuUfcUfgGfcAfgsusg	552
AD-66165	A-132422	CfsasGfaAfaGfaGfAfAfgUfgCfuUfuGfaAfL96	503	A-132423	usUfscAfaAfgCfaCfuucUfcUfuUfcUfgsgsc	553
AD-66155	A-132402	AfsgsAfaAfgAfgAfAfGfuGfcUfuUfgAfgAfL96	504	A-132403	usCfsuCfaAfaGfcAfcuuCfuCfuUfuCfusgsg	554
AD-66159	A-132410	AfsgsUfgCfuUfuGfAfGfcCfuCfaGfcUfuAfL96	505	A-132411	usAfsaGfcUfgAfgGfcucAfaAfgCfaCfususc	555
AD-66168	A-132428	UfsusCfcAfcAfaGfAfAfuGfaGfaUfuUfgAfL96	506	A-132429	usCfsaUfaUfcUfcAfuucUfuGfuGfgAfasasa	556
AD-66185	A-132462	UfscsCfaCfaAfgAfAfAfUfgAfgAfuAfuGfgUfL96	507	A-132463	asCfscAfuAfuCfuCfauuCfuUfgUfgGfasasa	557
AD-66156	A-132404	CfscsAfcAfaGfaAfUfGfaGfaUfaUfgGfuAfL96	508	A-132405	usAfscCfaUfaUfcUfcauUfcUfuGfuGfgsasa	558
AD-66113	A-132316	AfsasGfaAfuGfaGfAfUfaUfgGfuAfuAfgAfL96	509	A-132317	usCfsuAfuAfcCfaUfaucUfcAfuUfcUfusgsu	559
AD-66188	A-132468	UfsgsGfuAfuAfgAfAfCfuGfaGfcAfaGfcAfL96	510	A-132469	usGfscUfuGfcUfcAfguuCfuAfuAfcCfasusa	560
AD-66190	A-132472	GfsusAfuAfgAfaCfUfGfaGfcAfaGfcAfgAfL96	511	A-132473	usCfsuGfcUfuGfcUfcagUfuCfuAfuAfuAfcscsa	561
AD-66180	A-132452	AfsusAfgAfaCfuGfAfGfcAfaGfcAfgCfuAfL96	512	A-132453	usAfsgCfuGfcUfuGfcucAfgUfuCfuAfusasc	562
AD-66117	A-132324	CfscsAfgAfuGfcCfAfGfuGfcAfaGfgGfuAfL96	513	A-132325	usAfscCfcUfuGfcAfcugGfcAfuCfuGfgscsc	563
AD-66169	A-132430	GfscsCfaGfuGfcAfAfGfgGfuCfcUfgAfuAfL96	514	A-132431	usAfsuCfaGfgAfcCfcuuGfcAfcUfgGfcsasu	564
AD-66174	A-132440	CfsasGfuGfcAfaGfGfGfuCfcUfgAfuGfcAfL96	515	A-132441	usGfscAfuCfaGfgAfcccUfuGfcAfcUfggsgsc	565
AD-66175	A-132442	AfscsCfaAfgGfcAfAfGfcUfgCfuAfuGfaUfL96	516	A-132443	asUfscAfuAfgCfaGfcuuGfcCfuUfgGfusgsu	566

AD-66158	A-132408	CfscsAfaGfgCfaAfGfCfuGfcUfaUfgAfuAfL96	517	A-132409	usAfsuCfaUfaGfcAfgcuUfgCfcUfuGfgsusg	567
AD-66119	A-132328	AfsgsGfcUfuCfaUfGfUfcCfcAfcUfcAfuAfL96	518	A-132329	usAfsuGfaGfuGfgGfacaUfgAfaGfcCfusasg	568
AD-66187	A-132466	GfsgsCfuCfcGfcAfAfGfaGfuCfuGfuCfuUfL96	519	A-132467	asAfsgAfcAfgAfcUfcuuGfcGfgAfgCfcsgsc	569
AD-66163	A-132418	GfscsUfcCfgCfaAfGfAfgUfcUfgUfcUfuAfL96	520	A-132419	usAfsaGfaCfaGfaCfucuUfgCfgGfaGfcscsg	570
AD-66116	A-132322	CfscsGfcAfaGfaGfUfCfuGfuCfuGfcGfaUfL96	521	A-132323	asUfscGfaAfgAfcAfgacUfcUfuGfcGfgsasg	571
AD-66137	A-132364	GfsusUfcGfaGfgGfGfGfcUfgAfaGfaAfuAfL96	522	A-132365	usAfsuUfcUfuCfaGfcccCfcUfcGfaAfcsusg	572
AD-66183	A-132458	GfsgsAfaGfgCfaAfGfAfuUfgUfgUfcCfcAfL96	523	A-132459	usGfsgGfaCfaCfaAfucuUfgCfcUfuCfcsasu	573
AD-66164	A-132420	AfsgsGfcAfaGfaUfUfGfuGfuCfcCfaUfuAfL96	524	A-132421	usAfsaUfgGfgAfcAfcaaUfcUfuGfcCfususc	574
AD-66121	A-132332	AfsasCfuCfaAfuAfAfAfgUfgCfuUfuGfaAfL96	525	A-132333	usUfscAfaAfgCfaCfuuuAfuUfgAfgUfususc	575
AD-66126	A-132342	AfsasUfaAfaGfuGfCfUfuUfgAfaAfaCfgUfL96	526	A-132343	asCfsgUfuUfuCfaAfagcAfcUfuUfaUfusgsa	576
AD-66178	A-132448	AfsgsUfgCfuUfuGfAfAfaAfuGfcUfgAfgAfL96	527	A-132449	usCfsuCfaGfcAfuUfuucAfaAfgCfaCfususu	577

Example 4. *In vitro* screening of F12 siRNA duplexes

Cell culture and transfections

Hep3b or Primary Mouse Hepatocyte cells (PMH) (MSCP10, Lot# MC613) were transfected by adding 4.9µl of Opti-MEM plus 0.1µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a 384-well plate and incubated at room temperature for 15 minutes. Forty µl of DMEM (Hep3b) of William's E Medium (PMH) containing about 5 x10^3 cells was then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification.

Single dose experiments were performed at 10nM and 0.01nM final duplex concentration and dose response experiments were done over a range of doses from 10nM to 36fM final duplex concentration over 8, 6-fold dilutions.

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, 50µl of Lysis/Binding Buffer and 25µl of lysis buffer containing 3µl 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 150µl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150µl Elution Buffer, re-captured and the supernatant was removed.

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

Ten µl of a master mix containing 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl 10x Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H2O per reaction was added to RNA isolated as described above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 hours 37°C. Plates were then incubated at 81°C for 8 minutes.

Real time PCR:

Two µl of cDNA were added to a master mix containing 0.5µl of GAPDH TaqMan Probe (Hs99999905_m1 or 4352339E), 0.5µl F12 probe (Hs00166821 or Mm00491349) and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was performed using 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 10nM AD-1955, or mock transfected cells. IC_{50}s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955, a non-targeting control, or naïve cells.

The sense and antisense sequences of AD-1955 are:

SENSE: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO: 2343);

ANTISENSE: UCGAAGuACUcAGCGuAAGdTsdT (SEQ ID NO: 2344).

Table 11 shows the results of a single dose screen in Hep3b cells transfected with the indicated human F12 iRNAs. Table 12 shows the results of a single dose response screen in Hep3b cells transfected with the indicated human F12 iRNAs. Table 13 shows the results of a single dose screen in primary mouse hepatocytes transfected with the indicated mouse F12 iRNAs. Table 14 shows the results of a dose response screen in primary mouse hepatocytes transfected with the indicated human F12 iRNAs. Data are expressed as percent of mRNA remaining relative to AD-1955.

Table 11. F12 Single Dose Screen in Hep3bCells

DuplexId	10nM AVG	0.1nM AVG	10nM STDEV	0.1nM STDEV
AD-66186	33.1	88.4	5.3	12.6
AD-66157	62.2	85.3	9.6	13.2
AD-66118	47.4	59.4	2.9	10.6
AD-66115	54.8	73.9	4.8	3
AD-66170	31.6	57.3	3.9	12.5
AD-66166	74.7	88.8	14.3	15.8
AD-66173	22.3	58.5	7.6	11.8
AD-66177	52.9	86.7	6.9	6.3
AD-66161	50.3	59.9	7.9	10
AD-66114	42.1	82.3	5.3	8.5
AD-66179	78.4	101.4	14.3	16.1
AD-66160	45.4	82.3	13.4	18.5
AD-66171	74.8	126.2	12.1	28.2
AD-66189	49.3	78.1	16.6	9.1
AD-66122	47.2	94.9	7.4	7.5
AD-66176	42.7	69.4	5.2	7
AD-66125	46	91.8	7.5	17.4
AD-66112	60.4	136.8	11.4	14.4
AD-66172	34.9	70.2	13.1	11.1
AD-66127	39.5	73.3	8.5	12.4

AD-66162	79.1	93.6	13	24.7
AD-66181	59.8	101.7	1.2	5.4
AD-66184	34	72.9	7.8	14.9
AD-66182	47	101	8.8	7.9
AD-66167	30.3	60.2	2.6	5.9
AD-66165	44.3	63.2	11.4	22.3
AD-66155	45.3	72.8	13.5	16.1
AD-66159	49.6	98	8.4	31.2
AD-66168	25.5	52.9	5.8	16.6
AD-66185	40.8	81.7	3.8	11.5
AD-66156	30.8	75.6	4.4	5.4
AD-66113	42.1	76	8.1	5.9
AD-66188	43.9	82.1	9.1	15.4
AD-66190	40.2	74.9	9	8.3
AD-66180	34.6	83.1	6.6	23.3
AD-66117	48.9	108.1	4.1	9.5
AD-66169	64.9	89.4	9.8	1.9
AD-66174	55.4	107.6	7.9	23
AD-66175	37.9	104.7	4	19.7
AD-66158	55	107.3	14.7	31.7
AD-66119	27.6	69.8	3.4	4.3
AD-66187	53.3	105	19.6	9.6
AD-66163	33.6	53.9	5.1	4.9
AD-66116	33.9	57.4	10.4	12.6
AD-66137	103.4	136.7	6.6	15.9
AD-66183	36.5	91.9	8	12.7
AD-66164	31.3	78.2	5.1	6.4
AD-66121	26.5	72.1	2.7	18.3
AD-66126	33.2	56.7	2.6	12.6
AD-66178	51.1	72.1	6.3	16.5

Table 12. F12 Dose Response Screen in Hep3b Cells

DuplexId	IC_{50} (nM)
AD-66170	0.085
AD-66173	0.244
AD-66176	N/A
AD-66125	N/A
AD-66172	0.398
AD-66167	0.457
AD-66165	0.058
AD-66168	0.657
AD-66163	0.481
AD-66116	0.089
AD-66126	0.086

Table 13. F12 Single Dose Screen in Primary Mouse Hepatocytes

DuplexId	10nM AVG	0.1nM AVG	10nM STDEV	0.1nM STDEV
AD-66186	93.1	102.6	2	6.6
AD-66157	97.4	114.5	16.5	17
AD-66118	65.9	93	11.6	11.9
AD-66115	61.8	89	5.5	8.9
AD-66170	88	98.5	11.7	8.4
AD-66166	106.8	98.5	8.8	5.2
AD-66173	106.8	106	11.2	14.8
AD-66177	87.5	103	3.6	3.2
AD-66161	94.4	103.1	7	15.9
AD-66114	38.6	79.1	4.1	5
AD-66179	71.1	105.7	6.8	18.2
AD-66160	14.6	106.8	1.2	8.7
AD-66171	17.7	102.5	2.3	6.1
AD-66189	9.1	90.2	1.3	6.1
AD-66122	14.4	95.7	0.7	13.9
AD-66176	10.9	85.8	2.1	4.6
AD-66125	12.6	80.5	2.1	6.2
AD-66112	19.1	82	7.2	3.5
AD-66172	4.2	75.3	0.4	6.7
AD-66127	7.4	48.4	3.7	7.3
AD-66162	3.9	30.6	1.9	4.9

AD-66181	7.2	69.2	0.9	4.1
AD-66184	93.6	110.9	4.1	6.8
AD-66182	13.4	89.9	1.3	2
AD-66167	4.8	55.5	0.5	2.6
AD-66165	2.1	18.7	0.3	3.6
AD-66155	5.7	48	0.7	5.1
AD-66159	7.2	88.7	0.5	3.7
AD-66168	65.6	105.6	1.6	11.3
AD-66185	96	108.9	3.1	16
AD-66156	56.8	107.2	3.5	8.8
AD-66113	72.8	88.7	4.8	5.5
AD-66188	117.5	95.5	17.3	4.9
AD-66190	118.3	96.5	5.8	8.4
AD-66180	121.4	109.3	15.2	6.6
AD-66117	72.3	89.1	7.4	8.5
AD-66169	89.4	103.7	8.8	4.2
AD-66174	92	103.4	18.1	8.4
AD-66175	89.5	112.9	13.7	8.9
AD-66158	103.9	105.3	11.5	15.2
AD-66119	66.5	92	8.9	9
AD-66187	109.1	107	16.4	10.3
AD-66163	89.9	106	6.8	6.1
AD-66116	69.8	97	8.2	10.6
AD-66137	17.6	94.1	2.1	8.7
AD-66183	100.1	109.6	7.6	8.4
AD-66164	84	98.8	10.2	9.8
AD-66121	2.5	30.5	0.4	3.2
AD-66126	4.1	22.3	0.3	2.3
AD-66178	79.6	112.8	6.8	16.5

Table 14. F12 Dose Response Screen in Primary Mouse Hepatocytes

DuplexId	IC_{50} (nM)
AD-66170	N/A
AD-66173	N/A
AD-66176	3.571
AD-66125	14.962
AD-66172	1.104
AD-66167	1.013
AD-66165	0.231
AD-66168	N/A
AD-66163	N/A
AD-66116	N/A
AD-66121	0.119
AD-66126	0.045

Example 5. KNG1 iRNA Synthesis

Source of reagents

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

Transcripts

siRNA Design

A set of siRNAs targeting the human KNG1, "kininogen 1" (human: NCBI refseqID NM_001166451; NCBI GeneID: 3827), as well as toxicology-species KNG1 orthologs (cynomolgus monkey: XM_005545463; mouse: NM_001102409; rat, NM_012696) were designed using custom R and Python scripts. The human NM_001166451 REFSEQ mRNA has a length of 2035 bases. The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position position 235 through position 2035 (the coding region and 3' UTR was determined using a linear model that predicted the direct measure of mRNA knockdown based on the data of more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. Subsets of the KNG1 siRNAs were designed with perfect or near-perfect matches between human and cynomolgus monkey. A further subset was designed with perfect or near-perfect matches to mouse and rat KNG1 orthologs. For each strand of the siRNA, a custom Python script was used in a brute force search to measure the number and positions of mismatches between the

siRNA and all potential alignments in the target species transcriptome. Extra weight was given to mismatches in the seed region, defined here as positions 2-9 of the antisense oligonucleotide, as well the cleavage site of the siRNA, defined here as positions 10-11 of the antisense oligonucleotide. The relative weights for the mismatches were 2.8 for seed mismatches, 1.2 for cleavage site mismatches, and 1 mismatches in other positions up through antisense position 19. Mismatches in the first position were ignored. A specificity score was calculated for each strand by summing the value of each weighted mismatch. Preference was given to siRNAs whose antisense score in human and cynomolgus monkey was >= 3.0 and predicted efficacy was >= 70% knockdown of the NM_001166451 transcript.

A detailed list of the unmodified KNG1 sense and antisense strand sequences is shown in Table 15. A detailed list of the modified KNG1 sense and antisense strand sequences is shown in Table 16.

siRNA Synthesis

KNG1 siRNA sequences were synthesized at 1 μmol scale on a Mermade 192 synthesizer (BioAutomation) using the solid support mediated phosphoramidite chemistry. The solid support was controlled pore glass (500°A) loaded with custom GalNAc ligand or universal solid support (AM biochemical). Ancillary synthesis reagents, 2'-F and 2'-O-Methyl RNA and deoxy phosphoramidites were obtained from Thermo-Fisher (Milwaukee, WI) and Hongene (China). 2'F 2'-O-Methyl, GNA (glycol nucleic acids), 5'phosphate and other modifications were introduced using the corresponding phosphoramidites. Synthesis of 3' GalNAc conjugated single strands was performed on a GalNAc modified CPG support. Custom CPG universal solid support was used for the synthesis of antisense single strands. Coupling time for all phosphoramidites (100 mM in acetonitrile) was 5 min employing 5-Ethylthio-1H-tetrazole (ETT) as activator (0.6 M in acetonitrile). Phosphorothioate linkages were generated using a 50 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (1:1 v/v). Oxidation time was 3 minutes. All sequences were synthesized with final removal of the DMT group ("DMT off").

Upon completion of the solid phase synthesis, oligoribonucleotides were cleaved from the solid support and deprotected in sealed 96 deep well plates using 200 μL Aqueous Methylamine reagents at 60°C for 20 minutes. For sequences containing 2' ribo residues (2'-OH) that are protected with a tert-butyl dimethyl silyl (TBDMS) group, a second step deprotection was performed using TEA.3HF (triethylamine trihydro fluoride) reagent. To the methylamine deprotection solution, 200uL of dimethyl sulfoxide (DMSO) and 300ul

TEA.3HF reagent was added and the solution was incubated for additional 20min at 60°C. At the end of cleavage and deprotection step, the synthesis plate was allowed to come to room temperature and was precipitated by addition of 1mL of acetontile: ethanol mixture (9:1). The plates were cooled at -80 C for 2 hrs, superanatant decanted carefully with the aid of a multi channel pipette. The oligonucleotide pellet was re-suspended in 20mM NaOAc buffer and were desalted using a 5 mL HiTrap size exclusion column (GE Healthcare) on an AKTA Purifier System equipped with an A905 autosampler and a Frac 950 fraction collector. Desalted samples were collected in 96-well plates. Samples from each sequence were analyzed by LC-MS to confirm the identity, UV (260 nm) for quantification and a selected set of samples by IEX chromatography to determine purity.

Annealing of KNG1 single strands was performed on a Tecan liquid handling robot. Equimolar mixture of sense and antisense single strands were combined and annealed in 96 well plates. After combining the complementary single strands, the 96-well plate was sealed tightly and heated in an oven at 100°C for 10 minutes and allowed to come slowly to room temperature over a period 2-3 hours. The concentration of each duplex was normalized to 10μM in 1X PBS and then submitted for *in vitro* screening assays.

Table 15. KNG1 Unmodified Sequences

Duplex name	sense oligo name	Sense Sequence 5' to 3'	SEQ ID NO:	antis oligoname	Antisense Sequence 5' to 3'	SEQ ID NO:	Position in NM_001166451
AD-66259	A-132506	GAGGAAAUUGACUGCAAUGAA	578	A-132507	UUCAUUGCAGUCAAUUUCCUCGG	647	301_324
AD-66261	A-132510	CACGUUUUAUUCCUUCAAGUA	579	A-132511	UACUUGAAGGAAUAAAACGUGUC	648	438_461
AD-66262	A-132512	UACCUACUCAAUUGUGCAAAA	580	A-132513	UUUUGCACAAUUGAGUAGGUAAU	649	822_845
AD-66263	A-132514	CUUUCUAUUUCAAGAUUGACA	581	A-132515	UGUCAAUCUUGAAAUAGAAAGUU	650	1118_1141
AD-66260	A-132508	AACCACAUGUUCCAAGGAAAA	582	A-132509	UUUUCCUUGGAACAUGUGGUUUC	651	1206_1229
AD-66341	A-132670	AAUAAAAGAAGAAACAACUGU	583	A-132671	ACAGUUGUUUCUUCUUUUAUUUC	652	1416_1439
AD-66345	A-132678	AGAAGAAACAACUGUAAGUCA	584	A-132679	UGACUUACAGUUGUUUCUUCUUU	653	1422_1445
AD-66328	A-132644	CGGGAUUCAGGAAAAGAACAA	585	A-132645	UUGUUCUUUUCCUGAAUCCCGCU	654	1480_1503
AD-66317	A-132622	GGGAUUCAGGAAAAGAACAAA	586	A-132623	UUUGUUCUUUUCCUGAAUCCCGC	655	1481_1504
AD-66333	A-132654	GGAUUCAGGAAAAGAACAAGA	587	A-132655	UCUUGUUCUUUUCCUGAAUCCCG	656	1482_1505
AD-66338	A-132664	GAUUCAGGAAAAGAACAAGGA	588	A-132665	UCCUUGUUCUUUUCCUGAAUCCC	657	1483_1506
AD-66343	A-132674	AUUCAGGAAAAGAACAAGGGA	589	A-132675	UCCCUUGUUCUUUUCCUGAAUCC	658	1484_1507
AD-66319	A-132626	AAACAUGAACGUGACCAAGGA	590	A-132627	UCCUUGGUCACGUUCAUGUUUAU	659	1567_1590
AD-66346	A-132680	CACGAACAACAGCAUGGUCUU	591	A-132681	AAGACCAUGCUGUUGUUCGUGUC	660	1624_1647
AD-66329	A-132646	GGUCUUGGUCAUGGACAUAAA	592	A-132647	UUUAUGUCCAUGACCAAGACCAU	661	1639_1662
AD-66270	A-132528	CUUGGUCAUGGACAUAAGUUA	593	A-132529	UAACUUAUGUCCAUGACCAAGAC	662	1642_1665
AD-66279	A-132546	UUGGUCAUGGACAUAAGUUCA	594	A-132547	UGAACUUAUGUCCAUGACCAAGA	663	1643_1666
AD-66273	A-132534	GGUCAUGGACAUAAGUUCAAA	595	A-132535	UUUGAACUUAUGUCCAUGACCAA	664	1645_1668
AD-66264	A-132516	AUGGACAUAAGUUCAAACUUA	596	A-132517	UAAGUUUGAACUUAUGUCCAUGA	665	1649_1672
AD-66342	A-132672	GGACAUAAGUUCAAACUUGAU	597	A-132673	AUCAAGUUUGAACUUAUGUCCAU	666	1651_1674
AD-66278	A-132544	CAUAAGUUCAAACUUGAUGAU	598	A-132545	AUCAUCAAGUUUGAACUUAUGUC	667	1654_1677

AD-66277	A-132542	AUAAGUUCAAACUUGAUGAUA	599	A-132543	UAUCAUCAAGUUUGAACUUAUGU	668	1655_1678
AD-66267	A-132522	UUCAAACUUGAUGAUGAUCUU	600	A-132523	AAGAUCAUCAUCAAGUUUGAACU	669	1660_1683
AD-66325	A-132638	UCAAACUUGAUGAUGAUCUUA	601	A-132639	UAAGAUCAUCAUCAAGUUUGAAC	670	1661_1684
AD-66320	A-132628	AACUUGAUGAUGAUCUUGAAA	602	A-132629	UUUCAAGAUCAUCAUCAAGUUUG	671	1664_1687
AD-66336	A-132660	GUCCUUGACCAUGGACAUAAA	603	A-132661	UUUAUGUCCAUGGUCAAGGACAU	672	1699_1722
AD-66280	A-132548	GACCAUGGACAUAAGCAUAAA	604	A-132549	UUUAUGCUUAUGUCCAUGGUCAA	673	1705_1728
AD-66272	A-132532	AUGGACAUAAGCAUAAGCAUA	605	A-132533	UAUGCUUAUGCUUAUGUCCAUGG	674	1709_1732
AD-66275	A-132538	GAAUGGAAAGCACAAUGGUUA	606	A-132539	UAACCAUUGUGCUUUCCAUUCUU	675	1767_1790
AD-66348	A-132684	GAAAGCACAAUGGUUGGAAAA	607	A-132685	UUUUCCAACCAUUGUGCUUUCCA	676	1772_1795
AD-66340	A-132668	AAGCACAAUGGUUGGAAAACA	608	A-132669	UGUUUUCCAACCAUUGUGCUUUC	677	1774_1797
AD-66330	A-132648	AUGGUUGGAAAACAGAGCAUU	609	A-132649	AAUGCUCUGUUUUUCCAACCAUUG	678	1781_1804
AD-66306	A-132600	GGUUGGAAAACAGAGCAUUUA	610	A-132601	UAAAUGCUCUGUUUUUCCAACCAU	679	1783_1806
AD-66322	A-132632	AUUUGGCAAGCUCUUCUGAAA	611	A-132633	UUUCAGAAGAGCUUGCCAAAUGC	680	1799_1822
AD-66274	A-132536	UCUUCUGAAGACAGUACUACA	612	A-132537	UGUAGUACUGUCUUCAGAAGAGC	681	1810_1833
AD-66271	A-132530	CAGAGUGAUGACGAUUGGAUA	613	A-132531	UAUCCAAUCGUCAUCACUCUGUA	682	1975_1998
AD-66339	A-132666	CUUUCAUUUAACCCAAUAUCA	614	A-132667	UGAUAUUGGGUUAAAUGAAAGGC	683	2023_2046
AD-66276	A-132540	UUUCAUUUAACCCAAUAUCAA	615	A-132541	UUGAUAUUGGGUUAAAUGAAAGG	684	2024_2047
AD-66281	A-132550	UUUAACCCAAUAUCAGAUUUU	616	A-132551	AAAAUCUGAUAUUGGGUUAAAUG	685	2029_2052
AD-66313	A-132614	UUAACCCAAUAUCAGAUUUUA	617	A-132615	UAAAAUCUGAUAUUGGGUUAAAU	686	2030_2053
AD-66307	A-132602	GUGGCUAUGGGUAUUUCUUUA	618	A-132603	UAAAGAAAUACCCAUAGCCACUU	687	2172_2195
AD-66309	A-132606	UUUCUUUCAUACUUUAUUAAA	619	A-132607	UUUAAUAAAGUAUGAAAGAAAUA	688	2185_2208
AD-66316	A-132620	UUCUUUCAUACUUUAUUAAAA	620	A-132621	UUUUAAUAAAGUAUGAAAGAAAU	689	2186_2209
AD-66321	A-132630	UCUUUCAUACUUUAUUAAAGU	621	A-132631	ACUUUAAUAAAGUAUGAAAGAAA	690	2187_2210
AD-66323	A-132634	UUCAUACUUUAUUAAAGUAUA	622	A-132635	UAUACUUUAAUAAAGUAUGAAAG	691	2190_2213
AD-66315	A-132618	CUUUAUUAAAGUAUCAAUAUA	623	A-132619	UAUAUUGAUACUUUAAUAAAGUA	692	2196_2219

AD-66268	A-132524	UUUAUUAAAGUAUCAAUAUCA	624	A-132525	UGAUAUUGAUACUUUAAUAAAGU	693	2197_2220
AD-66332	A-132652	AAGUAUCAAUAUCCCUCUCUA	625	A-132653	UAGAGAGGGAUAUUGAUACUUUA	694	2204_2227
AD-66303	A-132594	CAUUGUCCAGAUGAAAAUAUA	626	A-132595	UAUAUUUUCAUCUGGACAAUGGA	695	2225_2248
AD-66334	A-132656	AUGAAAAUAUCCUGAUAUAAU	627	A-132657	AUUAUAUCAGGAUAUUUUCAUCU	696	2235_2258
AD-66331	A-132650	UCUCCACGGACUGCAUAAAAU	628	A-132651	AUUUUAUGCAGUCCGUGGAGACU	697	2327_2350
AD-66326	A-132640	CACGGACUGCAUAAAAUUGUA	629	A-132641	UACAAUUUUAUGCAGUCCGUGGA	698	2331_2354
AD-66312	A-132612	CUGCAAUUGGCUUCUCUGAUA	630	A-132613	UAUCAGAGAAGCCAAUUGCAGCA	699	2441_2464
AD-66304	A-132596	UGAUAACAAAUAUGUACCUUA	631	A-132597	UAAGGUACAUAUUUGUUAUCAGA	700	2457_2480
AD-66324	A-132636	UACCUUACAACAUAUGUCAUA	632	A-132637	UAUGACAUAUGUUGUAAGGUACA	701	2471_2494
AD-66266	A-132520	UACAACAUAUGUCAUGAAUUU	633	A-132521	AAAUUCAUGACAUAUGUUGUAAG	702	2476_2499
AD-66311	A-132610	AUUCUUGUCAUUCUUAAUAAA	634	A-132611	UUUAUUAAGAAUGACAAGAAUCU	703	2507_2530
AD-66335	A-132658	UUCUUGUCAUUCUUAAUAAAA	635	A-132659	UUUUAUUAAGAAUGACAAGAAUC	704	2508_2531
AD-66344	A-132676	UCUUGUCAUUCUUAAUAAACU	636	A-132677	AGUUUAUUAAGAAUGACAAGAAU	705	2509_2532
AD-66305	A-132598	AUUUGAAUGUGUGUGAAAAUA	637	A-132599	UAUUUUCACACACAUUCAAAUAC	706	2542_2565
AD-66318	A-132624	GAAUGUGUGUGAAAAUAAGGA	638	A-132625	UCCUUAUUUUCACACACAUUCAA	707	2546_2569
AD-66308	A-132604	AUGUGUGUGAAAAUAAGGGAA	639	A-132605	UUCCCUUAUUUUCACACACAUUC	708	2548_2571
AD-66327	A-132642	GUGUGUGAAAAUAAGGGAAGU	640	A-132643	ACUUCCCUUAUUUUCACACACAU	709	2550_2573
AD-66337	A-132662	GUGUGAAAAUAAGGGAAGUCA	641	A-132663	UGACUUCCCUUAUUUUCACACAC	710	2552_2575
AD-66347	A-132682	UGUGAAAAUAAGGGAAGUCAA	642	A-132683	UUGACUUCCCUUAUUUUCACACA	711	2553_2576
AD-66269	A-132526	GUGAAAAUAAGGGAAGUCAAA	643	A-132527	UUUGACUUCCCUUAUUUUCACAC	712	2554_2577
AD-66314	A-132616	AAUAAGGGAAGUCAAGAGAUU	644	A-132617	AAUCUCUUGACUUCCCUUAUUUU	713	2559_2582
AD-66265	A-132518	GGGAAGUCAAGAGAUUAAAUA	645	A-132519	UAUUUAAUCUCUUGACUUCCCUU	714	2564_2587
AD-66310	A-132608	UAAAUGCUGAACUUAUUAAUA	646	A-132609	UAUUAAUAAGUUCAGCAUUUAAU	715	2579_2602

Table 16. KNG1 Modified Sequences

Duplex name	sense oligo name	Sense Sequence 5' to 3'	SEQ ID NO:	antis oligoname	Antisense Sequence 5' to 3'	SEQ ID NO:
AD-66259	A-132506	GfsasGfgAfaAfuUfGfAfcUfgCfaAfuGfaAfL96	716	A-132507	usUfscAfuUfgCfaGfucaAfuUfuCfcUfcsgsg	785
AD-66261	A-132510	CfsasCfgUfuUfuAfUfUfcCfuUfcAfaGfuAfL96	717	A-132511	usAfscUfuGfaAfgGfaauAfaAfaCfgUfgsusc	786
AD-66262	A-132512	UfsasCfcUfaCfuCfAfAfuUfgUfgCfaAfaAfL96	718	A-132513	usUfsuUfgCfaCfaAfuugAfgUfaGfgUfasasu	787
AD-66263	A-132514	CfsusUfuCfuAfuUfUfCfaAfgAfuUfgAfcAfL96	719	A-132515	usGfsuCfaAfuCfuUfgaaAfuAfgAfaAfgsusu	788
AD-66260	A-132508	AfsasCfcAfcAfuGfUfUfcCfaAfgGfaAfaAfL96	720	A-132509	usUfsuUfcCfuUfgGfaacAfuGfuGfgUfususc	789
AD-66341	A-132670	AfsasUfaAfaAfgAfAfGfaAfaCfaAfcUfgUfL96	721	A-132671	asCfsaGfuUfgUfuUfcuuCfuUfuUfaUfususc	790
AD-66345	A-132678	AfsgsAfaGfaAfaCfAfAfcUfgUfaAfgUfcAfL96	722	A-132679	usGfsaCfuUfaCfaGfuugUfuUfcUfuCfususu	791
AD-66328	A-132644	CfsgsGfgAfuUfcAfGfGfaAfaAfgAfaCfaAfL96	723	A-132645	usUfsgUfuCfuUfuUfccuGfaAfuCfcCfgscsu	792
AD-66317	A-132622	GfsgsGfaUfuCfaGfGfAfaAfaGfaAfcAfaAfL96	724	A-132623	usUfsuGfuUfcUfuUfuccUfgAfaUfcCfcsgsc	793
AD-66333	A-132654	GfsgsAfuUfcAfgGfAfAfaAfgAfaCfaAfgAfL96	725	A-132655	usCfsuUfgUfuCfuUfuucCfuGfaAfuCfcscsg	794
AD-66338	A-132664	GfsasUfuCfaGfgAfAfAfaGfaAfcAfaGfgAfL96	726	A-132665	usCfscUfuGfuUfcUfuuuCfcUfgAfaUfcscsc	795
AD-66343	A-132674	AfsusUfcAfgGfaAfAfAfgAfaCfaAfgGfgAfL96	727	A-132675	usCfscCfuUfgUfuCfuuuUfcCfuGfaAfuscsc	796
AD-66319	A-132626	AfsasAfcAfuGfaAfCfGfuGfaCfcAfaGfgAfL96	728	A-132627	usCfscUfuGfgUfcAfcguUfcAfuGfuUfusasu	797
AD-66346	A-132680	CfsasCfgAfaCfaAfCfAfgCfaUfgGfuCfuUfL96	729	A-132681	asAfsgAfcCfaUfgCfuguUfgUfuCfgUfgsusc	798
AD-66329	A-132646	GfsgsUfcUfuGfgUfCfAfuGfgAfcAfuAfaAfL96	730	A-132647	usUfsuAfuGfuCfcAfugaCfcAfaGfaCfcsasu	799
AD-66270	A-132528	CfsusUfgGfuCfaUfGfGfaCfaUfaAfgUfuAfL96	731	A-132529	usAfsaCfuUfaUfgUfccaUfgAfcCfaAfgsasc	800
AD-66279	A-132546	UfsusGfgUfcAfuGfGfAfcAfuAfaGfuUfcAfL96	732	A-132547	usGfsaAfcUfuAfuGfuccAfuGfaCfcAfasgsa	801
AD-66273	A-132534	GfsgsUfcAfuGfgAfCfAfuAfaGfuUfcAfaAfL96	733	A-132535	usUfsuGfaAfcUfuAfuguCfcAfuGfaCfcsasa	802
AD-66264	A-132516	AfsusGfgAfcAfuAfAfGfuUfcAfaAfcUfuAfL96	734	A-132517	usAfsaGfuUfuGfaAfcuuAfuGfuCfcAfusgsa	803
AD-66342	A-132672	GfsgsAfcAfuAfaGfUfUfcAfaAfcUfuGfaUfL96	735	A-132673	asUfscAfaGfuUfuGfaacUfuAfuGfuCfcsasu	804
AD-66278	A-132544	CfsasUfaAfgUfuCfAfAfaCfuUfgAfuGfaUfL96	736	A-132545	asUfscAfuCfaAfgUfuugAfaCfuUfaUfgsusc	805

AD-66277	A-132542	AfsusAfaGfuUfcAfAfAfcUfuGfaUfgAfuAfL96	737	A-132543	usAfsuCfaUfcAfaGfuuuGfaAfcUfuAfusgsu	806
AD-66267	A-132522	UfsusCfaAfaCfuUfGfAfuGfaUfgAfuCfuUfL96	738	A-132523	asAfsgAfuCfaUfcAfucaAfgUfuUfgAfascsu	807
AD-66325	A-132638	UfscsAfaAfcUfuGfAfUfgAfuGfaUfcUfuAfL96	739	A-132639	usAfsaGfaUfcAfuCfaucAfaGfuUfuGfasasc	808
AD-66320	A-132628	AfsasCfuUfgAfuGfAfUfgAfuCfuUfgAfaAfL96	740	A-132629	usUfsuCfaAfgAfuCfaucAfuCfaAfgUfususg	809
AD-66336	A-132660	GfsusCfcUfuGfaCfCfAfuGfgAfcAfuAfaAfL96	741	A-132661	usUfsuAfuGfuCfcAfuggUfcAfaGfgAfcsasu	810
AD-66280	A-132548	GfsasCfcAfuGfgAfCfAfuAfaGfcAfuAfaAfL96	742	A-132549	usUfsuAfuGfcUfuAfuguCfcAfuGfgUfcsasa	811
AD-66272	A-132532	AfsusGfgAfcAfuAfAfGfcAfuAfaGfcAfuAfL96	743	A-132533	usAfsuGfcUfuAfuGfcuuAfuGfuCfcAfusgsg	812
AD-66275	A-132538	GfsasAfuGfgAfaAfGfCfaCfaAfuGfgUfuAfL96	744	A-132539	usAfsaCfcAfuUfgUfgcuUfuCfcAfuUfcsusu	813
AD-66348	A-132684	GfsasAfaGfcAfcAfAfUfgGfuUfgGfaAfaAfL96	745	A-132685	usUfsuUfcCfaAfcCfauuGfuGfcUfuUfcscsa	814
AD-66340	A-132668	AfsasGfcAfcAfaUfGfGfuUfgGfaAfaAfcAfL96	746	A-132669	usGfsuUfuUfcCfaAfccaUfuGfuGfcUfususc	815
AD-66330	A-132648	AfsusGfgUfuGfgAfAfAfaCfaGfaGfcAfuUfL96	747	A-132649	asAfsuGfcUfcUfgUfuuuCfcAfaCfcAfususg	816
AD-66306	A-132600	GfsgsUfuGfgAfaAfAfCfaGfaGfcAfuUfuAfL96	748	A-132601	usAfsaAfuGfcUfcUfguuUfuCfcAfaCfcsasu	817
AD-66322	A-132632	AfsusUfuGfgCfaAfGfCfuCfuUfcUfgAfaAfL96	749	A-132633	usUfsuCfaGfaAfgAfgcuUfgCfcAfaAfusgsc	818
AD-66274	A-132536	UfscsUfuCfuGfaAfGfAfcAfgUfaCfuAfcAfL96	750	A-132537	usGfsuAfgUfaCfuGfucuUfcAfgAfaGfasgsc	819
AD-66271	A-132530	CfsasGfaGfuGfaUfGfAfcGfaUfuGfgAfuAfL96	751	A-132531	usAfsuCfcAfaUfcGfucaUfcAfcUfcUfgsusa	820
AD-66339	A-132666	CfsusUfuCfaUfuUfAfAfcCfcAfaUfaUfcAfL96	752	A-132667	usGfsaUfaUfuGfgGfuuaAfaUfgAfaAfgsgsc	821
AD-66276	A-132540	UfsusUfcAfuUfuAfAfCfcCfaAfuAfuCfaAfL96	753	A-132541	usUfsgAfuAfuUfgGfguuAfaAfuGfaAfasgsg	822
AD-66281	A-132550	UfsusUfaAfcCfcAfAfUfaUfcAfgAfuUfuUfL96	754	A-132551	asAfsaAfuCfuGfaUfauuGfgGfuUfaAfasusg	823
AD-66313	A-132614	UfsusAfaCfcCfaAfUfAfuCfaGfaUfuUfuAfL96	755	A-132615	usAfsaAfaUfcUfgAfuauUfgGfgUfuAfasasu	824
AD-66307	A-132602	GfsusGfgCfuAfuGfGfGfuAfuUfuCfuUfuAfL96	756	A-132603	usAfsaAfgAfaAfuAfcccAfuAfgCfcAfcsusu	825
AD-66309	A-132606	UfsusUfcUfuUfcAfUfAfcUfuUfaUfuAfaAfL96	757	A-132607	usUfsuAfaUfaAfaGfuauGfaAfaGfaAfasusa	826
AD-66316	A-132620	UfsusCfuUfuCfaUfAfCfuUfuAfuUfaAfaAfL96	758	A-132621	usUfsuUfaAfuAfaAfguaUfgAfaAfgAfasasu	827
AD-66321	A-132630	UfscsUfuUfcAfuAfCfUfuUfaUfuAfaAfgUfL96	759	A-132631	asCfsuUfuAfaUfaAfaguAfuGfaAfaGfasasa	828
AD-66323	A-132634	UfsusCfaUfaCfuUfUfAfuUfaAfaGfuAfuAfL96	760	A-132635	usAfsuAfcUfuUfaAfuaaAfgUfaUfgAfasasg	829
AD-66315	A-132618	CfsusUfuAfuUfaAfAfGfuAfuCfaAfuAfuAfL96	761	A-132619	usAfsuAfuUfgAfuAfcuuUfaAfuAfaAfgsusa	830

AD-66268	A-132524	UfsusUfaUfuAfaAfGfUfaUfcAfaUfaUfcAfL96	762	A-132525	usGfsaUfaUfuGfaUfacuUfuAfaUfaAfasgsu	831
AD-66332	A-132652	AfsasGfuAfuCfaAfUfAfuCfcCfuCfuCfuAfL96	763	A-132653	usAfsgAfgAfgGfgAfuauUfgAfuAfcUfususa	832
AD-66303	A-132594	CfsasUfuGfuCfcAfGfAfuGfaAfaAfuAfuAfL96	764	A-132595	usAfsuAfuUfuUfcAfucuGfgAfcAfaUfgsgsa	833
AD-66334	A-132656	AfsusGfaAfaAfuAfUfCfcUfgAfuAfuAfaUfL96	765	A-132657	asUfsuAfuAfuCfaGfgauAfuUfuUfcAfuscsu	834
AD-66331	A-132650	UfscsUfcCfaCfgGfAfCfuGfcAfuAfaAfaUfL96	766	A-132651	asUfsuUfuAfuGfcAfgucCfgUfgGfaGfascsu	835
AD-66326	A-132640	CfsasCfgGfaCfuGfCfAfuAfaAfaUfuGfuAfL96	767	A-132641	usAfscAfaUfuUfuAfugcAfgUfcCfgUfgsgsa	836
AD-66312	A-132612	CfsusGfcAfaUfuGfGfCfuUfcUfcUfgAfuAfL96	768	A-132613	usAfsuCfaGfaGfaAfgccAfaUfuGfcAfgscsa	837
AD-66304	A-132596	UfsgsAfuAfaCfaAfAfUfaUfgUfaCfcUfuAfL96	769	A-132597	usAfsaGfgUfaCfaUfauuUfgUfuAfuCfasgsa	838
AD-66324	A-132636	UfsasCfcUfuAfcAfAfCfaUfaUfgUfcAfuAfL96	770	A-132637	usAfsuGfaCfaUfaUfguuGfuAfaGfgUfascsa	839
AD-66266	A-132520	UfsasCfaAfcAfuAfUfGfuCfaUfgAfaUfuUfL96	771	A-132521	asAfsaUfuCfaUfgAfcauAfuGfuUfgUfasasg	840
AD-66311	A-132610	AfsusUfcUfuGfuCfAfUfuCfuUfaAfuAfaAfL96	772	A-132611	usUfsuAfuUfaAfgAfaugAfcAfaGfaAfuscsu	841
AD-66335	A-132658	UfsusCfuUfgUfcAfUfUfcUfuAfaUfaAfaAfL96	773	A-132659	usUfsuUfaUfuAfaGfaauGfaCfaAfgAfasusc	842
AD-66344	A-132676	UfscsUfuGfuCfaUfUfCfuUfaAfuAfaAfcUfL96	774	A-132677	asGfsuUfuAfuUfaAfgaaUfgAfcAfaGfasasu	843
AD-66305	A-132598	AfsusUfuGfaAfuGfUfGfuGfuGfaAfaAfuAfL96	775	A-132599	usAfsuUfuUfcAfcAfcacAfuUfcAfaAfusasc	844
AD-66318	A-132624	GfsasAfuGfuGfuGfUfGfaAfaAfuAfaGfgAfL96	776	A-132625	usCfscUfuAfuUfuUfcacAfcAfcAfuUfcsasa	845
AD-66308	A-132604	AfsusGfuGfuGfuGfAfAfaAfuAfaGfgGfaAfL96	777	A-132605	usUfscCfcUfuAfuUfuucAfcAfcAfcAfususc	846
AD-66327	A-132642	GfsusGfuGfuGfaAfAfAfuAfaGfgGfaAfgUfL96	778	A-132643	asCfsuUfcCfcUfuAfuuuUfcAfcAfcAfcsasu	847
AD-66337	A-132662	GfsusGfuGfaAfaAfUfAfaGfgGfaAfgUfcAfL96	779	A-132663	usGfsaCfuUfcCfcUfuauUfuUfcAfcAfcsasc	848
AD-66347	A-132682	UfsgsUfgAfaAfaUfAfAfgGfgAfaGfuCfaAfL96	780	A-132683	usUfsgAfcUfuCfcCfuuaUfuUfuCfaCfascsa	849
AD-66269	A-132526	GfsusGfaAfaAfuAfAfGfgGfaAfgUfcAfaAfL96	781	A-132527	usUfsuGfaCfuUfcCfcuuAfuUfuUfcAfcsasc	850
AD-66314	A-132616	AfsasUfaAfgGfgAfAfGfuCfaAfgAfgAfuUfL96	782	A-132617	asAfsuCfuCfuUfgAfcuuCfcCfuUfaUfususu	851
AD-66265	A-132518	GfsgsGfaAfgUfcAfAfGfaGfaUfuAfaAfuAfL96	783	A-132519	usAfsuUfuAfaUfcUfcuuGfaCfuUfcCfcsusu	852
AD-66310	A-132608	UfsasAfaUfgCfuGfAfAfcUfuAfuUfaAfuAfL96	784	A-132609	usAfsuUfaAfuAfaGfuucAfgCfaUfuUfasasu	853

Example 6. *In vitro* screening of KNG1 siRNA duplexes

Cell culture and transfections

Hep3b were transfected by adding 4.9µl of Opti-MEM plus 0.1µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl of siRNA duplexes per well into a 384-well plate and incubated at room temperature for 15 minutes. Forty µl of DMEM (Hep3b) of William's E Medium (PMH) containing about 5 x10^3 cells was then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification.

Single dose experiments were performed at 10nM and 0.01nM final duplex concentration and dose response experiments were done over a range of doses from 10nM to 36fM final duplex concentration over 8, 6-fold dilutions.

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, 50µl of Lysis/Binding Buffer and 25µl of lysis buffer containing 3µl 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 150µl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150µl Elution Buffer, re-captured and the supernatant was removed.

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

Ten µl of a master mix containing 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl 10x Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H2O per reaction was added to RNA isolated as described above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 hours 37°C. Plates were then incubated at 81°C for 8 minutes.

Real time PCR:

Two µl of cDNA were added to a master mix containing 0.5µl of GAPDH TaqMan Probe (Hs99999905_m1 or 4352339E), 0.5µl F12 probe (Hs00166821 or Mm00491349) and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was performed using 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 10nM AD-1955, or mock transfected cells. IC_{50}s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955, a non-targeting control, or naïve cells.

The sense and antisense sequences of AD-1955 are:

SENSE: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO: 2343);

ANTISENSE: UCGAAGuACUcAGCGuAAGdTsdT (SEQ ID NO: 2344).

Table 17 shows the results of a single dose screen in Hep3b cells transfected with the indicated human KNG1 iRNAs. Table 18 shows the results of a dose response screen in Hep3b cells transfected with the indicated human KNG1 iRNAs. Data are expressed as percent of mRNA remaining relative to AD-1955.

Table 17. KNG1 Single Dose Screen in Hep3b

DuplexId	10nM AVG	0.1nM AVG	10 nM ST DEV	0.1 nM ST DEV
AD-66259	5	30.8	1.4	12.6
AD-66261	14.5	74.9	4.5	37.1
AD-66262	14.4	40.4	12.7	19.8
AD-66263	23.8	87.4	33.9	20.6
AD-66260	42.4	78.7	12.8	20.7
AD-66341	30	88.9	11.8	20.6
AD-66345	79.5	180	9.2	57.7
AD-66328	75.1	74.5	16.8	13.5
AD-66317	30.4	71.5	6.4	19.6
AD-66333	66	90.7	23.5	31.4
AD-66338	74.2	123.7	30.4	35.3
AD-66343	69	86.9	27.3	24
AD-66319	70.9	93.6	10.7	26.8
AD-66346	68.2	184.8	5.5	55.7
AD-66329	73.5	104.6	15.6	15.5
AD-66270	96.1	80.5	51.4	22.4
AD-66279	54.7	75.7	28.6	21.5
AD-66273	141.2	71.9	26.9	12.9
AD-66264	82.6	92.3	43.5	25.1
AD-66342	55.9	91.6	12.4	8.5
AD-66278	77.4	62.2	23.9	17.4
AD-66277	56.5	86	41.7	31.8
AD-66267	56.1	68.7	22.4	15.6

AD-66325	40.3	60.8	13.5	19.2
AD-66320	70.4	99.5	16.5	6.2
AD-66336	102.7	94.4	26.6	21.3
AD-66280	71.9	94.8	32.4	19.9
AD-66272	150.9	241.6	43.7	195.8
AD-66275	49.3	100.4	12	40.6
AD-66348	64.8	117.2	17.2	21.5
AD-66340	56.7	85.1	15.7	26.4
AD-66330	61.5	97.1	12.4	24.9
AD-66306	36.1	68.4	8.2	14
AD-66322	59.9	84.4	14.4	28.8
AD-66274	109.6	88.2	48	17.3
AD-66271	130.9	70.1	25.3	20.9
AD-66339	68.4	107.4	29.3	27.7
AD-66276	40.6	85	8.8	31
AD-66281	111.1	89.8	54.8	27.2
AD-66313	57.1	112.6	8.5	35.9
AD-66307	37.2	70.4	10	5.4
AD-66309	42.7	58.8	9.4	11.5
AD-66316	42.2	75.3	10.3	9.9
AD-66321	63.8	106.1	28	32.4
AD-66323	68.7	89	16.3	21.4
AD-66315	41.4	87.5	7.2	10.8
AD-66268	81.1	55	34.2	5.6
AD-66332	59.6	74.6	22.8	22.8
AD-66303	63.3	52.3	9.6	7.1
AD-66334	47.7	72.7	11.9	36.2
AD-66331	51.1	98.1	13.6	33.5
AD-66326	53	58.9	5.7	11.7
AD-66312	76	90.8	16.2	19.8
AD-66304	85.5	54.3	12.4	4.3
AD-66324	49.5	63.4	8.2	4.7
AD-66266	118.3	185.1	21.2	42.8
AD-66311	59	68.6	4.3	15.5
AD-66335	65.8	74.3	9.6	28.2
AD-66344	113.2	110.1	41.2	37.7
AD-66305	62.5	100.6	18.1	32.7
AD-66318	56.5	60.4	12.5	5.2
AD-66308	43.7	65.6	12	12.9
AD-66327	58.5	65.8	11.9	9.2

AD-66337	102.8	156.4	41.6	32.7
AD-66347	78.4	105.7	25.9	24.3
AD-66269	66.2	85.1	20.4	15
AD-66314	49.6	98.3	8.9	25.3
AD-66265	109.9	177.7	40.1	57.8
AD-66310	42.1	73.4	7	27.5

Table 18. KNG1 Dose Response Screen in Hep3b

DuplexId	IC_{50} (nM)
AD-66259	0.035
AD-66261	1.02
AD-66262	0.04
AD-66263	0.299
AD-66341	9.181

Example 7. *In Vivo* KLKB1, F12, and KNG1 Silencing in Wild-Type Mice

Three of the most active agents targeting KLKB1, described above, three of the most active agents targeting F12, described above, and two of the most active agents targeting KNG1, described above, were selected for further evaluation. In particular, additional agents targeting nucleotides 1661-1682, or nucleotides 1905-1926, or nucleotides 382-403 of NM_000892 (a KLKB1 gene) (Figure 7), additional agents targeting nucleotides 2017-2040, or nucleotides 315-338, or nucleotides 438-459 of NM_000505 (an F12 gene) (Figure 8), and additional agents targeting nucleotides 301-324 or nucleotides 822-845 of NM_001166451 (a KNG1 gene) (Figure 9) were synthesized as described above. The *in vivo* efficacy of these additional agents was assessed by administration of a single subcutaneous dose of the agent to wild-type C57BL/6 mice and determining the level of mRNA at 7-10 days post-dose. The unmodified nucleotide sequences of the sense and antisense strands of the agents depicted in Figure 7 targeting KLKB1 are provided in Table 19A, and the modified nucleotide sequences of the sense and antisense strands of the agents depicted in Figure 7 are provided in Table 19B. The unmodified nucleotide sequences of the sense and antisense strands of the agents depicted in Figure 8 targeting F12 are provided in Table 19C, and the modified nucleotide sequences of the sense and antisense strands of the agents depicted in Figure 8 are provided in Table 19D. The unmodified nucleotide sequences of the sense and antisense strands of the agents depicted in Figure 9 targeting KNG1 are provided in Table 19E, and the modified nucleotide sequences of the sense and antisense strands of the agents depicted in Figure 9 are provided in Table 19F.

In particular, with respect to the additional agents targeting a KLKB1 gene, wild-type C57BL/6 mice were administered a single 1 mg/kg or 3 mg/kg dose of the agent and the level of KLKB1 mRNA was determined at 7-10 days post-dose. The results of these assays are provided in Figure 1 which demonstrates that AD-66948 was the most efficiacious agent targeting a KLKB1 gene that was tested.

With respect to the additional agents targeting F12, wild-type C57BL/6 mice were administered either a single 1 mg/kg dose or a single 3 mg/kg dose, or a single 1 mg/kg dose or a single 10 mg/kg dose of the agent and the level of F12 mRNA was determined at 7-10 days post-dose. The results of these assays are provided in Figure 2 which demonstrates that AD-67244 was the most efficiacious agent targeting a F12 gene that was tested.

With respect to the additional agents targeting a KNG1 gene, wild-type C57BL/6 mice were administered a single 1 mg/kg or 3 mg/kg dose of the agent and the level of KNG1 mRNA was determined at 7-10 days post-dose. The results of these assays are provided in

Figure 3 which demonstrates that AD-67344 was the most efficiacious agent targeting a KNG1 gene that was tested.

Table 19A. Unmodified sense and antisense strand sequences of agents targeting KLKB1

Duplex Name	Target	Antisense Start Position on mRNA	Sense Position in GenBank Reference Sequence	Antisense Position in GenBank Reference Sequence	Sense Sequence	SEQ ID NO	Antisense Sequence	SEQ ID NO
AD-65077	KLKB1	1659	NM_000892.3_1661-1681_s	NM_000892.3_1659-1681_as	AAUCCAAAAUAUUCUACAAAA	854	UUUUGUAGAAUAUUUUGGAUUUC	863
AD-66944	KLKB1	1659	NM_000892.3_1661-1681_s	NM_000892.3_1659-1681_as	AAUCCAAAAUAUUCUACAAAA	855	UUUUGUAGAAUAUUUUGGAUUUC	864
AD-66945	KLKB1	1659	NM_000892.3_1661-1681_s	NM_000892.3_1659-1681_as	AAUCCAAAAUAUUCUACAAAA	856	UUUUGUAGAAUAUUUUGGAUUUC	865
AD-65087	KLKB1	1903	NM_000892.3_1905-1925_s	NM_000892.3_1903-1925_as	ACCAAAGUCGCUGAGUACAUA	857	UAUGUACUCAGCGACUUUGGUGU	866
AD-66946	KLKB1	1903	NM_000892.3_1905-1925_s	NM_000892.3_1903-1925_as	ACCAAAGUCGCUGAGUACAUA	858	UAUGUACUCAGCGACUUUGGUGU	867
AD-66947	KLKB1	1903	NM_000892.3_1905-1925_s	NM_000892.3_1903-1925_as	ACCAAAGUCGCUGAGUACAUA	859	UAUGUACUCAGCGACUUUGGUGU	868
AD-65103	KLKB1	380	NM_000892.3_382-402_s	NM_000892.3_380-402_as	GUGGUCAUCAAAUAAGUGCUU	860	AAGCACUUAUUUGAUGACCACAU	869
AD-66948	KLKB1	380	NM_000892.3_382-402_s	NM_000892.3_380-402_as	GUGGUCAUCAAAUAAGUGCUU	861	AAGCACUUAUUUGAUGACCACAU	870
AD-66949	KLKB1	380	NM_000892.3_382-402_s	NM_000892.3_380-402_as	GUGGUCAUCAAAUAAGUGCUU	862	AAGCACUUAUUUGAUGACCACAU	871

Table 19B. Modified sense and antisense strand sequences of agents targeting KLKB1

Duplex Name	Target	Antisense Start Position on mRNA	Sense Position in GenBank Reference Sequence	Antisense Position in GenBank Reference Sequence	Sense Sequence	SEQ ID NO	Antisense Sequence	SEQ ID NO
AD-65077	KLKB1	1659	NM_000892.3_1661-1681_s	NM_000892.3_1659-1681_as	AfsasUfcCfaAfaAfUfAfuUfcUfaCfaAfaAfL96	872	usUfsuUfgUfaGfaAfuauUfuUfgGfaUfususc	881
AD-66944	KLKB1	1659	NM_000892.3_1661-1681_s	NM_000892.3_1659-1681_as	asasuccaAfaAfUfAfuucuacaaaaL96	873	usUfsuugUfaGfAfauauUfuUfggauususc	882

AD-66945	KLKB1	1659	NM_000892.3_1661-1681_s	NM_000892.3_1659-1681_as	asasuccaAfaAfUfAfuucuacaaaaL96	874	UfsUfsuugUfaGfAfauauUfuUfggauususc	883
AD-65087	KLKB1	1903	NM_000892.3_1905-1925_s	NM_000892.3_1903-1925_as	AfscsCfaAfaGfuCfGfCfuGfaGfuAfcAfuAfL96	875	usAfsuGfuAfcUfcAfgcgAfcUfuUfgGfusgsu	884
AD-66946	KLKB1	1903	NM_000892.3_1905-1925_s	NM_000892.3_1903-1925_as	ascscaaaGfuCfGfCfugaguacauaL96	876	usAfsuguAfcUfCfagcgAfcUfuuggusgsu	885
AD-66947	KLKB1	1903	NM_000892.3_1905-1925_s	NM_000892.3_1903-1925_as	ascscaaaGfuCfGfCfugaguacauaL96	877	UfsAfsuguAfcUfCfagcgAfcUfuuggusgsu	886
AD-65103	KLKB1	380	NM_000892.3_382-402_s	NM_000892.3_380-402_as	GfsusGfgUfcAfuCfAfAfaUfaAfgUfgCfuUfL96	878	asAfsgCfaCfuUfaUfuugAfuGfaCfcAfcsasu	887
AD-66948	KLKB1	380	NM_000892.3_382-402_s	NM_000892.3_380-402_as	gsusggucAfuCfAfAfauaagugcuuL96	879	asAfsgcaCfuUfAfuuugAfuGfaccacsasu	888
AD-66949	KLKB1	380	NM_000892.3_382-402_s	NM_000892.3_380-402_as	gsusggucAfuCfAfAfauaagugcuuL96	880	AfsAfsgcaCfuUfAfuuugAfuGfaccacsasu	889

Table 19C. Unmodified sense and antisense strand sequences of agents targeting F12

Duplex Name	Target	Antisense Start Position on mRNA	Sense Position in GenBank Reference Sequence	Antisense Position in GenBank Reference Sequence	Sense Sequence	SEQ ID NO	Antisense Sequence	SEQ ID NO
AD-66121	F12	2018	NM_000505.3_2020-2040_s	NM_000505.3_2018-2040_as	AACUCAAUAAAGUGCUUUGAA	890	UUCAAAGCACUUUAUUGAGUUUC	898
AD-67244	F12	2018	NM_000505.3_2020-2040_s	NM_000505.3_2018-2040_as	AACUCAAUAAAGUGCUUUGAA	891	UUCAAAGCACUUUAUUGAGUUUC	899
AD-67245	F12	2018	NM_000505.3_2020-2040_s	NM_000505.3_2018-2040_as	AACUCAAUAAAGUGCUUUGAA	892	UUCAAAGCACUUUAUUGAGUUUC	900
AD-66125	F12	316	NM_000505.3_318-338_s	NM_000505.3_316-338_as	AGCCCAAGAAAGUGAAAGACA	893	UGUCUUUCACUUUCUUGGGCUCC	901
AD-67246	F12	2023	NM_000505.3_2025-2045_s	NM_000505.3_2023-2045_as	AAUAAAGUGCUUUGAAAACGU	894	ACGUUUUCAAAGCACUUUAUUGA	902
AD-67247	F12	2023	NM_000505.3_2025-2045_s	NM_000505.3_2023-2045_as	AAUAAAGUGCUUUGAAAACGU	895	ACGUUUUCAAAGCACUUUAUUGA	903
AD-67248	F12	438	NM_000029.3_440-460_s	NM_000029.3_438-460_as	CAGAAAGAGAAGUGCUUUGAA	896	UUCAAAGCACUUCUCUUUCUGGC	904
AD-67249	F12	438	NM_000029.3_440-460_s	NM_000029.3_438-460_as	CAGAAAGAGAAGUGCUUUGAA	897	UUCAAAGCACUUCUCUUUCUGGC	905

Table 19D. Modified sense and antisense strand sequences of agents targeting F12

Duplex Name	Target	Antisense Start Position on mRNA	Sense Position in GenBank Reference Sequence	Antisense Position in GenBank Reference Sequence	Sense Sequence	SEQ ID NO	Antisense Sequence	SEQ ID NO
AD-66121	F12	2018	NM_000505.3_2020-2040_s	NM_000505.3_2018-2040_as	AfsasCfuCfaAfuAfAfAfgUfgCfuUfuGfaAfL96	906	usUfscAfaAfgCfaCfuuuAfuUfgAfgUfususc	914
AD-67244	F12	2018	NM_000505.3_2020-2040_s	NM_000505.3_2018-2040_as	asascucaAfuAfAfAfgugcuuugaaL96	907	usUfscaaAfgCfAfcuuuAfuUfgaguususc	915
AD-67245	F12	2018	NM_000505.3_2020-2040_s	NM_000505.3_2018-2040_as	asascucaAfuAfAfAfgugcuuugaaL96	908	UfsUfscaaAfgCfAfcuuuAfuUfgaguususc	916
AD-66125	F12	316	NM_000505.3_318-338_s	NM_000505.3_316-338_as	AfsgsCfcCfaAfgAfAfAfgUfgAfaAfgAfcAfL96	909	usGfsuCfuUfuCfaCfuuuCfuUfgGfgCfuscsc	917
AD-67246	F12	2023	NM_000505.3_2025-2045_s	NM_000505.3_2023-2045_as	asasuaaaGfuGfCfUfuugaaaacguL96	910	asCfsguuUfuCfAfaagcAfcUfuuauusgsa	918
AD-67247	F12	2023	NM_000505.3_2025-2045_s	NM_000505.3_2023-2045_as	asasuaaaGfuGfCfUfuugaaaacguL96	911	AfsCfsguuUfuCfAfaagcAfcUfuuauusgsa	919
AD-67248	F12	438	NM_000029.3_440-460_s	NM_000029.3_438-460_as	csasgaaaGfaGfAfAfgugcuuugaaL96	912	usUfscaaAfgCfAfcuucUfcUfuucugsgsc	920
AD-67249	F12	438	NM_000029.3_440-460_s	NM_000029.3_438-460_as	csasgaaaGfaGfAfAfgugcuuugaaL96	913	UfsUfscaaAfgCfAfcuucUfcUfuucugsgsc	921

Table 19E. Unmodified sense and antisense strand sequences of agents targeting KNG1

Duplex Name	Target	Antisense Start Position on mRNA	Sense Position in GenBank Reference Sequence	Antisense Position in GenBank Reference Sequence	Sense Sequence	SEQ ID NO	Antisense Sequence	SEQ ID NO
AD-66259	KNG1	302	NM_000893.3_304-324_s	NM_000893.3_302-324_as	GAGGAAAUUGACUGCAAUGAA	922	UUCAUUGCAGUCAAUUUCCUCGG	928
AD-67344	KNG1	302	NM_000893.3_304-324_s	NM_000893.3_302-324_as	GAGGAAAUUGACUGCAAUGAA	923	UUCAUUGCAGUCAAUUUCCUCGG	929
AD-67345	KNG1	302	NM_000893.3_304-324_s	NM_000893.3_302-324_as	GAGGAAAUUGACUGCAAUGAA	924	UUCAUUGCAGUCAAUUUCCUCGG	930
AD-	KNG1	823	NM_000893.3	NM_000893.3_823-	UACCUACUCAAUUGUGCAAAA	925	UUUUGCACAAUUGAGUAGGUAAU	931

66262			_825-845_s	845_as			
AD-67346	KNG1	823	NM_000893.3_825-845_s	NM_000893.3_823-845_as	UACCUACUCAAUUGUGCAAAA	926	UUUUGCACAAUUGAGUAGGUAAU	932
AD-67347	KNG1	823	NM_000893.3_825-845_s	NM_000893.3_823-845_as	UACCUACUCAAUUGUGCAAAA	927	UUUUGCACAAUUGAGUAGGUAAU	933

Table 19F. Modified sense and antisense strand sequences of agents targeting KNG1

Duplex Name	Target	Antisense Start Position on mRNA	Sense Position in GenBank Reference Sequence	Antisense Position in GenBank Reference Sequence	Sense Sequence	SEQ ID NO	Antisense Sequence	SEQ ID NO
AD-66259	KNG1	302	NM_000893.3_304-324_s	NM_000893.3_30 2-324_as	GfsasGfgAfaAfuUfGfAfcUfgCfaAfuGfaA fL96	934	usUfscAfuUfgCfaGfucaAfuUfuCfcUfcsgsg	940
AD-67344	KNG1	302	NM_000893.3_304-324_s	NM_000893.3_30 2-324_as	gsasggaaAfuUfGfAfcugcaaugaaL96	935	usUfscauUfgCfAfgucaAfuUfuccucsgsg	941
AD-67345	KNG1	302	NM_000893.3_304-324_s	NM_000893.3_30 2-324_as	gsasggaaAfuUfGfAfcugcaaugaaL96	936	UfsUfscauUfgCfAfgucaAfuUfuccucsgsg	942
AD-66262	KNG1	823	NM_000893.3_825-845_s	NM_000893.3_82 3-845_as	UfsasCfcUfaCfuCfAfAfuUfgUfgCfaAfaA fL96	937	usUfsuUfgCfaCfaAfuugAfgUfaGfgUfasasu	943
AD-67346	KNG1	823	NM_000893.3_825-845_s	NM_000893.3_82 3-845_as	usasccuaCfuCfAfAfuugugcaaaaL96	938	usUfsuugCfaCfAfauugAfgUfagguasasu	944
AD-67347	KNG1	823	NM_000893.3_825-845_s	NM_000893.3_82 3-845_as	usasccuaCfuCfAfAfuugugcaaaaL96	939	UfsUfsuugCfaCfAfauugAfgUfagguasasu	945

Example 8. *In Vivo* KLKB1, F12, and KNG1 Silencing in ACE-Inhibitor Induced Vascular Permeability Mouse Model

To determine the *in vivo* efficacy of a single dose of a subset of the agents described above to reduce human KLKB1, F12, or KNG1 mRNA levels, wild-type C57BL/6 female mice were subcutaneously administered a single 0 mg/kg, 0.3 mg/kg 1 mg/kg, 3 mg/kg or 10 mg/kg dose of AD-66948 (targeting KLKB1), or a single 0 mg/kg, 0.1 mg/kg 0.3 mg/kg, 1 mg/kg or 3 mg/kg dose of AD-67244 (targeting F12), or a single 0 mg/kg, 0.3 mg/kg 1 mg/kg, 3 mg/kg or 10 mg/kg dose of AD-67344 (targeting KNG1). At day 7 post-dose, animals were intravenously administered 2.5 mg/kg of the angiotensin-converting enzyme (ACE) inhibitor, captopril, in order to induce vascular permeability. Fifteen minutes after administration of captopril, animals were intravenously administered 30 mg/kg Evans blue dye. Fifteen minutes after Evans Blue dye administration, animals were sacrificed and blood, intestine, and liver samples were collected. Evans Blue dye was extracted and quantified from the blood and intestine samples, and target mRNA levels were determined in the liver samples.

The results of these assays using an agent targeting KLKB1 (AD-66948) are shown in Figure 4. The results of these assays using an agent targeting F12 (AD-AD-67244) are shown in Figure 5. The results of these assays using an agent targeting KNG1 (AD-AD-67344) are shown in Figure 6.

Example 9. Synthesis and *In vitro* screening of F12 siRNA duplexes

Additional iRNA agents targeting F12 were designed, synsthesized and screened for *in vitro* efficacy, as described above. A detailed list of the additional unmodified F12 sense and antisense strand sequences is shown in Table 20. A detailed list of the additional modified F12 sense and antisense strand sequences is shown in Table 21. Table 22 shows the results of a single dose screen in Hep3b cells transfected with the indicated additional F12 iRNAs. Data are expressed as percent of mRNA remaining relative to AD-1955.

Table 20. F12 Unmodified Sequences

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Position in NM_000505.3	Antisense Sequence 5' to 3'	SEQ ID NO	Position in NM_000505.3
AD-70653	GACUCCUGGAUAGGCAGCU	946	12-30	AGCUGCCUAUCCAGGAGUC	1130	12-30
AD-70654	UAGGCAGCUGGACCAACGA	947	22-40	UCGUUGGUCCAGCUGCCUA	1131	22-40
AD-70655	ACCAACGGACGGAUGCCAU	948	33-51	AUGGCAUCCGUCCGUUGGU	1132	33-51
AD-70656	AUGCCAUGAGGGCUCUGCU	949	45-63	AGCAGAGCCCUCAUGGCAU	1133	45-63
AD-70657	GCUCUGCUGCUCCUGGGGU	950	56-74	ACCCCAGGAGCAGCAGAGC	1134	56-74
AD-70658	UCCUGGGGUUCCUGCUGGU	951	66-84	ACCAGCAGGAACCCCAGGA	1135	66-84
AD-70659	CUGCUGGUGAGCUUGGAGU	952	77-95	ACUCCAAGCUCACCAGCAG	1136	77-95
AD-70660	CUUGGAGUCAACACUUUCA	953	88-106	UGAAAGUGUUGACUCCAAG	1137	88-106
AD-70661	ACUUUCGAUUCCACCUUGA	954	100-118	UCAAGGUGGAAUCGAAAGU	1138	100-118
AD-70662	CCACCUUGGGAAGCCCCCA	955	110-128	UGGGGGCUUCCCAAGGUGG	1139	110-128
AD-70663	GCCCCCAAGGAGCAUAAGU	956	122-140	ACUUAUGCUCCUUGGGGGC	1140	122-140
AD-70664	CAUAAGUACAAAGCUGAAA	957	134-152	UUUCAGCUUUGUACUUAUG	1141	134-152
AD-70665	AAGCUGAAGAGCACACAGU	958	144-162	ACUGUGUGCUCUUCAGCUU	1142	144-162
AD-70666	ACACAGUCGUUCUCACUGU	959	156-174	ACAGUGAGAACGACUGUGU	1143	156-174
AD-70667	UUCUCACUGUCACCGGGGA	960	165-183	UCCCCGGUGACAGUGAGAA	1144	165-183
AD-70668	ACCGGGGAGCCCUGCCACU	961	176-194	AGUGGCAGGGCUCCCCGGU	1145	176-194
AD-70669	UGCCACUUCCCCUUCCAGU	962	188-206	ACUGGAAGGGGAAGUGGCA	1146	188-206
AD-70670	UUCCAGUACCACCGGCAGA	963	200-218	UCUGCCGGUGGUACUGGAA	1147	200-218
AD-70671	ACCGGCAGCUGUACCACAA	964	210-228	UUGUGGUACAGCUGCCGGU	1148	210-228
AD-70672	UACCACAAAUGUACCCACA	965	221-239	UGUGGGUACAUUUGUGGUA	1149	221-239
AD-70673	UACCCACAAGGGCCGGCCA	966	232-250	UGGCCGGCCCUUGUGGGUA	1150	232-250

AD-70674	GCCGGCCAGGCCCUCAGCA	967	243-261	UGCUGAGGGCCUGGCCGGC	1151	243-261
AD-70675	CUCAGCCCUGGUGUGCUAA	968	255-273	UUAGCACACCAGGGCUGAG	1152	255-273
AD-70676	UGUGCUACCACCCCCAACU	969	266-284	AGUUGGGGGUGGUAGCACA	1153	266-284
AD-70677	ACCCCCAACUUUGAUCAGA	970	275-293	UCUGAUCAAAGUUGGGGGU	1154	275-293
AD-70678	AUCAGGACCAGCGAUGGGA	971	288-306	UCCCAUCGCUGGUCCUGAU	1155	288-306
AD-70679	AGCGAUGGGGGAUACUGUUU	972	297-315	AAACAGUAUCCCCAUCGCU	1156	297-315
AD-70680	UACUGUUUGGAGCCCAAGA	973	308-326	UCUUGGGCUCCAAACAGUA	1157	308-326
AD-70681	CCAAGAAAGUGAAAGACCA	974	321-339	UGGUCUUUCACUUUCUUGG	1158	321-339
AD-70682	AAAGACCACUGCAGCAAAC	975	332-350	GUUUGCUGCAGUGGUCUUU	1159	332-350
AD-70683	UGCAGCAAACACAGCCCCU	976	341-359	AGGGGCUGUGUUUUGCUGCA	1160	341-359
AD-70684	AGCCCCUGCCAGAAAGGAA	977	353-371	UUCCUUUCUGGCAGGGGCU	1161	353-371
AD-70685	AGAAAGGAGGGACCUGUGU	978	363-381	ACACAGGUCCCUCCUUUCU	1162	363-381
AD-70686	ACCUGUGUGAACAUGCCAA	979	374-392	UUGGCAUGUUCACACAGGU	1163	374-392
AD-70687	AUGCCAAGCGGCCCCCACU	980	386-404	AGUGGGGGCCGCUUGGCAU	1164	386-404
AD-70688	GCCCCCACUGUCUCUGUCA	981	396-414	UGACAGAGACAGUGGGGGC	1165	396-414
AD-70689	CACCUCACUGGAAACCACU	982	419-437	AGUGGUUUCCAGUGAGGUG	1166	419-437
AD-70690	AACCACUGCCAGAAAGAGA	983	431-449	UCUCUUUCUGGCAGUGGUU	1167	431-449
AD-70691	CAGAAAGAGAAGUGCUUUA	984	440-458	UAAAGCACUUCUCUUUCUG	1168	440-458
AD-70692	UGCUUUGAGCCUCAGCUUA	985	452-470	UAAGCUGAGGCUCAAAGCA	1169	452-470
AD-70693	CAGCUUCUCCCGGUUUUUCA	986	464-482	UGAAAAACCGGAGAAGCUG	1170	464-482
AD-70694	CGGUUUUUCCCACAAGAAUA	987	473-491	UAUUCUUGUGGGAAAAACCG	1171	473-491
AD-70695	CAAGAAUGAGAGAUAUGGUAU	988	484-502	AUACCAUAUCUCAUUCUUG	1172	484-502
AD-70696	UAUGGUAUAGAACUGAGCA	989	495-513	UGCUCAGUUCUAUACCAUA	1173	495-513
AD-70697	UGAGCAAGCAGCUGUGGCA	990	508-526	UGCCACAGCUGCUUGCUCA	1174	508-526
AD-70698	GCUGUGGCCAGAUGCCAGU	991	518-536	ACUGGCAUCUGGCCACAGC	1175	518-536
AD-70699	AUGCCAGUGCAAGGGUCCU	992	529-547	AGGACCCUUGCACUGGCAU	1176	529-547

AD-70700	AAGGGUCCCUGAUGCCCACU	993	539-557	AGUGGGCAUCAGGACCCUU	1177	539-557
AD-70701	UGCCCCACUGCCAGCGGCUA	994	550-568	UAGCCGCUGGGCAGUGGGCA	1178	550-568
AD-70702	CGGCUGGGCCAGCCAGGCCU	995	563-581	AGGCCUGGCUGGCCAGCCG	1179	563-581
AD-70703	AGCCAGGCCUGCCGCACCA	996	572-590	UGGUGCGGCAGGCCUGGCU	1180	572-590
AD-70704	CGCACCAACCCGUGCCUCA	997	584-602	UGAGGCACGGGGUUGGUGCG	1181	584-602
AD-70705	UGCCUCCAUGGGGGUCGCU	998	596-614	AGCGACCCCCAUGGAGGCA	1182	596-614
AD-70706	GGGGUCGCUGCCUAGAGGU	999	606-624	ACCUCUAGGCAGCGACCCC	1183	606-624
AD-70707	CUAGAGGUGGAGGGCCACA	1000	617-635	UGUGGCCCUCCACCUCUAG	1184	617-635
AD-70708	AGGGCCACCGCCUGUGCCA	1001	627-645	UGGCACAGGCGGUGGCCCU	1185	627-645
AD-70709	UGUGCCACUGCCCGGUGGA	1002	639-657	UCCACCGGGCAGUGGCACA	1186	639-657
AD-70710	CGGUGGGCUACACCGGAGA	1003	651-669	UCUCCGGUGUAGCCCACCG	1187	651-669
AD-70711	ACCGGAGCCUUCUGCGACA	1004	662-680	UGUCGCAGAAGGCUCCGGU	1188	662-680
AD-70712	UUCUGCGACGUGGACACCA	1005	671-689	UGGUGUCCACGUCGCAGAA	1189	671-689
AD-70713	GACACCAAGGCAAGCUGCU	1006	683-701	AGCAGCUUGCCUUGGUGUC	1190	683-701
AD-70714	CAAGCUGCUAUGAUGGCCA	1007	693-711	UGGCCAUCAUAGCAGCUUG	1191	693-711
AD-70715	GAUGGCCGCGGCUCAGCU	1008	704-722	AGCUGAGCCCGCGGCCAUC	1192	704-722
AD-70716	UCAGCUACCGCGGCCUGGA	1009	717-735	UCCAGGCCGCGGUAGCUGA	1193	717-735
AD-70717	CGGCCUGGCCAGGACCACA	1010	727-745	UGUGGUCCUGGCCAGGCCG	1194	727-745
AD-70718	AGGACCACGCUCUCGGUA	1011	737-755	UACCCGAGAGCGUGGUCCU	1195	737-755
AD-70719	UCGGGUGCGCCCUGUCAGA	1012	749-767	UCUGACAGGGCGCACCCGA	1196	749-767
AD-70720	CUGUCAGCCGUGGGCCUCA	1013	760-778	UGAGGCCCACGGCUGACAG	1197	760-778
AD-70721	UGGGCCUCGGAGGCCACCU	1014	770-788	AGGUGGCCUCCCGAGGCCCA	1198	770-788
AD-70722	CCACCUACCGGAACGUGAA	1015	783-801	UUCACGUUCCGGUAGGUGG	1199	783-801
AD-70723	AACGUGACUGCCGAGCAAA	1016	794-812	UUUGCUCGGCAGUCACGUU	1200	794-812
AD-70724	CGAGCAAGCGCGGAACUGA	1017	805-823	UCAGUUCCGCGCUUGCUCG	1201	805-823
AD-70725	CGGAACUGGGGACUGGGCA	1018	815-833	UGCCCAGUCCCCCAGUUCCG	1202	815-833

AD-70726	GACUGGGCCGGCCACCGCCUU	1019	825-843	AAGGCGUGGCCGCCCAGUC	1203	825-843
AD-70727	ACGCCUUCUGCCGGAACCA	1020	837-855	UGGUUCCGGCAGAAGGCGU	1204	837-855
AD-70728	CGGAACCCGGACAACGACA	1021	848-866	UGUCGUUGUCCCGGGUUCCG	1205	848-866
AD-70729	AACGACAUCCGCCCGUGGU	1022	860-878	ACCACGGGCGGGAUGUCGUU	1206	860-878
AD-70730	GCCCGUGGUGCUUCGUGCU	1023	870-888	AGCACGAAGCACCACGGGC	1207	870-888
AD-70731	UUCGUGCUGAACCGCGACA	1024	881-899	UGUCGCGGUUCAGCACGAA	1208	881-899
AD-70732	ACCGCGACCGGCUGAGCUA	1025	891-909	UAGCUCAGCCGGUCGCGGU	1209	891-909
AD-70733	CUGAGCUGGGAGUACUGCA	1026	902-920	UGCAGUACUCCCAGCUCAG	1210	902-920
AD-70734	UACUGCGACCUGGCACAGU	1027	914-932	ACUGUGCCAGGUCGCAGUA	1211	914-932
AD-70735	UGGCACAGUGCCAGACCCA	1028	924-942	UGGGUCUGGCACUGUGCCA	1212	924-942
AD-70736	AGACCCCAACCCAGGCGGA	1029	936-954	UCCGCCUGGGUUGGGGUCU	1213	936-954
AD-70737	AGGCGGCGCCUCCGACCCA	1030	948-966	UGGGUCCGGAGGCGCCGCCU	1214	948-966
AD-70738	UCCGACCCCGGUGUCCCCU	1031	958-976	AGGGGACACCGGGGUCCGGA	1215	958-976
AD-70739	UGUCCCCUAGGCUUCAUGU	1032	969-987	ACAUGAAGCCUAGGGGACA	1216	969-987
AD-70740	UUCAUGUCCCACUCAUGCA	1033	981-999	UGCAUGAGUGGGGACAUGAA	1217	981-999
AD-70741	ACUCAUGCCCCGCGCAGCCA	1034	991-1009	UGGCUGCGCGGGGCAUGAGU	1218	991-1009
AD-70742	CGCAGCCGGCACCGCCGAA	1035	1002-1020	UUCGGCGGUGCCGGCUGCG	1219	1002-1020
AD-70743	ACCGCCGAAGCCUCAGCCA	1036	1012-1030	UGGCUGAGGCUUCGGCGGU	1220	1012-1030
AD-70744	UCAGCCCACGACCCGGACA	1037	1024-1042	UGUCCGGGUCGUGGGCUGA	1221	1024-1042
AD-70745	ACCCGGACCCCGCCUCAGU	1038	1034-1052	ACUGAGGCGGGGGUCCGGGU	1222	1034-1052
AD-70562	CCUCAGUCCCCAGACCCCGA	1039	1046-1064	UCGGGGUCUGGGGACUGAGG	1223	1046-1064
AD-70563	AGACCCCGGGAGCCUUGCA	1040	1056-1074	UGCAAGGCUCCCCGGGGUCU	1224	1056-1074
AD-70564	CCUUGCCGGCGAAGCGGGA	1041	1068-1086	UCCCGCUUCGCCGGCAAGG	1225	1068-1086
AD-70565	AAGCGGGAGCAGCCGCCUU	1042	1079-1097	AAGGCGGCUGCUCCCGCUU	1226	1079-1097
AD-70566	AGCCGCCUUCCCUGACCAA	1043	1089-1107	UUGGUCAGGGAAGGCGGCU	1227	1089-1107
AD-70567	UGACCAGGAACGGCCCACU	1044	1101-1119	AGUGGGCCGUUCCUGGUCA	1228	1101-1119

AD-70568	CGGCCCACUGAGCUGCGGA	1045	1111-1129	UCCGCAGCUCAGUGGGCCG	1229	1111-1129
AD-70569	UGCGGGCAGCGGCUCCGCA	1046	1124-1142	UGCGGAGCCGCUGCCCGCA	1230	1124-1142
AD-70570	CGGCUCCGCAAGAGUCUGU	1047	1133-1151	ACAGACUCUUGCGGAGCCG	1231	1133-1151
AD-70571	AGUCUGUCUUCGAUGACCA	1048	1145-1163	UGGUCAUCGAAGACAGACU	1232	1145-1163
AD-70572	CGAUGACCCGCGUCGUUGA	1049	1155-1173	UCAACGACGCGGGUCAUCG	1233	1155-1173
AD-70573	UCGUUGGCGGGCUGGUGGA	1050	1167-1185	UCCACCAGCCCCGCCAACGA	1234	1167-1185
AD-70574	UGGUGGCGCUACGCGGGGA	1051	1179-1197	UCCCCGCGUAGCGCCACCA	1235	1179-1197
AD-70575	UACGCGGGGCGCACCCCUA	1052	1188-1206	UAGGGGUGCGCCCCGCGUA	1236	1188-1206
AD-70576	ACCCCUACAUCGCCGCGCU	1053	1200-1218	AGCGCGGCGAUGUAGGGGU	1237	1200-1218
AD-70577	GCCGCGCUGUACUGGGGCA	1054	1211-1229	UGCCCCAGUACAGCGCGGC	1238	1211-1229
AD-70578	CUGGGGCCACAGUUUCUGA	1055	1222-1240	UCAGAAACUGUGGCCCCAG	1239	1222-1240
AD-70579	UUUCUGCGCCGGCAGCCUA	1056	1234-1252	UAGGCUGCCGGCGCAGAAA	1240	1234-1252
AD-70580	CGGCAGCCUCAUCGCCCCA	1057	1243-1261	UGGGGCGAUGAGGCUGCCG	1241	1243-1261
AD-70581	UCGCCCCCUGCUGGGUGCU	1058	1254-1272	AGCACCCAGCAGGGGGCGA	1242	1254-1272
AD-70582	UGGGUGCUGACGGCCGCUA	1059	1265-1283	UAGCGGCCGUCAGCACCCA	1243	1265-1283
AD-70583	GCCGCUCACUGCCUGCAGA	1060	1277-1295	UCUGCAGGCAGUGAGCGGC	1244	1277-1295
AD-70584	CUGCAGGACCGGCCCGCAA	1061	1289-1307	UUGCGGGCCGGUCCUGCAG	1245	1289-1307
AD-70585	GGCCCGCACCCGAGGAUCU	1062	1299-1317	AGAUCCUCGGGUGCGGGCC	1246	1299-1317
AD-70586	CGAGGAUCUGACGGUGGUA	1063	1309-1327	UACCACCGUCAGAUCCUCG	1247	1309-1327
AD-70587	GUGGUGCUCGGCCAGGAAA	1064	1322-1340	UUUCCUGGCCGAGCACCAC	1248	1322-1340
AD-70588	GCCAGGAACGCCGUAACCA	1065	1332-1350	UGGUUACGGCGUUCCUGGC	1249	1332-1350
AD-70589	CGUAACCACAGCUGUGAGA	1066	1343-1361	UCUCACAGCUGUGGUUACG	1250	1343-1361
AD-70590	UGUGAGCCGUGCCAGACGU	1067	1355-1373	ACGUCUGGCACGGCUCACA	1251	1355-1373
AD-70591	UGCCAGACGUUGGCCGUGA	1068	1364-1382	UCACGGCCAACGUCUGGCA	1252	1364-1382
AD-70592	GCCGUGCGCUCCUACCGCU	1069	1376-1394	AGCGGUAGGAGCGCACGGC	1253	1376-1394
AD-70593	UACCGCUUGCACGAGGCCU	1070	1388-1406	AGGCCUCGUGCAAGCGGUA	1254	1388-1406

AD-70594	ACGAGGCCUUCUCGCCCGU	1071	1398-1416	ACGGGCGAGAAGGCCUCGU	1255	1398-1416
AD-70595	UCGCCCGUCAGCUACCAGA	1072	1409-1427	UCUGGUAGCUGACGGGCGA	1256	1409-1427
AD-70596	CUACCAGCACGACCUGGCU	1073	1420-1438	AGCCAGGUCGUGCUGGUAG	1257	1420-1438
AD-70597	ACCUGGCUCUGUUGCGCCU	1074	1431-1449	AGGCGCAACAGAGCCAGGU	1258	1431-1449
AD-70598	UUGCGCCUUCAGGAGGAUA	1075	1442-1460	UAUCCUCCUGAAGGCGCAA	1259	1442-1460
AD-70599	GAGGAUGCGGACGGCAGCU	1076	1454-1472	AGCUGCCGUCCGCAUCCUC	1260	1454-1472
AD-70600	ACGGCAGCUGCGCGCUCCU	1077	1464-1482	AGGAGCGCGCAGCUGCCGU	1261	1464-1482
AD-70601	CGCUCCUGUCGCCUUACGU	1078	1476-1494	ACGUAAGGCGACAGGAGCG	1262	1476-1494
AD-70602	CCUUACGUUCAGCCGGUGU	1079	1487-1505	ACACCGGCUGAACGUAAGG	1263	1487-1505
AD-70603	AGCCGGUGUGCCUGCCAAA	1080	1497-1515	UUUGGCAGGCACACCGGCU	1264	1497-1515
AD-70604	UGCCAAGCGGCGCCGCGCA	1081	1509-1527	UGCGCGGCGCCGCUUGGCA	1265	1509-1527
AD-70605	GCGCCGCGCGACCCUCCGA	1082	1518-1536	UCGGAGGGUCGCGCGGCGC	1266	1518-1536
AD-70606	CCCUCCGAGACCACGCUCU	1083	1529-1547	AGAGCGUGGUCUCGGAGGG	1267	1529-1547
AD-70607	CGCUCUGCCAGGUGGCCGA	1084	1542-1560	UCGGCCACCUGGCAGAGCG	1268	1542-1560
AD-70608	AGGUGGCCGGCUGGGGCCA	1085	1551-1569	UGGCCCCAGCCGGCCACCU	1269	1551-1569
AD-70609	UGGGGCCACCAGUUCGAGA	1086	1562-1580	UCUCGAACUGGUGGCCCCA	1270	1562-1580
AD-70610	UUCGAGGGGGCGGAGGAAU	1087	1574-1592	AUUCCUCCGCCCCCUCGAA	1271	1574-1592
AD-70611	CGGAGGAAUAUGCCAGCUU	1088	1584-1602	AAGCUGGCAUAUUCCUCCG	1272	1584-1602
AD-70612	CAGCUUCCUGCAGGAGGCA	1089	1597-1615	UGCCUCCUGCAGGAAGCUG	1273	1597-1615
AD-70613	AGGAGGCGCAGGUACCGUU	1090	1608-1626	AACGGUACCUGCGCCUCCU	1274	1608-1626
AD-70614	AGGUACCGUUCCUCUCCCU	1091	1617-1635	AGGGAGAGGAACGGUACCU	1275	1617-1635
AD-70615	CUCUCCCUGGAGCGCUGCU	1092	1628-1646	AGCAGCGCUCCCAGGGAGAG	1276	1628-1646
AD-70616	CGCUGCUCAGCCCCCGGACA	1093	1640-1658	UGUCCCGGGGCUGAGCAGCG	1277	1640-1658
AD-70617	CCGGACGUGCACGGAUCCU	1094	1652-1670	AGGAUCCGUGCACGUCCGG	1278	1652-1670
AD-70618	CGGAUCCUCCAUCCUCCCA	1095	1663-1681	UGGGAGGAUGGAGGGAUCCG	1279	1663-1681
AD-70619	CAUCCUCCCCCGGCAUGCUA	1096	1672-1690	UAGCAUGCCGGGGAGGGAUG	1280	1672-1690

AD-70620	CAUGCUCUGCGCAGGGUUA	1097	1684-1702	UAACCCUGCGCAGAGCAUG	1281	1684-1702
AD-70621	AGGGUUCCUCGAGGGCGGA	1098	1696-1714	UCCGCCCUCGAGGAACCCU	1282	1696-1714
AD-70622	GAGGGCGGCACCGAUGCGU	1099	1706-1724	ACGCAUCGGUGCCGCCCUC	1283	1706-1724
AD-70623	GAUGCGUGCCAGGGUGAUU	1100	1718-1736	AAUCACCCUGGCACGCAUC	1284	1718-1736
AD-70624	AGGGUGAUUCCGGAGGCCA	1101	1728-1746	UGGCCUCCGGAAUCACCCU	1285	1728-1746
AD-70625	CGGAGGCCCGCUGGUGUGU	1102	1738-1756	ACACACCAGCGGGCCUCCG	1286	1738-1756
AD-70626	GGUGUGUGAGGACCAAGCU	1103	1750-1768	AGCUUGGUCCUCACACACC	1287	1750-1768
AD-70627	CCAAGCUGCAGAGCGCCGA	1104	1762-1780	UCGGCGCUCUGCAGCUUGG	1288	1762-1780
AD-70628	AGAGCGCCGGCUCACCCUA	1105	1771-1789	UAGGGUGAGCCGGCGCUCU	1289	1771-1789
AD-70629	UCACCCUGCAAGGCAUCAU	1106	1782-1800	AUGAUGCCUUGCAGGGUGA	1290	1782-1800
AD-70630	GGCAUCAUCAGCUGGGGAU	1107	1793-1811	AUCCCCAGCUGAUGAUGCC	1291	1793-1811
AD-70631	CUGGGGAUCGGGCUGUGGU	1108	1804-1822	ACCACAGCCCGAUCCCCAG	1292	1804-1822
AD-70632	UGUGGUGACCGCAACAAGA	1109	1817-1835	UCUUGUUGCGGUCACCACA	1293	1817-1835
AD-70633	CAACAAGCCAGGCGUCUAA	1110	1828-1846	UUAGACGCCUGGCUUGUUG	1294	1828-1846
AD-70634	AGGCGUCUACACCGAUGUA	1111	1837-1855	UACAUCGGUGUAGACGCCU	1295	1837-1855
AD-70635	GAUGUGGCCUACUACCUGA	1112	1850-1868	UCAGGUAGUAGGCCACAUC	1296	1850-1868
AD-70636	UACUACCUGGCCUGGAUCA	1113	1859-1877	UGAUCCAGGCCAGGUAGUA	1297	1859-1877
AD-70637	CUGGAUCCGGGAGCACACA	1114	1870-1888	UGUGUGCUCCCGGAUCCAG	1298	1870-1888
AD-70638	AGCACACCGUUUCCUGAUU	1115	1881-1899	AAUCAGGAAACGGUGUGCU	1299	1881-1899
AD-70639	UCCUGAUUGCUCAGGGACU	1116	1892-1910	AGUCCCUGAGCAAUCAGGA	1300	1892-1910
AD-70640	CAGGGACUCAUCUUUCCCU	1117	1903-1921	AGGGAAAGAUGAGUCCCUG	1301	1903-1921
AD-70641	UUUCCCUCCUUGGUGAUUA	1118	1915-1933	UAAUCACCAAGGAGGGAAA	1302	1915-1933
AD-70642	UGGUGAUUCCGCAGUGAGA	1119	1925-1943	UCUCACUGCGGAAUCACCA	1303	1925-1943
AD-70643	AGUGAGAGAGUGGCUGGGA	1120	1937-1955	UCCCAGCCACUCUCUCACU	1304	1937-1955
AD-70644	GCUGGGGCAUGGAAGGCAA	1121	1949-1967	UUGCCUUCCAUGCCCCAGC	1305	1949-1967
AD-70645	UGGAAGGCAAGAUUGUGUA	1122	1958-1976	UACACAAUCUUGCCUUCCA	1306	1958-1976

AD-70646	UUGUGUCCCAUUCCCCCAA	1123	1970-1988	UUGGGGGAAUGGGACACAA	1307	1970-1988
AD-70647	UCCCCCAGUGCGGCCAGCU	1124	1981-1999	AGCUGGCCGCACUGGGGGA	1308	1981-1999
AD-70648	GCCAGCUCCGCGCCAGGAU	1125	1993-2011	AUCCUGGCGCGGAGCUGGC	1309	1993-2011
AD-70649	GCCAGGAUGGCGCAGGAAA	1126	2004-2022	UUUCCUGCGCCAUCCUGGC	1310	2004-2022
AD-70650	GCAGGAACUCAAUAAAGUA	1127	2015-2033	UACUUUAUUGAGUUCCUGC	1311	2015-2033
AD-70651	AAUAAAGUGCUUUGAAAAU	1128	2025-2043	AUUUUCAAAGCACUUUAUU	1312	2025-2043
AD-70652	UUGAAAAUGCUGAGAAAAA	1129	2036-2054	UUUUUCUCAGCAUUUUCAA	1313	2036-2054

Table 21. F12 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-70653	GACUCCUGGAUAGGCAGCUdTdT	1314	AGCUGCCUAUCCAGGAGUCdTdT	1498	GACUCCUGGAUAGGCAGCU	1682
AD-70654	UAGGCAGCUGGACCAACGAdTdT	1315	UCGUUGGUCCAGCUGCCUAdTdT	1499	UAGGCAGCUGGACCAACGG	1683
AD-70655	ACCAACGGACGGAUGCCAUdTdT	1316	AUGGCAUCCGUCCGUUGGUdTdT	1500	ACCAACGGACGGAUGCCAU	1684
AD-70656	AUGCCAUGAGGGCUCUGCUdTdT	1317	AGCAGAGCCCUCAUGGCAUdTdT	1501	AUGCCAUGAGGGCUCUGCU	1685
AD-70657	GCUCUGCUGCUCCUGGGGUdTdT	1318	ACCCCAGGAGCAGCAGAGCdTdT	1502	GCUCUGCUGCUCCUGGGGU	1686
AD-70658	UCCUGGGGUUCCUGCUGGUdTdT	1319	ACCAGCAGGAACCCCAGGAdTdT	1503	UCCUGGGGUUCCUGCUGGU	1687
AD-70659	CUGCUGGUGAGCUUGGAGUdTdT	1320	ACUCCAAGCUCACCAGCAGdTdT	1504	CUGCUGGUGAGCUUGGAGU	1688
AD-70660	CUUGGAGUCAACACUUUCAdTdT	1321	UGAAAGUGUUGACUCCAAGdTdT	1505	CUUGGAGUCAACACUUUCG	1689
AD-70661	ACUUUCGAUUCCACCUUGAdTdT	1322	UCAAGGUGGAAUCGAAAGUdTdT	1506	ACUUUCGAUUCCACCUUGG	1690
AD-70662	CCACCUUGGGAAGCCCCCAdTdT	1323	UGGGGGCUUCCCAAGGUGGdTdT	1507	CCACCUUGGGAAGCCCCCA	1691
AD-70663	GCCCCCAAGGAGCAUAAGUdTdT	1324	ACUUAUGCUCCUUGGGGGCdTdT	1508	GCCCCCAAGGAGCAUAAGU	1692
AD-70664	CAUAAGUACAAAGCUGAAAdTdT	1325	UUUCAGCUUUGUACUUAUGdTdT	1509	CAUAAGUACAAAGCUGAAG	1693
AD-70665	AAGCUGAAGAGCACACAGUdTdT	1326	ACUGUGUGCUCUUCAGCUUdTdT	1510	AAGCUGAAGAGCACACAGU	1694
AD-70666	ACACAGUCGUUCUCACUGUdTdT	1327	ACAGUGAGAACGACUGUGUdTdT	1511	ACACAGUCGUUCUCACUGU	1695
AD-70667	UUCUCACUGUCACCGGGGAdTdT	1328	UCCCCGGUGACAGUGAGAAdTdT	1512	UUCUCACUGUCACCGGGGA	1696

AD-70668	ACCGGGGAGCCCUGCCACUdTdT	1329	AGUGGCAGGGCUCCCCCGGUdTdT	1513	ACCGGGGAGCCCUGCCACU	1697
AD-70669	UGCCACUUCCCCUUCCAGUdTdT	1330	ACUGGAAGGGGAAGUGGCAdTdT	1514	UGCCACUUCCCCUUCCAGU	1698
AD-70670	UUCCAGUACCACCGGCAGAdTdT	1331	UCUGCCGGUGGUACUGGAAdTdT	1515	UUCCAGUACCACCGGCAGC	1699
AD-70671	ACCGGCAGCUGUACCACAAdTdT	1332	UUGUGGUACAGCUGCCGGUdTdT	1516	ACCGGCAGCUGUACCACAA	1700
AD-70672	UACCACAAAUGUACCCACAdTdT	1333	UGUGGGUACAUUUGUGGUAdTdT	1517	UACCACAAAUGUACCCACA	1701
AD-70673	UACCCACAAGGGCCGGCCAdTdT	1334	UGGCCGGCCCUUGUGGGUAdTdT	1518	UACCCACAAGGGCCGGCCA	1702
AD-70674	GCCGGCCAGGCCCUCAGCAdTdT	1335	UGCUGAGGGCCUGGCCGGCdTdT	1519	GCCGGCCAGGCCCUCAGCC	1703
AD-70675	CUCAGCCCUGGUGUGCUAAdTdT	1336	UUAGCACACCAGGGCUGAGdTdT	1520	CUCAGCCCUGGUGUGCUAC	1704
AD-70676	UGUGCUACCACCCCCAACUdTdT	1337	AGUUGGGGGUGGUAGCACAdTdT	1521	UGUGCUACCACCCCCAACU	1705
AD-70677	ACCCCCAACUUUGAUCAGAdTdT	1338	UCUGAUCAAAGUUGGGGGUdTdT	1522	ACCCCCAACUUUGAUCAGG	1706
AD-70678	AUCAGGACCAGCGAUGGGAdTdT	1339	UCCCAUCGCUGGUCCUGAUdTdT	1523	AUCAGGACCAGCGAUGGGG	1707
AD-70679	AGCGAUGGGGAUACUGUUUUdTdT	1340	AAACAGUAUCCCCAUCGCUdTdT	1524	AGCGAUGGGGAUACUGUUU	1708
AD-70680	UACUGUUUGGAGCCCAAGAdTdT	1341	UCUUGGGCUCCAAACAGUAdTdT	1525	UACUGUUUGGAGCCCAAGA	1709
AD-70681	CCAAGAAAGUGAAAGACCAdTdT	1342	UGGUCUUUUCACUUUCUUGGdTdT	1526	CCAAGAAAGUGAAAGACCA	1710
AD-70682	AAAGACCACUGCAGCAAACdTdT	1343	GUUUGCUGCAGUGGUCUUUUdTdT	1527	AAAGACCACUGCAGCAAAC	1711
AD-70683	UGCAGCAAACACAGCCCCUdTdT	1344	AGGGGCUGUGUUUUGCUGCAdTdT	1528	UGCAGCAAACACAGCCCCU	1712
AD-70684	AGCCCCUGCCAGAAAGGAAdTdT	1345	UUCCUUUUCUGGCAGGGGCUdTdT	1529	AGCCCCUGCCAGAAAGGAG	1713
AD-70685	AGAAAGGAGGGACCUGUGUdTdT	1346	ACACAGGUCCCCUCUUUCUdTdT	1530	AGAAAGGAGGGACCUGUGU	1714
AD-70686	ACCUGUGUGAACAUGCCCAAdTdT	1347	UUGGCAUGUUCACACAGGUdTdT	1531	ACCUGUGUGAACAUGCCAA	1715
AD-70687	AUGCCAAGCGGCCCCCACUdTdT	1348	AGUGGGGGGCCGCUUGGCAUdTdT	1532	AUGCCAAGCGGCCCCCACU	1716
AD-70688	GCCCCCACUGUCUCUGUCAdTdT	1349	UGACAGAGACAGUGGGGGCdTdT	1533	GCCCCCACUGUCUCUGUCC	1717
AD-70689	CACCUCACUGGAAACCACUdTdT	1350	AGUGGUUUCCAGUGAGGUGdTdT	1534	CACCUCACUGGAAACCACU	1718
AD-70690	AACCACUGCCAGAAAGAGAdTdT	1351	UCUCUUUCUGGCAGUGGUUdTdT	1535	AACCACUGCCAGAAAGAGA	1719
AD-70691	CAGAAAGAGAAGUGCUUUAdTdT	1352	UAAAGCACUUUCUCUUUCUGdTdT	1536	CAGAAAGAGAAGUGCUUUG	1720
AD-70692	UGCUUUGAGCCUCAGCUUAdTdT	1353	UAAGCUGAGGCUCAAAGCAdTdT	1537	UGCUUUGAGCCUCAGCUUC	1721
AD-70693	CAGCUUCUCCGGUUUUUUCAdTdT	1354	UGAAAAAACCGGAGAAGCUGdTdT	1538	CAGCUUCUCCGGUUUUUCC	1722

AD-70694	CGGUUUUUCCACAAGAAUAdTdT	1355	UAUUCUUGUGGAAAAACCGdTdT	1539	CGGUUUUUCCACAAGAAUG	1723
AD-70695	CAAGAAUGAGAUAUGGUAUdTdT	1356	AUACCAUAUCUCAUUCUUGdTdT	1540	CAAGAAUGAGAUAUGGUAU	1724
AD-70696	UAUGGUAUAGAACUGAGCAdTdT	1357	UGCUCAGUUCUAUACCAUAdTdT	1541	UAUGGUAUAGAACUGAGCA	1725
AD-70697	UGAGCAAGCAGCUGUGGCAdTdT	1358	UGCCACAGCUGCUUGCUCAdTdT	1542	UGAGCAAGCAGCUGUGGCC	1726
AD-70698	GCUGUGGCCAGAUGCCAGUdTdT	1359	ACUGGCAUCUGGCCACAGCdTdT	1543	GCUGUGGCCAGAUGCCAGU	1727
AD-70699	AUGCCAGUGCAAGGGUCCUdTdT	1360	AGGACCCUUGCACUGGCAUdTdT	1544	AUGCCAGUGCAAGGGUCCU	1728
AD-70700	AAGGGUCCCUGAUGCCCACUdTdT	1361	AGUGGGCAUCAGGACCCUUdTdT	1545	AAGGGUCCCUGAUGCCCACU	1729
AD-70701	UGCCCACUGCCAGCGGCUAdTdT	1362	UAGCCGCUGGCAGUGGGCAdTdT	1546	UGCCCACUGCCAGCGGCUG	1730
AD-70702	CGGCUGGCCAGCCAGGCCUdTdT	1363	AGGCCUGGCUGGCCAGCCGdTdT	1547	CGGCUGGCCAGCCAGGCCU	1731
AD-70703	AGCCAGGCCUGCCGCACCAdTdT	1364	UGGUGCGGCAGGCCUGGCUdTdT	1548	AGCCAGGCCUGCCGCACCA	1732
AD-70704	CGCACCAACCCGUGCCUCAdTdT	1365	UGAGGCACGGGUUGGUGCGdTdT	1549	CGCACCAACCCGUGCCUCC	1733
AD-70705	UGCCUCCAUGGGGGUCGCUdTdT	1366	AGCGACCCCCCAUGGAGGCAdTdT	1550	UGCCUCCAUGGGGGUCGCU	1734
AD-70706	GGGGUCGCUGCCUAGAGGUdTdT	1367	ACCUCUAGGCAGCGACCCCCdTdT	1551	GGGGUCGCUGCCUAGAGGU	1735
AD-70707	CUAGAGGUGGAGGGCCACAdTdT	1368	UGUGGCCCUCCACCUCUAGdTdT	1552	CUAGAGGUGGAGGGCCACC	1736
AD-70708	AGGGCCACCGCCUGUGCCAdTdT	1369	UGGCACAGGCGGUGGCCCUdTdT	1553	AGGGCCACCGCCUGUGCCA	1737
AD-70709	UGUGCCACUGCCCGGUGGAdTdT	1370	UCCACCGGGCAGUGGCACAdTdT	1554	UGUGCCACUGCCCGGUGGG	1738
AD-70710	CGGUGGGCUACACCGGAGAdTdT	1371	UCUCCGGUGUAGCCCACCGdTdT	1555	CGGUGGGCUACACCGGAGC	1739
AD-70711	ACCGGAGCCUUCUGCGACAdTdT	1372	UGUCGCAGAAGGCUCCGGUdTdT	1556	ACCGGAGCCUUCUGCGACG	1740
AD-70712	UUCUGCGACGUGGACACCAdTdT	1373	UGGUGUCCACGUCGCAGAAdTdT	1557	UUCUGCGACGUGGACACCA	1741
AD-70713	GACACCAAGGCAAGCUGCUdTdT	1374	AGCAGCUUGCCUUGGUGUCdTdT	1558	GACACCAAGGCAAGCUGCU	1742
AD-70714	CAAGCUGCUAUGAUGGGCCAdTdT	1375	UGGCCAUCAUAGCAGCUUGdTdT	1559	CAAGCUGCUAUGAUGGCCG	1743
AD-70715	GAUGGCCGCGGGCUCAGCUdTdT	1376	AGCUGAGCCCGCGGCCAUCdTdT	1560	GAUGGCCGCGGGCUCAGCU	1744
AD-70716	UCAGCUACCGCGGCCUGGAdTdT	1377	UCCAGGCCGCGGUAGCUGAdTdT	1561	UCAGCUACCGCGGCCUGGC	1745
AD-70717	CGGCCUGGGCCAGGACCACAdTdT	1378	UGUGGUCCUGGCCAGGCCGdTdT	1562	CGGCCUGGGCCAGGACCACG	1746
AD-70718	AGGACCACGCUCUCGGGUAdTdT	1379	UACCCGAGAGGCGUGGUCCUdTdT	1563	AGGACCACGCUCUCGGGUG	1747
AD-70719	UCGGGUGCGCCCUGUCAGAdTdT	1380	UCUGACAGGGCGCACCCGAdTdT	1564	UCGGGUGCGCCCUGUCAGC	1748

AD-70720	CUGUCAGCCGUGGGCCUCAdTdT	1381	UGAGGCCCACGGCUGACAGdTdT	1565	CUGUCAGCCGUGGGCCUCG	1749
AD-70721	UGGGCCUCGGAGGCCACCUdTdT	1382	AGGUGGCCUCCCGAGGCCCAdTdT	1566	UGGGCCUCGGAGGCCACCU	1750
AD-70722	CCACCUACCGGAACGUGAAdTdT	1383	UUCACGUUCCGGUAGGUGGdTdT	1567	CCACCUACCGGAACGUGAC	1751
AD-70723	AACGUGACUGCCGAGCAAAAdTdT	1384	UUUGCUCGGCAGUCACGUUdTdT	1568	AACGUGACUGCCGAGCAAG	1752
AD-70724	CGAGCAAGCGCGGAACUGAdTdT	1385	UCAGUUCCGCGCUUGCUCGdTdT	1569	CGAGCAAGCGCGGAACUGG	1753
AD-70725	CGGAACUGGGGACUGGGCAdTdT	1386	UGCCCAGUCCCCCAGUUCCGdTdT	1570	CGGAACUGGGGACUGGGCG	1754
AD-70726	GACUGGGCGGCCACGCCUUdTdT	1387	AAGGCGUGGCCGCCCAGUCdTdT	1571	GACUGGGCGGCCACGCCUU	1755
AD-70727	ACGCCUUCUGCCGGAACCAdTdT	1388	UGGUUCCGGCAGAAGGCGUdTdT	1572	ACGCCUUCUGCCGGAACCC	1756
AD-70728	CGGAACCCGGACAACGACAdTdT	1389	UGUCGUUGUCCCGGGUUCCGdTdT	1573	CGGAACCCGGACAACGACA	1757
AD-70729	AACGACAUCCGCCCGUGGUdTdT	1390	ACCACGGGCGGAUGUCGUUdTdT	1574	AACGACAUCCGCCCGUGGU	1758
AD-70730	GCCCGUGGUGCUUCGUGCUdTdT	1391	AGCACGAAGCACCACGGGCdTdT	1575	GCCCGUGGUGCUUCGUGCU	1759
AD-70731	UUCGUGCUGAACCGCGACAdTdT	1392	UGUCGCGGUUCAGCACGAAdTdT	1576	UUCGUGCUGAACCGCGACC	1760
AD-70732	ACCGCGACCGGCUGAGCUAdTdT	1393	UAGCUCAGCCGGUCGCGGUdTdT	1577	ACCGCGACCGGCUGAGCUG	1761
AD-70733	CUGAGCUGGGAGUACUGCAdTdT	1394	UGCAGUACUCCCAGCUCAGdTdT	1578	CUGAGCUGGGAGUACUGCG	1762
AD-70734	UACUGCGACCUGGCACAGUdTdT	1395	ACUGUGCCAGGUCGCAGUAdTdT	1579	UACUGCGACCUGGCACAGU	1763
AD-70735	UGGCACAGUGCCAGACCCAdTdT	1396	UGGGUCUGGCACUGUGCCAdTdT	1580	UGGCACAGUGCCAGACCCC	1764
AD-70736	AGACCCCAACCCAGGCGGAdTdT	1397	UCCGCCUGGGUUGGGGUCUdTdT	1581	AGACCCCAACCCAGGCGGC	1765
AD-70737	AGGCGGCGCCUCCCGACCCAdTdT	1398	UGGGUCCGGAGGCGCCGCCUdTdT	1582	AGGCGGCGCCUCCCGACCCC	1766
AD-70738	UCCGACCCCGGUGUCCCCUdTdT	1399	AGGGGACACCGGGGUCGGAdTdT	1583	UCCGACCCCGGUGUCCCCU	1767
AD-70739	UGUCCCCUAGGCUUCAUGUdTdT	1400	ACAUGAAGCCUAGGGGACAdTdT	1584	UGUCCCCUAGGCUUCAUGU	1768
AD-70740	UUCAUGUCCCACUCAUGCAdTdT	1401	UGCAUGAGUGGGACAUGAAdTdT	1585	UUCAUGUCCCACUCAUGCC	1769
AD-70741	ACUCAUGCCCCGCGCAGCCAdTdT	1402	UGGCUGCGCGGGCAUGAGUdTdT	1586	ACUCAUGCCCCGCGCAGCCG	1770
AD-70742	CGCAGCCGGCACCGCCGAAdTdT	1403	UUCGGCCGGUGCCGGCUGCGdTdT	1587	CGCAGCCGGCACCGCCGAA	1771
AD-70743	ACCGCCGAAGCCUCAGCCAdTdT	1404	UGGCUGAGGCUUCGGCGGUdTdT	1588	ACCGCCGAAGCCUCAGCCC	1772
AD-70744	UCAGCCCACGACCCGGACAdTdT	1405	UGUCCCGGGUCGUGGGGCUGAdTdT	1589	UCAGCCCACGACCCGGACC	1773
AD-70745	ACCCGGACCCCCGCCUCAGUdTdT	1406	ACUGAGGCGGGGGUCCGGGUdTdT	1590	ACCCGGACCCCCGCCUCAGU	1774

AD-70562	CCUCAGUCCCAGACCCCGAdTdT	1407	UCGGGGUCUGGGACUGAGGdTdT	1591	CCUCAGUCCCAGACCCCGG	1775
AD-70563	AGACCCCGGGAGCCUUGCAdTdT	1408	UGCAAGGCUCCCCGGGGUCUdTdT	1592	AGACCCCGGGAGCCUUGCC	1776
AD-70564	CCUUGCCGGCGAAGCGGGAdTdT	1409	UCCCGCUUCGCCGGCAAGGdTdT	1593	CCUUGCCGGCGAAGCGGGA	1777
AD-70565	AAGCGGGAGCAGCCGCCUUdTdT	1410	AAGGCGGCUGCUCCCCGCUUdTdT	1594	AAGCGGGAGCAGCCGCCUU	1778
AD-70566	AGCCGCCUUCCCUGACCAAdTdT	1411	UUGGUCAGGGAAGGCGGCUdTdT	1595	AGCCGCCUUCCCCUGACCAG	1779
AD-70567	UGACCAGGAACGGCCCACUdTdT	1412	AGUGGGCCGUUCCUGGUCAdTdT	1596	UGACCAGGAACGGCCCACU	1780
AD-70568	CGGCCCACUGAGCUGCGGAdTdT	1413	UCCGCAGCUCAGUGGGCCGdTdT	1597	CGGCCCACUGAGCUGCGGG	1781
AD-70569	UGCGGGCAGCGGCUCCGCAdTdT	1414	UGCGGAGCCGCUGCCCCGCAdTdT	1598	UGCGGGCAGCGGCUCCGCA	1782
AD-70570	CGGCUCCGCAAGAGUCUGUdTdT	1415	ACAGACUCUUGCGGAGCCGdTdT	1599	CGGCUCCGCAAGAGUCUGU	1783
AD-70571	AGUCUGUCUUCGAUGACCAdTdT	1416	UGGUCAUCGAAGACAGACUdTdT	1600	AGUCUGUCUUCGAUGACCC	1784
AD-70572	CGAUGACCCGCGUCGUUGAdTdT	1417	UCAACGACGCGGGUCAUCGdTdT	1601	CGAUGACCCGCGUCGUUGG	1785
AD-70573	UCGUUGGCGGGCUGGUGGAdTdT	1418	UCCACCAGCCCGCCAACGAdTdT	1602	UCGUUGGCGGGCUGGUGGC	1786
AD-70574	UGGUGGCGCUACGCGGGGAdTdT	1419	UCCCCGCGUAGCGCCACCAdTdT	1603	UGGUGGCGCUACGCGGGGC	1787
AD-70575	UACGCGGGGCGCACCCCUAdTdT	1420	UAGGGGUGCGCCCCGCGUAdTdT	1604	UACGCGGGGCGCACCCCUA	1788
AD-70576	ACCCCUACAUCGCCGCGCUdTdT	1421	AGCGCGGCGAUGUAGGGGUdTdT	1605	ACCCCUACAUCGCCGCGCU	1789
AD-70577	GCCGCGCUGUACUGGGGCAdTdT	1422	UGCCCCAGUACAGCGCGGCdTdT	1606	GCCGCGCUGUACUGGGGCC	1790
AD-70578	CUGGGGCCACAGUUUCUGAdTdT	1423	UCAGAAACUGUGGCCCCAGdTdT	1607	CUGGGGCCACAGUUUCUGC	1791
AD-70579	UUUCUGCGCCGGCAGCCUAdTdT	1424	UAGGCUGCCCGGCGCAGAAAdTdT	1608	UUUCUGCGCCGGCAGCCUC	1792
AD-70580	CGGCAGCCUCAUCGCCCCAdTdT	1425	UGGGGCGAUGAGGCUGCCGdTdT	1609	CGGCAGCCUCAUCGCCCCC	1793
AD-70581	UCGCCCCCCUGCUGGGUGCUdTdT	1426	AGCACCCCAGCAGGGGGCGAdTdT	1610	UCGCCCCCCUGCUGGGUGCU	1794
AD-70582	UGGGUGCUGACGGCCGCUAdTdT	1427	UAGCGGCCGUCAGCACCCAdTdT	1611	UGGGUGCUGACGGCCGCUC	1795
AD-70583	GCCGCUCACUGCCUGCAGAdTdT	1428	UCUGCAGGCAGUGAGCGGCdTdT	1612	GCCGCUCACUGCCUGCAGG	1796
AD-70584	CUGCAGGACCGGCCCGCAAdTdT	1429	UUGCGGGCCGGUCCUGCAGdTdT	1613	CUGCAGGACCGGCCCGCAC	1797
AD-70585	GGCCCGCACCCGAGGAUCUdTdT	1430	AGAUCCUCCGGGUGCGGGCCdTdT	1614	GGCCCGCACCCGAGGAUCU	1798
AD-70586	CGAGGAUCUGACGGUGGUAdTdT	1431	UACCACCGUCAGAUCCUCGdTdT	1615	CGAGGAUCUGACGGUGGUG	1799
AD-70587	GUGGUGCUCGGCCAGGAAAdTdT	1432	UUUCCUGGCCGAGCACCACdTdT	1616	GUGGUGCUCGGCCAGGAAC	1800

AD-70588	GCCAGGAACGCCGUAACCAdTdT	1433	UGGUUACGGCGUUCCUGGCdTdT	1617	GCCAGGAACGCCGUAACCA	1801
AD-70589	CGUAACCACAGCUGUGAGAdTdT	1434	UCUCACAGCUGUGGUUACGdTdT	1618	CGUAACCACAGCUGUGAGC	1802
AD-70590	UGUGAGCCGUGCCAGACGUdTdT	1435	ACGUCUGGCACGGCUCACAdTdT	1619	UGUGAGCCGUGCCAGACGU	1803
AD-70591	UGCCAGACGUUGGCCGUGAdTdT	1436	UCACGGCCAACGUCUGGCAdTdT	1620	UGCCAGACGUUGGCCGUGC	1804
AD-70592	GCCGUGCGCUCCUACCGCUdTdT	1437	AGCGGUAGGAGCGCACGGCdTdT	1621	GCCGUGCGCUCCUACCGCU	1805
AD-70593	UACCGCUUGCACGAGGCCUdTdT	1438	AGGCCUCGUGCAAGCGGUAdTdT	1622	UACCGCUUGCACGAGGCCU	1806
AD-70594	ACGAGGCCUUCUCGCCCGUdTdT	1439	ACGGGCGAGAAGGCCUCGUdTdT	1623	ACGAGGCCUUCUCGCCCGU	1807
AD-70595	UCGCCCGUCAGCUACCAGAdTdT	1440	UCUGGUAGCUGACGGGCGAdTdT	1624	UCGCCCGUCAGCUACCAGC	1808
AD-70596	CUACCAGCACGACCUGGCUdTdT	1441	AGCCAGGUCGUGCUGGUAGdTdT	1625	CUACCAGCACGACCUGGCU	1809
AD-70597	ACCUGGCUCUGUUGCGCCUdTdT	1442	AGGCGCAACAGAGCCAGGUdTdT	1626	ACCUGGCUCUGUUGCGCCU	1810
AD-70598	UUGCGCCUUCAGGAGGAUAdTdT	1443	UAUCCUCCUGAAGGCGCAAdTdT	1627	UUGCGCCUUCAGGAGGAUG	1811
AD-70599	GAGGAUGCGGACGGCAGCUdTdT	1444	AGCUGCCGUCCGCAUCCUCdTdT	1628	GAGGAUGCGGACGGCAGCU	1812
AD-70600	ACGGCAGCUGCGCGCUCCUdTdT	1445	AGGAGCGCGCAGCUGCCGUdTdT	1629	ACGGCAGCUGCGCGCUCCU	1813
AD-70601	CGCUCCUGUCGCCUUACGUdTdT	1446	ACGUAAGGCGACAGGAGCGdTdT	1630	CGCUCCUGUCGCCUUACGU	1814
AD-70602	CCUUACGUUCAGCCGGUGUdTdT	1447	ACACCGGCUGAACGUAAGGdTdT	1631	CCUUACGUUCAGCCGGUGU	1815
AD-70603	AGCCGGUGUGCCUGCCAAAdTdT	1448	UUUGGCAGGCACACCGGCUdTdT	1632	AGCCGGUGUGCCUGCCAAG	1816
AD-70604	UGCCAAGCGGCGCCGCGCAdTdT	1449	UGCGCGGCGCCGCUUGGCAdTdT	1633	UGCCAAGCGGCGCCGCGCG	1817
AD-70605	GCGCCGCGCGACCCUCCGAdTdT	1450	UCGGAGGGUCGCGCGGCGCdTdT	1634	GCGCCGCGCGACCCUCCGA	1818
AD-70606	CCCUCCGAGACCACGCUCUdTdT	1451	AGAGCGUGGUCUCCGGAGGGdTdT	1635	CCCUCCGAGACCACGCUCU	1819
AD-70607	CGCUCUGCCAGGUGGCCGAdTdT	1452	UCGGCCACCUGGCAGAGCGdTdT	1636	CGCUCUGCCAGGUGGCCGG	1820
AD-70608	AGGUGGCCGGCUGGGGCCAdTdT	1453	UGGCCCCCAGCCGGCCACCUdTdT	1637	AGGUGGCCGGCUGGGGCCA	1821
AD-70609	UGGGGCCACCAGUUCGAGAdTdT	1454	UCUCGAACUGGUGGGCCCCAdTdT	1638	UGGGGCCACCAGUUCGAGG	1822
AD-70610	UUCGAGGGGGGCGGAGGAAUdTdT	1455	AUUCCUCCCGCCCCCCUCGAAdTdT	1639	UUCGAGGGGGGCGGAGGAAU	1823
AD-70611	CGGAGGAAUAUGCCAGCUUdTdT	1456	AAGCUGGCAUAUUCCUCCGGdTdT	1640	CGGAGGAAUAUGCCAGCUU	1824
AD-70612	CAGCUUCCUGCAGGAGGCAdTdT	1457	UGCCUCCUGCAGGAAGCUGdTdT	1641	CAGCUUCCUGCAGGAGGCG	1825
AD-70613	AGGAGGCGCAGGUACCGUUdTdT	1458	AACGGUACCUGCGCCUCCUdTdT	1642	AGGAGGCGCAGGUACCGUU	1826

AD-70614	AGGUACCGUUCCUCCUCCCUdTdT	1459	AGGGAGAGGAACGGUACCUdTdT	1643	AGGUACCGUUCCUCCUCCCU	1827
AD-70615	CUCUCCCUGGAGCGCUGCUdTdT	1460	AGCAGCGCUCCCAGGGAGAGdTdT	1644	CUCUCCCUGGAGCGCUGCU	1828
AD-70616	CGCUGCUCAGCCCCGGACAdTdT	1461	UGUCCGGGGCUGAGCAGCGdTdT	1645	CGCUGCUCAGCCCCGGACG	1829
AD-70617	CCGGACGUGCACGGAUCCUdTdT	1462	AGGAUCCGUGCACGUCCGGdTdT	1646	CCGGACGUGCACGGAUCCU	1830
AD-70618	CGGAUCCUCCAUCCUCCCAdTdT	1463	UGGGAGGAUGGAGGAUCCGdTdT	1647	CGGAUCCUCCAUCCUCCCC	1831
AD-70619	CAUCCUCCCCCGGCAUGCUAdTdT	1464	UAGCAUGCCGGGGAGGAUGdTdT	1648	CAUCCUCCCCGGCAUGCUC	1832
AD-70620	CAUGCUCUGCGCAGGGUUAdTdT	1465	UAACCCUGCGCAGAGCAUGdTdT	1649	CAUGCUCUGCGCAGGGUUC	1833
AD-70621	AGGGUUCCUCGAGGGCGGAdTdT	1466	UCCGCCCUCGAGGAACCCUdTdT	1650	AGGGUUCCUCGAGGGCGGC	1834
AD-70622	GAGGGCGGCACCGAUGCGUdTdT	1467	ACGCAUCGGUGCCGCCCUCdTdT	1651	GAGGGCGGCACCGAUGCGU	1835
AD-70623	GAUGCGUGCCAGGGUGAUUdTdT	1468	AAUCACCCUGGCACGCAUCdTdT	1652	GAUGCGUGCCAGGGUGAUU	1836
AD-70624	AGGGUGAUUCCGGAGGCCAdTdT	1469	UGGCCUCCGGAAUCACCCUdTdT	1653	AGGGUGAUUCCGGAGGCCC	1837
AD-70625	CGGAGGCCCGCUGGGUGUGUdTdT	1470	ACACACCAGCGGGCCUCCGdTdT	1654	CGGAGGCCCGCUGGUGUGU	1838
AD-70626	GGUGUGUGAGGACCAAGCUdTdT	1471	AGCUUGGUCCUCACACACCdTdT	1655	GGUGUGUGAGGACCAAGCU	1839
AD-70627	CCAAGCUGCAGAGCGCCGAdTdT	1472	UCGGCGCUCUGCAGCUUGGdTdT	1656	CCAAGCUGCAGAGCGCCGG	1840
AD-70628	AGAGCGCCGGCUCACCCUAdTdT	1473	UAGGGUGAGCCGGCGCUCUdTdT	1657	AGAGCGCCGGCUCACCCUG	1841
AD-70629	UCACCCUGCAAGGCAUCAUdTdT	1474	AUGAUGCCUUGCAGGGUGAdTdT	1658	UCACCCUGCAAGGCAUCAU	1842
AD-70630	GGCAUCAUCAGCUGGGGAUdTdT	1475	AUCCCCAGCUGAUGAUGCCdTdT	1659	GGCAUCAUCAGCUGGGGAU	1843
AD-70631	CUGGGGAUCGGGCUGUGGUdTdT	1476	ACCACAGCCCGAUCCCCCAGdTdT	1660	CUGGGGAUCGGGCUGUGGU	1844
AD-70632	UGUGGUGACCGCAACAAGAdTdT	1477	UCUUGUUGCGGUCACCACAdTdT	1661	UGUGGUGACCGCAACAAGC	1845
AD-70633	CAACAAGCCAGGCGUCUAAdTdT	1478	UUAGACGCCUGGCUUGUUGdTdT	1662	CAACAAGCCAGGCGUCUAC	1846
AD-70634	AGGCGUCUACACCGAUGUAdTdT	1479	UACAUCGGUGUAGACGCCUdTdT	1663	AGGCGUCUACACCGAUGUG	1847
AD-70635	GAUGUGGCCUACUACCUGAdTdT	1480	UCAGGUAGUAGGCCACAUCdTdT	1664	GAUGUGGCCUACUACCUGG	1848
AD-70636	UACUACCUGGCCUGGAUCAdTdT	1481	UGAUCCAGGCCAGGUAGUAdTdT	1665	UACUACCUGGCCUGGAUCC	1849
AD-70637	CUGGAUCCGGGAGCACACACAdTdT	1482	UGUGUGCUCCCCGGAUCCAGdTdT	1666	CUGGAUCCGGGAGCACACC	1850
AD-70638	AGCACACCGUUUCCUGAUUdTdT	1483	AAUCAGGAAACGGUGUGCUdTdT	1667	AGCACACCGUUUCCUGAUU	1851
AD-70639	UCCUGAUUGCUCAGGGACUdTdT	1484	AGUCCCCUGAGCAAUCAGGAdTdT	1668	UCCUGAUUGCUCAGGGACU	1852

AD-70640	CAGGGACUCAUCUUUCCCCUdTdT	1485	AGGGAAAGAUGAGUCCCUGdTdT	1669	CAGGGACUCAUCUUUCCCU	1853
AD-70641	UUUCCCUCCCUUGGUGAUUAdTdT	1486	UAAUCACCAAGGAGGGAAAdTdT	1670	UUUCCCUCCUUGGUGAUUC	1854
AD-70642	UGGUGAUUCCGCAGUGAGAdTdT	1487	UCUCACUGCGGAAUCACCAdTdT	1671	UGGUGAUUCCGCAGUGAGA	1855
AD-70643	AGUGAGAGAGUGGCUGGGAdTdT	1488	UCCCAGCCACUCUCUCACUdTdT	1672	AGUGAGAGAGUGGCUGGGG	1856
AD-70644	GCUGGGGCAUGGAAGGCAAdTdT	1489	UUGCCUUCCAUGCCCCAGCdTdT	1673	GCUGGGGCAUGGAAGGCAA	1857
AD-70645	UGGAAGGCAAGAUUGUGUAdTdT	1490	UACACAAUCUUGCCUUCCAdTdT	1674	UGGAAGGCAAGAUUGUGUC	1858
AD-70646	UUGUGUCCCCAUUCCCCCCAAdTdT	1491	UUGGGGGAAUGGGACACAAdTdT	1675	UUGUGUCCCAUUCCCCCCAG	1859
AD-70647	UCCCCCCAGUGCGGCCAGCUdTdT	1492	AGCUGGCCGCACUGGGGGAdTdT	1676	UCCCCCCAGUGCGGCCAGCU	1860
AD-70648	GCCAGCUCCGCGCCAGGAUdTdT	1493	AUCCUGGCGCGGAGCUGGCdTdT	1677	GCCAGCUCCGCGCCAGGAU	1861
AD-70649	GCCAGGAUGGCGCAGGAAAdTdT	1494	UUUCCCUGCGCCAUCCUGGCdTdT	1678	GCCAGGAUGGCGCAGGAAC	1862
AD-70650	GCAGGAACUCAAUAAAGUdTdT	1495	UACUUUAUUGAGUUCCUGCdTdT	1679	GCAGGAACUCAAUAAAGUG	1863
AD-70651	AAUAAAGUGCUUUGAAAAUdTdT	1496	AUUUUCAAAGCACUUUAUUdTdT	1680	AAUAAAGUGCUUUGAAAAU	1864
AD-70652	UUGAAAAUGCUGAGAAAAAdTdT	1497	UUUUUCUCAGCAUUUUCAAdTdT	1681	UUGAAAAUGCUGAGAAAAA	1865

Table 22. F12 Single Dose Screen in Hep3b Cells

Duplex Name	AVG	STDEV
AD-70653	75.05	21.99
AD-70654	59.86	17.07
AD-70655	49.58	5.13
AD-70656	42.85	9.76
AD-70657	40.2	6.21
AD-70658	52.43	13.02
AD-70659	34.67	3.33
AD-70660	33.59	8.28
AD-70661	53.13	11.32
AD-70662	61.89	7.76
AD-70663	48.43	6.92
AD-70664	34.42	4.01
AD-70665	33.22	4.21
AD-70666	33.44	5.89
AD-70667	47.6	10.96
AD-70668	125.01	38.32
AD-70669	64.78	12.71
AD-70670	57.49	5.4
AD-70671	30.06	7.8
AD-70672	54.95	2.39
AD-70673	79.79	10.29
AD-70674	88.3	12.07
AD-70675	55.83	14.88
AD-70676	61.99	12.96
AD-70677	50.27	9.84
AD-70678	65.84	10.37
AD-70679	51.1	8.97
AD-70680	64.71	10.54
AD-70681	41.02	6.75
AD-70682	60.65	9.01
AD-70683	96.74	6.29
AD-70684	71.16	13.22
AD-70685	99.97	12.48
AD-70686	45.51	6.21
AD-70687	68.37	5.36
AD-70688	65.68	6.4

AD-70689	63.41	5.72
AD-70690	54.1	7.23
AD-70691	43.79	11.91
AD-70692	51.36	8.64
AD-70693	43.25	7.81
AD-70694	51.13	4.52
AD-70695	47.38	4.76
AD-70696	63.08	3.96
AD-70697	49.53	6.44
AD-70698	56.12	8.22
AD-70699	53.68	4.62
AD-70700	68.45	12.64
AD-70701	94.45	11.32
AD-70702	70.82	8.36
AD-70703	93.79	7.87
AD-70704	35.84	4.09
AD-70705	87.79	5.74
AD-70706	59.21	9.08
AD-70707	64.22	10.1
AD-70708	49.55	3
AD-70709	87.37	7.17
AD-70710	76.54	11.55
AD-70711	62.4	4.69
AD-70712	80.45	8.12
AD-70713	76.68	16.28
AD-70714	61.92	15.07
AD-70715	85.76	8.24
AD-70716	97.67	8.1
AD-70717	70.83	2.72
AD-70718	50.19	9.69
AD-70719	77.23	4.82
AD-70720	69.02	6.52
AD-70721	84.91	12.03
AD-70722	42.64	6.44
AD-70723	56.77	6.73
AD-70724	50.28	7.37
AD-70725	73.06	14.77
AD-70726	69.29	8.43
AD-70727	68.98	5.88

AD-70728	59.51	5.26
AD-70729	77.31	11.18
AD-70730	48.22	9.04
AD-70731	63.52	3.78
AD-70732	60.89	6.26
AD-70733	55.56	13.83
AD-70734	110.37	7.09
AD-70735	70.96	1.41
AD-70736	72.71	4.28
AD-70737	66.94	4.75
AD-70738	104.61	9.8
AD-70739	87.48	8.44
AD-70740	69.08	9.31
AD-70741	67.82	3.49
AD-70742	92.93	14.66
AD-70743	59.32	9.95
AD-70744	81.97	6.05
AD-70745	54.96	7.81
AD-70562	46.21	8.44
AD-70563	44.88	5.69
AD-70564	67.82	20.32
AD-70565	52.32	12.39
AD-70566	53.22	10.43
AD-70567	46.28	10.21
AD-70568	41.84	3.91
AD-70569	46.27	10.51
AD-70570	37.31	7.6
AD-70571	55.84	13.93
AD-70572	64.38	6.03
AD-70573	75.03	17.72
AD-70574	61.2	7.6
AD-70575	55.54	18.99
AD-70576	48.67	7.52
AD-70577	34.12	10.23
AD-70578	56.62	6.22
AD-70579	58.22	17.32
AD-70580	64.99	8.66
AD-70581	86.55	15.76
AD-70582	72.76	11.98

AD-70583	47.99	20.51
AD-70584	54	14.12
AD-70585	43.72	6.69
AD-70586	55.96	12.05
AD-70587	64.82	18.43
AD-70588	66.06	13.08
AD-70589	56.65	10.27
AD-70590	77.82	4.75
AD-70591	68.65	9.93
AD-70592	37.1	9.84
AD-70593	50.14	17.24
AD-70594	50.16	13.61
AD-70595	60.63	13.54
AD-70596	80.78	12.29
AD-70597	60.74	21.94
AD-70598	70.51	8.48
AD-70599	67.75	7.59
AD-70600	68.09	31.51
AD-70601	53.28	21.16
AD-70602	44.03	10.56
AD-70603	87.08	40.51
AD-70604	69.39	9.62
AD-70605	86.92	27.74
AD-70606	62.19	7.28
AD-70607	67.55	19.57
AD-70608	98.46	10.23
AD-70609	77.67	10.72
AD-70610	108.45	21.97
AD-70611	73.02	19.12
AD-70612	97.49	26.26
AD-70613	65.22	19.24
AD-70614	96.69	21.51
AD-70615	76.53	7.96
AD-70616	69.73	12.06
AD-70617	58.38	10.85
AD-70618	73.89	22.5
AD-70619	85.32	25.92
AD-70620	72.03	33.04
AD-70621	83.22	24.59

AD-70622	108.98	14.93
AD-70623	71.28	32.49
AD-70624	67.8	25.27
AD-70625	52.08	10.91
AD-70626	40.94	13.75
AD-70627	33.55	3.35
AD-70628	52.37	10.46
AD-70629	53.46	4.07
AD-70630	47	8.42
AD-70631	64.51	42.23
AD-70632	30.66	4.32
AD-70633	33.64	12.21
AD-70634	65.42	6.92
AD-70635	45.84	6.76
AD-70636	47.83	6.63
AD-70637	64.39	8.42
AD-70638	38.91	8.35
AD-70639	40.87	7.79
AD-70640	50.87	13.34
AD-70641	49.64	5.85
AD-70642	44.04	8.02
AD-70643	61.04	11.12
AD-70644	50.03	9.07
AD-70645	67.35	28.98
AD-70646	50.93	6
AD-70647	83.29	5.96
AD-70648	53.57	15.44
AD-70649	46.35	8.99
AD-70650	52.06	7.83
AD-70651	64.65	9.04
AD-70652	100.8	9.21

Example 11. *In Vivo* F12 Silencing in Mustard Oil-Induced Vascular Permeability Mouse Model

As discussed above and demonstrated in Figures 2 and 5, AD-67244 was the most efficacious agent targeting an F12 gene that was tested, resulting in robust, dose-dependent reduction of F12 mRNA and plasma F12 protein in wild-type mice, and normalization of vascular permeability in a bradykinin-induced vascular leakage mouse model of HAE (the ACE-inhibitor-induced mouse model).

The *in vivo* efficacy of AD-67244 was also assessed in a second mouse model of HAE. In particular, the ability of AD-67244 to rescue mustard oil-induced vascular permeability in C1-INH deficient mice was determined by subcutaneously administering CD-1 female mice (n=10/group) a single 3 mg/kg, 0.5 mg/kg, or 0.1 mg/kg dose of AD-67244 in combination with a single10 mg/kg dose of a double stranded RNA agent targeting C1-INH at day -7 . On Day 0, Evans Blue dye (30 mg/kg) was injected into the tail vein of the animals and a 5% solution of mustard oil was topically applied to the right ear of each animal (the left ear was left untreated and served as a control) . Thirty minutes later, the animals were sacrificed, each ear was collected for dye extravasation to determine vascular permeability, and livers were collected for F12 and C1-INH mRNA measurements.

As shown in Figure 10A, administration of a single 3 mg/kg, 0.5 mg/kg, or 0.1 mg/kg dose of AD-67244 normalized vascular permeability in these mice and, as shown in Figure 10B, this administration resulted in robust, dose-dependent reduction of F12 mRNA in the livers of these animals. The level of C1-INH in the livers of these animals was less than 0.01% of the level of C1-INH in the livers of the control group administered. These data demonstrate that AD-67244 can mitigate excess bradykinin stimulation.

Example 12. *In Vivo* F12 Silencing in Non-Human Primates

To determine the efficacy of AD-67244 in non-human primates, female *Cynomolgus* monkeys (n=3 per group) were subcutaneously administered a single 3 mg/kg, 1 mg/kg, 0.3 mg/kg, or 0.1 mg/kg dose of AD-67244. The level of *Cynomolgus* F12 plasma protein levels was measured by ELISA at days -5, -3, -1, 3, 7, 10, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 112, 126, and 140 post-dose. Figure 11 demonstrates that administration of a single 0.3 mg/kg dose of AD-67244 resulted in greater than 85% reduction in F12 protein. Figure 11 also demonstrates that this reduction in F12 protein was durable with greater than 70% and 50% reduction at 2 and 3 months post-dose, respectively.

Example 13. Effect of 5' Modification of AD-67244 on Potency in Mice

The effect of modifying the 5' antisense phosphate of AD-67244 with a vinylphosphate (VP) on the potency of the agent was determined in mice. Wild-type mice (n=3/group) were administered a single 0.5 mg/kg dose of either AD-67244 (sense: 5' – asascucaAfuAfAfAfgugcuuugaa – 3' (SEQ ID NO: 1866); antisense: 5' – usUfscaaAfgCfAfcuuuAfuUfgaguususc – 3' (SEQ ID NO: 1867); ALN-F12) or AD-74841 (sense: 5' – asascucaAfuAfAfAfgugcuuugaa – 3' (SEQ ID NO: 1868); antisense: 5' – VP - usUfscaaAfgCfAfcuuuAfuUfgaguususc – 3' (SEQ ID NO: 1869); ALN-F12-VP). The plasma level of F12 protein was determined by ELISA at days 0, 3, 7, 15, and 21 post-dose. Figure 12 demonstrates that 5' modification of the antisense phosphate group with a vinylphosphate moderately increased the potency of AD-67244.

Example 14. Synthesis and *In vitro* screening of F12 siRNA duplexes

Additional iRNA agents targeting F12, *e.g.,* targeting about nucleotides 2000-2060 of SEQ ID NO:9, were designed, synsthesized, and screened for *in vitro* efficacy, as described above. A detailed list of the additional unmodified F12 sense and antisense strand sequences is shown in Table 23. A detailed list of the additional modified F12 sense and antisense strand sequences is shown in Table 24. Table 25 provides the results of a single dose screen in Hep3b cells transfected with the indicated additional F12 iRNAs. Data are expressed as percent of mRNA remaining relative to AD-1955.

Table 23: F12 Unmodified Sequences

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Range in SEQ ID NO:9	Antisense Sequence 5' to 3'	SEQ ID NO	Range in SEQ ID NO:9
AD-70649.2	GCCAGGAUGGCGCAGGAAA	1870	2004-2022	UUUCCUGCGCCAUCCUGGC	1908	2004-2022
AD-75921.1	CCAGGAUGGCGCAGGAACU	1871	2005-2023	AGUUCCUGCGCCAUCCUGG	1909	2005-2023
AD-75920.1	CAGGAUGGCGCAGGAACUA	1872	2006-2024	UAGUUCCUGCGCCAUCCUG	1910	2006-2024
AD-75919.1	AGGAUGGCGCAGGAACUCA	1873	2007-2025	UGAGUUCCUGCGCCAUCCU	1911	2007-2025
AD-75918.1	GGAUGGCGCAGGAACUCAA	1874	2008-2026	UUGAGUUCCUGCGCCAUCC	1912	2008-2026
AD-75917.1	GAUGGCGCAGGAACUCAAU	1875	2009-2027	AUUGAGUUCCUGCGCCAUC	1913	2009-2027
AD-75916.1	AUGGCGCAGGAACUCAAUA	1876	2010-2028	UAUUGAGUUCCUGCGCCAU	1914	2010-2028
AD-75915.1	UGGCGCAGGAACUCAAUAA	1877	2011-2029	UUAUUGAGUUCCUGCGCCA	1915	2011-2029
AD-75914.1	GGCGCAGGAACUCAAUAAA	1878	2012-2030	UUUAUUGAGUUCCUGCGCC	1916	2012-2030
AD-75913.1	GCGCAGGAACUCAAUAAAA	1879	2013-2031	UUUUAUUGAGUUCCUGCGC	1917	2013-2031
AD-75912.1	CGCAGGAACUCAAUAAAGU	1880	2014-2032	ACUUUAUUGAGUUCCUGCG	1918	2014-2032
AD-70650.2	GCAGGAACUCAAUAAAGUA	1881	2015-2033	UACUUUAUUGAGUUCCUGC	1919	2015-2033
AD-75911.1	CAGGAACUCAAUAAAGUGA	1882	2016-2034	UCACUUUAUUGAGUUCCUG	1920	2016-2034
AD-75910.1	AGGAACUCAAUAAAGUGCU	1883	2017-2035	AGCACUUUAUUGAGUUCCU	1921	2017-2035
AD-75909.1	GGAACUCAAUAAAGUGCUU	1884	2018-2036	AAGCACUUUAUUGAGUUCC	1922	2018-2036
AD-75908.1	GAACUCAAUAAAGUGCUUU	1885	2019-2037	AAAGCACUUUAUUGAGUUC	1923	2019-2037
AD-75907.1	AACUCAAUAAAGUGCUUUA	1886	2020-2038	UAAAGCACUUUAUUGAGUU	1924	2020-2038
AD-75906.1	ACUCAAUAAAGUGCUUUGA	1887	2021-2039	UCAAAGCACUUUAUUGAGU	1925	2021-2039
AD-75922.1	UCAAUAAAGUGCUUUGAAA	1888	2023-2041	UUUCAAAGCACUUUAUUGA	1926	2023-2041
AD-75923.1	CAAUAAAGUGCUUUGAAAA	1889	2024-2042	UUUUCAAAGCACUUUAUUG	1927	2024-2042
AD-70651.2	AAUAAAGUGCUUUGAAAAU	1890	2025-2043	AUUUUCAAAGCACUUUAUU	1928	2025-2043
AD-75924.1	AUAAAGUGCUUUGAAAAUA	1891	2026-2044	UAUUUUCAAAGCACUUUAU	1929	2026-2044
AD-75925.1	UAAAGUGCUUUGAAAAUGA	1892	2027-2045	UCAUUUUCAAAGCACUUUA	1930	2027-2045
AD-75926.1	AAAGUGCUUUGAAAAUGCU	1893	2028-2046	AGCAUUUUCAAAGCACUUU	1931	2028-2046
AD-75927.1	AAGUGCUUUGAAAAUGCUA	1894	2029-2047	UAGCAUUUUCAAAGCACUU	1932	2029-2047
AD-75928.1	AGUGCUUUGAAAAUGCUGA	1895	2030-2048	UCAGCAUUUUCAAAGCACU	1933	2030-2048

AD-75929.1	GUGCUUUGAAAAUGCUGAA	1896	2031-2049	UUCAGCAUUUUCAAAGCAC	1934	2031-2049
AD-75930.1	UGCUUUGAAAAUGCUGAGA	1897	2032-2050	UCUCAGCAUUUUCAAAGCA	1935	2032-2050
AD-75931.1	GCUUUGAAAAUGCUGAGAA	1898	2033-2051	UUCUCAGCAUUUUCAAAGC	1936	2033-2051
AD-75932.1	CUUUGAAAAUGCUGAGAAA	1899	2034-2052	UUUCUCAGCAUUUUCAAAG	1937	2034-2052
AD-75933.1	UUUGAAAAUGCUGAGAAAA	1900	2035-2053	UUUUCUCAGCAUUUUCAAA	1938	2035-2053
AD-70652.2	UUGAAAAUGCUGAGAAAAA	1901	2036-2054	UUUUUCUCAGCAUUUUCAA	1939	2036-2054
AD-75934.1	UGAAAAUGCUGAGAAAAAA	1902	2037-2055	UUUUUUCUCAGCAUUUUCA	1940	2037-2055
AD-75935.1	GAAAAUGCUGAGAAAAAAA	1903	2038-2056	UUUUUUUCUCAGCAUUUUC	1941	2038-2056
AD-75936.1	AAAAUGCUGAGAAAAAAAA	1904	2039-2057	UUUUUUUUCUCAGCAUUUU	1942	2039-2057
AD-75937.1	AAAUGCUGAGAAAAAAAAA	1905	2040-2058	UUUUUUUUUCUCAGCAUUU	1943	2040-2058
AD-75938.1	AAUGCUGAGAAAAAAAAAA	1906	2041-2059	UUUUUUUUUUCUCAGCAUU	1944	2041-2059
AD-75939.1	AUGCUGAGAAAAAAAAAAA	1907	2042-2060	UUUUUUUUUUUCUCAGCAU	1945	2042-2060

Table 24: F12 Modified Sequences

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Antisense Sequence 5' to 3'	SEQ ID NO	mRNA target sequence 5' to 3'	SEQ ID NO	mRNA target site in SEQ ID NO:9
AD-70649	GCCAGGAUGGCGCAGGAAAd TdT	1946	UUUCCUGCGCCAUCCUGGCd TdT	1984	GCCAGGAUGGCGCAGGA AC	2022	2004-2022
AD-75921	CCAGGAUGGCGCAGGAACUd TdT	1947	AGUUCCUGCGCCAUCCUGGd TdT	1985	CCAGGAUGGCGCAGGAA CU	2023	2005-2023
AD-75920	CAGGAUGGCGCAGGAACUAd TdT	1948	UAGUUCCUGCGCCAUCCUGd TdT	1986	CAGGAUGGCGCAGGAAC UC	2024	2006-2024
AD-75919	AGGAUGGCGCAGGAACUCAd TdT	1949	UGAGUUCCUGCGCCAUCCUd TdT	1987	AGGAUGGCGCAGGAACU CA	2025	2007-2025
AD-75918	GGAUGGCGCAGGAACUCAAd TdT	1950	UUGAGUUCCUGCGCCAUCCd TdT	1988	GGAUGGCGCAGGAACUC AA	2026	2008-2026
AD-75917	GAUGGCGCAGGAACUCAAUd	1951	AUUGAGUUCCUGCGCCAUC	1989	GAUGGCGCAGGAACUCA	2027	2009-2027

	TdT		dTdT		AU		
AD-75916	AUGGCGCAGGAACUCAAUAd TdT	1952	UAUUGAGUUCCUGCGCCAU dTdT	1990	AUGGCGCAGGAACUCAA UA	2028	2010-2028
AD-75915	UGGCGCAGGAACUCAAUAAd TdT	1953	UUAUUGAGUUCCUGCGCCA dTdT	1991	UGGCGCAGGAACUCAAU AA	2029	2011-2029
AD-75914	GGCGCAGGAACUCAAUAAAd TdT	1954	UUUAUUGAGUUCCUGCGCC dTdT	1992	GGCGCAGGAACUCAAUA AA	2030	2012-2030
AD-75913	GCGCAGGAACUCAAUAAAAd TdT	1955	UUUUAUUGAGUUCCUGCGC dTdT	1993	GCGCAGGAACUCAAUAA AG	2031	2013-2031
AD-75912	CGCAGGAACUCAAUAAAGUd TdT	1956	ACUUUAUUGAGUUCCUGCG dTdT	1994	CGCAGGAACUCAAUAAA GU	2032	2014-2032
AD-70650	GCAGGAACUCAAUAAAGUAd TdT	1957	UACUUUAUUGAGUUCCUGC dTdT	1995	GCAGGAACUCAAUAAAG UG	2033	2015-2033
AD-75911	CAGGAACUCAAUAAAGUGAd TdT	1958	UCACUUUAUUGAGUUCCUG dTdT	1996	CAGGAACUCAAUAAAGU GC	2034	2016-2034
AD-75910	AGGAACUCAAUAAAGUGCUd TdT	1959	AGCACUUUAUUGAGUUCCU dTdT	1997	AGGAACUCAAUAAAGUG CU	2035	2017-2035
AD-75909	GGAACUCAAUAAAGUGCUUd TdT	1960	AAGCACUUUAUUGAGUUCC dTdT	1998	GGAACUCAAUAAAGUGC UU	2036	2018-2036
AD-75908	GAACUCAAUAAAGUGCUUUd TdT	1961	AAAGCACUUUAUUGAGUUC dTdT	1999	GAACUCAAUAAAGUGCU UU	2037	2019-2037
AD-75907	AACUCAAUAAAGUGCUUUAd TdT	1962	UAAAGCACUUUAUUGAGUU dTdT	2000	AACUCAAUAAAGUGCUU UG	2038	2020-2038
AD-75906	ACUCAAUAAAGUGCUUUGAd TdT	1963	UCAAAGCACUUUAUUGAGU dTdT	2001	ACUCAAUAAAGUGCUUU GA	2039	2021-2039
AD-75922	UCAAUAAAGUGCUUUGAAAd TdT	1964	UUUCAAAGCACUUUAUUGA dTdT	2002	UCAAUAAAGUGCUUUGA AA	2040	2023-2041
AD-75923	CAAUAAAGUGCUUUGAAAAd TdT	1965	UUUUCAAAGCACUUUAUUG dTdT	2003	CAAUAAAGUGCUUUGAA AA	2041	2024-2042
AD-70651	AAUAAAGUGCUUUGAAAAUd TdT	1966	AUUUUCAAAGCACUUUAUU dTdT	2004	AAUAAAGUGCUUUGAAA AU	2042	2025-2043
AD-75924	AUAAAGUGCUUUGAAAAUAd TdT	1967	UAUUUUCAAAGCACUUUAU dTdT	2005	AUAAAGUGCUUUGAAAA UG	2043	2026-2044
AD-75925	UAAAGUGCUUUGAAAAUGAd	1968	UCAUUUUCAAAGCACUUUA	2006	UAAAGUGCUUUGAAAAU	2044	2027-2045

	TdT		dTdT		GC		
AD-75926	AAAGUGCUUUGAAAAUGCUd TdT	1969	AGCAUUUUCAAAGCACUUU dTdT	2007	AAAGUGCUUUGAAAAUG CU	2045	2028-2046
AD-75927	AAGUGCUUUGAAAAUGCUAd TdT	1970	UAGCAUUUUCAAAGCACUU dTdT	2008	AAGUGCUUUGAAAAUGC UG	2046	2029-2047
AD-75928	AGUGCUUUGAAAAUGCUGAd TdT	1971	UCAGCAUUUUCAAAGCACU dTdT	2009	AGUGCUUUGAAAAUGCU GA	2047	2030-2048
AD-75929	GUGCUUUGAAAAUGCUGAAd TdT	1972	UUCAGCAUUUUCAAAGCAC dTdT	2010	GUGCUUUGAAAAUGCUG AG	2048	2031-2049
AD-75930	UGCUUUGAAAAUGCUGAGAd TdT	1973	UCUCAGCAUUUUCAAAGCA dTdT	2011	UGCUUUGAAAAUGCUGA GA	2049	2032-2050
AD-75931	GCUUUGAAAAUGCUGAGAAd TdT	1974	UUCUCAGCAUUUUCAAAGC dTdT	2012	GCUUUGAAAAUGCUGAG AA	2050	2033-2051
AD-75932	CUUUGAAAAUGCUGAGAAAd TdT	1975	UUUCUCAGCAUUUUCAAAG dTdT	2013	CUUUGAAAAUGCUGAGA AA	2051	2034-2052
AD-75933	UUUGAAAAUGCUGAGAAAAd TdT	1976	UUUUCUCAGCAUUUUCAAA dTdT	2014	UUUGAAAAUGCUGAGAA AA	2052	2035-2053
AD-70652	UUGAAAAUGCUGAGAAAAAd TdT	1977	UUUUUCUCAGCAUUUUCAA dTdT	2015	UUGAAAAUGCUGAGAAA AA	2053	2036-2054
AD-75934	UGAAAAUGCUGAGAAAAAAd TdT	1978	UUUUUUCUCAGCAUUUUCA dTdT	2016	UGAAAAUGCUGAGAAAA AA	2054	2037-2055
AD-75935	GAAAAUGCUGAGAAAAAAAd TdT	1979	UUUUUUUCUCAGCAUUUUC dTdT	2017	GAAAAUGCUGAGAAAAA AA	2055	2038-2056
AD-75936	AAAAUGCUGAGAAAAAAAAd TdT	1980	UUUUUUUUCUCAGCAUUUU dTdT	2018	AAAAUGCUGAGAAAAAA AA	2056	2039-2057
AD-75937	AAAUGCUGAGAAAAAAAAAd TdT	1981	UUUUUUUUUCUCAGCAUUU dTdT	2019	AAAUGCUGAGAAAAAAA AA	2057	2040-2058
AD-75938	AAUGCUGAGAAAAAAAAAAd TdT	1982	UUUUUUUUUUCUCAGCAUU dTdT	2020	AAUGCUGAGAAAAAAAA AA	2058	2041-2059
AD-75939	AUGCUGAGAAAAAAAAAAAd TdT	1983	UUUUUUUUUUUCUCAGCAU dTdT	2021	AUGCUGAGAAAAAAAAA AA	2059	2042-2060

Table 25. F12 Single Dose Screen in Hep3b Cells

Duplex ID	10nM_AVG	10nM_SD	0.1nM_AVG	0.1nM_SD
AD-70649.2	28.65	6.26	41.38	9.60
AD-75921.1	29.32	7.31	41.14	10.86
AD-75920.1	30.91	5.90	45.92	15.18
AD-75919.1	32.12	14.45	66.98	17.31
AD-75918.1	28.51	14.34	57.71	21.51
AD-75917.1	22.80	1.02	33.45	5.13
AD-75916.1	27.48	7.88	34.62	6.73
AD-75915.1	50.58	28.39	56.95	39.88
AD-75914.1	28.22	5.74	54.70	9.80
AD-75913.1	38.35	11.58	32.08	9.74
AD-75912.1	27.06	9.92	39.41	14.48
AD-70650.2	31.86	12.64	40.42	11.08
AD-75911.1	28.50	5.83	53.54	9.61
AD-75910.1	34.12	6.44	47.93	22.85
AD-75909.1	35.13	13.76	51.88	42.23
AD-75908.1	38.17	7.67	66.18	59.34
AD-75907.1	40.80	20.27	62.36	20.96
AD-75906.1	49.29	8.64	58.20	26.56
AD-75922.1	25.51	3.58	45.53	20.00
AD-75923.1	49.08	13.60	49.27	11.54
AD-70651.2	55.60	32.34	94.24	39.01
AD-75924.1	46.27	14.11	53.33	11.68
AD-75925.1	37.21	8.81	46.28	17.48
AD-75926.1	27.13	6.82	39.29	8.19
AD-75927.1	47.80	14.67	62.71	21.77
AD-75928.1	34.40	6.27	70.89	29.90
AD-75929.1	43.65	16.80	54.91	4.67
AD-75930.1	72.67	33.09	81.86	17.63
AD-75931.1	85.60	17.39	88.98	12.61
AD-75932.1	46.69	3.04	68.57	12.35
AD-75933.1	75.04	4.59	97.52	8.55
AD-70652.2	104.50	12.08	84.12	4.74
AD-75934.1	83.25	19.97	82.77	10.51
AD-75935.1	65.87	3.46	84.47	11.66
AD-75936.1	97.74	3.66	93.48	10.33
AD-75937.1	112.45	30.62	98.91	29.75
AD-75938.1	125.12	33.83	110.47	33.87
AD-75939.1	112.95	24.79	93.19	18.21

Example 15. Evaluation of 5'-End Modifications of F12 siRNA duplexes

Additional iRNA agents targeting F12 comprising a nucleotide comprising a 5'-phosphate mimic, *i.e.*, a vinyl phosphate, were designed, synsthesized, and screened for *in vitro* efficacy, as described above. Agents comprising the same unmodified and modified nucleotide sequences of these agents but without the 5'-antisense strand vinyl phosphate modification were also designed, synthesized and screened, as described above. A detailed list of all of these additional unmodified F12 sense and antisense strand sequences is shown in Table 26. A detailed list of all of these additional modified F12 sense and antisense strand sequences is shown in Table 27. Table 28 provides the results of a single dose screen in primary mouse hepatocytes cells transfected with the indicated F12 dsRNA agents.

The *in vivo* efficacy of a subset of these compounds was also assessed by subcutaneously administering wild-type mice a single 0.5 mg/kg dose of an agent and determining the level of F12 protein in the plasma of the animals at days 3, 7, and 15 post-dose. Figure 13 depicts the results of these assays and demonstrates that the addition of a 5'vinyl phosphate to the antisense strands has a moderate effect on the *in vivo* efficacy of the indicated agents.

Table 26. F12 Unmodified F12 Sequences

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Antisense Sequence 5' to 3'	SEQ ID NO	Range in SEQ ID NO:9
AD-73610	GGAGCCCAAGAAAGUGAAAGA	2060	UCUUUCACUUUCUUGGGCUCCAA	2105	299-321
AD-73633	GGAGCCCAAGAAAGUGAAAGA	2061	UCUUUCACUUUCUUGGGCUCCAA	2106	299-321
AD-73604	GAGCCCAAGAAAGUGAAAGAA	2062	UUCUUUCACUUUCUUGGGCUCCA	2107	300-322
AD-73627	GAGCCCAAGAAAGUGAAAGAA	2063	UUCUUUCACUUUCUUGGGCUCCA	2108	300-322
AD-73595	GCCCAAGAAAGUGAAAGACCA	2064	UGGUCUUUCACUUUCUUGGGCUC	2109	302-324
AD-73617	GCCCAAGAAAGUGAAAGACCA	2065	UGGUCUUUCACUUUCUUGGGCUC	2110	302-324
AD-73606	CCCAAGAAAGUGAAAGACCAA	2066	UUGGUCUUUCACUUUCUUGGGCU	2111	303-325
AD-73629	CCCAAGAAAGUGAAAGACCAA	2067	UUGGUCUUUCACUUUCUUGGGCU	2112	303-325
AD-73609	AAAGAGAAAUGCUUUGAGCCA	2068	UGGCUCAAAGCAUUUCUCUUUCU	2113	426-448
AD-73632	AAAGAGAAAUGCUUUGAGCCA	2069	UGGCUCAAAGCAUUUCUCUUUCU	2114	426-448
AD-73599	AAGAGAAAUGCUUUGAGCCUA	2070	UAGGCUCAAAGCAUUUCUCUUUC	2115	427-449
AD-73621	AAGAGAAAUGCUUUGAGCCUA	2071	UAGGCUCAAAGCAUUUCUCUUUC	2116	427-449
AD-73597	AGAGAAAUGCUUUGAGCCUCA	2072	UGAGGCUCAAAGCAUUUCUCUUU	2117	428-450
AD-73619	AGAGAAAUGCUUUGAGCCUCA	2073	UGAGGCUCAAAGCAUUUCUCUUU	2118	428-450
AD-73596	GAGAAAUGCUUUGAGCCUCAA	2074	UUGAGGCUCAAAGCAUUUCUCUU	2119	429-451
AD-73618	GAGAAAUGCUUUGAGCCUCAA	2075	UUGAGGCUCAAAGCAUUUCUCUU	2120	429-451
AD-73614	AGAAAUGCUUUGAGCCUCAGA	2076	UCUGAGGCUCAAAGCAUUUCUCU	2121	430-452
AD-73637	AGAAAUGCUUUGAGCCUCAGA	2077	UCUGAGGCUCAAAGCAUUUCUCU	2122	430-452
AD-73611	AAAUGCUUUGAGCCUCAGCUA	2078	UAGCUGAGGCUCAAAGCAUUUCU	2123	432-454
AD-73634	AAAUGCUUUGAGCCUCAGCUA	2079	UAGCUGAGGCUCAAAGCAUUUCU	2124	432-454
AD-73605	AUGCUUUGAGCCUCAGCUUCA	2080	UGAAGCUGAGGCUCAAAGCAUUU	2125	434-456

AD-73628	AUGCUUUGAGCCUCAGCUUCA	2081	UGAAGCUGAGGCUCAAAGCAUUU	2126	434-456
AD-73601	UGCUUUGAGCCUCAGCUUCUA	2082	UAGAAGCUGAGGCUCAAAGCAUU	2127	435-457
AD-73624	UGCUUUGAGCCUCAGCUUCUA	2083	UAGAAGCUGAGGCUCAAAGCAUU	2128	435-457
AD-73613	GCUUUGAGCCUCAGCUUCUCA	2084	UGAGAAGCUGAGGCUCAAAGCAU	2129	436-458
AD-73636	GCUUUGAGCCUCAGCUUCUCA	2085	UGAGAAGCUGAGGCUCAAAGCAU	2130	436-458
AD-73616	ACUCCACCUUCCUGCAGGAGA	2086	UCUCCUGCAGGAAGGUGGAGUAU	2131	1522-1544
AD-73639	ACUCCACCUUCCUGCAGGAGA	2087	UCUCCUGCAGGAAGGUGGAGUAU	2132	1522-1544
AD-73603	CACAGAAACUCAAUAAAGUGA	2088	UCACUUUAUUGAGUUUCUGUGCC	2133	1927-1949
AD-73626	CACAGAAACUCAAUAAAGUGA	2089	UCACUUUAUUGAGUUUCUGUGCC	2134	1927-1949
AD-73607	ACAGAAACUCAAUAAAGUGCA	2090	UGCACUUUAUUGAGUUUCUGUGC	2135	1928-1950
AD-73630	ACAGAAACUCAAUAAAGUGCA	2091	UGCACUUUAUUGAGUUUCUGUGC	2136	1928-1950
AD-73600	CAGAAACUCAAUAAAGUGCUA	2092	UAGCACUUUAUUGAGUUUCUGUG	2137	1929-1951
AD-73622	CAGAAACUCAAUAAAGUGCUA	2093	UAGCACUUUAUUGAGUUUCUGUG	2138	1929-1951
AD-73615	AGAAACUCAAUAAAGUGCUUA	2094	UAAGCACUUUAUUGAGUUUCUGU	2139	1930-1952
AD-73638	AGAAACUCAAUAAAGUGCUUA	2095	UAAGCACUUUAUUGAGUUUCUGU	2140	1930-1952
AD-73598	GAAACUCAAUAAAGUGCUUUA	2096	UAAAGCACUUUAUUGAGUUUCUG	2141	1931-1953
AD-73620	GAAACUCAAUAAAGUGCUUUA	2097	UAAAGCACUUUAUUGAGUUUCUG	2142	1931-1953
AD-73602	AAACUCAAUAAAGUGCUUUGA	2098	UCAAAGCACUUUAUUGAGUUUCU	2143	1932-1954
AD-73625	AAACUCAAUAAAGUGCUUUGA	2099	UCAAAGCACUUUAUUGAGUUUCU	2144	1932-1954
AD-73608	ACUCAAUAAAGUGCUUUGAAA	2100	UUUCAAAGCACUUUAUUGAGUUU	2145	1934-1956
AD-73631	ACUCAAUAAAGUGCUUUGAAA	2101	UUUCAAAGCACUUUAUUGAGUUU	2146	1934-1956
AD-73612	UCAAUAAAGUGCUUUGAAAAA	2102	UUUUUCAAAGCACUUUAUUGAGU	2147	1936-1958
AD-73635	UCAAUAAAGUGCUUUGAAAAA	2103	UUUUUCAAAGCACUUUAUUGAGU	2148	1936-1958
AD-73623	AACUCAAUAAAGUGCUUUGAA	2104	UUCAAAGCACUUUAUUGAGUUUC	2149	1933-1955
AD-74838	AAUAAAGUGCUUUGAAAACGA	2333	UCGUUUUCAAAGCACUUUAUUGA	2335	1938-1960
AD-74842	AAUAAAGUGCUUUGAAAACGA	2334	UCGUUUUCAAAGCACUUUAUUGA	2336	1938-1960

Table 27. Modified F12 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-73610	gsgsagccCfaAfGfAfaagugaaagaL96	2150	usCfsuuuCfaCfUfuucuUfgGfgcuccsasa	2195	UUGGAGCCCAAGAAAGUGAAAGA	2240
AD-73633	gsgsagccCfaAfGfAfaagugaaagaL96	2151	PusCfsuuuCfaCfUfuucuUfgGfgcuccsasa	2196	UUGGAGCCCAAGAAAGUGAAAGA	2241
AD-73604	gsasgcccAfaGfAfAfagugaaagaaL96	2152	usUfscuuUfcAfCfuuucUfuGfggcucscsa	2197	UGGAGCCCAAGAAAGUGAAAGAC	2242
AD-73627	gsasgcccAfaGfAfAfagugaaagaaL96	2153	PusUfscuuUfcAfCfuuucUfuGfggcucscsa	2198	UGGAGCCCAAGAAAGUGAAAGAC	2243
AD-73595	gscsccaaGfaAfAfGfugaaagaccaL96	2154	usGfsgucUfuUfCfacuuUfcUfugggcsusc	2199	GAGCCCAAGAAAGUGAAAGACCA	2244
AD-73617	gscsccaaGfaAfAfGfugaaagaccaL96	2155	PusGfsgucUfuUfCfacuuUfcUfugggcsusc	2200	GAGCCCAAGAAAGUGAAAGACCA	2245
AD-73606	cscscaagAfaAfGfUfgaaagaccaaL96	2156	usUfsgguCfuUfUfcacuUfuCfuugggscsu	2201	AGCCCAAGAAAGUGAAAGACCAU	2246
AD-73629	cscscaagAfaAfGfUfgaaagaccaaL96	2157	PusUfsgguCfuUfUfcacuUfuCfuugggscsu	2202	AGCCCAAGAAAGUGAAAGACCAU	2247
AD-73609	asasagagAfaAfUfGfcuuugagccaL96	2158	usGfsgcuCfaAfAfgcauUfuCfucuuuscsu	2203	AGAAAGAGAAAUGCUUUGAGCCU	2248
AD-73632	asasagagAfaAfUfGfcuuugagccaL96	2159	PusGfsgcuCfaAfAfgcauUfuCfucuuuscsu	2204	AGAAAGAGAAAUGCUUUGAGCCU	2249
AD-73599	asasgagaAfaUfGfCfuuugagccuaL96	2160	usAfsggcUfcAfAfagcaUfuUfcucuususc	2205	GAAAGAGAAAUGCUUUGAGCCUC	2250
AD-73621	asasgagaAfaUfGfCfuuugagccuaL96	2161	PusAfsggcUfcAfAfagcaUfuUfcucuususc	2206	GAAAGAGAAAUGCUUUGAGCCUC	2251
AD-73597	asgsagaaAfuGfCfUfuugagccucaL96	2162	usGfsaggCfuCfAfaagcAfuUfucucususu	2207	AAAGAGAAAUGCUUUGAGCCUCA	2252
AD-73619	asgsagaaAfuGfCfUfuugagccucaL96	2163	PusGfsaggCfuCfAfaagcAfuUfucucususu	2208	AAAGAGAAAUGCUUUGAGCCUCA	2253
AD-73596	gsasgaaaUfgCfUfUfugagccucaaL96	2164	usUfsgagGfcUfCfaaagCfaUfuucucsusu	2209	AAGAGAAAUGCUUUGAGCCUCAG	2254
AD-73618	gsasgaaaUfgCfUfUfugagccucaaL96	2165	PusUfsgagGfcUfCfaaagCfaUfuucucsusu	2210	AAGAGAAAUGCUUUGAGCCUCAG	2255
AD-73614	asgsaaauGfcUfUfUfgagccucagaL96	2166	usCfsugaGfgCfUfcaaaGfcAfuuucuscsu	2211	AGAGAAAUGCUUUGAGCCUCAGC	2256
AD-73637	asgsaaauGfcUfUfUfgagccucagaL96	2167	PusCfsugaGfgCfUfcaaaGfcAfuuucuscsu	2212	AGAGAAAUGCUUUGAGCCUCAGC	2257
AD-73611	asasaugcUfuUfGfAfgccucagcuaL96	2168	usAfsgcuGfaGfGfcucaAfaGfcauuuscsu	2213	AGAAAUGCUUUGAGCCUCAGCUU	2258
AD-73634	asasaugcUfuUfGfAfgccucagcuaL96	2169	PusAfsgcuGfaGfGfcucaAfaGfcauuuscsu	2214	AGAAAUGCUUUGAGCCUCAGCUU	2259
AD-73605	asusgcuuUfgAfGfCfcucagcuucaL96	2170	usGfsaagCfuGfAfggcuCfaAfagcaususu	2215	AAAUGCUUUGAGCCUCAGCUUCU	2260
AD-73628	asusgcuuUfgAfGfCfcucagcuucaL96	2171	PusGfsaagCfuGfAfggcuCfaAfagcaususu	2216	AAAUGCUUUGAGCCUCAGCUUCU	2261

AD-73601	usgscuuuGfaGfCfCfucagcuucuaL96	2172	usAfsgaaGfcUfGfaggcUfcAfaagcasusu	2217	AAUGCUUUGAGCCUCAGCUUCUC	2262
AD-73624	usgscuuuGfaGfCfCfucagcuucuaL96	2173	PusAfsgaaGfcUfGfaggcUfcAfaagcasusu	2218	AAUGCUUUGAGCCUCAGCUUCUC	2263
AD-73613	gscsuuugAfgCfCfUfcagcuucucaL96	2174	usGfsagaAfgCfUfgaggCfuCfaaagcsasu	2219	AUGCUUUGAGCCUCAGCUUCUCA	2264
AD-73636	gscsuuugAfgCfCfUfcagcuucucaL96	2175	PusGfsagaAfgCfUfgaggCfuCfaaagcsasu	2220	AUGCUUUGAGCCUCAGCUUCUCA	2265
AD-73616	ascsuccaCfcUfUfCfcugcaggagaL96	2176	usCfsuccUfgCfAfggaaGfgUfggagusasu	2221	AUACUCCACCUUCCUGCAGGAGG	2266
AD-73639	ascsuccaCfcUfUfCfcugcaggagaL96	2177	PusCfsuccUfgCfAfggaaGfgUfggagusasu	2222	AUACUCCACCUUCCUGCAGGAGG	2267
AD-73603	csascagaAfaCfUfCfaauaaagugaL96	2178	usCfsacuUfuAfUfugagUfuUfcugugscsc	2223	GGCACAGAAACUCAAUAAAGUGC	2268
AD-73626	csascagaAfaCfUfCfaauaaagugaL96	2179	PusCfsacuUfuAfUfugagUfuUfcugugscsc	2224	GGCACAGAAACUCAAUAAAGUGC	2269
AD-73607	ascsagaaAfcUfCfCfAfauaaagugcaL96	2180	usGfscacUfuUfAfuugaGfuUfucugusgsc	2225	GCACAGAAACUCAAUAAAGUGCU	2270
AD-73630	ascsagaaAfcUfCfCfAfauaaagugcaL96	2181	PusGfscacUfuUfAfuugaGfuUfucugusgsc	2226	GCACAGAAACUCAAUAAAGUGCU	2271
AD-73600	csasgaaaCfuCfAfAfuaaagugcuaL96	2182	usAfsgcaCfuUfUfauugAfgUfuucugsusg	2227	CACAGAAACUCAAUAAAGUGCUU	2272
AD-73622	csasgaaaCfuCfAfAfuaaagugcuaL96	2183	PusAfsgcaCfuUfUfauugAfgUfuucugsusg	2228	CACAGAAACUCAAUAAAGUGCUU	2273
AD-73615	asgsaaacUfcAfAfUfaaagugcuuaL96	2184	usAfsagcAfcUfUfuauuGfaGfuuucusgsu	2229	ACAGAAACUCAAUAAAGUGCUUU	2274
AD-73638	asgsaaacUfcAfAfUfaaagugcuuaL96	2185	PusAfsagcAfcUfUfuauuGfaGfuuucusgsu	2230	ACAGAAACUCAAUAAAGUGCUUU	2275
AD-73598	gsasaacuCfaAfUfAfaagugcuuuaL96	2186	usAfsaagCfaCfUfuuauUfgAfguuucsusg	2231	CAGAAACUCAAUAAAGUGCUUUG	2276
AD-73620	gsasaacuCfaAfUfAfaagugcuuuaL96	2187	PusAfsaagCfaCfUfuuauUfgAfguuucsusg	2232	CAGAAACUCAAUAAAGUGCUUUG	2277
AD-73602	asasacucAfaUfAfAfagugcuuugaL96	2188	usCfsaaaGfcAfCfuuuaUfuGfaguuucsu	2233	AGAAACUCAAUAAAGUGCUUUGA	2278
AD-73625	asasacucAfaUfAfAfagugcuuugaL96	2189	PusCfsaaaGfcAfCfuuuaUfuGfaguuucsu	2234	AGAAACUCAAUAAAGUGCUUUGA	2279
AD-73608	ascsucaaUfaAfAfGfugcuuugaaaL96	2190	usUfsucaAfaGfCfacuuUfaUfugagususu	2235	AAACUCAAUAAAGUGCUUUGAAA	2280
AD-73631	ascsucaaUfaAfAfGfugcuuugaaaL96	2191	PusUfsucaAfaGfCfacuuUfaUfugagususu	2236	AAACUCAAUAAAGUGCUUUGAAA	2281
AD-73612	uscsaauaAfaGfUfGfcuuugaaaaaL96	2192	usUfsuuuCfaAfAfgcacUfuUfauugasgsu	2237	ACUCAAUAAAGUGCUUUGAAAAC	2282
AD-73635	uscsaauaAfaGfUfGfcuuugaaaaaL96	2193	PusUfsuuuCfaAfAfgcacUfuUfauugasgsu	2238	ACUCAAUAAAGUGCUUUGAAAAC	2283
AD-73623	asascucaAfuAfAfAfgugcuuugaaL96	2194	PusUfscaaAfgCfAfcuuuAfuUfgaguususc	2239	GAAACUCAAUAAAGUGCUUUGAA	2284
AD-74838	asasuaaaGfuGfCfUfuugaaaacgaL96	2337	usCfsguuUfuCfAfaagcAfcUfuuauusgsa	2339	UCAAUAAAGUGCUUUGAAAACGA	2341
AD-74842	asasuaaaGfuGfCfUfuugaaaacgaL96	2338	PusCfsguuUfuCfAfaagcAfcUfuuauusgsa	2340	UCAAUAAAGUGCUUUGAAAACGA	2342

Table 28. F12 Single Dose Screen in Primary Mouse Hepatocytes

	Activity			
	10nM		0.1nM*	
Duplex ID	Avg	SD	Avg	SD
AD-67244	7.5	2.3	69.5	4.6
AD-73610	46.8	14.1	104.7	10.1
AD-73633	18.0	6.8	69.2	13.3
AD-73604	21.0	6.3	100.3	10.0
AD-73627	10.5	3.2	55.5	5.7
AD-73595	29.0	7.6	96.1	4.8
AD-73617	12.4	4.9	66.1	10.5
AD-73606	11.8	3.6	93.2	4.1
AD-73629	14.9	4.6	57.8	6.4
AD-73609	35.2	4.7	89.6	5.0
AD-73632	3.3	0.6	46.5	8.0
AD-73599	11.7	2.2	84.4	10.9
AD-73621	5.9	1.7	34.8	4.4
AD-73597	9.4	1.8	60.6	3.0
AD-73619	5.0	1.7	21.0	7.4
AD-73596	7.3	3.1	53.2	10.9
AD-73618	4.6	2.4	29.4	8.2
AD-73614	24.0	8.8	96.0	4.8
AD-73637	7.1	2.2	47.3	6.9
AD-73611	17.3	3.8	92.5	4.0
AD-73634	7.1	3.5	54.5	12.5
AD-73605	10.2	2.1	88.7	6.0
AD-73628	5.7	0.4	23.5	8.1
AD-73601	6.4	2.4	67.4	9.9
AD-73624	3.0	0.5	28.0	5.9
AD-73613	16.4	5.3	92.6	8.8
AD-73636	4.8	1.5	22.5	7.2
AD-73616	99.7	8.0	97.3	3.2
AD-73639	35.5	4.8	100.3	6.7
AD-73603	12.8	5.0	87.7	7.2
AD-73626	2.2	0.8	19.8	4.3
AD-73607	17.4	5.6	90.0	6.4
AD-73630	3.9	1.2	25.0	7.3
AD-73600	2.7	1.5	24.9	4.9
AD-73622	1.5	0.2	16.2	2.3
AD-73615	7.6	3.6	51.9	4.8
AD-73638	3.7	1.5	17.6	5.6

AD-73598	2.0	0.5	18.7	7.5
AD-73620	2.0	0.3	9.5	3.4
AD-73602	4.4	1.9	48.7	8.4
AD-73625	3.3	1.4	9.8	2.0
AD-73608	5.5	1.3	65.4	10.7
AD-73631	2.1	0.4	11.1	1.9
AD-73612	5.4	1.4	49.1	7.3
AD-73635	3.5	0.7	13.0	2.5
AD-73623	2.5	0.4	7.2	1.2

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Factor XII (Hageman Factor) (F12), wherein said dsRNA agent comprises a sense strand and an antisense strand, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:9 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:10.

2. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Factor XII (Hageman Factor) (F12) , wherein said dsRNA agent 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 9, 10, 19C, 19D, 20, 21, 23, 24, 26, and 27.

3. A double stranded ribonucleic acid (RNAi) agent for inhibiting expression of a Factor XII (Hageman Factor) (F12) gene, wherein the double stranded RNAi agent 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 nucleotides 2000-2060 of SEQ ID NO:9.

4. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Kallikrein B, Plasma (Fletcher Factor) 1 (KLKB1), wherein said dsRNA agent comprises a sense strand and an antisense strand, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2.

5. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of a Kallikrein B, Plasma (Fletcher Factor) 1 (KLKB1), wherein said dsRNA agent 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, 19A, or 19B.

6. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Kininogen 1 (KNG1), wherein said dsRNA agent comprises a sense strand and an antisense strand, wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:17 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:18.

7. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of a Kininogen 1 (KNG1), wherein said dsRNA agent 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 15, 16, 19E or 19F.

8. The dsRNA agent of any one of claims 1-7, wherein said dsRNA agent comprises at least one modified nucleotide.

9. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Factor XII (Hageman Factor) (F12), wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:9 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:10,

wherein substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand are modified nucleotides, and

wherein said sense strand is conjugated to a ligand attached at the 3'-terminus.

10. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Kallikrein B, Plasma (Fletcher Factor) 1 (KLKB1), wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2,

wherein substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand are modified nucleotides, and

wherein said sense strand is conjugated to a ligand attached at the 3'-terminus.

11. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of Kininogen 1 (KNG1), wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region,

wherein said sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:17 and said antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:18,

wherein substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand are modified nucleotides, and

wherein said sense strand is conjugated to a ligand attached at the 3'-terminus.

12. The dsRNA agent of any one of claims 9-11, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a modification.

13. The dsRNA agent of any one of claims 8-12, wherein at least one of said modified nucleotides is selected from the group consisting of 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 locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, and a nucleotide comprising a 5'-phosphate mimic.

14. The dsRNA agent of any of claims 8-12, wherein at least one of said modified nucleotides is selected from the group consisting of 2'-O-methyl and 2'fluoro modifications.

15. The dsRNA agent of claim 13 or 14, further comprising at least one phosphorothioate internucleotide linkage.

16. The dsRNA agent of claim 15, wherein the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages.

17. The dsRNA agent of any one of claims 1-7, wherein the region of complementarity is at least 17 nucleotides in length.

18. The dsRNA agent of any one of claims 1-7, wherein the region of complementarity is 19 to 30 nucleotides in length.

19. The dsRNA agent of claim 18, wherein the region of complementarity is 21 nucleotides in length.

20. The dsRNA agent of claim 18, wherein the region of complementarity is 21 to 23 nucleotides in length.

21. The dsRNA agent of claim 18, wherein the region of complementarity is 19 nucleotides in length.

22. The dsRNA agent of any one of claims 1-21, wherein each strand is no more than 30 nucleotides in length.

23. The dsRNA agent of any one of claims 1-21, wherein each strand is independently 19-30 nucleotides in length.

24. The dsRNA agent of any one of claims 1-20, wherein each strand is independently 19-25 nucleotides in length.

25. The dsRNA agent of any one of claims 1-23, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

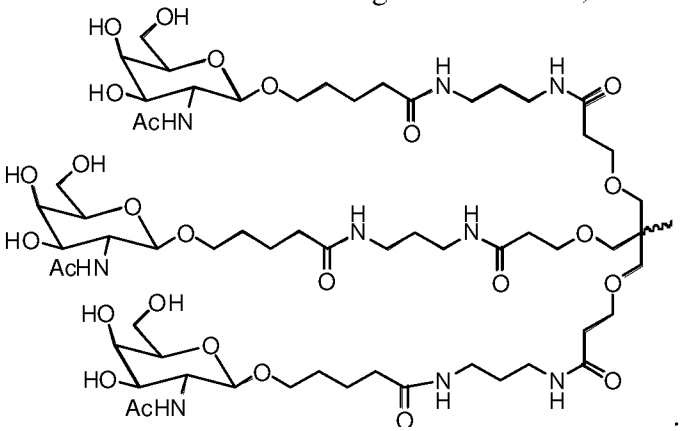
26. The dsRNA agent of any one of claims 1-23, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

27. The dsRNA agent of any one of claims 1-7, further comprising a ligand.

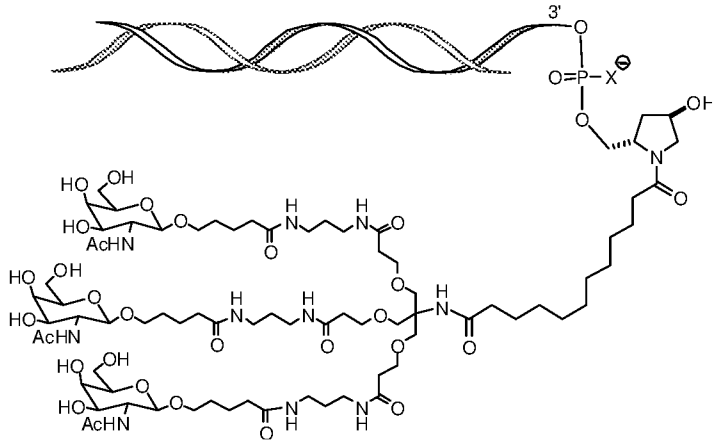
28 The dsRNA agent of claim 27, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

29. The dsRNA agent of any one of claims 9-11 and 28, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

30. The dsRNA agent of claim 29, wherein the ligand is



31. The dsRNA agent of claim 30, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

32. The dsRNA agent of claim 31, wherein the X is O.

33. The dsRNA agent of claim 1, wherein the agent is selected from the group consisting of AD-66170, AD-66173, AD-66176, AD-66125, AD-66172 , AD-66167 , AD-66165, AD-66168, AD-66163, AD-66116, AD-66126, and AD-67244.

34. The dsRNA agent of claim 4, wherein the agent is selected from the group consisting of AD-65077, AD-65170, AD-65103, AD-65083, AD-65087, AD-65149, AD-64652, AD-65162, AD-65153, AD-65084, AD-65099, and AD-66948.

35. The dsRNA agent of claim 6, wherein the agent is selected from the group consisting of AD-66259, AD-66261, AD-66262, AD-66263, AD-6634, and AD-67344.

36. A cell containing the dsRNA agent of any one of claims 1-35.

37. A vector encoding at least one strand of of the dsRNA agent of any one of claims 1-3.

38. A vector encoding at least one strand of of the dsRNA agent of claim 4 or 5.

39. A vector encoding at least one strand of of the dsRNA agent of claim 6 or 7.

40. A pharmaceutical composition for inhibiting expression of a KLKB1 gene comprising the dsRNA agent of any one of claims 4, 5, and 10, or the vector of claim 38.

41. A pharmaceutical composition for inhibiting expression of a F12 gene comprising the dsRNA agent of any one of claims 1-3 and 9, or the vector of claim 37.

42. A pharmaceutical composition for inhibiting expression of a KNG1 gene comprising the dsRNA agent of any one of claims 6, 7, and 11 or the vector of claim 39.

43. The pharmaceutical composition of any one of claims 40-42, wherein dsRNA agent is administered in an unbuffered solution.

44. The pharmaceutical composition of claim 43, wherein said unbuffered solution is saline or water.

45. The pharmaceutical composition of any one of claims 40-42, wherein said dsRNA agent is administered with a buffer solution.

46. The pharmaceutical composition of claim 45, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

47. The pharmaceutical composition of claim 45, wherein said buffer solution is phosphate buffered saline (PBS).

48. A pharmaceutical composition comprising the dsRNA agent of any one of claims 1-35, and a lipid formulation.

49. A method of inhibiting KLKB1 expression in a cell, the method comprising:

(a) contacting the cell with the dsRNA agent of any one of claims 4, 5, and 10, or a pharmaceutical composition of any one of claims 40 and 43-48; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a KLKB1 gene, thereby inhibiting expression of the KLKB1 gene in the cell.

50. A method of inhibiting F12 expression in a cell, the method comprising:

(a) contacting the cell with the dsRNA agent of any one of claims 1-3 and 9, or a pharmaceutical composition of any one of claims 41 and 43-48; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a F12 gene, thereby inhibiting expression of the F12 gene in the cell.

51. A method of inhibiting KNG1 expression in a cell, the method comprising:

(a) contacting the cell with the dsRNA agent of any one of claims 6, 7, and 11, or a pharmaceutical composition of any one of claims 42-48; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a KNG1 gene, thereby inhibiting expression of the KNG1 gene in the cell.

52. The method of any one of claims 49-51, wherein said cell is within a subject.

53. The method of claim 50, wherein the subject is a human.

54. The method of any one of claims 49, 52, and 53, wherein the KLKB1 expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

55. The method of any one of claims 50, 52, and 53, wherein the F12 expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

56. The method of any one of claims 51-53, wherein the KNG1 expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98% or about 100%.

57. A method of treating a subject having a disease or disorder that would benefit from reduction in expression of a contact activation pathway gene, the method comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-11, or a pharmaceutical composition of any one of claims 40-48, thereby treating said subject.

58. A method of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in expression of a contact activation pathway gene, the method comprising administering to the subject a prophylactically effective amount of the dsRNA agent of any one of claims 1-11, or a pharmaceutical composition of any one of claims 40-48, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in expression of a contact activation pathway gene.

59. The method of claim 57 or 58, wherein the administration of the dsRNA to the subject causes a decrease in bradykinin levels or a decrease in coagulation factor XII activity.

60. The method of claim 57 or 58, wherein the disorder is a contact activation pathway-associated disease.

61. The method of claim 59, wherein the contact activation pathway-associated disease is thrombophilia.

62. The method of claim 60, wherein the contact activation pathway-associated disease is hereditary angioedema (HAE).

63. The method of claim 60, wherein the contact activation pathway-associated disease is Flectcher Factor Deficiency.

64. The method of claim 60, wherein the contact activation pathway-associated disease is essential hypertension.

65. The method of claim 58, wherein the at least one symptom is an angioedema attack.

66. The method of claim 58, wherein the at least one symptom is thrombus formation.

67. The method of claim 57 or 58, wherein the subject is human.

68. The method of any one of claims 57-67, further comprising administering an anti-KLKB1 antibody, or antigen-binding fragment thereof, to the subject.

69. The method of claim 57, wherein the method comprises administering to the subject a therapeutically effective amount of a dsRNA agent of any one of claims 1-3 or 9, and the method further comprises administering to the subject a dsRNA agent of any one of claims 4, 5, and 10.

70. The method of claim 57, wherein the method comprises administering to the subject a therapeutically effective amount of a dsRNA agent of any one of claims 1-3 or 9, and the method further comprises administering to the subject a dsRNA agent of any one of claims 6, 7, and 11.

71. The method of claim 58, wherein the method comprises administering to the subject a prophylactically effective amount of a dsRNA agent of any one of claims 1-3 or 9, and the method further comprises administering to the subject a dsRNA agent of any one of claims 4, 5, and 10.

72. The method of claim 58, wherein the method comprises administering to the subject a prophylactically effective amount of a dsRNA agent of any one of claims 1-3 or 9, and the method further comprises administering to the subject a dsRNA agent of any one of claims 6, 7, and 11.

73. The method of claim 57 or 58, further comprising measuring bradykinin and/or coagulation factor XII levels in said subject.

74. A method of treating a subject having a thrombophilia, the method comprising administering to the subject a therapeutically effective amount of a dsRNA agent of any one of claims 1-3 or 9, or the pharmaceutical composition of any one of claims 41 and 43-48, thereby treating said subject.

75. A method of preventing at least one symptom in a subject having a thrombophilia, the method comprising administering to the subject a prophylactically effective amount of a dsRNA agent of any one of claims 1-3 or 9, or a pharmaceutical composition of any one of claims 41 and 43-48, thereby preventing at least one symptom in the subject.

76. The method of claim 72 or 73, further comprising administering to the subject a dsRNA agent of any one of claims 4, 5, or 10.

77. The method of claim 72 or 73, further comprising administering to the subject a dsRNA agent of any one of claims 6, 7, or 11.

78. A method of treating a subject having hereditary angioedema (HAE), the method comprising administering to the subject a therapeutically effective amount of a dsRNA agent of any one of claims 1-3 or 9, or a pharmaceutical composition of any one of claims 41 and 43-48, thereby treating said subject.

79. A method of preventing at least one symptom in a subject havinghereditary angioedema (HAE), the method comprising administering to the subject a prophylactically effective amount of a dsRNA agent of any one of claims 1-3 or 9, or a pharmaceutical composition of any one of claims 41 and 43-48, thereby preventing at least one symptom in the subject.

80. The method of claim 76 or 77, further comprising administering to the subject a dsRNA agent of any one of claims 4, 5, or 10.

81. The method of claim 76 or 77, further comprising administering to the subject a dsRNA agent of any one of claims 6, 7, or 11.

82. A method of inhibiting the expression of KLKB1 in a subject, the method comprising

administering to said subject a therapeutically effective amount of a dsRNA agent of any one of claims 4, 5, or 10, thereby inhibiting the expression of KLKB1 in said subject.

83. A method of inhibiting the expression of F12 in a subject, the method comprising

administering to said subject a therapeutically effective amount of a dsRNA agent of any one of claims 1-3 or 9 thereby inhibiting the expression of F12 in said subject.

84. A method of inhibiting the expression of KNG1 in a subject, the method comprising

administering to said subject a therapeutically effective amount of a dsRNA agent of any one of claims 6, 7, or 11, thereby inhibiting the expression of KNG1 in said subject.

85. A method of preventing the formation of a thrombus in a subject at risk of forming a thrombus, comprising administering to the subject a prophylactically effective amount of a dsRNA agent of any one of claims 1-3 or 9, or a pharmaceutical composition of any one of claims 41 and 43-48, thereby inhibiting formation of a thrombus in the subject at risk of forming a thrombus.

86. The method of claim 85, wherein the subject at risk of forming a thrombus has a contact activation pathway-associated disease or disorder.

87. The method of claim 86, wherein the contact activation pathway-associated disease is thrombophilia.

88. The method of claim 86, wherein the contact activation pathway-associated disease is hereditary angioedema (HAE).

89. The method of claim 86, wherein the contact activation pathway-associated disease is Flectcher Factor Deficiency.

90. The method of claim 86, wherein the contact activation pathway-associated disease is essential hypertension.

91. The method of claim 85, wherein the subject at risk of forming a thrombus is selected from the group consisting of a surgical patient; a medical patient; a pregnant subject; a postpartum subject; a subject that has previously had a thrombus; a subject undergoing hormone replacement therapy; a subject sitting for long periods; and an obese subject.

92. The method of claim 83, further comprising administering to the subject a dsRNA agent of any one of claims 4, 5, or 10.

93. The method of claim 83, further comprising administering to the subject a dsRNA agent of any one of claims 6, 7, or 11.

94. A method of preventing an angioedema attack in a subject having heriditary angioedema (HAE) , comprising administering to the subject a prophylactically effective amount of a dsRNA agent of any one of claims 1-3 or 9, or a pharmaceutical composition of any one of claims 41 and 43-48, thereby preventing an angioedema attack in the subject.

95. The method of claim 94, further comprising administering to the subject a dsRNA agent of any one of claims 4, 5, or 10.

96. The method of claim 94, further comprising administering to the subject a dsRNA agent of any one of claims 6, 7, or 11.

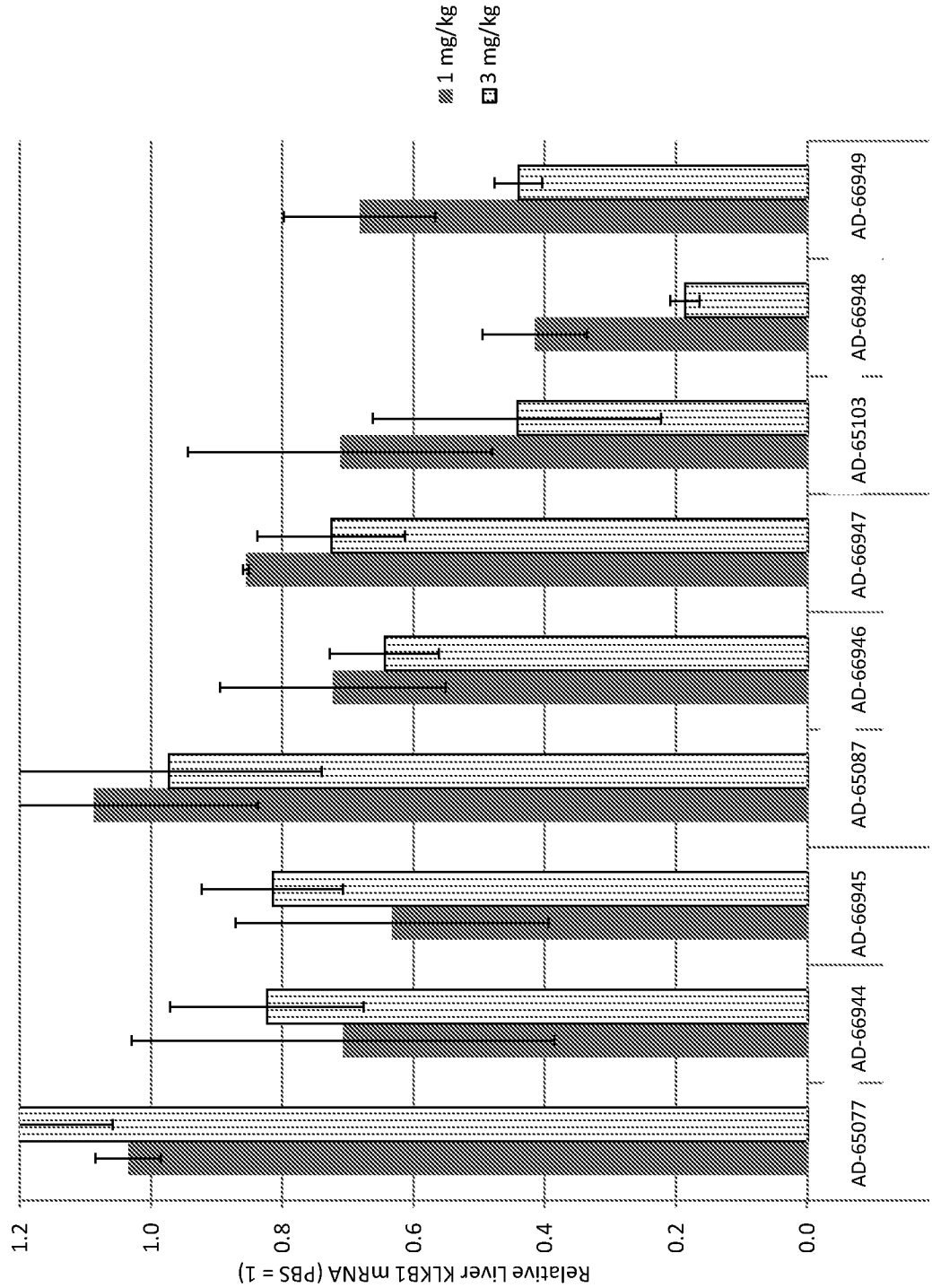

1 mg/kg
3 mg/kg
Relative Liver KLKB1 mRNA (PBS = 1)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
AD-65077
AD-66944
AD-66945
AD-65087
AD-66946
AD-66947
AD-65103
AD-66948
AD-66949

Figure 1

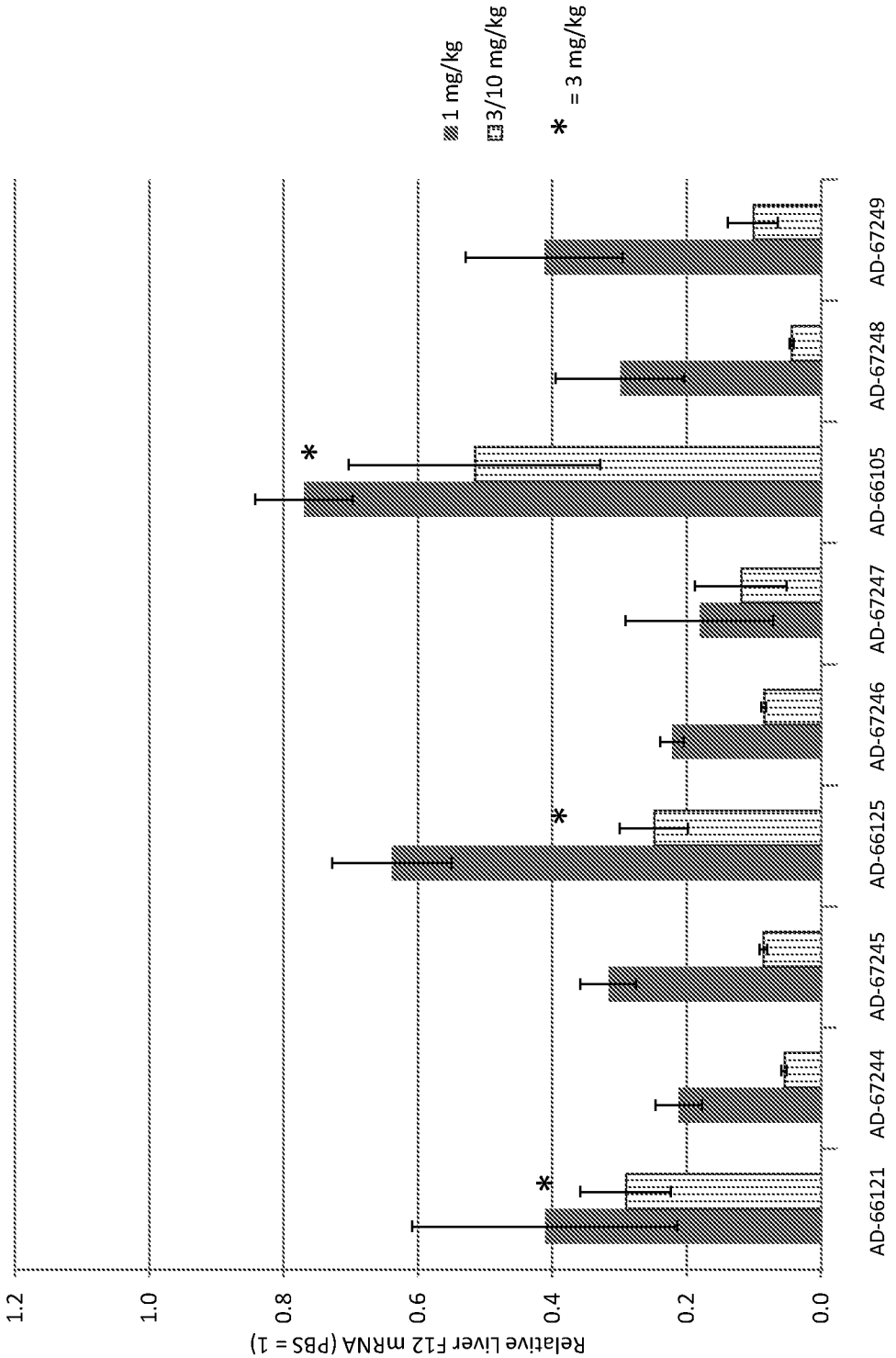

1 mg/kg
3/10 mg/kg
* = 3 mg/kg
Relative Liver F12 mRNA (PBS = 1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
AD-66121
AD-67244
AD-67245
AD-66125
AD-67246
AD-67247
AD-66105
AD-67248
AD-67249

Figure 2

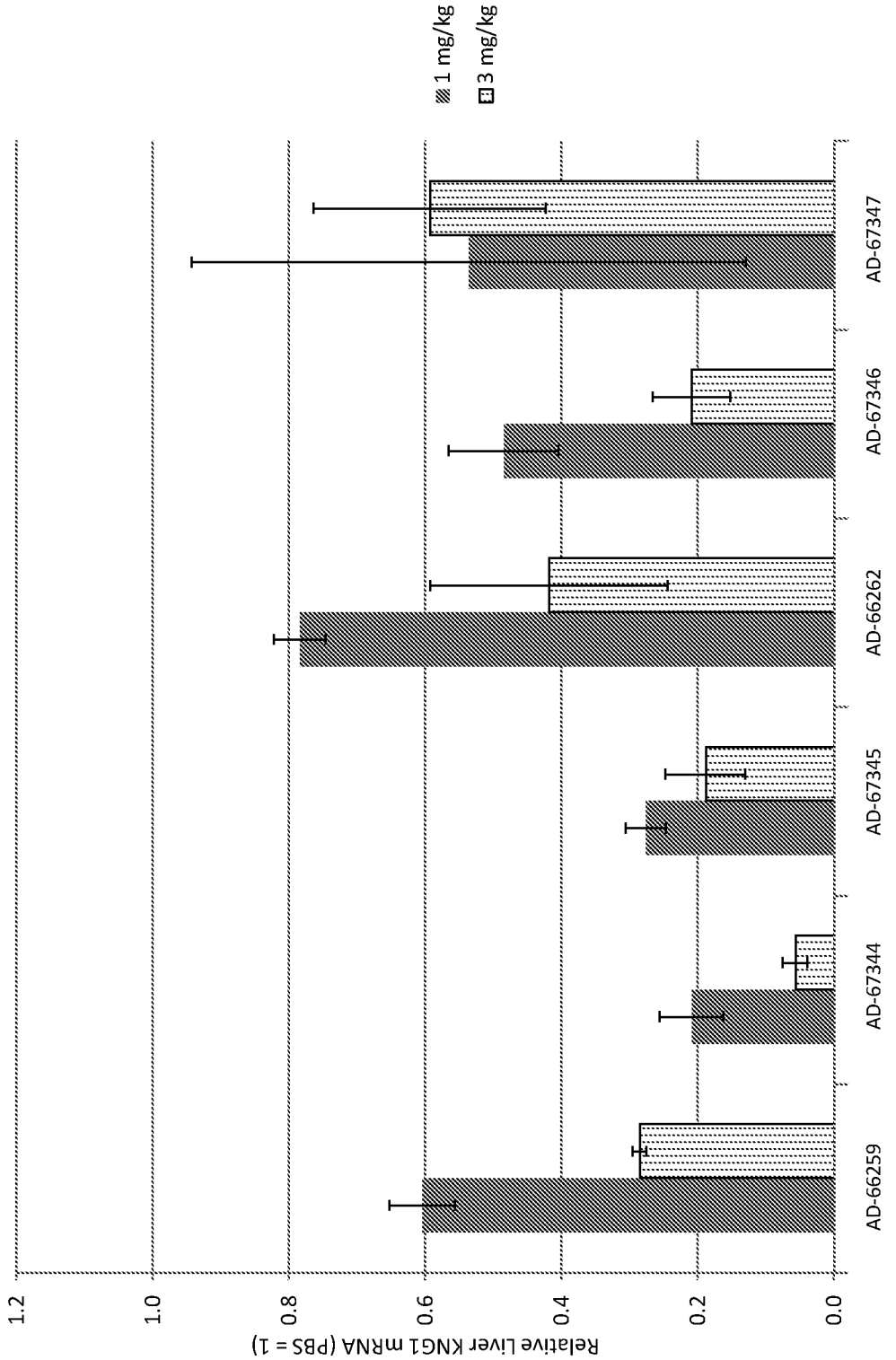

1 mg/kg
3 mg/kg
Relative Liver KNG1 mRNA (PBS = 1)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
AD-66259
AD-67344
AD-67345
AD-66262
AD-67346
AD-67347

Figure 3

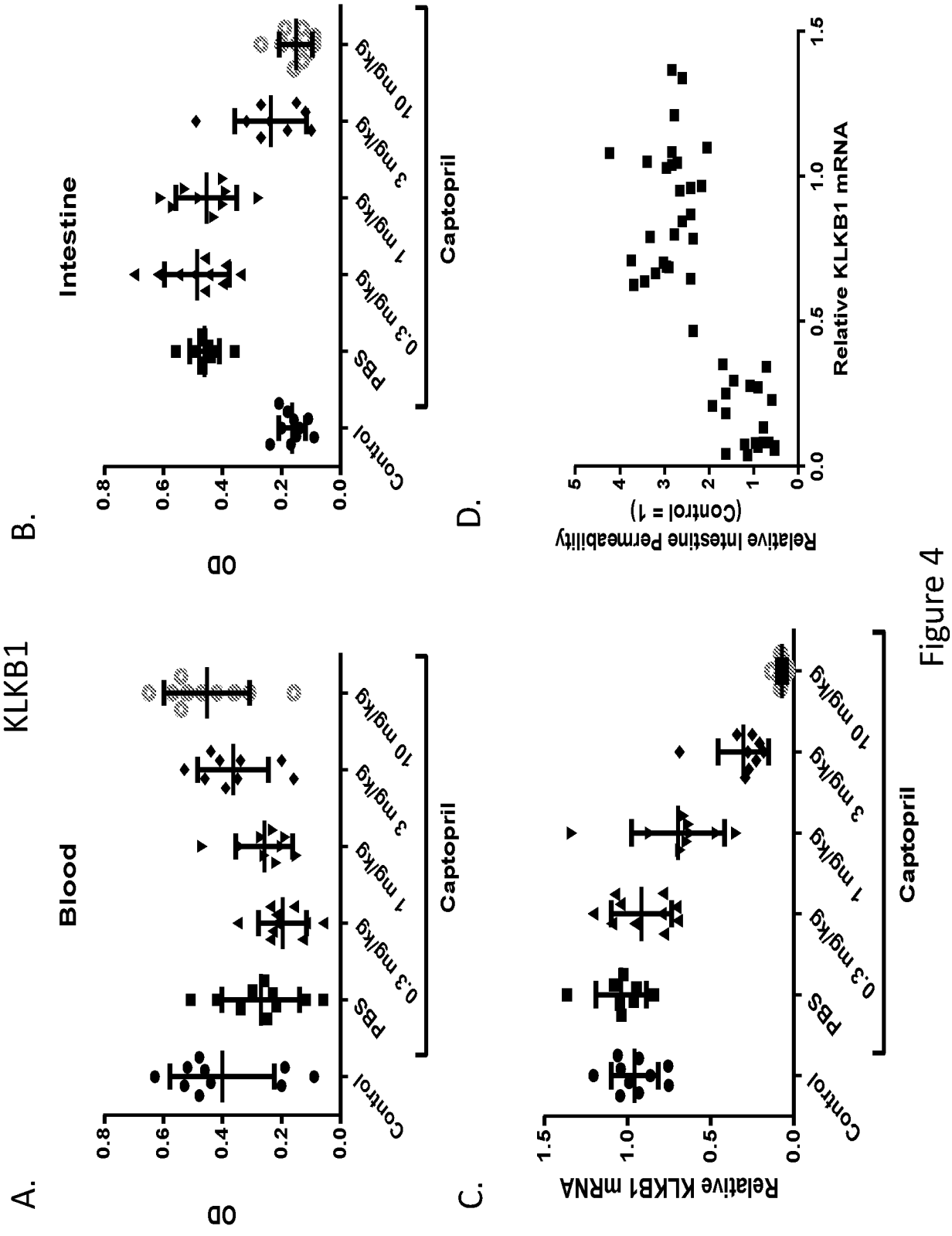

A.
KLKB1
Blood
OD
0.0
0.2
0.4
0.6
0.8
Control
PBS
0.3 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
Captopril
B.
Intestine
OD
0.0
0.2
0.4
0.6
0.8
Control
PBS
0.3 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
Captopril
C.
Relative KLKB1 mRNA
0.0
0.5
1.0
1.5
Control
PBS
0.3 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
Captopril
D.
Relative Intestine Permeability
(Control = 1)
0
1
2
3
4
5
0.0
0.5
1.0
1.5
Relative KLKB1 mRNA

Figure 4

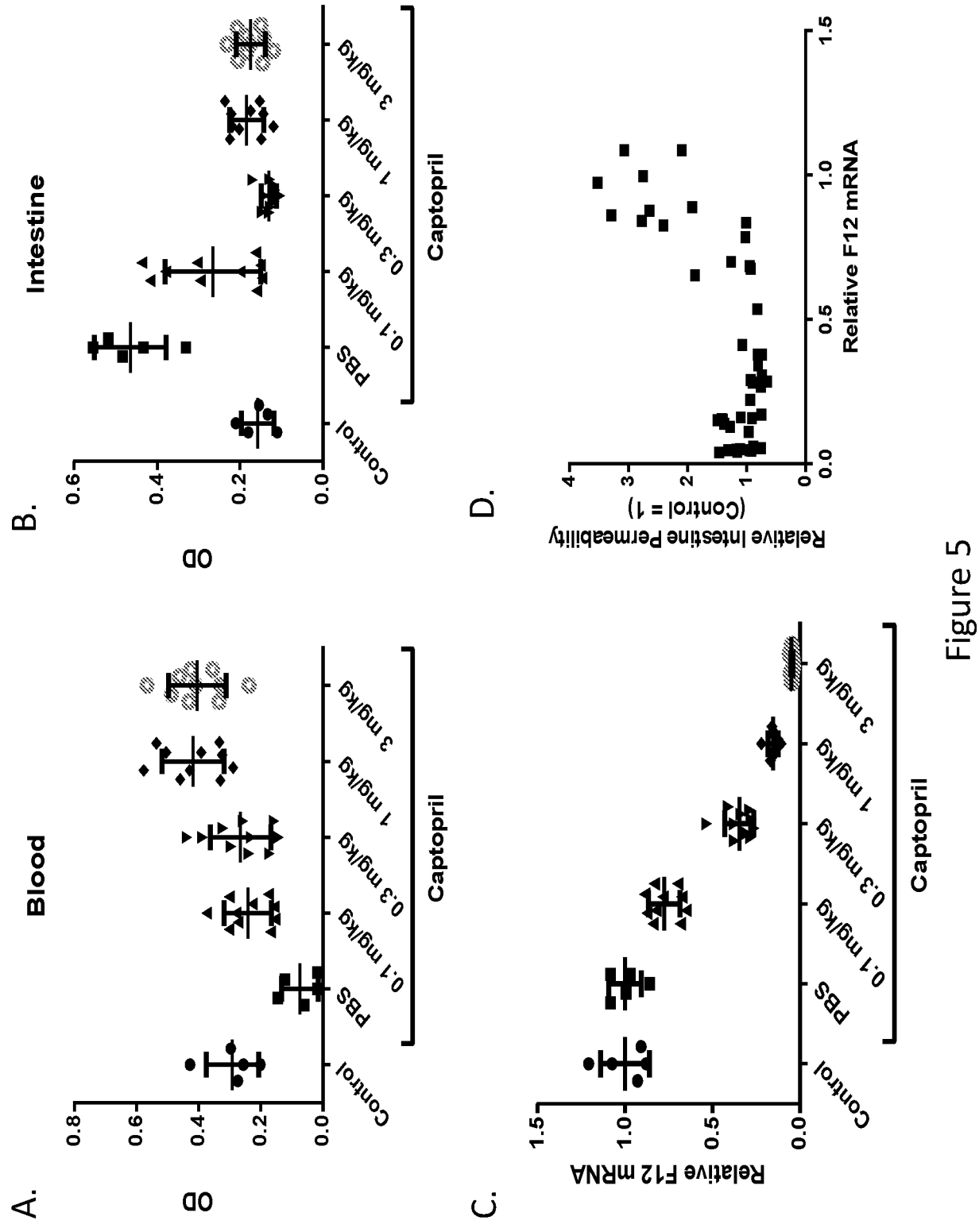

F12
A.
Blood
OD
0.0
0.2
0.4
0.6
0.8
Control
PBS
0.1 mg/kg
0.3 mg/kg
1 mg/kg
3 mg/kg
Captopril
B.
Intestine
OD
0.0
0.2
0.4
0.6
Control
PBS
0.1 mg/kg
0.3 mg/kg
1 mg/kg
3 mg/kg
Captopril
C.
Relative F12 mRNA
0.0
0.5
1.0
1.5
Control
PBS
0.1 mg/kg
0.3 mg/kg
1 mg/kg
3 mg/kg
Captopril
D.
Relative Intestine Permeability
(Control = 1)
0
1
2
3
4
0.0
0.5
1.0
1.5
Relative F12 mRNA

Figure 5

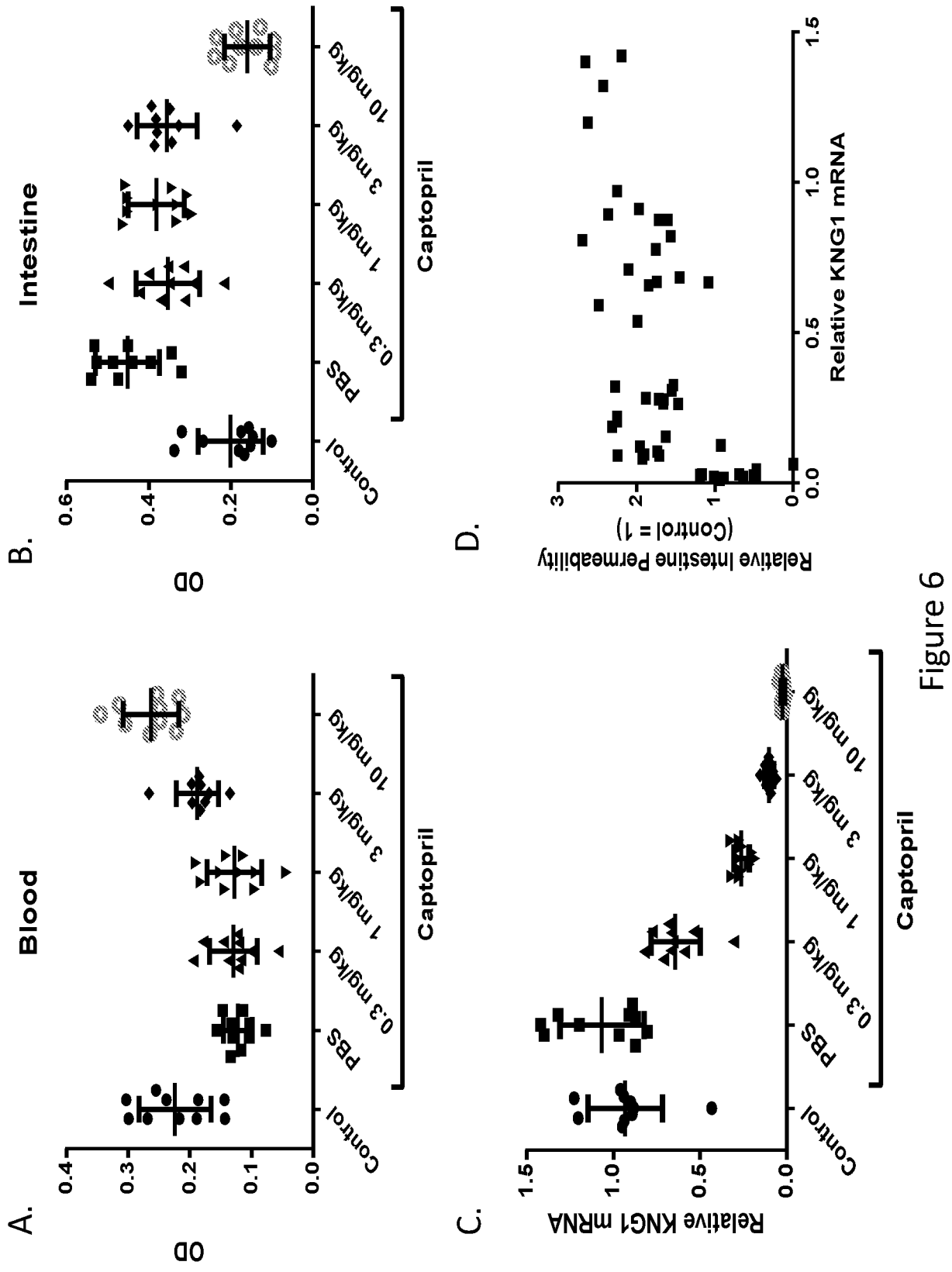

KNG1
A.
Blood
OD
0.4
0.3
0.2
0.1
0.0
Control
PBS
0.3 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
Captopril
B.
Intestine
OD
0.6
0.4
0.2
0.0
Control
PBS
0.3 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
Captopril
C.
Relative KNG1 mRNA
1.5
1.0
0.5
0.0
Control
PBS
0.3 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
Captopril
D.
Relative Intestine Permeability
(Control = 1)
3
2
1
0
0.0
0.5
1.0
1.5
Relative KNG1 mRNA

Figure 6

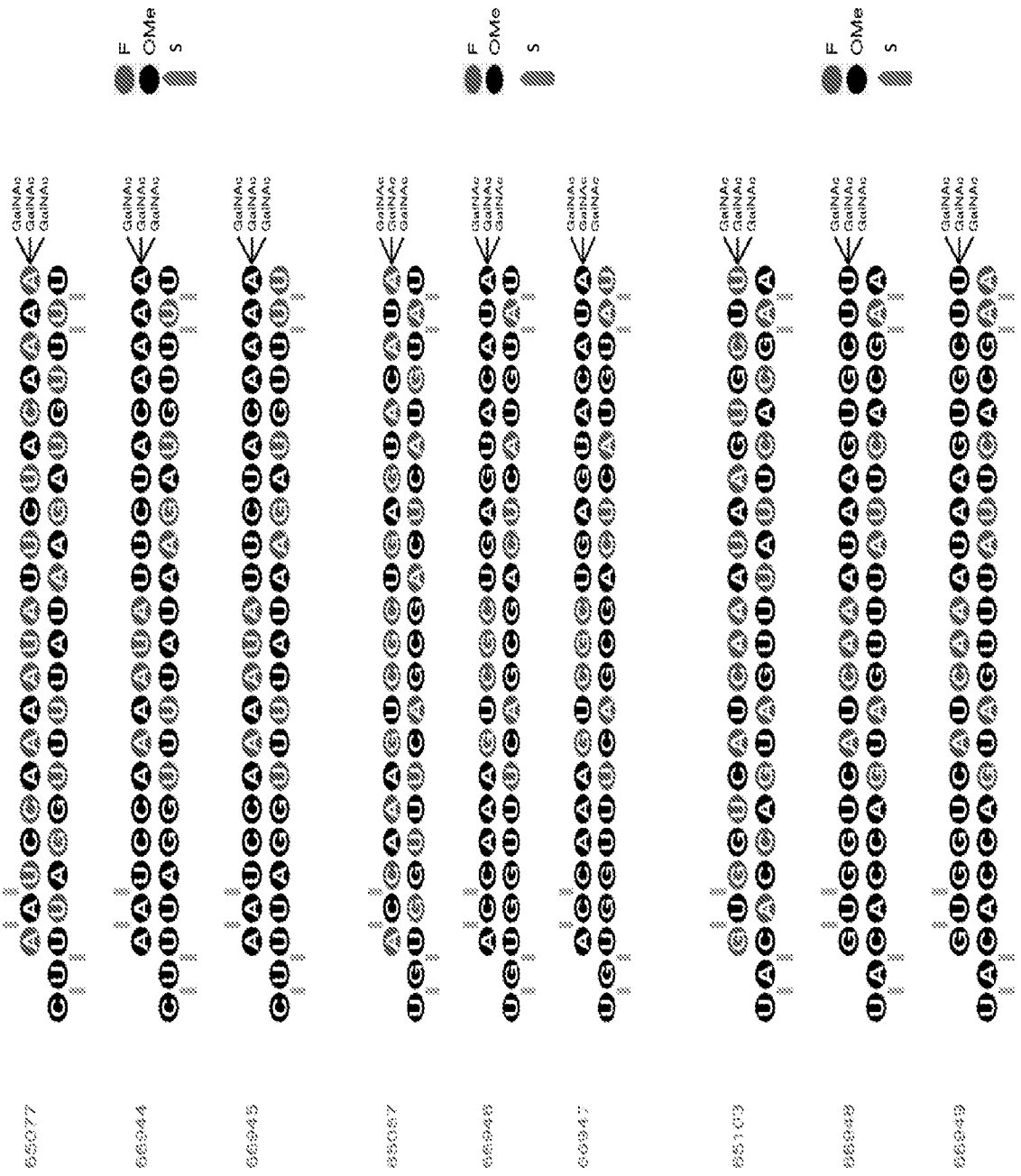


Figure 7

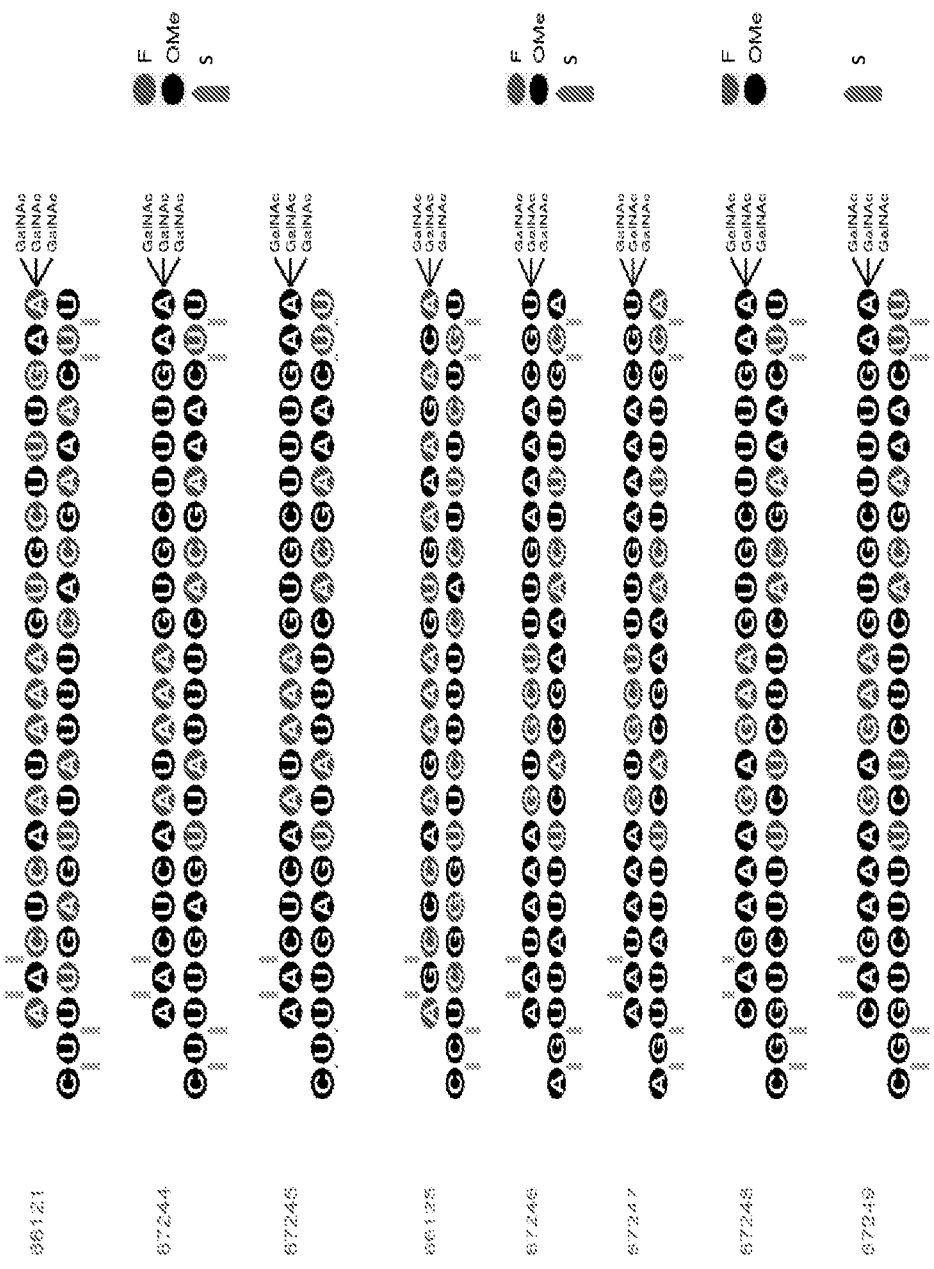

F
OMe
S
GalNAc
GalNAc
GalNAc
661121
672244
672245
661125
672246
672247
672248
672249

Figure 8

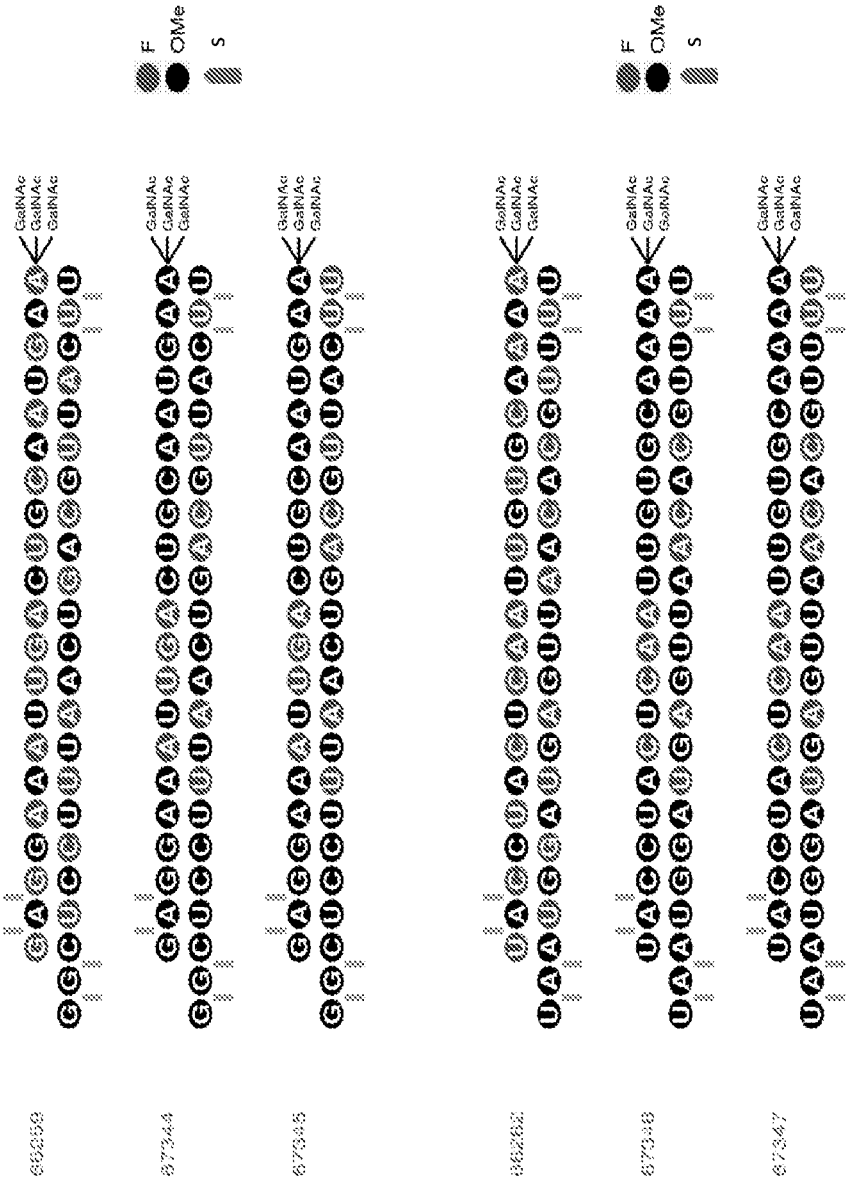

F
OMe
S
GalNAc
GalNAc
GalNAc
662359
673344
673345
662362
673348
673347

Figure 9

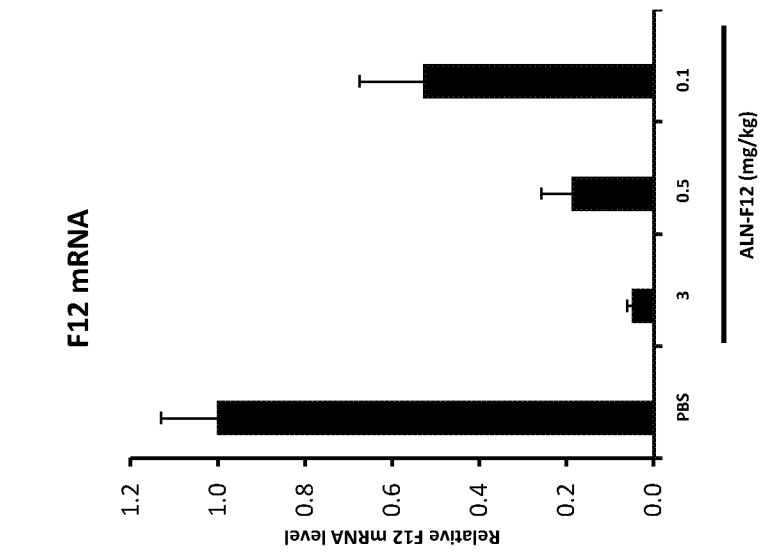


Figure 10B

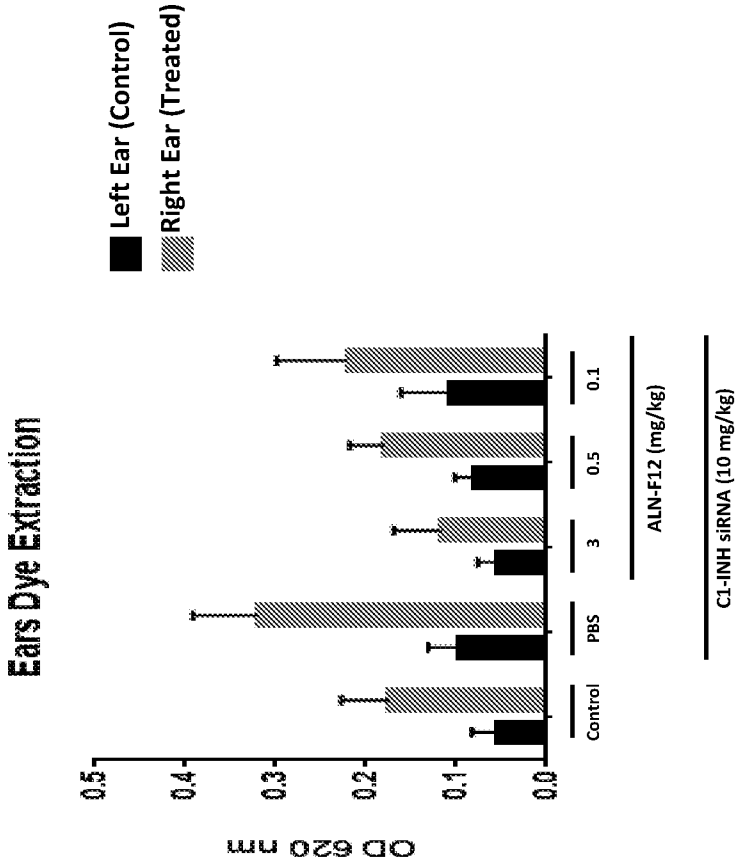


Figure 10A

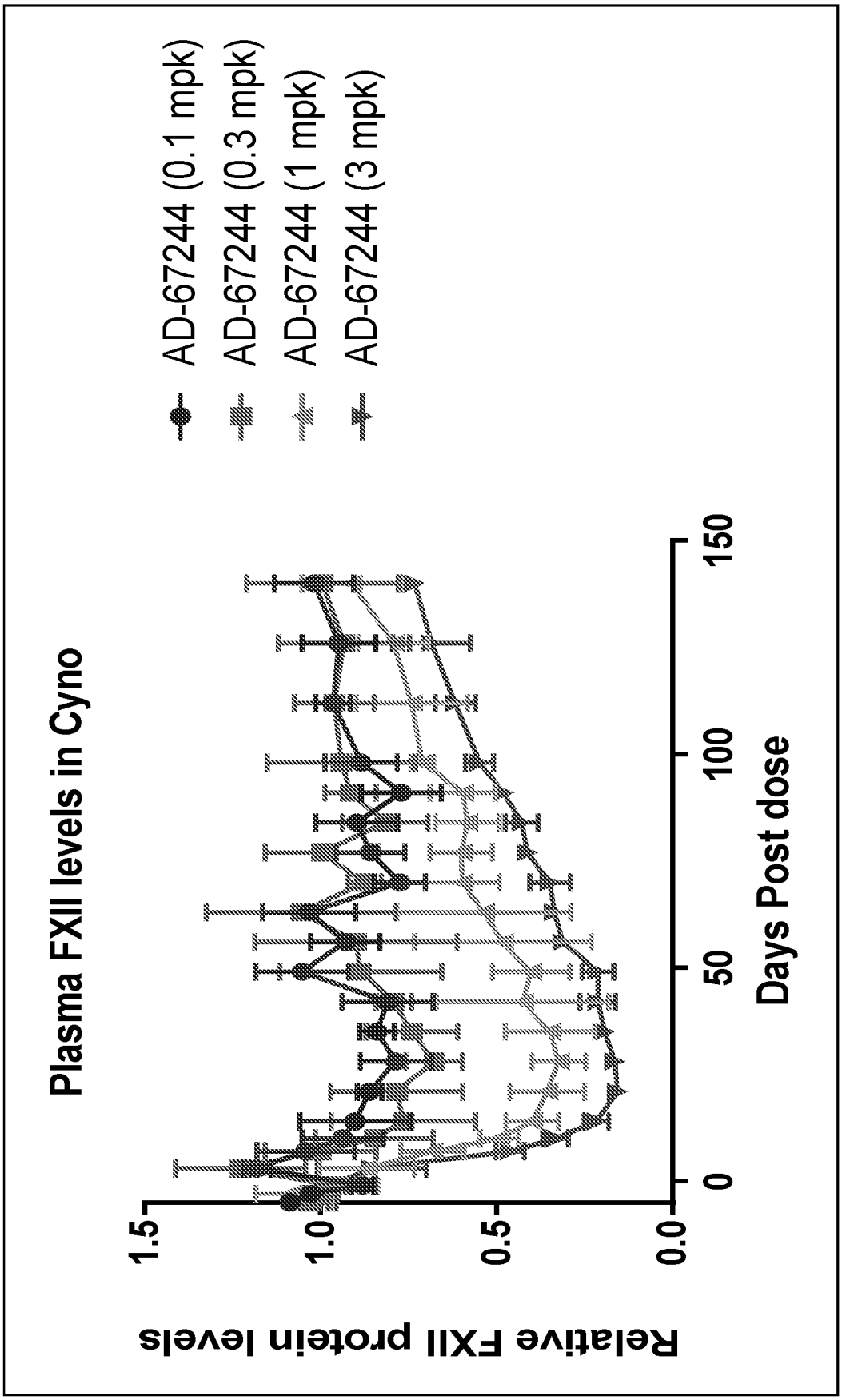

Plasma FXII levels in Cyno
AD-67244 (0.1 mpk)
AD-67244 (0.3 mpk)
AD-67244 (1 mpk)
AD-67244 (3 mpk)
Relative FXII protein levels
1.5
1.0
0.5
0.0
0
50
100
150
Days Post dose

Figure 11

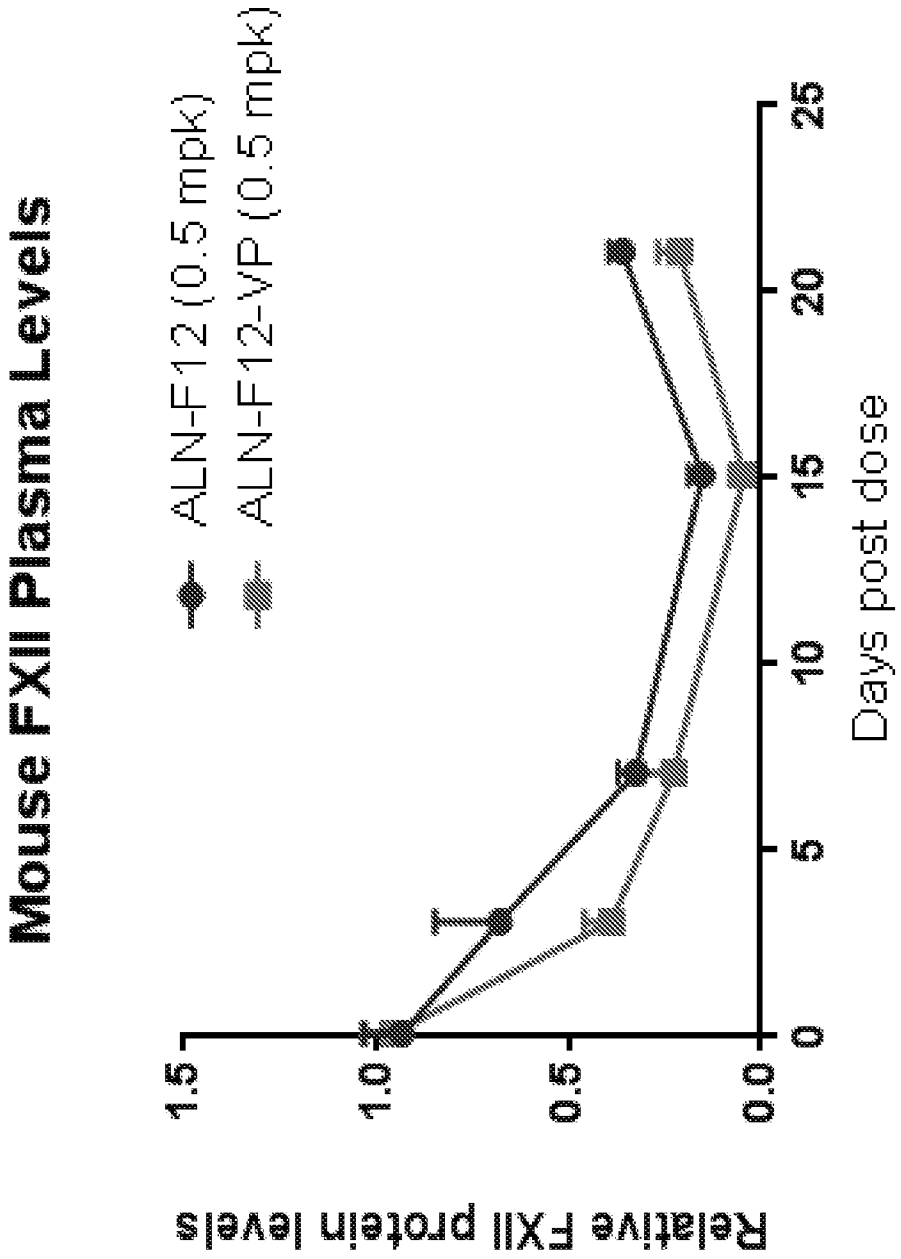

Mouse FXII Plasma Levels
ALN-F12 (0.5 mpk)
ALN-F12-VP (0.5 mpk)
Relative FXII protein levels
1.5
1.0
0.5
0.0
0
5
10
15
20
25
Days post dose

Figure 12

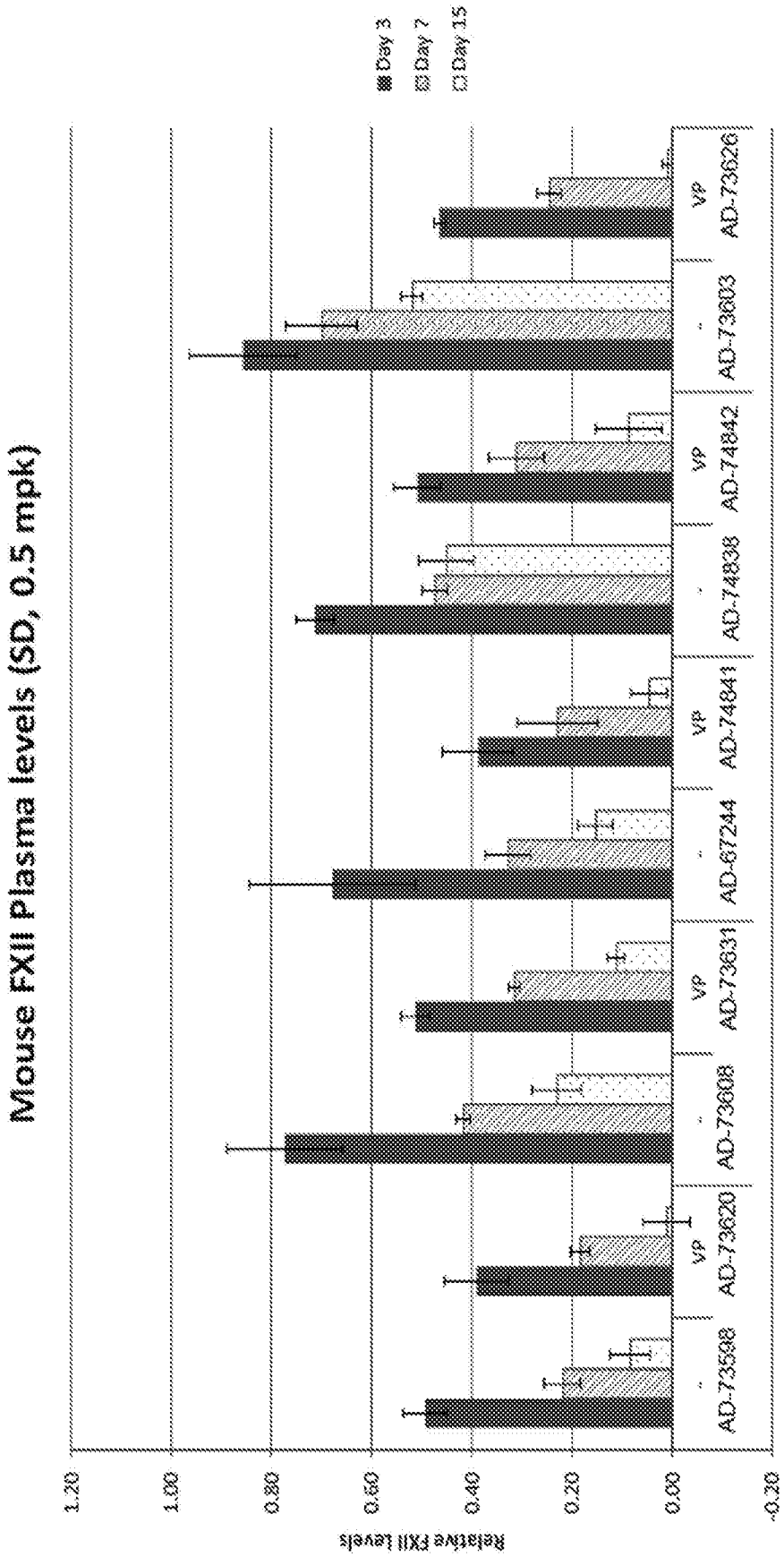

Mouse FXII Plasma levels (SD, 0.5 mpk)
Day 3
Day 7
Day 15
Relative FXII levels
1.20
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
AD-73598
VP
AD-73620
AD-73608
VP
AD-73631
AD-67244
VP
AD-74841
AD-74838
VP
AD-74842
AD-73603
VP
AD-73626

Figure 13