

RNAi Agents for Inhibiting Expression of Activin Receptor-Like Kinase 7 (ALK7), Compositions Thereof, and Methods of Use

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to United States Provisional Patent Application Serial No. 63/612,531, filed on December 20, 2023, United States Provisional Patent Application Serial No. 63/634,860, filed on April 16, 2024, and United States Provisional Patent Application Serial No. 63/683,214, filed on August 14, 2024, the contents of each of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present disclosure relates to RNA interference (RNAi) agents, e.g., double stranded RNAi agents such as chemically modified small interfering RNAs (siRNAs), for inhibition of Activin Receptor-Like Kinase 7 (“ALK7”) gene expression, compositions that include ALK7 RNAi agents, and methods of use thereof.

SEQUENCE LISTING

[0003] This application contains a Sequence Listing (in compliance with Standard ST26), which has been submitted in xml format and is hereby incorporated by reference in its entirety. The xml sequence listing file is named 30730-WO_SeqListing.xml, created December 6, 2024, and is 3427 kb in size.

BACKGROUND

[0004] As a key type I receptor for TGF- β family members, activin receptor-like kinase 7 (ALK7), also referred to as Acvr1c, has emerged as the determinate receptor for nodal and activin, both of which are implicated in various diseases. ALK7 is specifically expressed during the late phase of adipocyte differentiation. Adipocyte specific ALK7 dysfunction causes increased lipolysis, which leads to decreased fat accumulation, while ALK7 is also associated with multiple factors implicated in metabolic disease. ALK7 decreases inflammatory adipocytokine secretion and improves glucose tolerance and insulin sensitivity, and also protects against the development of pathological cardiac hypertrophy. Cheng et al. ALK7 Acts as a Positive Regulator of Macrophage Activation through Down-Regulation of

PPAR γ Expression. J Atheroscler Thromb. 2021 Apr 1;28(4):375-384. doi: 10.5551/jat.54445. Epub 2020 Jul 9. PMID: 32641645; PMCID: PMC8147563.

[0005] ALK7 is known to be predominantly expressed in adipose tissue compared with other tissues. Whole body ALK7-KO mice show reduced fat accumulation when under a high-fat diet. JCI Insight. 2023;8(4):e161229. <https://doi.org/10.1172/jci.insight.161229>.

SUMMARY

[0006] There exists a need for novel RNA interference (RNAi) agents (termed RNAi agents, RNAi triggers, or triggers), *e.g.*, double stranded RNAi agents such as siRNAs, that are able to selectively and efficiently inhibit the expression of an ALK7 gene, including for use as a therapeutic or medicament. Further, there exists a need for compositions of novel ALK7-specific RNAi agents for the treatment of diseases or disorders associated with ALK7 gene expression and/or disorders that can be mediated at least in part by a reduction in ALK7 gene expression.

[0007] The nucleotide sequences and chemical modifications of the ALK7 RNAi agents disclosed herein, as well as their combination with certain specific pharmacokinetic and pharmacodynamic (PK/PD) modulators suitable for selectively and efficiently delivering the ALK7 RNAi agents to adipose tissue *in vivo*, differ from those previously disclosed or known in the art. The ALK7 RNAi agents disclosed herein provide for highly potent and efficient inhibition of the expression of an ALK7 gene.

[0008] In general, the present disclosure features ALK7 gene-specific RNAi agents, compositions that include ALK7 RNAi agents, and methods for inhibiting expression of an ALK7 gene *in vitro* and/or *in vivo* using the ALK7 RNAi agents and compositions that include ALK7 RNAi agents described herein. The ALK7 RNAi agents described herein are able to selectively and efficiently decrease expression of an ALK7 gene, and thereby reduce the expression of the ALK7 protein.

[0009] The described ALK7 RNAi agents can be used in methods for therapeutic treatment (including preventative or prophylactic treatment) of symptoms and diseases including, but not limited to obesity, diabetes, or insulin resistance.

[0010] In one aspect, the disclosure features RNAi agents for inhibiting expression of an ALK7 gene, wherein the RNAi agent includes a sense strand (also referred to as a passenger strand) and an antisense strand (also referred to as a guide strand). The sense strand and the antisense strand can be partially, substantially, or fully complementary to each other. The length of the

RNAi agent sense strands described herein each can be 15 to 49 nucleotides in length. The length of the RNAi agent antisense strands described herein each can be 18 to 49 nucleotides in length. In some embodiments, the sense and antisense strands are independently 18 to 26 nucleotides in length. The sense and antisense strands can be either the same length or different lengths. In some embodiments, the sense and antisense strands are independently 21 to 26 nucleotides in length. In some embodiments, the sense and antisense strands are independently 21 to 24 nucleotides in length. In some embodiments, both the sense strand and the antisense strand are 21 nucleotides in length. In some embodiments, the antisense strands are independently 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In some embodiments, the sense strands are independently 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, or 49 nucleotides in length. The RNAi agents described herein, upon delivery to adipose tissue, inhibit the expression of one or more ALK7 gene variants *in vivo* and/or *in vitro*.

[0011] The ALK7 RNAi agents disclosed herein target a human ALK7 gene (*see, e.g.*, SEQ ID NO:1). In some embodiments, the ALK7 RNAi agents disclosed herein target a portion of an ALK7 gene having the sequence of any of the sequences disclosed in Table 1.

[0012] In another aspect, the disclosure features compositions, including pharmaceutical compositions, that include one or more of the disclosed ALK7 RNAi agents that are able to selectively and efficiently decrease expression of an ALK7 gene. The compositions that include one or more ALK7 RNAi agents described herein can be administered to a subject, such as a human or animal subject, for the treatment (including prophylactic treatment or inhibition) of symptoms and diseases associated with ALK7 protein levels.

[0013] Examples of ALK7 RNAi agent sense strands and antisense strands that can be used in an ALK7 RNAi agent are provided in Tables 3, 4, 5, and 6. Examples of ALK7 RNAi agent duplexes are provided in Tables 7A, 7B, 8, 9A, and 10. Examples of 19-nucleotide core stretch sequences that may consist of or may be included in the sense strands and antisense strands of certain ALK7 RNAi agents disclosed herein, are provided in Table 2.

[0014] In another aspect, the disclosure features methods for delivering ALK7 RNAi agents to adipose tissue in a subject, such as a mammal, *in vivo*. Also described herein are compositions for use in such methods. In some embodiments, disclosed herein are methods for delivering ALK7 RNAi agents to adipose tissue to a subject *in vivo*. In some embodiments, the subject is a human subject.

[0015] The methods disclosed herein include the administration of one or more ALK7 RNAi agents to a subject, e.g., a human or animal subject, by any suitable means known in the art. The pharmaceutical compositions disclosed herein that include one or more ALK7 RNAi agents can be administered in a number of ways depending upon whether local or systemic treatment is desired. Administration can be, but is not limited to, for example, intravenous, intraarterial, subcutaneous, intraperitoneal, subdermal (e.g., via an implanted device), and intraparenchymal administration. In some embodiments, the pharmaceutical compositions described herein are administered by subcutaneous or intravenous injection.

[0016] In some embodiments, it is desired that the ALK7 RNAi agents described herein inhibit the expression of an ALK7 gene in adipose tissue.

[0017] The one or more ALK7 RNAi agents can be delivered to target cells or tissues using any oligonucleotide delivery technology known in the art. In some embodiments, an ALK7 RNAi agent is delivered to cells or tissues by covalently linking the RNAi agent to a targeting group or a lipid moiety.

[0018] A PK/PD modulator can be linked to the 3' or 5' end of a sense strand or an antisense strand of an ALK7 RNAi agent. In some embodiments, a PK/PD modulator is linked to the 3' or 5' end of the sense strand. In some embodiments, a PK/PD modulator is linked to the 5' end of the sense strand. In some embodiments, a PK/PD modulator is linked internally to a nucleotide on the sense strand and/or the antisense strand of the RNAi agent. In some embodiments, a PK/PD modulator is linked to the RNAi agent via a linker.

[0019] In another aspect, the disclosure features compositions that include one or more ALK7 RNAi agents that have the duplex structures disclosed in Tables 7A, 7B, 8, 9A, and 10.

[0020] The use of ALK7 RNAi agents provides methods for therapeutic (including prophylactic) treatment of diseases or disorders for which a reduction in ALK7 protein levels can provide a therapeutic benefit. The ALK7 RNAi agents disclosed herein can be used to treat various metabolic diseases, including obesity, diabetes, or insulin resistance. Such methods of treatment include administration of an ALK7 RNAi agent to a human being or animal having elevated ALK7 protein beyond desirable levels.

Definitions

[0021] As used herein, the terms “oligonucleotide” and “polynucleotide” mean a polymer of linked nucleosides each of which can be independently modified or unmodified.

[0022] As used herein, an “RNAi agent” (also referred to as an “RNAi trigger”) means a chemical composition of matter that contains an RNA or RNA-like (e.g., chemically modified RNA) oligonucleotide molecule that is capable of degrading or inhibiting (e.g., degrades or inhibits under appropriate conditions) translation of messenger RNA (mRNA) transcripts of a target mRNA in a sequence specific manner. As used herein, RNAi agents may operate through the RNA interference mechanism (i.e., inducing RNA interference through interaction with the RNA interference pathway machinery (RNA-induced silencing complex or RISC) of mammalian cells), or by any alternative mechanism(s) or pathway(s). While it is believed that RNAi agents, as that term is used herein, operate primarily through the RNA interference mechanism, the disclosed RNAi agents are not bound by or limited to any particular pathway or mechanism of action. RNAi agents disclosed herein are comprised of a sense strand and an antisense strand, and include, but are not limited to: small (or short) interfering RNAs (siRNAs), double stranded RNAs (dsRNA), micro RNAs (miRNAs), short hairpin RNAs (shRNA), and dicer substrates. The antisense strand of the RNAi agents described herein is at least partially complementary to the mRNA being targeted (i.e. ALK7 mRNA). RNAi agents can include one or more modified nucleotides and/or one or more non-phosphodiester linkages.

[0023] As used herein, the terms “silence,” “reduce,” “inhibit,” “down-regulate,” or “knockdown” when referring to expression of a given gene, mean that the expression of the gene, as measured by the level of RNA transcribed from the gene or the level of polypeptide, protein, or protein subunit translated from the mRNA in a cell, group of cells, tissue, organ, or subject in which the gene is transcribed, is reduced when the cell, group of cells, tissue, organ, or subject is treated with the RNAi agents described herein as compared to a second cell, group of cells, tissue, organ, or subject that has not or have not been so treated.

[0024] As used herein, the terms “sequence” and “nucleotide sequence” mean a succession or order of nucleobases or nucleotides, described with a succession of letters using standard nomenclature.

[0025] As used herein, a “base,” “nucleotide base,” or “nucleobase,” is a heterocyclic pyrimidine or purine compound that is a component of a nucleotide, and includes the primary purine bases adenine and guanine, and the primary pyrimidine bases cytosine, thymine, and uracil. A nucleobase may further be modified to include, without limitation, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. (*See, e.g., Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-*

VCH, 2008). The synthesis of such modified nucleobases (including phosphoramidite compounds that include modified nucleobases) is known in the art.

[0026] As used herein, and unless otherwise indicated, the term “complementary,” when used to describe a first nucleobase or nucleotide sequence (e.g., RNAi agent sense strand or targeted mRNA) in relation to a second nucleobase or nucleotide sequence (e.g., RNAi agent antisense strand or a single-stranded antisense oligonucleotide), means the ability of an oligonucleotide or polynucleotide including the first nucleotide sequence to hybridize (form base pair hydrogen bonds under mammalian physiological conditions (or otherwise suitable *in vivo* or *in vitro* conditions)) and form a duplex or double helical structure under certain standard conditions with an oligonucleotide that includes the second nucleotide sequence. The person of ordinary skill in the art would be able to select the set of conditions most appropriate for a hybridization test. Complementary sequences include Watson-Crick base pairs or non-Watson-Crick base pairs and include natural or modified nucleotides or nucleotide mimics, at least to the extent that the above hybridization requirements are fulfilled. Sequence identity or complementarity is independent of modification. For example, a and Af, as defined herein, are complementary to U (or T) and identical to A for the purposes of determining identity or complementarity.

[0027] As used herein, “perfectly complementary” or “fully complementary” means that in a hybridized pair of nucleobase or nucleotide sequence molecules, all (100%) of the bases in a contiguous sequence of a first oligonucleotide will hybridize with the same number of bases in a contiguous sequence of a second oligonucleotide. The contiguous sequence may comprise all or a part of a first or second nucleotide sequence.

[0028] As used herein, “partially complementary” means that in a hybridized pair of nucleobase or nucleotide sequence molecules, at least 70%, but not all, of the bases in a contiguous sequence of a first oligonucleotide will hybridize with the same number of bases in a contiguous sequence of a second oligonucleotide. The contiguous sequence may comprise all or a part of a first or second nucleotide sequence.

[0029] As used herein, “substantially complementary” means that in a hybridized pair of nucleobase or nucleotide sequence molecules, at least 85%, but not all, of the bases in a contiguous sequence of a first oligonucleotide will hybridize with the same number of bases in a contiguous sequence of a second oligonucleotide. The contiguous sequence may comprise all or a part of a first or second nucleotide sequence.

[0030] As used herein, the terms “complementary,” “fully complementary,” “partially complementary,” and “substantially complementary” are used with respect to the nucleobase

or nucleotide matching between the sense strand and the antisense strand of an RNAi agent, or between the antisense strand of an RNAi agent and a sequence of an ALK7 mRNA.

[0031] As used herein, the term “substantially identical” or “substantial identity,” as applied to a nucleic acid sequence means the nucleotide sequence (or a portion of a nucleotide sequence) has at least about 85% sequence identity or more, e.g., at least 90%, at least 95%, or at least 99% identity, compared to a reference sequence. Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window. The percentage is calculated by determining the number of positions at which the same type of nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. The inventions disclosed herein encompass nucleotide sequences substantially identical to those disclosed herein.

[0032] As used herein, the terms “treat,” “treatment,” and the like, mean the methods or steps taken to provide relief from or alleviation of the number, severity, and/or frequency of one or more symptoms of a disease in a subject. As used herein, “treat” and “treatment” may include the prevention, management, prophylactic treatment, and/or inhibition or reduction of the number, severity, and/or frequency of one or more symptoms of a disease in a subject.

[0033] As used herein, the phrase “introducing into a cell,” when referring to an RNAi agent, means functionally delivering the RNAi agent into a cell. The phrase “functional delivery,” means delivering the RNAi agent to the cell in a manner that enables the RNAi agent to have the expected biological activity, e.g., sequence-specific inhibition of gene expression.

[0034] Unless stated otherwise, use of the symbol  as used herein means that any group or groups may be linked thereto that is in accordance with the scope of the inventions described herein.

[0035] As used herein, the term “isomers” refers to compounds that have identical molecular formulae, but that differ in the nature or the sequence of bonding of their atoms or in the arrangement of their atoms in space. Isomers that differ in the arrangement of their atoms in space are termed “stereoisomers.” Stereoisomers that are not mirror images of one another are termed “diastereoisomers,” and stereoisomers that are non-superimposable mirror images are termed “enantiomers,” or sometimes optical isomers. A carbon atom bonded to four non-identical substituents is termed a “chiral center.”

[0036] As used herein, unless specifically identified in a structure as having a particular conformation, for each structure in which asymmetric centers are present and thus give rise to enantiomers, diastereomers, or other stereoisomeric configurations, each structure disclosed herein is intended to represent all such possible isomers, including their optically pure and racemic forms. For example, the structures disclosed herein are intended to cover mixtures of diastereomers as well as single stereoisomers.

[0037] As used in a claim herein, the phrase “consisting of” excludes any element, step, or ingredient not specified in the claim. When used in a claim herein, the phrase “consisting essentially of” limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claimed invention.

[0038] The person of ordinary skill in the art would readily understand and appreciate that the compounds and compositions disclosed herein may have certain atoms (e.g., N, O, or S atoms) in a protonated or deprotonated state, depending upon the environment in which the compound or composition is placed. Accordingly, as used herein, the structures disclosed herein envisage that certain functional groups, such as, for example, OH, SH, or NH, may be protonated or deprotonated. The disclosure herein is intended to cover the disclosed compounds and compositions regardless of their state of protonation based on the environment (such as pH), as would be readily understood by the person of ordinary skill in the art. Correspondingly, compounds described herein with labile protons or basic atoms should also be understood to represent salt forms of the corresponding compound. Compounds described herein may be in a free acid, free base, or salt form. Pharmaceutically acceptable salts of the compounds described herein should be understood to be within the scope of the invention.

[0039] As used herein, the term “linked” or “conjugated” when referring to the connection between two compounds or molecules means that two compounds or molecules are joined by a covalent bond. Unless stated, the terms “linked” and “conjugated” as used herein may refer to the connection between a first compound and a second compound either with or without any intervening atoms or groups of atoms.

[0040] As used herein, the term “including” is used to herein 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 the context clearly indicates otherwise.

[0041] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing

of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0042] Other objects, features, aspects, and advantages of the invention will be apparent from the following detailed description, accompanying figures, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] FIG. 1A shows the average body weights of mice administered a control formulation, RNAi agent, GLP1 agonist, or combination thereof according to Example 20.

[0044] FIG. 1B shows the body composition of mice administered a control formulation, RNAi agent, GLP1 agonist, or combination thereof according to Example 20.

[0045] FIG 2 shows a Proposed Clinical Study Schema for Part 1 (Cohorts 1-4) of a Phase 1/2a dose-escalating study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of single and multiple doses of an ALK7 RNAi agent, showing dosing for adult volunteers with obesity (*see* Example 21 herein). “ARO-ALK7” represents an ALK7 RNAi agent-conjugate in accordance with the disclosure herein.

[0046] FIG 3 shows Proposed Clinical Study Schema for Part 2 of a Phase 1/2a dose-escalating study to evaluate the safety, tolerability, and pharmacodynamics of repeat doses of an ALK7 RNAi agent in combination with a GLP-1/GIP agonist (tirzepatide (TZP)), showing dosing for adult volunteers with obesity with and without type 2 diabetes mellitus (*see* Example 21 herein). “ARO-ALK7” represents an ALK7 RNAi agent-conjugate in accordance with the disclosure herein.

[0047] FIGs 4A-4D show a chemical structure representation of ALK7 RNAi agent AC006188 (SEQ ID NOs: 269/535), shown with PK/PD modulators linked to the 5' and 3' ends of the sense strand, shown as a free acid.

[0048] FIGs 5A-5D show a chemical structure representation of ALK7 RNAi agent AC006189 (SEQ ID NOs: 270/536), shown with PK/PD modulators linked to the 5' and 3' ends of the sense strand, shown as a free acid.

[0049] FIGs 6A-6D show a chemical structure representation of ALK7 RNAi agent AC006648 (SEQ ID NOs: 320/534), shown with PK/PD modulators linked to the 5' and 3' ends of the sense strand, shown as a free acid.

[0050] FIGs 7A-7D show a chemical structure representation of ALK7 RNAi agent AC006661 (SEQ ID NOs: 328/533), shown with PK/PD modulators linked to the 5' and 3' ends of the sense strand, shown as a free acid.

[0051] FIGs 8A-8D show a chemical structure representation of ALK7 RNAi agent AC006663 (SEQ ID NOs: 330/533), shown with PK/PD modulators linked to the 5' and 3' ends of the sense strand, shown as a free acid.

[0052] FIGs 9A-9D show a chemical structure representation of ALK7 RNAi agent AC006188 (SEQ ID NOs: 269/535), shown with PK/PD modulators linked to the 5' and 3' ends of the sense strand, shown as a sodium salt.

[0053] FIGs 10A-10D show a chemical structure representation of ALK7 RNAi agent AC006189 (SEQ ID NOs: 270/536), shown with PK/PD modulators linked to the 5' and 3' ends of the sense strand, shown as a sodium salt.

[0054] FIGs 11A-11D show a chemical structure representation of ALK7 RNAi agent AC006648 (SEQ ID NOs: 320/534), shown with PK/PD modulators linked to the 5' and 3' ends of the sense strand, shown as a sodium salt.

[0055] FIGs 12A-12D show a chemical structure representation of ALK7 RNAi agent AC006661 (SEQ ID NOs: 328/533), shown with PK/PD modulators linked to the 5' and 3' ends of the sense strand, shown as a sodium salt.

[0056] FIGs 13A-13D show a chemical structure representation of ALK7 RNAi agent AC006663 (SEQ ID NOs: 330/533), shown with PK/PD modulators linked to the 5' and 3' ends of the sense strand, shown as a sodium salt.

DETAILED DESCRIPTION

RNAi Agents

[0057] Described herein are RNAi agents for inhibiting expression of the ALK7 (or Acvr1c) gene (referred to herein as ALK7 RNAi agents or ALK7 RNAi triggers). Each ALK7 RNAi agent disclosed herein comprises a sense strand and an antisense strand. The sense strand can be 15 to 49 nucleotides in length. The antisense strand can be 18 to 30 nucleotides in length. The sense and antisense strands can be either the same length or they can be different lengths. In some embodiments, the sense and antisense strands are each independently 18 to 27 nucleotides in length. In some embodiments, both the sense and antisense strands are each 21-26 nucleotides in length. In some embodiments, the sense and antisense strands are each 21-24 nucleotides in length. In some embodiments, the sense and antisense strands are each

independently 19-21 nucleotides in length. In some embodiments, the sense strand is about 19 nucleotides in length while the antisense strand is about 21 nucleotides in length. In some embodiments, the sense strand is about 21 nucleotides in length while the antisense strand is about 23 nucleotides in length. In some embodiments, a sense strand is 23 nucleotides in length and an antisense strand is 21 nucleotides in length. In some embodiments, both the sense and antisense strands are each 21 nucleotides in length. In some embodiments, the RNAi agent sense strands are each independently 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, or 49 nucleotides in length. In some embodiments, the RNAi agent antisense strands are each independently 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In some embodiments, a double-stranded RNAi agent has a duplex length of about 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides.

[0058] Examples of nucleotide sequences used in forming ALK7 RNAi agents are provided in Tables 2, 3, 4, 5, 6, and 10. Examples of RNAi agent duplexes, that include the sense strand and antisense strand sequences in Tables 2, 3, 4, 5, 6, are shown in Tables 7A, 7B, 8, 9A, and 10.

[0059] In some embodiments, the region of perfect, substantial, or partial complementarity between the sense strand and the antisense strand is 16-26 (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) nucleotides in length and occurs at or near the 5' end of the antisense strand (e.g., this region may be separated from the 5' end of the antisense strand by 0, 1, 2, 3, or 4 nucleotides that are not perfectly, substantially, or partially complementary).

[0060] A sense strand of the ALK7 RNAi agents described herein includes at least 15 consecutive nucleotides that have at least 85% identity to a core stretch sequence (also referred to herein as a "core stretch" or "core sequence") of the same number of nucleotides in an ALK7 mRNA. In some embodiments, a sense strand core stretch sequence is 100% (perfectly) complementary or at least about 85% (substantially) complementary to a core stretch sequence in the antisense strand, and thus the sense strand core stretch sequence is typically perfectly identical or at least about 85% identical to a nucleotide sequence of the same length (sometimes referred to, e.g., as a target sequence) present in the ALK7 mRNA target. In some embodiments, this sense strand core stretch is 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. In some embodiments, this sense strand core stretch is 17 nucleotides in length. In some embodiments, this sense strand core stretch is 19 nucleotides in length.

[0061] An antisense strand of an ALK7 RNAi agent described herein includes at least 16 consecutive nucleotides that have at least 85% complementarity to a core stretch of the same number of nucleotides in an ALK7 mRNA and to a core stretch of the same number of nucleotides in the corresponding sense strand. In some embodiments, an antisense strand core stretch is 100% (perfectly) complementary or at least about 85% (substantially) complementary to a nucleotide sequence (*e.g.*, target sequence) of the same length present in the ALK7 mRNA target. In some embodiments, this antisense strand core stretch is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. In some embodiments, this antisense strand core stretch is 19 nucleotides in length. In some embodiments, this antisense strand core stretch is 17 nucleotides in length. A sense strand core stretch sequence can be the same length as a corresponding antisense core sequence or it can be a different length.

[0062] The ALK7 RNAi agent sense and antisense strands anneal to form a duplex. A sense strand and an antisense strand of an ALK7 RNAi agent can be partially, substantially, or fully complementary to each other. Within the complementary duplex region, the sense strand core stretch sequence is at least 85% complementary or 100% complementary to the antisense core stretch sequence. In some embodiments, the sense strand core stretch sequence contains a sequence of at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, or at least 23 nucleotides that is at least 85% or 100% complementary to a corresponding 16, 17, 18, 19, 20, 21, 22, or 23 nucleotide sequence of the antisense strand core stretch sequence (*i.e.*, the sense and antisense core stretch sequences of an ALK7 RNAi agent have a region of at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, or at least 23 nucleotides that is at least 85% base paired or 100% base paired.)

[0063] In some embodiments, the antisense strand of an ALK7 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the antisense strand sequences in Table 2 or Table 3. In some embodiments, the sense strand of an ALK7 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the sense strand sequences in Table 2, Table 4, Table 5, Table 6, Table 6a, or Table 10.

[0064] In some embodiments, the sense strand and/or the antisense strand can optionally and independently contain an additional 1, 2, 3, 4, 5, or 6 nucleotides (extension) at the 3' end, the 5' end, or both the 3' and 5' ends of the core stretch sequences. The antisense strand additional nucleotides, if present, may or may not be complementary to the corresponding sequence in the ALK7 mRNA. The sense strand additional nucleotides, if present, may or may not be identical to the corresponding sequence in the ALK7 mRNA. The antisense strand additional

nucleotides, if present, may or may not be complementary to the corresponding sense strand's additional nucleotides, if present.

[0065] As used herein, an extension comprises 1, 2, 3, 4, 5, or 6 nucleotides at the 5' and/or 3' end of the sense strand core stretch sequence and/or antisense strand core stretch sequence. The extension nucleotides on a sense strand may or may not be complementary to nucleotides, either core stretch sequence nucleotides or extension nucleotides, in the corresponding antisense strand. Conversely, the extension nucleotides on an antisense strand may or may not be complementary to nucleotides, either core stretch nucleotides or extension nucleotides, in the corresponding sense strand. In some embodiments, both the sense strand and the antisense strand of an RNAi agent contain 3' and 5' extensions. In some embodiments, one or more of the 3' extension nucleotides of one strand base pairs with one or more 5' extension nucleotides of the other strand. In other embodiments, one or more of 3' extension nucleotides of one strand do not base pair with one or more 5' extension nucleotides of the other strand. In some embodiments, an ALK7 RNAi agent has an antisense strand having a 3' extension and a sense strand having a 5' extension. In some embodiments, the extension nucleotide(s) are unpaired and form an overhang. As used herein, an "overhang" refers to a stretch of one or more unpaired nucleotides located at a terminal end of either the sense strand or the antisense strand that does not form part of the hybridized or duplexed portion of an RNAi agent disclosed herein.

[0066] In some embodiments, an ALK7 RNAi agent comprises an antisense strand having a 3' extension of 1, 2, 3, 4, 5, or 6 nucleotides in length. In other embodiments, an ALK7 RNAi agent comprises an antisense strand having a 3' extension of 1, 2, or 3 nucleotides in length. In some embodiments, one or more of the antisense strand extension nucleotides comprise nucleotides that are complementary to the corresponding ALK7 mRNA sequence. In some embodiments, one or more of the antisense strand extension nucleotides comprise nucleotides that are not complementary to the corresponding ALK7 mRNA sequence.

[0067] In some embodiments, an ALK7 RNAi agent comprises a sense strand having a 3' extension of 1, 2, 3, 4, or 5 nucleotides in length. In some embodiments, one or more of the sense strand extension nucleotides comprises adenosine, uracil, or thymidine nucleotides, AT dinucleotide, or nucleotides that correspond to or are the identical to nucleotides in the ALK7 mRNA sequence. In some embodiments, the 3' sense strand extension includes or consists of one of the following sequences, but is not limited to: T, UT, TT, UU, UUT, TTT, or TTTT (each listed 5' to 3').

[0068] A sense strand can have a 3' extension and/or a 5' extension. In some embodiments, an ALK7 RNAi agent comprises a sense strand having a 5' extension of 1, 2, 3, 4, 5, or 6 nucleotides in length. In some embodiments, one or more of the sense strand extension nucleotides comprise nucleotides that correspond to or are identical to nucleotides in the ALK7 mRNA sequence.

[0069] Examples of sequences used in forming ALK7 RNAi agents are provided in Tables 2, 3, 4, 5, 6, and 10. In some embodiments, an ALK7 RNAi agent antisense strand includes a sequence of any of the sequences in Tables 2, 3, or 10. In certain embodiments, an ALK7 RNAi agent antisense strand comprises or consists of any one of the modified sequences in Table 3. In some embodiments, an ALK7 RNAi agent antisense strand includes the sequence of nucleotides (from 5' end → 3' end) 1-17, 2-15, 2-17, 1-18, 2-18, 1-19, 2-19, 1-20, 2-20, 1-21, or 2-21, of any of the sequences in Tables 2 or 3. In some embodiments, an ALK7 RNAi agent sense strand includes the sequence of any of the sequences in Tables 2, 4, 5, or 6. In some embodiments, an ALK7 RNAi agent sense strand includes the sequence of nucleotides (from 5' end → 3' end) 1-18, 1-19, 1-20, 1-21, 2-19, 2-20, 2-21, 3-20, 3-21, or 4-21 of any of the sequences in Tables 2, 4, 5, or 6. In certain embodiments, an ALK7 RNAi agent sense strand comprises or consists of a modified sequence of any one of the modified sequences in Table 4, 5, 6, or 10.

[0070] In some embodiments, the sense and antisense strands of the RNAi agents described herein contain the same number of nucleotides. In some embodiments, the sense and antisense strands of the RNAi agents described herein contain different numbers of nucleotides. In some embodiments, the sense strand 5' end and the antisense strand 3' end of an RNAi agent form a blunt end. In some embodiments, the sense strand 3' end and the antisense strand 5' end of an RNAi agent form a blunt end. In some embodiments, both ends of an RNAi agent form blunt ends. In some embodiments, neither end of an RNAi agent is blunt-ended. As used herein a "blunt end" refers to an end of a double stranded RNAi agent in which the terminal nucleotides of the two annealed strands are complementary (form a complementary base-pair).

[0071] In some embodiments, the sense strand 5' end and the antisense strand 3' end of an RNAi agent form a frayed end. In some embodiments, the sense strand 3' end and the antisense strand 5' end of an RNAi agent form a frayed end. In some embodiments, both ends of an RNAi agent form a frayed end. In some embodiments, neither end of an RNAi agent is a frayed end. As used herein a frayed end refers to an end of a double stranded RNAi agent in which the terminal nucleotides of the two annealed strands form a pair (i.e., do not form an overhang) but

are not complementary (i.e. form a non-complementary pair). In some embodiments, one or more unpaired nucleotides at the end of one strand of a double stranded RNAi agent form an overhang. The unpaired nucleotides may be on the sense strand or the antisense strand, creating either 3' or 5' overhangs. In some embodiments, the RNAi agent contains: a blunt end and a frayed end, a blunt end and 5' overhang end, a blunt end and a 3' overhang end, a frayed end and a 5' overhang end, a frayed end and a 3' overhang end, two 5' overhang ends, two 3' overhang ends, a 5' overhang end and a 3' overhang end, two frayed ends, or two blunt ends. Typically, when present, overhangs are located at the 3' terminal ends of the sense strand, the antisense strand, or both the sense strand and the antisense strand.

[0072] The ALK7 RNAi agents disclosed herein may also be comprised of one or more modified nucleotides. In some embodiments, substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand of the ALK7 RNAi agent are modified nucleotides. The ALK7 RNAi agents disclosed herein may further be comprised of one or more modified internucleoside linkages, e.g., one or more phosphorothioate or phosphorodithioate linkages. In some embodiments, an ALK7 RNAi agent contains one or more modified nucleotides and one or more modified internucleoside linkages. In some embodiments, a 2'-modified nucleotide is combined with modified internucleoside linkage.

[0073] In some embodiments, an ALK7 RNAi agent is prepared or provided as a salt, mixed salt, or a free-acid. In some embodiments, an ALK7 RNAi agent is prepared as a pharmaceutically acceptable salt. In some embodiments, an ALK7 RNAi agent is prepared as a pharmaceutically acceptable sodium salt. Such forms that are well known in the art are within the scope of the inventions disclosed herein.

Modified Nucleotides

[0074] Modified nucleotides, when used in various oligonucleotide constructs, can preserve activity of the compound in cells while at the same time increasing the serum stability of these compounds, and can also minimize the possibility of activating interferon activity in humans upon administration of the oligonucleotide construct.

[0075] In some embodiments, an ALK7 RNAi agent contains one or more modified nucleotides. As used herein, a "modified nucleotide" is a nucleotide other than a ribonucleotide (2'-hydroxyl nucleotide). In some embodiments, at least 50% (e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, or 100%) of the nucleotides are modified nucleotides. As used herein, modified nucleotides can include, but

are not limited to, deoxyribonucleotides, nucleotide mimics, abasic nucleotides, 2'-modified nucleotides, inverted nucleotides, modified nucleobase-comprising nucleotides, bridged nucleotides, peptide nucleic acids (PNAs), 2',3'-seco nucleotide mimics (unlocked nucleobase analogues), locked nucleotides, 3'-O-methoxy (2' internucleoside linked) nucleotides, 2'-F-Arabino nucleotides, 5'-Me, 2'-fluoro nucleotide, morpholino nucleotides, vinyl phosphonate deoxyribonucleotides, vinyl phosphonate containing nucleotides, and cyclopropyl phosphonate containing nucleotides. 2'-modified nucleotides (*i.e.*, a nucleotide with a group other than a hydroxyl group at the 2' position of the five-membered sugar ring) include, but are not limited to, 2'-O-methyl nucleotides (also referred to herein or in the art as 2'-methoxy nucleotides), 2'-fluoro nucleotides (also referred to herein or in the art as 2'-deoxy-2'-fluoro nucleotides), 2'-deoxy nucleotides, 2'-methoxyethyl (2'-O-2-methoxyethyl) nucleotides (also referred herein or in the art as 2'-MOE nucleotides), 2'-amino nucleotides, and 2'-alkyl nucleotides. It is not necessary for all positions in a given compound to be uniformly modified. Conversely, more than one modification can be incorporated in a single ALK7 RNAi agent or even in a single nucleotide thereof. The ALK7 RNAi agent sense strands and antisense strands can be synthesized and/or modified by methods known in the art. Modification at one nucleotide is independent of modification at another nucleotide.

[0076] Modified nucleobases include synthetic and natural nucleobases, such as 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, (e.g., 2-aminopropyladenine, 5-propynyluracil, or 5-propynylcytosine), 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, inosine, xanthine, hypoxanthine, 2-aminoadenine, 6-alkyl (e.g., 6-methyl, 6-ethyl, 6-isopropyl, or 6-n-butyl) derivatives of adenine and guanine, 2-alkyl (e.g., 2-methyl, 2-ethyl, 2-isopropyl, or 2-n-butyl) and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine, 2-thiocytosine, 5-halouracil, cytosine, 5-propynyl uracil, 5-propynyl cytosine, 6-azo uracil, 6-azo cytosine, 6-azo thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-sulfhydryl, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo (e.g., 5-bromo), 5-trifluoromethyl, and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, and 3-deazaadenine.

[0077] In some embodiments, the 5' and/or 3' end of the antisense strand can include abasic residues (Ab), which can also be referred to as an "abasic site" or "abasic nucleotide." An abasic residue (Ab) is a nucleotide or nucleoside that lacks a nucleobase at the 1' position of the sugar moiety. (*See, e.g.*, U.S. Patent No. 5,998,203). In some embodiments, an abasic

residue can be placed internally in a nucleotide sequence. In some embodiments, Ab or AbAb can be added to the 3' end of the antisense strand. In some embodiments, the 5' end of the sense strand can include one or more additional abasic residues (e.g., (Ab) or (AbAb)). In some embodiments, UUAb, UAb, or Ab are added to the 3' end of the sense strand. In some embodiments, an abasic (deoxyribose) residue can be replaced with a ribitol (abasic ribose) residue.

[0078] In some embodiments, all or substantially all of the nucleotides of an RNAi agent are modified nucleotides. As used herein, an RNAi agent wherein substantially all of the nucleotides present are modified nucleotides is an RNAi agent having four or fewer (i.e., 0, 1, 2, 3, or 4) nucleotides in both the sense strand and the antisense strand being ribonucleotides (i.e., unmodified). As used herein, a sense strand wherein substantially all of the nucleotides present are modified nucleotides is a sense strand having two or fewer (i.e., 0, 1, or 2) nucleotides in the sense strand being unmodified ribonucleotides. As used herein, an antisense strand wherein substantially all of the nucleotides present are modified nucleotides is an antisense strand having two or fewer (i.e., 0, 1, or 2) nucleotides in the antisense strand being unmodified ribonucleotides. In some embodiments, one or more nucleotides of an RNAi agent is an unmodified ribonucleotide. Chemical structures for certain modified nucleotides are set forth in Table 11 herein.

Modified Internucleoside Linkages

[0079] In some embodiments, one or more nucleotides of an ALK7 RNAi agent are linked by non-standard linkages or backbones (i.e., modified internucleoside linkages or modified backbones). Modified internucleoside linkages or backbones include, but are not limited to, phosphorothioate groups (represented herein as a lower case "s"), chiral phosphorothioates, thiophosphates, phosphorodithioates (represented herein as lower case "ss"), phosphotriesters, aminoalkyl-phosphotriesters, alkyl phosphonates (e.g., methyl phosphonates or 3'-alkylene phosphonates), chiral phosphonates, phosphinates, phosphoramidates (e.g., 3'-amino phosphoramidate, aminoalkylphosphoramidates, or thionophosphoramidates), thionoalkyl-phosphonates, thionoalkylphosphotriesters, morpholino linkages, boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of boranophosphates, or boranophosphates having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. In some embodiments, a modified internucleoside linkage or backbone lacks a phosphorus atom. Modified internucleoside linkages lacking a phosphorus atom include, but

are not limited to, short chain alkyl or cycloalkyl inter-sugar linkages, mixed heteroatom and alkyl or cycloalkyl inter-sugar linkages, or one or more short chain heteroatomic or heterocyclic inter-sugar linkages. In some embodiments, modified internucleoside backbones include, but are not limited to, siloxane backbones, sulfide backbones, sulfoxide backbones, 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 other backbones having mixed N, O, S, and CH₂ components.

[0080] In some embodiments, a sense strand of an ALK7 RNAi agent can contain 1, 2, 3, 4, 5, or 6 phosphorothioate or phosphorodithioate linkages, an antisense strand of an ALK7 RNAi agent can contain 1, 2, 3, 4, 5, or 6 phosphorothioate or phosphorodithioate linkages, or both the sense strand and the antisense strand independently can contain 1, 2, 3, 4, 5, or 6 phosphorothioate or phosphorodithioate linkages. In some embodiments, a sense strand of an ALK7 RNAi agent can contain 1, 2, 3, or 4 phosphorothioate or phosphorodithioate linkages, an antisense strand of an ALK7 RNAi agent can contain 1, 2, 3, or 4 phosphorothioate or phosphorodithioate linkages, or both the sense strand and the antisense strand independently can contain 1, 2, 3, or 4 phosphorothioate or phosphorodithioate linkages.

[0081] In some embodiments, an ALK7 RNAi agent sense strand contains at least two phosphorothioate internucleoside linkages. In some embodiments, the phosphorothioate internucleoside linkages are between the nucleotides at positions 1-3 from the 3' end of the sense strand. In some embodiments, one phosphorothioate internucleoside linkage is at the 5' end of the sense strand nucleotide sequence, and another phosphorothioate or phosphorodithioate linkage is at the 3' end of the sense strand nucleotide sequence. In some embodiments, two phosphorothioate internucleoside linkage are located at the 5' end of the sense strand, and another phosphorothioate or phosphorodithioate linkage is at the 3' end of the sense strand. In some embodiments, the sense strand does not include any phosphorothioate internucleoside linkages between the nucleotides, but contains one, two, or three phosphorothioate or phosphorodithioate linkages between the terminal nucleotides on both the 5' and 3' ends and the optionally present inverted abasic residue terminal caps. In some embodiments, a targeting ligand is linked to the sense strand via a phosphorothioate or phosphorodithioate linkage.

[0082] In some embodiments, an ALK7 RNAi agent antisense strand contains four phosphorothioate internucleoside linkages. In some embodiments, the four phosphorothioate

internucleoside linkages are between the nucleotides at positions 1-3 from the 5' end of the antisense strand and between the nucleotides at positions 19-21, 20-22, 21-23, 22-24, 23-25, or 24-26 from the 5' end. In some embodiments, three phosphorothioate internucleoside linkages are located between positions 1-4 from the 5' end of the antisense strand, and a fourth phosphorothioate internucleoside linkage is located between positions 20-21 from the 5' end of the antisense strand. In some embodiments, an ALK7 RNAi agent contains at least three or four phosphorothioate internucleoside linkages in the antisense strand.

Capping Residues or Moieties

[0083] In some embodiments, the sense strand may include one or more capping residues or moieties, sometimes referred to in the art as a “cap,” a “terminal cap,” or a “capping residue.” As used herein, a “capping residue” is a non-nucleotide compound or other moiety that can be incorporated at one or more termini of a nucleotide sequence of an RNAi agent disclosed herein. A capping residue can provide the RNAi agent, in some instances, with certain beneficial properties, such as, for example, protection against exonuclease degradation. In some embodiments, inverted abasic residues (invAb) (also referred to in the art as “inverted abasic sites”) are added as capping residues (*see* Table 11). (See, e.g., F. Czauderna, *Nucleic Acids Res.*, 2003, 31(11), 2705-16). Capping residues are generally known in the art, and include, for example, inverted abasic residues as well as carbon chains such as a terminal C₃H₇ (propyl), C₆H₁₃ (hexyl), or C₁₂H₂₅ (dodecyl) groups. In some embodiments, a capping residue is present at either the 5' terminal end, the 3' terminal end, or both the 5' and 3' terminal ends of the sense strand. In some embodiments, the 5' end and/or the 3' end of the sense strand may include more than one inverted abasic deoxyribose moiety as a capping residue.

[0084] In some embodiments, one or more inverted abasic residues (invAb) are added to the 3' end of the sense strand. In some embodiments, one or more inverted abasic residues (invAb) are added to the 5' end of the sense strand. In some embodiments, one or more inverted abasic residues or inverted abasic sites are inserted between a targeting ligand and the nucleotide sequence of the sense strand of the RNAi agent. In some embodiments, the inclusion of one or more inverted abasic residues or inverted abasic sites at or near the terminal end or terminal ends of the sense strand of an RNAi agent allows for enhanced activity or other desired properties of an RNAi agent.

[0085] In some embodiments, one or more inverted abasic residues (invAb) are added to the 5' end of the sense strand. In some embodiments, one or more inverted abasic residues can be

inserted between a targeting ligand and the nucleotide sequence of the sense strand of the RNAi agent. The inverted abasic residues may be linked via phosphate, phosphorothioate (e.g., shown herein as (invAb)s)), or other internucleoside linkages. In some embodiments, the inclusion of one or more inverted abasic residues at or near the terminal end or terminal ends of the sense strand of an RNAi agent may allow for enhanced activity or other desired properties of an RNAi agent. In some embodiments, an inverted abasic (deoxyribose) residue can be replaced with an inverted ribitol (abasic ribose) residue. In some embodiments, the 3' end of the antisense strand core stretch sequence, or the 3' end of the antisense strand sequence, may include an inverted abasic residue. The chemical structures for inverted abasic deoxyribose residues are shown in Table 11 below.

ALK7 RNAi Agents

[0086] The ALK7 RNAi agents disclosed herein are designed to target specific positions on an ALK7 gene (e.g., SEQ ID NO:1 (NM_145259.3)). As defined herein, an antisense strand sequence is designed to target an ALK7 gene at a given position on the gene when the 5' terminal nucleobase of the antisense strand is aligned with a position that is 21 nucleotides downstream (towards the 3' end) from the position on the gene when base pairing to the gene. For example, as illustrated in Tables 1 and 2 herein, an antisense strand sequence designed to target an ALK7 gene at position 304 requires that when base pairing to the gene, the 5' terminal nucleobase of the antisense strand is aligned with position 324 of an ALK7 gene.

[0087] As provided herein, an ALK7 RNAi agent does not require that the nucleobase at position 1 (5' → 3') of the antisense strand be complementary to the gene, provided that there is at least 85% complementarity (e.g., at least 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% complementarity) of the antisense strand and the gene across a core stretch sequence of at least 16 consecutive nucleotides. For example, for an ALK7 RNAi agent disclosed herein that is designed to target position 304 of an ALK7 gene, the 5' terminal nucleobase of the antisense strand of the ALK7 RNAi agent must be aligned with position 324 of the gene; however, the 5' terminal nucleobase of the antisense strand may be, but is not required to be, complementary to position 324 of an ALK7 gene, provided that there is at least 85% complementarity (e.g., at least 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% complementarity) of the antisense strand and the gene transcript across a core stretch sequence of at least 16 consecutive nucleotides. As shown by, among other things, the various examples disclosed herein, the specific site of binding of the gene by the antisense strand of

the ALK7 RNAi agent (e.g., whether the ALK7 RNAi agent is designed to target an ALK7 gene at position 304, at position 264, at position 785, or at some other position) is an important factor to the level of inhibition achieved by the ALK7 RNAi agent. (See, e.g., Kamola et al., *The siRNA Non-seed Region and Its Target Sequences are Auxiliary Determinants of Off-Target Effects*, PLOS Computational Biology, 11(12), Figure 1 (2015)).

[0088] In some embodiments, the ALK7 RNAi agents disclosed herein target an ALK7 gene at or near the positions of the ALK7 sequence shown in Table 1. In some embodiments, the antisense strand of an ALK7 RNAi agent disclosed herein includes a core stretch sequence that is fully, substantially, or at least partially complementary to a target ALK7 19-mer sequence disclosed in Table 1.

Table 1. ALK7 19-mer mRNA Target Sequences (taken from *homo sapiens* Activin Receptor-Like Kinase 7 (ALK7) transcript, GenBank NM_145259.3 (SEQ ID NO:1))

SEQ ID No.	ALK7 19-mer Target Sequences (5' → 3')	Corresponding Positions of Sequence on SEQ ID NO: 1	Targeted Gene Position (as referred to herein)
2	ACUGAAGUGUGUAUGUCUU	295-313	293
3	AACAGAAGGAGCAUGUUGG	343-361	341
4	GUCAUGCUAACCAUGGAA	368-386	366
5	CUUCUGUCAUAGUCCAAC	439-457	437
6	CUGUCAUAGUCCAACAAU	442-460	440
7	UGGUAUCUGAAUAUCAUGA	1023-1041	1021
8	AACAGGGCUCCUUAUAUGA	1041-1059	1039
9	GUCUGGCACACCUUCAUAU	1125-1143	1123
10	CUGGCACACCUUCAUAUGG	1127-1145	1125
11	GGCACACCUUCAUAUGGAG	1129-1147	1127
12	ACAAGGUAACCGUCUAUU	1159-1177	1157
13	AGGUAACCGUCUAUUGCU	1162-1180	1160
14	GUGCCAUAGCGGACUUAGG	1230-1248	1228
15	CUGUGAAGCAUGAUUCAAU	1254-1272	1252
16	UGUGAAGCAUGAUUCAUAU	1255-1273	1253
17	AAUACUGAACACUAUCGAC	1270-1288	1268
18	UCGACAUACCUCAGAAUCC	1284-1302	1282
19	UGAAAUGCUUGAUGAUACA	1333-1351	1331
20	AAUGCUUGAUGAUACAAUG	1336-1354	1334
21	CGAGCUGACAUCUAUUCUG	1382-1400	1380
22	CUGUUGGUCUGGUUUACUG	1398-1416	1396
23	GUUGAGGAGUACCAAUUGC	1451-1469	1449
24	UCAGAUCCCUCGAUAGAGG	1490-1508	1488
25	AUCCUCGAUAGAGGAAAU	1494-1512	1492
26	AAGUUUCGACCAAGUAUCC	1535-1553	1533

SEQ ID No.	ALK7 19-mer Target Sequences (5' → 3')	Corresponding Positions of Sequence on SEQ ID NO: 1	Targeted Gene Position (as referred to herein)
27	ACUGCUCUUCGUUAAGA	1640-1658	1638

[0089] *Homo sapiens* activin A receptor type 1C, ACVR1C, also known as ALK7, GenBank NM_145259.3 (SEQ ID NO:1), gene transcript (8853 bases):

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1 agtggcagga ggcgcgcga cgcagccg cagggggcgt gggatggggg cggccgggga
61 gggggggccc cactgact agagccaacc gcgcactca aaaggggtgc ggtgccgcgc
121 tccctcccg cggcccggga acttcaaage gggccgtgct gcccggctg cctcgtctg
181 ctctggggcc tcgcagcccc ggcgcggcgc cctggtggcg atgaccggg cgctctgctc
241 agcgctccgc caggctctcc tctgctcgc agcgccgcgc gagctctgc caggactgaa
301 gtgtgtatgt ctttgtgtg atttcaaa cttcacctgc caacagaag gagcatgttg
361 ggcatcagtc atgctaacca atggaaaaga gcaggtgatc aaatcctgtg tctcccttc
421 agaactgaat gctcaagtct tctgcatag ttcaacaat gttacaaaa ccgaatgctg
481 cttcacagat tttgcaaca acataacact gcacctcca acagcatcac caaatgcccc
541 aaaacttga cccatggagc tggccatcat tattactgtg cctgtttgcc tctgtccat
601 agctgcgatg ctgacagtat gggcatgcca ggtcgacag tgctctaca ggaagaaaaa
661 gagacaaat gtggaggaa cactctctga gtgcaatctg gtaaatgctg gaaaaactc
721 gaaagatctg atttatgatg tgaccgcctc tggatctggc tctggtctac ctctgttgg
781 tcaaaggaca attgcaagga cgattgtgct tcaggaaata gtaggaaaag gtagatttgg
841 tgaggtgtgg catggaagat ggtgtgggga agatgtggct gtgaaaatat tctcctcag
901 agatgaaaga tcttggtttc gtgaggcaga aatttaccag acggtcatgc tgcgacatga
961 aaacatcctt ggtttcattg ctgctgaca caaagataat ggaacttga ctcaacttg
1021 gctggtatct gaatatcatg aacagggctc cttatatgac tattgataa gaaatatagt
1081 gaccgtggct ggaatgatca agctggcgc ctcaattgct agtggctgg cacacctca
1141 tatggagatt gttggtacac aaggtaaacc tgctattgct catcgagaca taaaatcaaa
1201 gaatatctta gtgaaaaagt gtgaaactg tgccatagcg gacttagggg ttgctgtgaa
1261 gcatgattca atactgaaca ctatcgacat acctcagaat cctaaagtgg gaaccaagag
1321 gtatatggct cctgaaatgc ttgatgatac aatgaatgtg aatatctttg agtcctcaa
1381 acgagctgac atctattctg ttggtctggt ttactgggaa atagcccgga ggtgttcagt
1441 cggaggaatt gttgaggagt accaattgcc ttattatgac atggtgcctt cagatccctc
1501 gatagaggaa atgagaaagg ttgtttgtga ccagaagttt cgaccaagta tcccaaacca
1561 gtggcaaagt tgtgaagcac tccgagtcac ggggagaata atgcgtgagt gttggtatgc

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1621 caacggagcg gcccgccctaa ctgctcttcg tattaagaag actatatctc aactttgtgt
1681 caaagaagac tgcaaagcct aatgatgata attatgttaa aaagaaatct ctcatagctt
1741 tcttttccat ttccccttt atgtgaatgt ttttgccatt tttttttgt tctacctcaa
1801 agataagaca gtacagtatt taagtgccca taaggcagca tgaaaagata actctaaagt
1861 taagcatggg caggagtga cttcatccaa tctctatgtt atgtttaatt ttatttgaa
1921 agcaaacct caactcatct ttttattaa taaggaagaa atatattaca aaagtataaa
1981 ataagctcta taaaaatgtt atagtcatta agtttttatt ttactgaac caagagcaca
2041 tgaatgaaca ggaaaagatg taaaacatt ttttctgag atgaaaacat attaattaa
2101 catgcaaatt agagcatgct atcttaggt gatgcaatct atgttcccc cttttaagt
2161 tagcaggact ttttaaaat aaatattgct ctaaacttta atatatcgaa cgtgagagt
2221 gagctgctta gtggaagatg taagtgggt ggggtgccca tgtgcttgggt cccccctt
2281 gctgttctcc tgttctcat aatccactac tgcagcagtc cctgaaccac taaacttgt
2341 cctttcattt acaaaagaga tacctgacat cctgagacac tgagaaatgt cctgaagtca
2401 cacagctaag ggcagaactg gcactaggtc caaatctgt gataatgaac accgtaaggt
2461 tagctagctt cctacttcc ctgaaatgt gcttttctcc ctatgtaata tcttttatta
2521 tgatatttgt ggtttagaag gcatattgag ttattttgca gaatcataat ggacccgcac
2581 aaaatctcag aaccatatct gttgacattt ttctcatag aaatatcatg gttacccat
2641 ttgttaatga gcattaatgt ttctgaaca cttccaaaga ttaatcaaac ataaatattc
2701 attgtctgaa aatgtcttta agatacaatt cagaggctcc tatttcttt gtacatacac
2761 acttagaaag aaaagacaga aaaggaagag gaaggaagga aatattttga gaatatattg
2821 agaagaatta agaaaactct tcaatgaagt gtaacaacc aaacctaca gacggtatca
2881 gaaacagcaa atagatatc ctctaccctt tcacagtgag tgagtgaagta cagaagaatg
2941 ctcatgatag tttgccttc attctactt ctgtggacac agagtaatga atatttaag
3001 ggacattaaa tatgcccttc aaatctataa tttactttg gtaaacgaga tttacatga
3061 tgtctttat gctcctaaaa catcttttt caaactccat tcttagaac attctctac
3121 tgagatgac caagacaaa agtgttctt ggtacttgc tataaagtga tagtacatgt
3181 tagcatataa tgtattttga agagtgaagt aaatgctatt gataacagta aaaaaaaaaa
3241 aaaaaactaa taacagtaaa gaaatgctac ttgattttt tttaaactgg attgctcaga
3301 ttacctgac gtggtggaac ccttttatta agaggagggg aaactttta ctatccata
3361 tttactgtt ctataagca aagcacagct tgggtaatag cgttctgaag gatatacttc
3421 tgtattttct catagagtac aatttagtga ttatgcttca ttactatg gaaatatgtt
3481 actgaatcta tcttcattt actgagtga aataaggaag gcaaaaaact gacagctatg
3541 gagtttgcgt gtacttccat actcgtaat gctctcatcc acttattaaa taatcataga

3601 gcacccatat cttgcttgcc acaatatcgg gtacaagagg gaatacaaag atagataggt
 3661 cctgccctca gggattttaa agtctaattt gggaatggga atagggatgt gagtgtgtgg
 3721 gggaagagat taaattgaca gataaaatac gaagtgggat gtcttgagtt ctgcatgaca
 3781 gtgggtttct aggataggtc tgaaaattgc ttctattgc aacacattta gaaagtagct
 3841 ttatttggat attacagaca atctaaatat atcatcagtt tttaaagtgt cctatgtgaa
 3901 gtgattttaa aaaagagcct atgtgaagg gtaatcttgc ttgttctgt tactaatttc
 3961 tcatagattg ttttctgca tatataagaa cgaaattatt tatttactat gggtgtacgt
 4021 gcctcaaata aacaagaatg atatttctg ttttattac ttatgtggg taaatatgt
 4081 tattgaattt ttaagagagg atttttacc atctccattt ttctgtcat tatgtttgt
 4141 agcttatttg aggggtgcta aatataattt catattttat tgggtcaact ttactctga
 4201 agaaatccgt atgttagtac attttgaggt attttcttg ttctgtgtt gttaactat
 4261 gactcctaac tgagtagtct tatattcaa ttacaaaata catttttaa gaaagggat
 4321 agagcagcaa aatgataag gaaaatgta aaagtgtta tatttcctt actcttaaca
 4381 ggattatata tagaacatgc tcaactaca aaataggatg atgaagtta gagcataagg
 4441 caggettctt gtatatactt atgctgtcaa atgttatatt gttttaatg gagtccatt
 4501 gtgtaattt tatttctttt acattttgtt ataagcaaaa aaaaaaaaaa ttctcctag
 4561 gttatgtca gagtatcagt gtctttact ccttacagat atttggctt tgggtataa
 4621 tacaagactt gggaaaacac tattatgaat ttctagtact gtataaagt gtgatggg
 4681 ttaaatgcag catcactttc tgaaaataag agaaacatta ttgtgtca gtatttcagc
 4741 atgaactgt tgcctgtta atttgcctt taagtttga atggtagag attctgtgt
 4801 atgctttct ctatgtctaa aatatttggc atgtcacatc tagaattctt aatttatgt
 4861 ctgacttgag agttaagtga aacatgactg tctgtcacta ttttaggcat agcacttgct
 4921 ttcatcttt atactttca ttaactttgc attttaaatt tccatgattg tatgaaaata
 4981 gtaacctggt tgcagtatct gaaaaaata aaattagctt ttattaaaa atgaaaatct
 5041 acaatagatt cattaggta gaagtccag aataatttat tattttatta cacctactat
 5101 tgtagaatta ctaataaaac aaaacaaaac tactctatt ttctggaat ttgccacca
 5161 tgtgacttat tggggcagag aaaactcagg gtgtctttg agtctgcaca aaagcaccag
 5221 ggaacctgct tagcaaatcg tctgaaaaca gggagctgat gtttgccatt atacaaagt
 5281 tgagtaaaca acttaaaatt gcttgtagg gcatagtctt tgattgaaat aagtatgaga
 5341 atgtatttgg ctaataaaat gtatttaaaa tatacaaatt ttatttccca ctggaaaatt
 5401 aagaaagtag cagtacaaa tgaataaaag ctggcagttg atgtcttcaa taatcattcc
 5461 tttaaaataa attcacaac acatcattac aagctactta gaaatgtta gtattcgtat
 5521 cttaaatgag caccattgtg gtttcaaag atattttaa catcttttgt gaaacatact

5581 atttctgttt gacaatgctt tgttctagct ctgtgtgctg atacctacat ggagtttttc
5641 tgctatttta gtgaaaacag taattctttt atcttgagag ctgcttctaa ataacaaaaa
5701 agttaattgg aatgtaagtt ttaaaaaat gtaaatatta aatagaattt ttataatatg
5761 ggcatTTTTT aaacatttt aatgaaaata tatagatatt tgactttctt ttttcatct
5821 agccttgctt tatatttcat tattttctc acttttttc ttaatttcc ctactatct
5881 ttcaacatta tcattagttc ctaattttaa aaataactct tatttaactt aaccgtctgg
5941 aatttagcct tctaataaaa gagaatccct tagtactctc agagattata taaagtattt
6001 ccaattttgt gtagatgacg aaaccaagga tcagagatta aatgactact agttactagc
6061 agaactctta tgaaagcctg gtggtgacac ccagcactgt tccctgccat atccccaact
6121 ttactgatt aaaaattaat tcgcatgcat ctaaacctac ctggaatgca cactaacgta
6181 atgtgccatt caatgatgat gataaaatcc cacttttctt tgggtttcta ctaaaataat
6241 ttactcactc agagttaggt caatgagaaa aactaaacta ggctaagaaa gagttgtaga
6301 atggttgttg agctaagtag gcaaccactg ggggtgctgat aaaattaatg gataaaattt
6361 aggaactgtg agagtataga atttcttagt gcaagtaatg attagagaag ctctcgaca
6421 tccccacccc ttctgtaag gactgttctt tctctttgta ccttggaatg ggggtgaaag
6481 gtgatttgat agctgaattt gggactatgt ccagtgggat attatctaac ttttctctt
6541 ttctcttttt ttccccca gtttctcatt agttgtctt tggcattcat ctcttttag
6601 tgcattaaaa aatgtttggc agatctcacc aatcccaagt cactctataa ttctgtatt
6661 tctttagttg tcgttaacc tgtccaaact tctacacaat gaacttcta acaagatttt
6721 aagttctctg tataagaggt ttcacctcat aacttctcca gtaatcttc atttggcact
6781 atagagtatt tgttaatggc agagatgatt ttcttttaa aacctaaaaa gactagctgt
6841 tatttgatt ctactttta gctaacatat aaagaatgct tacttttgct ttaatgctaa
6901 attccgcttg agaaatagta actgggaaag acaatttgaa atatatgccc cataatgtga
6961 ttgttaaatt tatttctgct gttccatacc attgctttgt ttgcttttg aaaaactaag
7021 ccattatgct attagtttgg aatataatac tacagcaaaa ttaggtaaca atccctattt
7081 taaaattccc ctaacaataa tagaactgcc agcatacttt tctcttcag ttgtagatga
7141 atacattcga gagaatatga gctgtatttc atcctagatt ttaatatttt cagatgtgac
7201 tgtatttctt gatcattggt ccaagttgct ctaaaagaaa ttttctctc cagacctaac
7261 agttttaact gcaagagttt actgtgggtt atgttaatct gaattttaat agggccacta
7321 agaactctgag tgccttagga gattaccctt ataccactg ccatcacatc cagttaggcc
7381 tgtgtgctc tatataaatc ttcccagctg aggggcaggt gcgggctaaa atccactgc
7441 aattggctcc cagacataat ttatatattt acagagaagc atcttattgg ctatatgtg
7501 tttaaagaat ggtctggctt atacatctc agaaaatgag aattaaag tcaaaataat

7561 tcttgacatc tacagattga acaaagaact tagaagaaat aatactttat cttttcatcc
 7621 tggcattcct gagagaagag aaattgattg ttatcatgt tggtttaatt ttcaacca
 7681 gacaatctgc agcaaggcac atggacccca atttgatat cgtccataca gtttcattc
 7741 tatgcatgga gctaattact gactttgcct gtaaagagag gattgtgtgc ctaaatittg
 7801 tctaacaat gcaagcgtag aatgacattt actaatattt ctatttcttc cataggctaa
 7861 ataatagtaa ctaagtattt ttaaggacac agccctttt ttctctttat acaaatagag
 7921 agtatctgag ccaaaatatt aaattctagt tctttccgc aatgactagt gtcaagctca
 7981 tgtactcttc tgattctaga ctggagaaga ttattcaaac ttgatctgtg ttcaggttt
 8041 ttaaatgtcc taaaacaga aaattagatt cagatctcaa aaaaggaatt ttggattgac
 8101 ttcaaagta ctaatactaa ttatactttt cttttggtag cgtgactctt ctataccta
 8161 agaacatatt acaaatgtca aaaccattgc attttgacat tgcaaaacat gccttgaact
 8221 ctgtaactac tgtgaaaaga atcaccgttg taaagacttt ttgtaagcta gctgatactc
 8281 ttaagtatgt aaaaagattg tctttcagcc gacaggccca aaggaaatgta tataaggaag
 8341 gaatatgaaa aaataaatta ggttttaaaa taggaattgg gcaataaact gtatcaaaaa
 8401 tatgtatgag gattttagta gttgtaattt aaatgtggaa ggtgaagaga atttcaaact
 8461 ccaaagagaa atgaatgata ttcagatgtt tcattaattt ctagtctgtg aaaatatgca
 8521 ttttatagta atatgtatag acttatttta tttagaaata atagtgtttt agaatttatt
 8581 aaaaactcag tgatagcctt tatacaaaaa tgtttaactt taccaacagc aagtcataaa
 8641 agtatttatt ttaaagcttt ttaattattt cgtgtaactt tcactgtctc tcagatgtaa
 8701 ataattatct gcctaaatgt tatattttta tgtatgcatt ttctgaaaaat gtattgtttt
 8761 gtaaagtggg aaagataata aatcaagcac ttctgcact tgtttctgtg aagcatatag
 8821 aactctattt taaataaagg aagatgtgtc gta

[0090] In some embodiments, an ALK7 RNAi agent includes an antisense strand wherein position 19 of the antisense strand (5'→3') is capable of forming a base pair with position 1 of a 19-mer target sequence disclosed in Table 1. In some embodiments, an ALK7 agent includes an antisense strand wherein position 1 of the antisense strand (5'→3') is capable of forming a base pair with position 19 of a 19-mer target sequence disclosed in Table 1.

[0091] In some embodiments, an ALK7 agent includes an antisense strand wherein position 2 of the antisense strand (5' → 3') is capable of forming a base pair with position 18 of a 19-mer target sequence disclosed in Table 1. In some embodiments, an ALK7 agent includes an antisense strand wherein positions 2 through 18 of the antisense strand (5' → 3') are capable of

forming base pairs with each of the respective complementary bases located at positions 18 through 2 of the 19-mer target sequence disclosed in Table 1.

[0092] For the RNAi agents disclosed herein, the nucleotide at position 1 of the antisense strand (from 5' end → 3' end) can be perfectly complementary to an ALK7 gene, or can be non-complementary to an ALK7 gene. In some embodiments, the nucleotide at position 1 of the antisense strand (from 5' end → 3' end) is a U, A, or dT. In some embodiments, the nucleotide at position 1 of the antisense strand (from 5' end → 3' end) forms an A:U or U:A base pair with the sense strand.

[0093] In some embodiments, an ALK7 RNAi agent antisense strand comprises the sequence of nucleotides (from 5' end → 3' end) 2-18 or 2-19 of any of the antisense strand sequences in Table 2 or Table 3. In some embodiments, an ALK7 RNAi sense strand comprises the sequence of nucleotides (from 5' end → 3' end) 1-17, 1-18, or 2-18 of any of the sense strand sequences in Table 2, Table 4, Table 5, Table 6, or Table 6a.

[0094] In some embodiments, an ALK7 RNAi agent is comprised of (i) an antisense strand comprising the sequence of nucleotides (from 5' end → 3' end) 2-18 or 2-19 of any of the antisense strand sequences in Table 2 or Table 3, and (ii) a sense strand comprising the sequence of nucleotides (from 5' end → 3' end) 1-17 or 1-18 of any of the sense strand sequences in Table 2, Table 4, Table 5, Table 6, or Table 6a.

[0095] In some embodiments, the ALK7 RNAi agents include core 19-mer nucleotide sequences shown in the following Table 2.

[0096] **Table 2.** ALK7 RNAi Agent Antisense Strand and Sense Strand Core Stretch Base Sequences (N=any nucleobase; I = inosine (hypoxanthine nucleobase))

SEQ ID NO:.	Antisense Strand Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO:.	Sense Strand Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	Corresponding Positions of Identified Sequence on SEQ ID NO: 1	Targeted Gene Position
28	AAGACAUACACACUUCAGU	145	ACUGAAGUGUGUAUGUCUU	295-313	293
29	UAGACAUACACACUUCAGU	146	ACUGAAGUGUGUAUGUCUA	295-313	293
30	NAGACAUACACACUUCAGU	147	ACUGAAGUGUGUAUGUCUN	295-313	293
31	NAGACAUACACACUUCAGN	148	NCUGAAGUGUGUAUGUCUN	295-313	293
32	CCAACAUGCUCUUCUGUU	149	AACAGAAGGAGCAUGUUG	343-361	341
33	UCAACAUGCUCUUCUGUU	150	AACAGAAGGAGCAUGUUGA	343-361	341
34	ACAACAUGCUCUUCUGUU	151	AACAGAAGGAGCAUGUUGU	343-361	341
35	NCAACAUGCUCUUCUGUU	152	AACAGAAGGAGCAUGUUGN	343-361	341
36	NCAACAUGCUCUUCUGUN	153	NACAGAAGGAGCAUGUUGN	343-361	341
37	UCCAUAUGGUUAGCAUGAC	154	GUCAUGCUAACCA AUGGAA	368-386	366
38	AUCCAUAUGGUUAGCAUGAC	155	GUCAUGCUAACCA AUGGAU	368-386	366
39	NUCCAUAUGGUUAGCAUGAC	156	GUCAUGCUAACCA AUGGAN	368-386	366
40	NUCCAUAUGGUUAGCAUGAN	157	NUCAUGCUAACCA AUGGAN	368-386	366
41	GUUGGAACUAUGACAGAG	158	CUUCUGUCAUAGUCC AAC	439-457	437
42	UUUGGAACUAUGACAGAG	159	CUUCUGUCAUAGUCC AAA	439-457	437
43	AUUGGAACUAUGACAGAG	160	CUUCUGUCAUAGUCC AAU	439-457	437
44	NUUGGAACUAUGACAGAG	161	CUUCUGUCAUAGUCC AAN	439-457	437
45	NUUGGAACUAUGACAGAGN	162	NUUCUGUCAUAGUCC AAN	439-457	437
46	AUUGUUGGAACUAUGACAG	163	CUGUCAUAGUCC AACAAU	442-460	440
47	UUUGUUGGAACUAUGACAG	164	CUGUCAUAGUCC AACAAA	442-460	440
48	NUUGUUGGAACUAUGACAG	165	CUGUCAUAGUCC AACAAAN	442-460	440
49	NUUGUUGGAACUAUGACAN	166	NUGUCAUAGUCC AACAAAN	442-460	440
50	UCAUGAUUAUCAGAUACCA	167	UGGUAUCUGAAUAUCAUGA	1023-1041	1021

SEQ ID NO:.	Antisense Strand Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO:.	Sense Strand Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	Corresponding Positions of Identified Sequence on SEQ ID NO: 1	Targeted Gene Position
51	ACAUGAUUUCAGAUACCA	168	UGGU AUCUGAAUAUCAUGU	1023-1041	1021
52	NCAUGAUUUCAGAUACCA	169	UGGU AUCUGAAUAUCAUGN	1023-1041	1021
53	NCAUGAUUUCAGAUACCN	170	NGGU AUCUGAAUAUCAUGN	1023-1041	1021
54	UCAUAUAAGGAGCCCGUU	171	AACAGGGCUCUUUAUAUGA	1041-1059	1039
55	ACAUUAAGGAGCCCGUU	172	AACAGGGCUCUUUAUAUGU	1041-1059	1039
56	NCAUAUAAGGAGCCCGUU	173	AACAGGGCUCUUUAUAUGN	1041-1059	1039
57	NCAUAUAAGGAGCCCGUN	174	NACAGGGCUCUUUAUAUGN	1041-1059	1039
58	AUAUGAAGGUGGCCAGAC	175	GUCUGGCACACCUUCAUAU	1125-1143	1123
59	UUAUGAAGGUGGCCAGAC	176	GUCUGGCACACCUUCAUAA	1125-1143	1123
60	NUAUGAAGGUGGCCAGAC	177	GUCUGGCACACCUUCAUAN	1125-1143	1123
61	NUAUGAAGGUGGCCAGAN	178	NUCUGGCACACCUUCAUAN	1125-1143	1123
62	CCAUAUGAAGGUGGCCAG	179	CUGGCACACCUUCAUUGG	1127-1145	1125
63	UCAUAUGAAGGUGGCCAG	180	CUGGCACACCUUCAUAUGA	1127-1145	1125
64	ACAUAUGAAGGUGGCCAG	181	CUGGCACACCUUCAUAUGU	1127-1145	1125
65	NCAUAUGAAGGUGGCCAG	182	CUGGCACACCUUCAUAUGN	1127-1145	1125
66	NCAUAUGAAGGUGGCCAN	183	NUGGCACACCUUCAUAUGN	1127-1145	1125
67	CUCCAUAUGAAGGUGGCC	184	GGCACACCUUCAUAUGGAG	1129-1147	1127
68	UCCAUAUGAAGGUGGCC	185	GGCACACCUUCAUAUGGAA	1129-1147	1127
69	AUCCAUAUGAAGGUGGCC	186	GGCACACCUUCAUAUGGAU	1129-1147	1127
70	NUCCAUAUGAAGGUGGCC	187	GGCACACCUUCAUAUGGAN	1129-1147	1127
71	NUCCAUAUGAAGGUGGCN	188	NGCACACCUUCAUAUGGAN	1129-1147	1127
72	AAUAGCAGGUUUACCUUGU	189	ACAAGGUAAACCCUGCUAUU	1159-1177	1157
73	UAUAGCAGGUUUACCUUGU	190	ACAAGGUAAACCCUGCUAUA	1159-1177	1157
74	NAUAGCAGGUUUACCUUGU	191	ACAAGGUAAACCCUGCUAUN	1159-1177	1157
75	NAUAGCAGGUUUACCUUGN	192	NCAAGGUAAACCCUGCUAUN	1159-1177	1157
76	AGCAAUAGCAGGUUUACCU	193	AGGUAAACCCUGCUAUUGCU	1162-1180	1160

SEQ ID NO.:	Antisense Strand Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.:	Sense Strand Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	Corresponding Positions of Identified Sequence on SEQ ID NO: 1	Targeted Gene Position
77	UGCAAUAGCAGGUUUACCU	194	AGGUAAACCUGCUAUUUGCA	1162-1180	1160
78	NGCAAUAGCAGGUUUACCU	195	AGGUAAACCUGCUAUUUGCN	1162-1180	1160
79	NGCAAUAGCAGGUUUACCN	196	NGGUAAACCUGCUAUUUGCN	1162-1180	1160
80	CCUAAAGUCCGCUAUGGCAC	197	GUGCCAUAGCGGACUUAGG	1230-1248	1228
81	UCUAAAGUCCGCUAUGGCAC	198	GUGCCAUAGCGGACUUAGA	1230-1248	1228
82	ACUAAAGUCCGCUAUGGCAC	199	GUGCCAUAGCGGACUUAGU	1230-1248	1228
83	NCUAAAGUCCGCUAUGGCAC	200	GUGCCAUAGCGGACUUAGN	1230-1248	1228
84	NCUAAAGUCCGCUAUGGCAN	201	NUGCCAUAGCGGACUUAGN	1230-1248	1228
85	AUUGAAUCAUGCUUCACAG	202	CUGUGAAGCAUGAUUCAAU	1254-1272	1252
86	UUUGAAUCAUGCUUCACAG	203	CUGUGAAGCAUGAUUCAA	1254-1272	1252
87	NUUGAAUCAUGCUUCACAG	204	CUGUGAAGCAUGAUUCAAN	1254-1272	1252
88	NUUGAAUCAUGCUUCACAN	205	NUGUGAAGCAUGAUUCAAN	1254-1272	1252
89	UAUUGAAUCAUGCUUCACA	206	UGUGAAGCAUGAUUCAUA	1255-1273	1253
90	AAUUGAAUCAUGCUUCACA	207	UGUGAAGCAUGAUUCAAUU	1255-1273	1253
91	NAUUGAAUCAUGCUUCACA	208	UGUGAAGCAUGAUUCAAUN	1255-1273	1253
92	NAUUGAAUCAUGCUUCACN	209	NGUGAAGCAUGAUUCAAUN	1255-1273	1253
93	GUCGAUAGUGUUCAGUAUU	210	AAUACUGAACACUACUCGAC	1270-1288	1268
94	UUCGAUAGUGUUCAGUAUU	211	AAUACUGAACACUACUCGAA	1270-1288	1268
95	AUCGAUAGUGUUCAGUAUU	212	AAUACUGAACACUACUCGAU	1270-1288	1268
96	NUCGAUAGUGUUCAGUAUU	213	AAUACUGAACACUACUCGAN	1270-1288	1268
97	NUCGAUAGUGUUCAGUAUN	214	NAUACUGAACACUACUCGAN	1270-1288	1268
98	GGAUUCUGAGGUUUGUCGA	215	UCGACAUACCUACAGAAUCC	1284-1302	1282
99	UGAUUCUGAGGUUUGUCGA	216	UCGACAUACCUACAGAAUCA	1284-1302	1282
100	AGAUUCUGAGGUUUGUCGA	217	UCGACAUACCUACAGAAUCU	1284-1302	1282
101	NGAUUCUGAGGUUUGUCGA	218	UCGACAUACCUACAGAAUCN	1284-1302	1282
102	NGAUUCUGAGGUUUGUCGN	219	NCGACAUACCUACAGAAUCN	1284-1302	1282

SEQ ID NO:.	Antisense Strand Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO:.	Sense Strand Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	Corresponding Positions of Identified Sequence on SEQ ID NO: 1	Targeted Gene Position
103	UGUAUCAUCAAGCAUUUCA	220	UGAAUGCUUGAUGAUACA	1333-1351	1331
104	AGUAUCAUCAAGCAUUUCA	221	UGAAUGCUUGAUGAUACU	1333-1351	1331
105	NGUAUCAUCAAGCAUUUCA	222	UGAAUGCUUGAUGAUACN	1333-1351	1331
106	NGUAUCAUCAAGCAUUUCN	223	NGAAUGCUUGAUGAUACN	1333-1351	1331
107	CAUUGUAUCAUCAAGCAUU	224	AAUGCUUGAUGAUACA AUG	1336-1354	1334
108	UAUUGUAUCAUCAAGCAUU	225	AAUGCUUGAUGAUACAUA	1336-1354	1334
109	AAUUGUAUCAUCAAGCAUU	226	AAUGCUUGAUGAUACA AU	1336-1354	1334
110	NAUUGUAUCAUCAAGCAUU	227	AAUGCUUGAUGAUACA AUN	1336-1354	1334
111	NAUUGUAUCAUCAAGCAUN	228	NAUGCUUGAUGAUACA AUN	1336-1354	1334
112	CAGAAUAGAUGUCAGCUCG	229	CGAGCUGACAUCUAUUCUG	1382-1400	1380
113	UAGAAUAGAUGUCAGCUCG	230	CGAGCUGACAUCUAUUCUA	1382-1400	1380
114	AAGAAUAGAUGUCAGCUCG	231	CGAGCUGACAUCUAUUCU	1382-1400	1380
115	NAGAAUAGAUGUCAGCUCG	232	CGAGCUGACAUCUAUUCUN	1382-1400	1380
116	NAGAAUAGAUGUCAGCUCN	233	NGAGCUGACAUCUAUUCUN	1382-1400	1380
117	CAGUAAACCAGACCAACAG	234	CUGUUGGUCUGGUUUACUG	1398-1416	1396
118	UAGUAAACCAGACCAACAG	235	CUGUUGGUCUGGUUUACUA	1398-1416	1396
119	AAGUAAACCAGACCAACAG	236	CUGUUGGUCUGGUUUACUU	1398-1416	1396
120	NAGUAAACCAGACCAACAG	237	CUGUUGGUCUGGUUUACUN	1398-1416	1396
121	NAGUAAACCAGACCAACAN	238	NUGUUGGUCUGGUUUACUN	1398-1416	1396
122	GCAAUUGGUACUCCUCAAC	239	GUUGAGGAGUACCAAUUGC	1451-1469	1449
123	UCAAUUGGUACUCCUCAAC	240	GUUGAGGAGUACCAAUUGA	1451-1469	1449
124	ACAAUUGGUACUCCUCAAC	241	GUUGAGGAGUACCAAUUGU	1451-1469	1449
125	NCAAUUGGUACUCCUCAAC	242	GUUGAGGAGUACCAAUUGN	1451-1469	1449
126	NCAAUUGGUACUCCUCAAN	243	NUUGAGGAGUACCAAUUGN	1451-1469	1449
127	CCUCUAUCGAGGGAUCUGA	244	UCAGAUCUCCUGAUAGAGG	1490-1508	1488
128	UCUCUAUCGAGGGAUCUGA	245	UCAGAUCUCCUGAUAGAGA	1490-1508	1488

SEQ ID NO:.	Antisense Strand Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO:.	Sense Strand Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	Corresponding Positions of Identified Sequence on SEQ ID NO: 1	Targeted Gene Position
129	ACUCUAUCGAGGGAUCUGA	246	UCAGAUCUCCUCGAUAGAGU	1490-1508	1488
130	NCUCUAUCGAGGGAUCUGA	247	UCAGAUCUCCUCGAUAGAGN	1490-1508	1488
131	NCUCUAUCGAGGGAUCUGN	248	NCAGAUCUCCUCGAUAGAGN	1490-1508	1488
132	AUUUCCUCUAUCGAGGGAU	249	AUCCUCUGAUAGAGGAAAU	1494-1512	1492
133	UUUUCCUCUAUCGAGGGAU	250	AUCCUCUGAUAGAGGAAAA	1494-1512	1492
134	UUUUCCUCUAUCGAGGGAU	251	AUCCUCUGAUAGAGGAAAN	1494-1512	1492
135	UUUUCCUCUAUCGAGGGAU	252	NUCCUCUGAUAGAGGAAAN	1494-1512	1492
136	GGAUACUUUGGUCGAAACUU	253	AAGUUUCGACCAAGUAUCC	1535-1553	1533
137	UGAUACUUUGGUCGAAACUU	254	AAGUUUCGACCAAGUAUCA	1535-1553	1533
138	AGAUACUUUGGUCGAAACUU	255	AAGUUUCGACCAAGUAUCU	1535-1553	1533
139	NGAUACUUUGGUCGAAACUU	256	AAGUUUCGACCAAGUAUCN	1535-1553	1533
140	NGAUACUUUGGUCGAAACUN	257	NAGUUUCGACCAAGUAUCN	1535-1553	1533
141	UCUUAUUAACGAAGAGCAGU	258	ACUGCUCUUCGUUUUAAGA	1640-1658	1638
142	ACUUAUUAACGAAGAGCAGU	259	ACUGCUCUUCGUUUUAAGU	1640-1658	1638
143	NCUUAUUAACGAAGAGCAGU	260	ACUGCUCUUCGUUUUAAGN	1640-1658	1638
144	NCUUAUUAACGAAGAGCAGN	261	NCUGCUCUUCGUUUUAAGN	1640-1658	1638

N = any nucleobase

[0097] The ALK7 RNAi agent sense strands and antisense strands that comprise or consist of the nucleotide sequences in Table 2 can be modified nucleotides or unmodified nucleotides. In some embodiments, the ALK7 RNAi agents having the sense and antisense strand sequences that comprise or consist of any of the nucleotide sequences in Table 2 are all or substantially all modified nucleotides.

[0098] In some embodiments, the antisense strand of an ALK7 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the antisense strand sequences in Table 2. In some embodiments, the sense strand of an ALK7 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the sense strand sequences in Table 2.

[0099] As used herein, each N listed in a sequence disclosed in Table 2 may be independently selected from any and all nucleobases (including those found on both modified and unmodified nucleotides). In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is complementary to the N nucleotide at the corresponding position on the other strand. In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is not complementary to the N nucleotide at the corresponding position on the other strand. In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is the same as the N nucleotide at the corresponding position on the other strand. In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is different from the N nucleotide at the corresponding position on the other strand.

[0100] Certain modified ALK7 RNAi agent sense and antisense strands are provided in Table 3, Table 4, Table 5, Table 6, Table 6a, and Table 10. Certain modified ALK7 RNAi agent antisense strands, as well as their underlying unmodified nucleobase sequences, are provided in Table 3. Certain modified ALK7 RNAi agent sense strands, as well as their underlying unmodified nucleobase sequences, are provided in Tables 4, 5, and 6. In forming ALK7 RNAi agents, each of the nucleotides in each of the underlying base sequences listed in Tables 3, 4, 5, and 6, as well as in Table 2, above, can be a modified nucleotide.

[0101] The ALK7 RNAi agents described herein are formed by annealing an antisense strand with a sense strand. A sense strand containing a sequence listed in Table 2, Table 4, Table 5, Table 6, Table 6a can be hybridized to any antisense strand containing a sequence listed in Table 2 or Table 3, provided the two sequences have a region of at least 85% complementarity over a contiguous 16, 17, 18, 19, 20, or 21 nucleotide sequence.

[0102] In some embodiments, an ALK7 RNAi agent antisense strand comprises a nucleotide sequence of any of the sequences in Table 2 or Table 3.

[0103] In some embodiments, an ALK7 RNAi agent comprises or consists of a duplex having the nucleobase sequences of the sense strand and the antisense strand of any of the sequences in Table 2, Table 3, Table 4, Table 5, Table 6, Table 6a, or Table 10.

[0104] Examples of antisense strands containing modified nucleotides are provided in Table 3. Examples of sense strands containing modified nucleotides are provided in Tables 4, 5 and 6.

[0105] As used in Tables 3, 4, 5, 6, 6a, and 10, the following notations are used to indicate modified nucleotides, targeting groups, and linking groups:

A	=	adenosine-3'-phosphate
C	=	cytidine-3'-phosphate
G	=	guanosine-3'-phosphate
U	=	uridine-3'-phosphate
I	=	inosine-3'-phosphate
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
i	=	2'-O-methylinosine-3'-phosphate
is	=	2'-O-methylinosine-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
Af	=	2'-fluoroadenosine-3'-phosphate
Afs	=	2'-fluoroadenosine-3'-phosphorothioate
Cf	=	2'-fluorocytidine-3'-phosphate
Cfs	=	2'-fluorocytidine-3'-phosphorothioate
Gf	=	2'-fluoroguanosine-3'-phosphate
Gfs	=	2'-fluoroguanosine-3'-phosphorothioate
Tf	=	2'-fluoro-5'-methyluridine-3'-phosphate
Tfs	=	2'-fluoro-5'-methyluridine-3'-phosphorothioate
Uf	=	2'-fluorouridine-3'-phosphate
Ufs	=	2'-fluorouridine-3'-phosphorothioate
dT	=	2'-deoxythymidine-3'-phosphate
AUNA	=	2',3'-seco-adenosine-3'-phosphate
AUNAS	=	2',3'-seco-adenosine-3'-phosphorothioate
CUNA	=	2',3'-seco-cytidine-3'-phosphate

CUNAS	=	2',3'-seco-cytidine-3'-phosphorothioate
GUNA	=	2',3'-seco-guanosine-3'-phosphate
GUNAS	=	2',3'-seco-guanosine-3'-phosphorothioate
UUNA	=	2',3'-seco-uridine-3'-phosphate
UUNAS	=	2',3'-seco-uridine-3'-phosphorothioate
a_2N	=	see Table 11
a_2Ns	=	see Table 11
(invAb)	=	inverted abasic deoxyribonucleotide-5'-phosphate, see Table 11
(invAb)s	=	inverted abasic deoxyribonucleotide-5'-phosphorothioate, see Table 11
s	=	phosphorothioate linkage
ss	=	phosphorodithioate linkage
p	=	terminal phosphate (as synthesized)
vpdN	=	vinyl phosphonate deoxyribonucleotide
cPrpa	=	5'-cyclopropyl phosphonate-2'-O-methyladenosine-3'-phosphate (see Table 11)
cPrpas	=	5'-cyclopropyl phosphonate-2'-O-methyladenosine-3'-phosphorothioate (see Table 11)
cPrpi	=	5'-cyclopropyl phosphonate-2'-O-methylinosine-3'-phosphate (see Table 11)
cPrpis	=	5'-cyclopropyl phosphonate-2'-O-methylinosine-3'-phosphorothioate (see Table 11)
cPrpu	=	5'-cyclopropyl phosphonate-2'-O-methyluridine-3'-phosphate (see Table 11)
cPrpus	=	5'-cyclopropyl phosphonate-2'-O-methyluridine-3'-phosphorothioate (see Table 11)
(C6-SS-C6)	=	see Table 11
(C6-S)	=	see Table 11
(6-SS-6)	=	see Table 11
(NH2-C6)	=	see Table 11
(NH2-C6)s	=	see Table 11
(NH-C6)	=	see Table 11
(NH-C6)s	=	see Table 11
L6	=	see Table 11
LP-371-a	=	see Table 11
LP-379-a	=	see Table 11
LP-371-p	=	see Table 11
LP-379-p	=	see Table 11
(NAG37)	=	see Table 11
(NAG37)s	=	see Table 11

[0106] As the person of ordinary skill in the art would readily understand, unless otherwise indicated by the sequence (such as, for example, by a phosphorothioate linkage “s” or phosphorodithioate linkage “ss”), when present in an oligonucleotide, the nucleotide monomers are mutually linked by 5’-3’-phosphodiester bonds. As the person of ordinary skill in the art would clearly understand, the inclusion of a phosphorothioate or phosphorodithioate linkage as shown in the modified nucleotide sequences disclosed herein replaces the phosphodiester linkage typically present in oligonucleotides. Further, the person of ordinary skill in the art would readily understand that the terminal nucleotide at the 3’ end of a given oligonucleotide sequence would typically have a hydroxyl (-OH) group at the respective 3’ position of the given monomer instead of a phosphate moiety *ex vivo*. Additionally, for the embodiments disclosed herein, when viewing the respective strand 5’ → 3’, the inverted abasic residues are inserted such that the 3’ position of the deoxyribose is linked at the 3’ end of the preceding monomer on the respective strand (*see, e.g.*, Table 11). Moreover, as the person of ordinary skill would readily understand and appreciate, while the phosphorothioate chemical structures depicted herein typically show the anion on the sulfur atom, the inventions disclosed herein encompass all phosphorothioate tautomers (e.g., where the sulfur atom has a double-bond and the anion is on an oxygen atom). Unless expressly indicated otherwise herein, such understandings of the person of ordinary skill in the art are used when describing the ALK7 RNAi agents and compositions of ALK7 RNAi agents disclosed herein.

[0107] Certain examples of PK/PD modulators and linking groups used with the ALK7 RNAi agents disclosed herein are included in the chemical structures provided below in Table 11. Each sense strand and/or antisense strand can have any PK/PD modulators or linking groups listed herein, as well as other targeting groups, PK/PD modulators, linking groups, conjugated to the 5’ and/or 3’ end of the sequence.

Table 3. ALK7 RNAi Agent Antisense Strand Sequences

AS Strand ID	Modified Antisense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CA005127	cPrpusAfsugauAfgaaUCfgUfgCfuucasc	262	UAUGAUAGAAUCGUGCUUCAC	551
CA005129	cPrpusAfsuggauUfcuggGfgUfaUfgucusc	263	UAGGAUUCUGGGGU AUGUCUC	552
CA005131	cPrpusCfsgaaUfngaUfcAfgCfucgcsu	264	UCGAUAUGAUGUCAGCUCGCU	553
CA005139	cPrpasAfsagacaUfacacAfcUfuCfagucsc	265	AAGACAUAACACACUUCAGUCC	554
CA005141	cPrpusCfsaacaUfgcucCfuUfcUfguuusg	266	UCAACAUGCUCCUUCUGUUUG	555
CA005143	cPrpusUfscacuUfgguUfgCfaUfgacug	267	UCCAUUGGUUAGCAUGACUG	556
CA005145	cPrpusUfsuggaAfcuauGfaCfaGfaagasc	268	UUUGAACUAUGACAGAAAGAC	557
CA005147	cPrpasUfsuguuGfgaacUfaUfgAfcagasc	269	AUUGUUGGAACUAUGACAGAC	558
CA005149	cPrpusCfsaugaUfaucAfgAfuAfcagasc	270	UCAUGAUUUUCAGAUACCAGC	559
CA005151	cPrpusCfsauauAfgaggaGfcCfcUfguucsa	271	UCAUAUAAGGAGCCCUUUA	560
CA005153	cPrpasUfsaugaAfggugUfgCfcAfgaccsa	272	AUAUGAAGGUGGCCAGACCA	561
CA005155	cPrpusCfsauauGfaaggUfgUfgCfcagasc	273	UCAUAUGAAGGUGGCCAGAC	562
CA005157	cPrpusUfscacuAfuagaaGfgUfgUfgccasc	274	UCCCAUAUGAAGGUGUGCCAG	563
CA005159	cPrpasAfsuagcAfgguUfaCfcUfugugsu	275	AAUAGCAGGUUUACCUUGUGU	564
CA005161	cPrpasGfscacuAfgcagGfuUfuAfcuug	276	AGCAAUAGCAGGUUUACCUUG	565
CA005163	cPrpusCfsuaagUfccgcUfaUfgGfcacasa	277	UCUAAGUCCGCUAUGGCACAA	566
CA005165	cPrpasUfsugaaUfcaugCfuUfcAfcagcsc	278	AUUGAAUCAUGCUUCACAGCC	567
CA005167	cPrpusAfsuugaAfucauGfcUfuCfagcsc	279	UAUUGAAUCAUGCUUCACAGC	568
CA005169	cPrpusUfscgauAfguguUfcAfgUfaugsc	280	UUCGAUAGUUAUGAUUUGC	569
CA005171	cPrpusGfsauucUfgaggUfaUfgUfcgausc	281	UGAUUCUGAGGU AUGUGAUC	570
CA005173	cPrpusGfsuaucafucaaaGfcAfuUfucagsg	282	UGUAUCAUCAAGCAUUUCAGG	571
CA005175	cPrpusAfsuuguAfucauCfaAfgCfaunuusc	283	UAUUGUAUCAUCAAGCAUUUC	572
CA005177	cPrpusAfsagauAfgaugUfcAfgCfucgusu	284	UAGAAUAGAUGUCAGCUUGUU	573
CA005179	cPrpusAfsguuaAfcagAfcCfaAfcagasc	285	UAGUAAACCAGACC AACAGAC	574
CA005181	cPrpusCfsauuuGfguacUfcCfuCfacasc	286	UCAUUUGGUACUCCUCAAACAC	575
CA005183	cPrpusCfsucuaUfcgagGfgAfuCfugaasg	287	UCUCUAUCGAGGGAUCUGAAG	576

AS Strand ID	Modified Antisense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CA005185	cPrpasUfsuuccUfcuauCfG Afg Gfgaucsu	288	AUUUCCUCUAUCGAGGGGAUCU	577
CA005187	cPrpusGfsauacUfugguCfG AfA Afcuuesu	289	UGAUACUUGGUCGAAACUUCU	578
CA005189	cPrpusCfsuuuaUfacgaAfgAfG Cfaguusc	290	UCUUAUAACGAAGACGAGUUC	579
CA006102	cPrpasAfsugauagaauCfG Ufg Cfuucasc	291	UAUGAUAGAUAUCGUUCUUCAC	551
CA007125	cPrpusUfsuggaAfcuauGfaCfaGfaagsasc	292	UUUGGAACUAUGACAGAAAGAC	557
CA007130	cPrpusUfsuggaacuauGfaCfagaagasc	293	UUUGGAACUAUGACAGAAAGAC	557
CA007131	cPrpusUfsUfggacuauGfaCfaGfaagasc	294	UUUGGAACUAUGACAGAAAGAC	557
CA007132	cPrpusUfsuGfgaacuauGfaCfaGfaagasc	295	UUUGGAACUAUGACAGAAAGAC	557
CA007133	cPrpusUfsuggAfacuauGfaCfaGfaagasc	296	UUUGGAACUAUGACAGAAAGAC	557
CA007134	cPrpusUfsuggAfaCfuAfuGfaCfaGfaagasc	297	UUUGGAACUAUGACAGAAAGAC	557
CA007135	cPrpasUfsuguuGfgaacUfaUfgAfcagasc	298	AUUGUUGGAACUAUGACAGAC	558
CA007137	cPrpusUfsuguuGfgaacUfaUfgAfcagasc	299	UUUGUUGGAACUAUGACAGAC	580
CA007142	cPrpasUfsuguunggaacUfaUfgacagasc	300	AUUGUUGGAACUAUGACAGAC	558
CA007143	cPrpasUfsUfguunggaacUfaUfgAfcagasc	301	AUUGUUGGAACUAUGACAGAC	558
CA007144	cPrpasUfsuGfnguugaacUfaUfgAfcagasc	302	AUUGUUGGAACUAUGACAGAC	558
CA007145	cPrpasUfsuguUfggaaCUfaUfgAfcagasc	303	AUUGUUGGAACUAUGACAGAC	558
CA007147	cPrpusCfsaugaUfauucAfgAfuAfccagsc	304	UCAUGAUUAUUCAGAUACCAGC	559
CA007152	cPrpusCfsaugauauucAfgAfuaccagsc	305	UCAUGAUUAUUCAGAUACCAGC	559
CA007153	cPrpusCfsAfugauauucAfgAfuAfccagsc	306	UCAUGAUUAUUCAGAUACCAGC	559
CA007154	cPrpusCfsaUfgauauucAfgAfuAfccagsc	307	UCAUGAUUAUUCAGAUACCAGC	559
CA007155	cPrpusCfsaugAfuauucAfgAfuAfccagsc	308	UCAUGAUUAUUCAGAUACCAGC	559
CA007156	cPrpusCfsaugAfuAfuUfcAfgAfuAfccagsc	309	UCAUGAUUAUUCAGAUACCAGC	559
CA007379	cPrpisUfsugnuGfgaacUfaUfgAfcagasc	310	IUUGUUGGAACUAUGACAGAC	581
CA007380	cPrpisUfsugnuGfgaacUfaUfgAfcagasc	311	IUUGUUGGAACUAUGACAGAC	581
CA007563	cPrpusUfscgauAfguguUfcAfgUfaungsc	312	UUCGAUAGUUGUUCAGUAUUGC	569
CA007568	cPrpusUfscgauaguguUfcAfguaungsc	313	UUCGAUAGUUGUUCAGUAUUGC	569
CA007569	cPrpusUfsCfgauaguguUfcAfgUfaungsc	314	UUCGAUAGUUGUUCAGUAUUGC	569

AS Strand ID	Modified Antisense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CA007570	cPrpusUfscGfauaguguUfcAfgUfauugsc	315	UUCGAUAGUGUUCAGUAUUGC	569
CA007571	cPrpusUfscgaUfaguguUfcAfgUfauugsc	316	UUCGAUAGUGUUCAGUAUUGC	569
CA007572	cPrpusUfscgaUfaGfuGfuUfcAfgUfauugsc	317	UUCGAUAGUGUUCAGUAUUGC	569
CA007573	cPrpusUfscGfaUfaGfuGfuUfcAfgUfauugsc	318	UUCGAUAGUGUUCAGUAUUGC	569
CA007574	cPrpusGfsuaucaAfucaaGfcAfuUfucagsg	319	UGUAUCAUCAAGCAUUUCAGG	571
CA007579	cPrpusGfsuaucaucaaaGfcAfuUfucagsg	320	UGUAUCAUCAAGCAUUUCAGG	571
CA007580	cPrpusGfsUfaucaucaaaGfcAfuUfucagsg	321	UGUAUCAUCAAGCAUUUCAGG	571
CA007581	cPrpusGfsuAfucaucaaaGfcAfuUfucagsg	322	UGUAUCAUCAAGCAUUUCAGG	571
CA007582	cPrpusGfsuauCfaucaaaGfcAfuUfucagsg	323	UGUAUCAUCAAGCAUUUCAGG	571
CA007583	cPrpusGfsuauCfaUfcAfaGfcAfuUfucagsg	324	UGUAUCAUCAAGCAUUUCAGG	571
CA007584	cPrpusGfsuAfuCfaUfcAfaGfcAfuUfucagsg	325	UGUAUCAUCAAGCAUUUCAGG	571
CA007586	cPrpusAfsuuguAfucauCfaAfgCfauususc	326	UAUUGUAUCAUCAAGCAUUUC	572
CA007591	cPrpusAfsuuguaucauCfaAfgcauuusc	327	UAUUGUAUCAUCAAGCAUUUC	572
CA007592	cPrpusAfsUfuguaucauCfaAfgCfauuusc	328	UAUUGUAUCAUCAAGCAUUUC	572
CA007593	cPrpusAfsuUfguaucauCfaAfgCfauuusc	329	UAUUGUAUCAUCAAGCAUUUC	572
CA007594	cPrpusAfsuugUfaucauCfaAfgCfauuusc	330	UAUUGUAUCAUCAAGCAUUUC	572
CA007595	cPrpusAfsuugUfaUfcAfuCfaAfgCfauuusc	331	UAUUGUAUCAUCAAGCAUUUC	572
CA007596	cPrpusAfsuUfgUfaUfcAfuCfaAfgCfauuusc	332	UAUUGUAUCAUCAAGCAUUUC	572
CA008042	cPrpusGfsuaUfcaucaaaGfcAfuUfucagsg	333	UGUAUCAUCAAGCAUUUCAGG	571
CA008047	cPrpusGfsuaUfcaucaaaGfcAfuUfucagsgsg	334	UGUAUCAUCAAGCAUUUCAGG	571
CA008050	cPrpisUfsugUfuggaacUfaUfgAfcagasc	335	IUUGUUGGAACUAUGACAGAC	581
CA008052	cPrpusCfsauGfauauuucAfgAfuAfcagasc	336	UCAUGAUAUUCAGAUACCCAGC	559
CA008053	cPrpusCfsauGfauauuucAfgAfuAfcagsgsc	337	UCAUGAUAUUCAGAUACCCAGC	559
CA008054	cPrpusUfscgAfuaguguUfcAfgUfauugsc	338	UUCGAUAGUGUUCAGUAUUGC	569
CA008055	cPrpusUfscgAfuaguguUfcAfgUfauugscsc	339	UUCGAUAGUGUUCAGUAUUGC	569
CA008087	cPrpisUfsugUfuggaacUfaUfgAfcagasc	340	IUUGUUGGAACUAUGACAGAC	581
CA008542	cPrpusUfsuggaAfcuauGfaUfaGfaagasc	341	UUUGGAACUAUGAUAGAAGAC	582

AS Strand ID	Modified Antisense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CA008544	cPrpusUfsuggaAfcuauGfaCfaGfaggasc	342	UUUGGAACUAUGACAGAGGAC	583
CA008546	cPrpusUfsuggaAfcuauGfaCfaGfaaggsc	343	UUUGGAACUAUGACAGAGGC	584
CA008547	cPrpusUfsuggaAfcuauGfaUfaGfaggasc	344	UUUGGAACUAUGAUAGAGGAC	585
CA008548	cPrpusUfsuggaAfcuauGfaUfaGfaaggsc	345	UUUGGAACUAUGAUAGAAAGGC	586
CA008550	cPrpusUfsuggaAfcuauGfaCfaGfggg	346	UUUGGAACUAUGACAGGGG	587
CA008551	cPrpusUfsuggaAfcuauGfaUfaGfggg	347	UUUGGAACUAUGAUAGGGG	588
CA008553	cPrpusUfsuGfuuggaacUfaUfgAfcagasc	348	UUUGUUGGAACUAUGACAGAC	580
CA008554	cPrpisUfsuGfuuggaacUfaUfgAfcagasc	349	IUUGUUGGAACUAUGACAGAC	581
CA008555	cPrpisUfsuGfuuggaacUfaUfgAfcagasc	350	IUUGUUGGAACUAUGACAGAC	581
CA008556	cPrpisUfsuguuGfgaacUfaUfgAfcagasc	351	IUUGUUGGAACUAUGACAGAC	581
CA009908	cPrpusUfsuggaAfcuauGfaCfaGfaagasc	352	UUUGGAACUAUGACAGAAAGAC	557
CA009909	cPrpusUfsugggAfcuauGfaCfaGfaagasc	353	UUUGGGACUAUGACAGAAAGAC	589
CA009910	cPrpusUfsuggG _{UNA} AfcuauGfaCfaGfaagasc	354	UUUGGGACUAUGACAGAAAGAC	589
CA009911	cPrpusUfsuggG _{UNA} AfcuauGfaCfaGfaggasc	355	UUUGGGACUAUGACAGAGGAC	590
CA009912	cPrpusUfsuggaGfcuauGfaCfaGfaagasc	356	UUUGGAGCUAUGACAGAAAGAC	591
CA009913	cPrpusUfsuggaG _{UNA} AcuauGfaCfaGfaggasc	357	UUUGGAGCUAUGACAGAGGAC	592
CA009914	cPrpusUfsuiigaAfcuauGfaCfaGfaagasc	358	UUUIGAACUAUGACAGAAAGAC	593
CA009915	cPrpusUfsugiaAfcuauGfaCfaGfaagasc	359	UUUGIAACUAUGACAGAAAGAC	594

I = inosine (hypoxanthine nucleobase) nucleotide

Table 4. ALK7 Agent Sense Strand Sequences (Shown Without Linkers, Conjugates, Capping Moieties, or Terminal dT)

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CS005138-NL	ggacugaaGfUfGfuguaugucuu	360	GGACUGAAAGUGUGUAUGUCUU	595
CS005140-NL	caaacagaAfGfGfagcauguuga	361	CAAAcAGAAGGAGCAUGUUGA	596
CS005142-NL	cagucaugCfUfAfaccaauggaa	362	CAGUCAUGCUAAACCAAUGGAA	597
CS005144-NL	gucuucugUfCfAfuguuuccaaa	363	GUCUUCUGUCAUAGUUCCAA	598
CS005146-NL	gucugucaUfAfGfuuuccaacaau	364	GUCUGUCAUAGUUCCAACAAU	599
CS005148-NL	gcugguauCfUfGfaauaucauga	365	GCUGGUAUCUGAAUAUCAUGA	600
CS005150-NL	ugaacaggGfCfUfccuuauauga	366	UGAACAGGGCUCUUAUAUGA	601
CS005152-NL	uggucuggCfAfCfaccuucuaau	367	UGGUCUGGCACACCUUCAUUAU	602
CS005154-NL	gucuggcaCfAfCfeuucuauga	368	GUCUGGCACACCUUCAUUGA	603
CS005156-NL	cuggcacaCfCfUfucuauggaa	369	CUGGCACACCUUCAUUGGAA	604
CS005158-NL	acacaaggUfAfAfaccugcuaau	370	ACACAAGGUAACCUGCUAUU	605
CS005160-NL	caagguaaAfCfCfugcuaauugcu	371	CAAGGUAACCUGCUAUUGCU	606
CS005162-NL	uuuguccaUfAfGfeggacuuauga	372	UUGUGCCAUAGCGGACUUAGA	607
CS005164-NL	ggcugugaAfGfCfanguauucaau	373	GGCUGUGAAGCAUGAUUCAAU	608
CS005166-NL	gcugugaaGfCfAfugauucaaua	374	GCUGUGAAGCAUGAUUCAAU	609
CS005168-NL	gcaauacuGfAfAfcaucuaucgaa	375	GCAAUACUGAACACUAUCGAA	610
CS005170-NL	gaucgacaUfAfCfucagagaauca	376	GAUCGACAUACCUCAGAAUCA	611
CS005172-NL	ccugaaauGfCfUfugaugauaca	377	CCUGAAAUAGCUUGAUGAUACA	612
CS005174-NL	ga_2NaaugcuUfGfAfugauacaa_2Nua	378	G(A ^{2N})AAUGCUUGAUGAUACA(A ^{2N})UA	613
CS005176-NL	aacgagcuGfAfCfaucauauuca	379	AACGAGCUGACAUCUAUUCUA	614
CS005178-NL	gucuguugGfUfCfugguuuacua	380	GUCUGUUGGUCUGGUUUAUAUA	615
CS005180-NL	guguugagGfAfGfuaaccauuga	381	GUGUUGAGGAGUACCAAUUGA	616
CS005182-NL	cuucagauCfCfCfucgagauagaga	382	CUUCAGAUCCCUCGUAUAGAGA	617
CS005184-NL	agaucuccuCfGfAfuaagaggaaau	383	AGAUCCCUCCGAUAGAGGAAAU	618
CS005186-NL	a_2NgaaguuuUfGfAfccaagauca	384	(A ^{2N})GAAGUUUCGACCAAGUAUCA	619

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CS005188-NL	gaacugcuCfUfUfcguuuuaga	385	GAACUGCUCUUCGUUUUAGA	620
CS005209-NL	ggacugaaGfUfGfuguaugucuu	386	GGACUGAAAGUGUGUAUGUCUU	595
CS005210-NL	caaacagaAfGfGfagcauguuga	387	CAAAACAGAAAGGAGCAUGUUUGA	596
CS005211-NL	cagucaugCfUfAfaccaauggaa	388	CAGUCAUGCUAACCAAUUGGAA	597
CS005212-NL	gucuucugUfCfAfuguuuccaaa	389	GUCUUCUGUCAUAGUUCCAA	598
CS005213-NL	gucugucaUfAfGfuuuccaacaau	390	GUCUGUCAUAGUUCCAACA AU	599
CS005214-NL	gcugguauCfUfGfaauaucauga	391	GCUGGUUAUCUGAAUAUCAUGA	600
CS005215-NL	ugaacaggGfCfUfccuuauuga	392	UGAACAGGGCUCUUAUAUGA	601
CS005216-NL	uggucuggCfAfCfaccuucauu	393	UGGUUGGCACACCUUCAU AU	602
CS005217-NL	gucuggcaCfAfCfcuucauuga	394	GUCUGGCACACCUUCAU AUGA	603
CS005218-NL	cuggcacaCfCfUfucuuauuggaa	395	CUGGCACACCUUCAU AUGGAA	604
CS005219-NL	acacaaggUfAfAfaccugcuuuu	396	ACACAAGGUAAACCUGCU AUU	605
CS005220-NL	caagguuaaAfCfCfugcuauugcu	397	CAAGGUAAACCUUGCU AUUGCU	606
CS005221-NL	uuuguccaUfAfGfcggacuuga	398	UUGUGCCAUAGCGGACUUA GA	607
CS005222-NL	ggcugugaAfGfCfaugaauucau	399	GGCUGUGAAGCAUGAUUCA AU	608
CS005223-NL	gcugugaaGfCfAfugauucaua	400	GCUGUGAAGCAUGAUUCA AU	609
CS005224-NL	gcaauacuGfAfAfcacuaucgaa	401	GCAAUACUGAAACACUAUCGAA	610
CS005225-NL	gaucgacaUfAfCfcucagaauc	402	GAUCGACAUACCUCCAGAAU CA	611
CS005226-NL	ccugaaauGfCfUfugaugauaca	403	CCUGAAAUUCUUGAUGAUACA	612
CS005227-NL	ga_2NaugcuUfGfAfugauacaa_2Nua	404	G(A ^{2N})AAUGCUUGAUGAUACA(A ^{2N})UA	613
CS005228-NL	aacgagcuGfAfCfaucuaucua	405	AACGAGCUGACAUCAUUCUA	614
CS005229-NL	gucugungGfUfCfugguuuacua	406	GUCUGUUGGUCUGGUUUA CU	615
CS005230-NL	guguugagGfAfGfuaaccauuga	407	GUGUUGAGGAGUACCAAUUGA	616
CS005231-NL	cuucagauCfCfCfucgauagaga	408	CUUCAGAUCCCCUCGAUAGAGA	617
CS005232-NL	agaucucuCfGfAfuaagaggaa	409	AGAUCCCUCCGAUAGAGGAA AU	618
CS005233-NL	a_2NgaaguuuCfGfAfccagaugauca	410	(A ^{2N})GAAGUUUCGACCAAGUAUCA	619

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CS005234-NL	gaacugcuCfUfUfcguuuuaga	411	GAACUGCUCUUCGUUUUAGA	620
CS007126-NL	gucuucugUfcAfuAfguuccaaa	412	GUCUUCUGUCAUAGUUCCAA	598
CS007127-NL	gucuucugUfCfaUfaguuccaaa	413	GUCUUCUGUCAUAGUUCCAA	598
CS007128-NL	gucuucUfguCfAfuaguuccaaa	414	GUCUUCUGUCAUAGUUCCAA	598
CS007129-NL	gucuucUfgUfcAfuaguuccaaa	415	GUCUUCUGUCAUAGUUCCAA	598
CS007136-NL	gucugucaUfAfGfuuuccaaca	416	GUCUGUCAUAGUUCCAACAA	621
CS007138-NL	gucugucaUfaGfuUfccacaau	417	GUCUGUCAUAGUUCCAACAA	599
CS007139-NL	gucugucaUfAfgUfuuccacaau	418	GUCUGUCAUAGUUCCAACAA	599
CS007140-NL	gucuguCfauAfGfuuuccacaau	419	GUCUGUCAUAGUUCCAACAA	599
CS007141-NL	gucuguCfaUfaGfuuuccacaau	420	GUCUGUCAUAGUUCCAACAA	599
CS007148-NL	gcugguauCfuGfaAfuaucauga	421	GCUGGUUUCUGAAUUAUCAUGA	600
CS007149-NL	gcugguauCfUfgAfauaucauga	422	GCUGGUUUCUGAAUUAUCAUGA	600
CS007150-NL	gcugguAfucUfGfaauaucauga	423	GCUGGUUUCUGAAUUAUCAUGA	600
CS007151-NL	gcugguAfucUfGfaauaucauga	424	GCUGGUUUCUGAAUUAUCAUGA	600
CS007564-NL	gcaauacuGfaAfcAfcuaucgaa	425	GCAAUACUGAACACUUAUCGAA	610
CS007565-NL	gcaauacuGfAfaCfacuaucgaa	426	GCAAUACUGAACACUUAUCGAA	610
CS007566-NL	gcaauaCfugAfAfcuaucgaa	427	GCAAUACUGAACACUUAUCGAA	610
CS007567-NL	gcaauaCfuGfaAfcuaucgaa	428	GCAAUACUGAACACUUAUCGAA	610
CS007578-NL	ccugaaAfuGfcUfugaugauaca	429	CCUGAAUAGCUUGAUGAUACA	612
CS007711-NL	gaaugcuUfGfAfugaauacaaua	430	GAAUAGCUUGAUGAUACAUA	622
CS007712-NL	ga_2NaaugcuUfgAfuGfaauaca_2Nua	431	G(A ^{2N})AAUGCUUGAUGAUACA(A ^{2N})UA	613
CS007713-NL	ga_2NaaugcuUfGfaUfgauaca_2Nua	432	G(A ^{2N})AAUGCUUGAUGAUACA(A ^{2N})UA	613
CS007714-NL	ga_2NaaugCfuGfAfugaauaca_2Nua	433	G(A ^{2N})AAUGCUUGAUGAUACA(A ^{2N})UA	613
CS007715-NL	ga_2NaaugCfuUfgAfugaauaca_2Nua	434	G(A ^{2N})AAUGCUUGAUGAUACA(A ^{2N})UA	613
CS007716-NL	ccugaaauGfcUfuGfaugauaca	435	CCUGAAUAGCUUGAUGAUACA	612
CS007717-NL	ccugaaauGfCfuUfgaugauaca	436	CCUGAAUAGCUUGAUGAUACA	612

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CS007718-NL	ccugaaAfugCfUfugaugauaca	437	CCUGAAAUGCUUUGAUGAUACA	612
CS007719-NL	ccugaaAfuGfcUfugaugauaca	438	CCUGAAAUGCUUUGAUGAUACA	612
CS008543-NL	guccucugUfcAfuAfguuccaaa	439	GUCCUCUGUCAUAGUUCCAAA	623
CS008545-NL	gccuucugUfcAfuAfguuccaaa	440	GCCUUCUGUCAUAGUUCCAAA	624
CS008549-NL	cccucgUfcAfuAfguuccaaa	441	CCCCUGUCAUAGUUCCAAA	625
CS008552-NL	gucugucaUfaGfuUfccacaaca	442	GUCUGUCAUAGUUCCACAACA	621
CS011689-NL	ccugaaAfuGfcUfugaugauaca	443	CCUGAAAUGCUUUGAUGAUACA	612
CS011691-NL	ccugaaAfuGfcUfugaugauaca	444	CCUGAAAUGCUUUGAUGAUACA	612

I = inosine (hypoxanthine nucleobase) nucleotide

(A^{2N}) = 2-aminoadenosine nucleotide

Table 5. ALK7 Agent Sense Strand Sequences (Shown With (NH2-C6) Linker, (NAG37)s ligand, (invAb) end cap, or terminal dT (see Table 11 for structure information.))

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CS005138	(NH2-C6)s(invAb)sggacugaaGfUfGfugauugucuu(s(invAb))-(C6-SS)-MeC5-dT	445	GGACUGAAGUGUGUAUGUCUUT	626
CS005140	(NH2-C6)s(invAb)scaaacagaAfGfGfagcaugaugas(invAb))-(C6-SS)-MeC5-dT	446	CAAAACAGAAAGGAGCAUGUUGAT	627
CS005142	(NH2-C6)s(invAb)scagucaugCfUfAfaccaauggaas(invAb))-(C6-SS)-MeC5-dT	447	CAGUCAUGCUAAACCAUUGGAAT	628
CS005144	(NH2-C6)s(invAb)sgucuuucugUfCfAfuguuuccaaas(invAb))-(C6-SS)-MeC5-dT	448	GUCUUCUGUCAUAGUUCCAAAT	629
CS005146	(NH2-C6)s(invAb)sgucugucaUfAfGfuccaacaauas(invAb))-(C6-SS)-MeC5-dT	449	GUCUGUCAUAGUUCCAACAAUT	630
CS005148	(NH2-C6)s(invAb)sgcugguauCfUfGfaaucaugas(invAb))-(C6-SS)-MeC5-dT	450	GCUGGUUUCUGAAUAUCAUGAT	631
CS005150	(NH2-C6)s(invAb)sugaacaggGfCfUfccuuauaugas(invAb))-(C6-SS)-MeC5-dT	451	UGAACAGGGCUCUUAUAUGAT	632
CS005152	(NH2-C6)s(invAb)suggucuggCfAfCfaccuucauauas(invAb))-(C6-SS)-MeC5-dT	452	UGGUCUGGCACACCUUCAUAUT	633
CS005154	(NH2-C6)s(invAb)sgucuggcaCfAfCfuccuauaugas(invAb))-(C6-SS)-MeC5-dT	453	GUCUGGCACACCUUCAUAUGAT	634
CS005156	(NH2-C6)s(invAb)scuggcacaCfCfUfucuuauuggaas(invAb))-(C6-SS)-MeC5-dT	454	CUGGCACACCUUCAUAUGGAAT	635
CS005158	(NH2-C6)s(invAb)sacacaaggUfAfAfaccugcuauus(invAb))-(C6-SS)-MeC5-dT	455	ACACAAGGUAAACCUGCUAUUT	636
CS005160	(NH2-C6)s(invAb)scaagguaaAfCfCfugcuauugcus(invAb))-(C6-SS)-MeC5-dT	456	CAAGGUAAACCUGCUAUUGCUT	637

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CS005162	(NH2-C6)s(invAb)suugugccaUfAfGfcggacuuagas(invAb)-(C6-SS)-MeC5-dT	457	UUGUGCCCAUAGCGGACUUAGAT	638
CS005164	(NH2-C6)s(invAb)sggcugugaAfGfCfagauucaaus(invAb)-(C6-SS)-MeC5-dT	458	GGCUGUGAAGCAUGAUUCAAUT	639
CS005166	(NH2-C6)s(invAb)sgcugugaaGfCfAfugauucaauas(invAb)-(C6-SS)-MeC5-dT	459	GCUGUGAAGCAUGAUUCAAUT	640
CS005168	(NH2-C6)s(invAb)sgcaauacuGfAfAfcacuaucgaas(invAb)-(C6-SS)-MeC5-dT	460	GCAAUACUGAAGCAUUCGAAT	641
CS005170	(NH2-C6)s(invAb)sgaucgacaUfAfCfcucagaucaucas(invAb)-(C6-SS)-MeC5-dT	461	GAUCGACAUACCCUCAGAAUCAT	642
CS005172	(NH2-C6)s(invAb)scugaaaUfGfUfugaucaucas(invAb)-(C6-SS)-MeC5-dT	462	CCUGAAAUGCUUGAUGAUACAT	643
CS005174	(NH2-C6)s(invAb)sga_2NaaugcuUfGfAfugaucaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	463	G(A ^{2N})AAUGCUUGAUGAUACA(A ^{2N})UA _T	644
CS005176	(NH2-C6)s(invAb)saacgagcuGfAfCfaucauuuacuas(invAb)-(C6-SS)-MeC5-dT	464	AACGAGCUGACAUUUAUUCUAT	645
CS005178	(NH2-C6)s(invAb)sgucuguugGfUfCfugguuuuacuas(invAb)-(C6-SS)-MeC5-dT	465	GUCUGUUGGUCUGGUUUAUUCUAT	646
CS005180	(NH2-C6)s(invAb)sguguugagGfAfGfuaccaauagagas(invAb)-(C6-SS)-MeC5-dT	466	GUGUUGAGGAGUACCAAUUGAT	647
CS005182	(NH2-C6)s(invAb)scuucagauCfCfCfucgauagagas(invAb)-(C6-SS)-MeC5-dT	467	CUUCAGAUCCCCUCGAUAGAGAT	648
CS005184	(NH2-C6)s(invAb)sagaucuccuGfGfAfugaggaaus(invAb)-(C6-SS)-MeC5-dT	468	AGAUCCCUCGAUAGAGGAAAUT	649
CS005186	(NH2-C6)s(invAb)sa_2NgaaguuuCfGfAfccaaguacuas(invAb)-(C6-SS)-MeC5-dT	469	(A ^{2N})GAAGUUUCGACCAAGUAUCAT	650

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CS005188	(NH2-C6)s(invAb)sgaacugcuCfUfUfcguaauaagas(invAb)-(C6-SS)-MeC5-dT	470	GAAACUGCUCUUCGUUAUAAAGAT	651
CS005209	(NAG37)s(invAb)sggacugaaGfUfGfuguaugucuuus(invAb)	471	GGACUGAAGUGUGUAUGUCUU	595
CS005210	(NAG37)s(invAb)scaaacagaAfGfGfagcauguugas(invAb)	472	CAAAACAGAAAGGAGCAUGUUUA	596
CS005211	(NAG37)s(invAb)scagucaugCfUfAfaccaauggaas(invAb)	473	CAGUCAUGCUAACCCAAUGGAA	597
CS005212	(NAG37)s(invAb)sgucuuucugUfCfAfaguuccaas(invAb)	474	GUCUUCUGUCAUAGUUCCAA	598
CS005213	(NAG37)s(invAb)sgucugucaUfAfGfuucaacaas(invAb)	475	GUCUGUCAUAGUUCCAACAAU	599
CS005214	(NAG37)s(invAb)sgcugguauCfUfGfaauaaucaugas(invAb)	476	GCUGGUAUCUGAAUAUCAUGA	600
CS005215	(NAG37)s(invAb)sugaacaggGfCfUfcuuauaugas(invAb)	477	UGAACAGGGCUCUUAUAUGA	601
CS005216	(NAG37)s(invAb)suggucuggCfAfCfaccuucauauus(invAb)	478	UGGUCUGGCACACCUUCAUAU	602
CS005217	(NAG37)s(invAb)sgucuggcaCfAfCfuucauauaugas(invAb)	479	GUCUGGCACACCUUCAUAUGA	603
CS005218	(NAG37)s(invAb)scuggcacaCfCfUfucuaauuggaas(invAb)	480	CUGGCACACCUUCAUAUGGAA	604
CS005219	(NAG37)s(invAb)sacacaaggUfAfAfaccugeuauus(invAb)	481	ACACAAGGUAACCCUGCUAUU	605
CS005220	(NAG37)s(invAb)scaagguaaAfCfCfugcuauugcus(invAb)	482	CAAGGUAACCCUGCUAUUGCU	606
CS005221	(NAG37)s(invAb)suugugccaUfAfGfeggacuuagag(invAb)	483	UUGUGCCAUAGCGGACUUAGA	607
CS005222	(NAG37)s(invAb)sggcugugaAfGfCf fauguaucauauus(invAb)	484	GGCUGUGAAGCAUGAUUCAAU	608
CS005223	(NAG37)s(invAb)sgcugugaaGfCfAfugauucauauus(invAb)	485	GCUGUGAAGCAUGAUUCAUAU	609
CS005224	(NAG37)s(invAb)sgcaauacuGfAfAfcauauuggaas(invAb)	486	GCAAUACUGAAACACUAUCGAA	610
CS005225	(NAG37)s(invAb)sgaucgacaUfAfCfucacagaucas(invAb)	487	GAUCGACAUACCCUCAGAAUCA	611
CS005226	(NAG37)s(invAb)scugaaauGfCfUfugaugauacac(invAb)	488	CCUGAAAUGCUUGAUGAUACA	612
CS005227	(NAG37)s(invAb)sga_2NaaugcuUfGfAfugauacaa_2Nuas(invAb)	489	G(A ^{2N})AAUGCUUGAUGAUACA(A ^{2N})UA	613
CS005228	(NAG37)s(invAb)saacgagcuGfAfCfaucauauucuas(invAb)	490	AACGAGCUGACAUCAUUCUA	614
CS005229	(NAG37)s(invAb)sgucugungGfUfCfugguuucuas(invAb)	491	GUCUGUUGGUCUGGUUUAUA	615
CS005230	(NAG37)s(invAb)sguguungagGfAfGfuaaccauugag(invAb)	492	GUGUUGAGGAGUACCAAUUGA	616

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CS005231	(NAG37)s(invAb)scuucagauCfCfCfucgauagagas(invAb)	493	CUUCAGAUCCCUCGUAUAGAGA	617
CS005232	(NAG37)s(invAb)sagauccuCfGfAfuagaggaaus(invAb)	494	AGAUCCCUCGUAUAGAGGAAAU	618
CS005233	(NAG37)s(invAb)sa_2NgaaguuuCFgAfcaaguauacas(invAb)	495	(A ^{2N})GAAGUUUCGACCAAGUAUCA	619
CS005234	(NAG37)s(invAb)sgaacugcuCfUfUfguaauagagas(invAb)	496	GAACUGCUCUUCGUUUAAGA	620
CS007126	(NAG37)s(invAb)sgucuuucgUfcAfuAfgnuccaas(invAb)	497	GUCUUCUGUCAUAGUUCCAA	598
CS007127	(NAG37)s(invAb)sgucuuucgUfcCfaUfaguuccaaas(invAb)	498	GUCUUCUGUCAUAGUUCCAA	598
CS007128	(NAG37)s(invAb)sgucuuucUfguCfAfuaguuccaaas(invAb)	499	GUCUUCUGUCAUAGUUCCAA	598
CS007129	(NAG37)s(invAb)sgucuuucUfgUfcAfuaguuccaaas(invAb)	500	GUCUUCUGUCAUAGUUCCAA	598
CS007136	(NAG37)s(invAb)sgucugucaUfAfGfuuccaacaas(invAb)	501	GUCUGUCAUAGUUCCAACAAA	621
CS007138	(NAG37)s(invAb)sgucugucaUfaGfuUfccacaas(invAb)	502	GUCUGUCAUAGUUCCAACAAU	599
CS007139	(NAG37)s(invAb)sgucugucaUfAfgUfuccacaas(invAb)	503	GUCUGUCAUAGUUCCAACAAU	599
CS007140	(NAG37)s(invAb)sgucugucfauAfGfuuccaacaas(invAb)	504	GUCUGUCAUAGUUCCAACAAU	599
CS007141	(NAG37)s(invAb)sgucugucfauUfaGfuuccaacaas(invAb)	505	GUCUGUCAUAGUUCCAACAAU	599
CS007148	(NAG37)s(invAb)sgcugguauCfuGfaAfuaucaugas(invAb)	506	GCUGGUAUCUGAAUAUCAUGA	600
CS007149	(NAG37)s(invAb)sgcugguauCfUfgAfauaucaugas(invAb)	507	GCUGGUAUCUGAAUAUCAUGA	600
CS007150	(NAG37)s(invAb)sgcugguAfuCfUfgfaauaucaugas(invAb)	508	GCUGGUAUCUGAAUAUCAUGA	600
CS007151	(NAG37)s(invAb)sgcugguAfuCfuGfaauaucaugas(invAb)	509	GCUGGUAUCUGAAUAUCAUGA	600
CS007564	(NAG37)s(invAb)sgcaauacuGfaAfcAfcuaucgaas(invAb)	510	GCAAUACUGAACACUAUCGAA	610
CS007565	(NAG37)s(invAb)sgcaauacuGfAfaCfacuaucgaas(invAb)	511	GCAAUACUGAACACUAUCGAA	610
CS007566	(NAG37)s(invAb)sgcaauaCfugAfAfcacuaucgaas(invAb)	512	GCAAUACUGAACACUAUCGAA	610
CS007567	(NAG37)s(invAb)sgcaauaCfuGfaAfcacuaucgaas(invAb)	513	GCAAUACUGAACACUAUCGAA	610
CS007578	(NAG37)s(invAb)scugaaAfuGfcUfugaugauacas(invAb)	514	CCUGAAAUAGCUUGAUGAUACA	612
CS007711	(NH2-C6)s(invAb)sgaaugcuUfGfAfugaucacaas(invAb)-(C6-SS)-MeC5-dT	515	GAAAUAGCUUGAUGAUACAUAUAT	652

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CS007712	(NH2-C6)s(invAb)sga_2NaaugcuUfgAfuGfauacaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	516	G(A ^{2N})AAUGCUUUGAUGAUACA(A ^{2N})UA _T	644
CS007713	(NH2-C6)s(invAb)sga_2NaaugcuUfGfaUfgauacaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	517	G(A ^{2N})AAUGCUUUGAUGAUACA(A ^{2N})UA _T	644
CS007714	(NH2-C6)s(invAb)sga_2NaaugCfuGfAfuGauacaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	518	G(A ^{2N})AAUGCUUUGAUGAUACA(A ^{2N})UA _T	644
CS007715	(NH2-C6)s(invAb)sga_2NaaugCfuUfgAfuGauacaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	519	G(A ^{2N})AAUGCUUUGAUGAUACA(A ^{2N})UA _T	644
CS007716	(NH2-C6)s(invAb)scgugaaUfGfuGfauacaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	520	CCUGAAAUGCUUUGAUGAUACAT	643
CS007717	(NH2-C6)s(invAb)scgugaaUfGfuGfauacaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	521	CCUGAAAUGCUUUGAUGAUACAT	643
CS007718	(NH2-C6)s(invAb)scgugaaUfGfuGfauacaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	522	CCUGAAAUGCUUUGAUGAUACAT	643
CS007719	(NH2-C6)s(invAb)scgugaaUfGfuGfauacaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	523	CCUGAAAUGCUUUGAUGAUACAT	643
CS008543	(NAG37)s(invAb)sguccucugUfcAfuAfguuccaaas(invAb)	524	GUCUCUGUCAUAGUUCCAA	623
CS008545	(NAG37)s(invAb)sguccucugUfcAfuAfguuccaaas(invAb)	525	GCCUUCUGUCAUAGUUCCAA	624
CS008549	(NAG37)s(invAb)sguccucugUfcAfuAfguuccaaas(invAb)	526	CCCUGUCAUAGUUCCAA	625
CS008552	(NAG37)s(invAb)sguccucugUfcAfuAfguuccaaas(invAb)	527	GUCUGUCAUAGUUCCAA	621
CS011689	(NH2-C6)-L6-C6-sccugaaAfuGfuUfugauacaa-(C6-SS)-MeC5-dT	528	CCUGAAAUGCUUUGAUGAUACAT	643
CS011691	(NH2-C6)scgugaaAfuGfuUfugauacaa-(C6-SS)-MeC5-dT	529	CCUGAAAUGCUUUGAUGAUACAT	643

I = inosine (hypoxanthine nucleobase) nucleotide
(A^{2N}) = 2-aminoadenosine nucleotide

Table 6. ALK7 Agent Sense Strand Sequences (Shown with lipid moiety.) The structures of the lipid moieties are shown in Table 11.

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Corresponding Sense Strand ID Number Without Linker or Conjugate (See Table 4)
CS005429	LP-379-a-L6-(NH-C6)s(invAb)sgugaagcaCfGfAfucauaucauas(invAb)-(C6-S)-LP-371-a	530	
CS005430	LP-379-a-L6-(NH-C6)s(invAb)sgagacauaCfCfCfagaauccuas(invAb)-(C6-S)-LP-371-a	531	
CS005431	LP-379-a-L6-(NH-C6)s(invAb)sagcgagcuGfAcfaucauucgas(invAb)-(C6-S)-LP-371-a	532	
CS007081	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugcuUfGfAfuGauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	533	CS005174-NL
CS007082	LP-379-a-L6-(NH-C6)s(invAb)sccugaaauGfCfUfugaugauacac(invAb)-(C6-S)-LP-371-a	534	CS005172-NL
CS007437	LP-379-a-L6-(NH-C6)s(invAb)sgucugucaUfAfGfuuccaacaas(invAb)-(C6-S)-LP-371-a	535	CS005146-NL
CS007438	LP-379-a-L6-(NH-C6)s(invAb)sgcugguauCfUfGfaaucaugac(invAb)-(C6-S)-LP-371-a	536	CS005148-NL
CS007966	LP-379-a-L6-(NH-C6)s(invAb)sccugaaauGfcUfuGfauGauacac(invAb)-(C6-S)-LP-371-a	537	CS007716-NL
CS007967	LP-379-a-L6-(NH-C6)s(invAb)sccugaaauGfCfUfugaugauacac(invAb)-(C6-S)-LP-371-a	538	CS007717-NL
CS007968	LP-379-a-L6-(NH-C6)s(invAb)sccugaaAfugCfUfugaugauacac(invAb)-(C6-S)-LP-371-a	539	CS007718-NL
CS007969	LP-379-a-L6-(NH-C6)s(invAb)sccugaaAfuGfcUfugaugauacac(invAb)-(C6-S)-LP-371-a	540	CS007719-NL
CS007970	LP-379-a-L6-(NH-C6)s(invAb)sgaaaugcuUfGfAfuGauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	541	CS007711-NL
CS007971	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugcuUfGfAfuGfauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	542	CS007712-NL
CS007972	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugcuUfGfAfuGfauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	543	CS007713-NL
CS007973	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugCfuUfGfAfuGfauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	544	CS007714-NL

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Corresponding Sense Strand ID Number Without Linker or Conjugate (See Table 4)
CS007974	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugCfuUfgAfugauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	545	CS007715-NL
CS009133	LP-379-a-L6-(NH-C6)s(invAb)sgucucugUfCfAfaguuccaas(invAb)-(C6-S)-LP-371-a	546	CS005144-NL
CS010070	LP-379-a-L6-(NH-C6)s(invAb)sgucugucaUfAfGfuuccaacaas(invAb)-(C6-SS)-MeC5-dT	547	CS005146-NL
CS011693	LP-379-a-L6-(NH-C6)scugaaAfuGfcUfugaugauacas-(C6-SS)-MeC5-dT	548	CS007578-NL
CS012040	LP-379-a-L6-(NH-C6)scugaaAfuGfcUfugaugauacas-(C6-S)-LP-371-a	549	CS007578-NL
CS015443	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugcuUfGfAfugauacaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	550	CS005174-NL

I = inosine (hypoxanthine nucleobase) nucleotide

(A^{2N}) = 2-aminoadenosine nucleotide

Table 6a. ALK7 Agent Sense Strand Sequences (Shown with lipid moiety.) The structures of the lipid moieties are shown in Table 11.

Strand ID	Modified Sense Strand (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3') (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
CS005429	LP-379-a-L6-(NH-C6)s(invAb)sgugaagcaCfGfAfuucuaucuas(invAb)-(C6-S)-LP-371-a	530	GUGAAGCACGAUUCUAUCAUA	653
CS005430	LP-379-a-L6-(NH-C6)s(invAb)sgagacauaCfCfCfcagaauccuas(invAb)-(C6-S)-LP-371-a	531	GAGACAUACCCCAGAAUCCUA	654

CS005431	LP-379-a-L6-(NH-C6)s(invAb)sagcgagcuGfAfCfaucuaucgas(invAb)-(C6-S)-LP-371-a	532	AGCGAGCUGACAUCUAUUCGA	655
CS007081	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugcuUfGfAfugauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	533	G(A ^{2N})AAUGCUUGAUGAUACA(A ^{2N})UA	613
CS007082	LP-379-a-L6-(NH-C6)s(invAb)scugaaauGfCfUfugaugauacas(invAb)-(C6-S)-LP-371-a	534	CCUGAAAUGCUUGAUGAUACA	612
CS007437	LP-379-a-L6-(NH-C6)s(invAb)sgucugucaUfAfGfuccaacaas(invAb)-(C6-S)-LP-371-a	535	GUUCUGUCAUAGUUCACAAU	599
CS007438	LP-379-a-L6-(NH-C6)s(invAb)sgcugguauCfUfGfaaucaugas(invAb)-(C6-S)-LP-371-a	536	GCUGGUAUCUGAAUAUCAUGA	600
CS007966	LP-379-a-L6-(NH-C6)s(invAb)scugaaauGfcUfuGfaugauacas(invAb)-(C6-S)-LP-371-a	537	CCUGAAAUGCUUGAUGAUACA	612
CS007967	LP-379-a-L6-(NH-C6)s(invAb)scugaaauGfCfuUfugaugauacas(invAb)-(C6-S)-LP-371-a	538	CCUGAAAUGCUUGAUGAUACA	612
CS007968	LP-379-a-L6-(NH-C6)s(invAb)scugaaAfugCfUfugaugauacas(invAb)-(C6-S)-LP-371-a	539	CCUGAAAUGCUUGAUGAUACA	612
CS007969	LP-379-a-L6-(NH-C6)s(invAb)scugaaAfUGfCfUfugaugauacas(invAb)-(C6-S)-LP-371-a	540	CCUGAAAUGCUUGAUGAUACA	612
CS007970	LP-379-a-L6-(NH-C6)s(invAb)sgaaugcuUfGfAfugauacaauas(invAb)-(C6-S)-LP-371-a	541	GAAAUUGCUUGAUGAUACAUA	622

CS007971	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugcuUfgAfuGfauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	542	G(A ^{2N})AAUGCUUGAUACA(A ^{2N})UA	613
CS007972	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugcuUfgAfuGfauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	543	G(A ^{2N})AAUGCUUGAUACA(A ^{2N})UA	613
CS007973	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugCfuGfAfugauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	544	G(A ^{2N})AAUGCUUGAUACA(A ^{2N})UA	613
CS007974	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugCfuUfgAfugauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	545	G(A ^{2N})AAUGCUUGAUACA(A ^{2N})UA	613
CS009133	LP-379-a-L6-(NH-C6)s(invAb)sgucucugUfCfAfuaguuccaas(invAb)-(C6-S)-LP-371-a	546	GUCUUCUGUCAUAGUUCCAAA	598
CS010070	LP-379-a-L6-(NH-C6)s(invAb)sgucugucaUfAfGfuuccaacaas(invAb)-(C6-SS)-MeC5-dT	547	GUCUGUCAUAGUUCCAACAUT	630
CS011693	LP-379-a-L6-(NH-C6)sccugaaAfuGfcUfugauacaa-(C6-SS)-MeC5-dT	548	CCUGAAAUGCUUGAUACAT	643
CS012040	LP-379-a-L6-(NH-C6)sccugaaAfuGfcUfugauacaa-(C6-S)-LP-371-a	549	CCUGAAAUGCUUGAUACA	612
CS015443	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugcuUfGfAfugauacaa_2Nuas(invAb)-(C6-SS)-MeC5-dT	550	G(A ^{2N})AAUGCUUGAUACA(A ^{2N})UA _T	644

I = inosine (hypoxanthine nucleobase) nucleotide

(A^{2N}) = 2-aminoadenosine nucleotide

[0108] The ALK7 RNAi agents disclosed herein are formed by annealing an antisense strand with a sense strand. A sense strand containing a sequence listed in Table 2, Table 4, Table 5, Table 6, or Table 6a can be hybridized to any antisense strand containing a sequence listed in Table 2 or Table 3, provided the two sequences have a region of at least 85% complementarity over a contiguous 16, 17, 18, 19, 20, or 21 nucleotide sequence.

[0109] As shown in Table 5 above, certain of the example ALK7 RNAi agent nucleotide sequences are shown to further include reactive linking groups at one or both of the 5' terminal end and the 3' terminal end of the sense strand. For example, many of the ALK7 RNAi agent sense strand sequences shown in Table 5 above have a (NH₂-C6) linking group at the 5' end of the nucleotide sequence. Other linking groups, such as a (6-SS-6) linking group or a (C6-SS-C6) linking group, may be present as well or alternatively in certain embodiments. Such reactive linking groups are positioned to facilitate the linking of targeting ligands, targeting groups, and/or PK/PD modulators to the ALK7 RNAi agents disclosed herein. Linking or conjugation reactions are well known in the art and provide for formation of covalent linkages between two molecules or reactants. Suitable conjugation reactions for use in the scope of the inventions herein include, but are not limited to, amide coupling reaction, Michael addition reaction, hydrazone formation reaction, inverse-demand Diels-Alder cycloaddition reaction, oxime ligation, and Copper (I)- catalyzed or strain-promoted azide-alkyne cycloaddition reaction.

[0110] In some embodiments, targeting ligands, can be synthesized as activated esters, such as tetrafluorophenyl (TFP) esters, which can be displaced by a reactive amino group (e.g., NH₂-C₆) to attach the targeting ligand to the ALK7 RNAi agents disclosed herein. In some embodiments, targeting ligands are synthesized as azides, which can be conjugated to a propargyl or DBCO group, for example, via Copper (I)- catalyzed or strain-promoted azide-alkyne cycloaddition reaction.

[0111] Additionally, certain of the nucleotide sequences can be synthesized with a dT nucleotide at the 3' terminal end of the sense strand, followed by (3' → 5') a linker (e.g., C6-SS-C6). The linker can, in some embodiments, facilitate the linkage to additional components, such as, for example, a lipid or one or more targeting ligands. As described herein, the disulfide bond of C6-SS-C6 is first reduced, removing the dT from the molecule, which can then facilitate the conjugation of the desired component. The terminal dT nucleotide therefore is not a part of the fully conjugated construct.

[0112] In some embodiments, the antisense strand of an ALK7 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the antisense strand sequences in Table 3 or Table 10. In some embodiments, the sense strand of an ALK7 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the sense strand sequences in Table 4, Table 5, Table 6, Table 6a, or Table 10.

[0113] In some embodiments, an ALK7 RNAi agent antisense strand comprises a nucleotide sequence of any of the sequences in Table 2 or Table 3. In some embodiments, an ALK7 RNAi agent antisense strand comprises the sequence of nucleotides (from 5' end → 3' end) 1-17, 2-17, 1-18, 2-18, 1-19, 2-19, 1-20, 2-20, 1-21, 2-21, 1-22, 2-22, 1-23, 2-23, 1-24, or 2-24 of any of the sequences in Table 2, Table 3, or Table 10. In certain embodiments, an ALK7 RNAi agent antisense strand comprises or consists of a modified sequence of any one of the modified sequences in Table 3 or Table 10.

[0114] In some embodiments, an ALK7 RNAi agent sense strand comprises the nucleotide sequence of any of the sequences in Table 2 or Table 4. In some embodiments, an ALK7 RNAi agent sense strand comprises the sequence of nucleotides (from 5' end → 3' end) 1-17, 2-17, 3-17, 4-17, 1-18, 2-18, 3-18, 4-18, 1-19, 2-19, 3-19, 4-19, 1-20, 2-20, 3-20, 4-20, 1-21, 2-21, 3-21, 4-21, 1-22, 2-22, 3-22, 4-22, 1-23, 2-23, 3-23, 4-23, 1-24, 2-24, 3-24, or 4-24, of any of the sequences in Table 2, Table 4, Table 5, Table 6, Table 6a or Table 10. In certain embodiments, an ALK7 RNAi agent sense strand comprises or consists of a modified sequence of any one of the modified sequences in Table 3 or Table 10.

[0115] For the RNAi agents disclosed herein, the nucleotide at position 1 of the antisense strand (from 5' end → 3' end) can be perfectly complementary to an ALK7 gene, or can be non-complementary to an ALK7 gene. In some embodiments, the nucleotide at position 1 of the antisense strand (from 5' end → 3' end) is a U, A, or dT (or a modified version of U, A or dT). In some embodiments, the nucleotide at position 1 of the antisense strand (from 5' end → 3' end) forms an A:U or U:A base pair with the sense strand.

[0116] In some embodiments, an ALK7 RNAi agent antisense strand comprises the sequence of nucleotides (from 5' end → 3' end) 2-18 or 2-19 of any of the antisense strand sequences in Table 2, Table 3, or Table 10. In some embodiments, an ALK7 RNAi sense strand comprises the sequence of nucleotides (from 5' end → 3' end) 1-17 or 1-18 of any of the sense strand sequences in Table 2, Table 4, Table 5, Table 6, Table 6a or Table 10.

[0117] In some embodiments, an ALK7 RNAi agent includes (i) an antisense strand comprising the sequence of nucleotides (from 5' end → 3' end) 2-18 or 2-19 of any of the antisense strand

sequences in Table 2, Table 3, or Table 10, and (ii) a sense strand comprising the sequence of nucleotides (from 5' end → 3' end) 1-17 or 1-18 of any of the sense strand sequences in Table 2, Table 4, Table 5, Table 6, Table 6a or Table 10.

[0118] A sense strand containing a sequence listed in Table 2 or Table 4 can be hybridized to any antisense strand containing a sequence listed in Table 2 or Table 3 provided the two sequences have a region of at least 85% complementarity over a contiguous 16, 17, 18, 19, 20, or 21 nucleotide sequence. In some embodiments, the ALK7 RNAi agent has a sense strand consisting of the modified sequence of any of the modified sequences in Table 4, Table 5, Table 6, Table 6a, or Table 10, and an antisense strand consisting of the modified sequence of any of the modified sequences in Table 3 or Table 10. Certain representative sequence pairings are exemplified by the Duplex ID Nos. shown in Tables 7A, 7B, 8, and 9A.

[0119] In some embodiments, an ALK7 RNAi agent comprises, consists of, or consists essentially of a duplex represented by any one of the Duplex ID Nos. presented herein. In some embodiments, an ALK7 RNAi agent consists of any of the Duplex ID Nos. presented herein. In some embodiments, an ALK7 RNAi agent comprises the sense strand and antisense strand nucleotide sequences of any of the Duplex ID Nos. presented herein. In some embodiments, an ALK7 RNAi agent comprises the sense strand and antisense strand nucleotide sequences of any of the Duplex ID Nos. presented herein and a targeting group, linking group, PK/PD modulator and/or other non-nucleotide group wherein the targeting group, linking group, PK/PD modulator and/or other non-nucleotide group is covalently linked (i.e., conjugated) to the sense strand or the antisense strand. In some embodiments, an ALK7 RNAi agent includes the sense strand and antisense strand modified nucleotide sequences of any of the Duplex ID Nos. presented herein. In some embodiments, an ALK7 RNAi agent comprises the sense strand and antisense strand modified nucleotide sequences of any of the Duplex ID Nos. presented herein and a targeting group, linking group, and/or other non-nucleotide group, wherein the targeting group, linking group, PK/PD modulator and/or other non-nucleotide group is covalently linked to the sense strand or the antisense strand.

[0120] In some embodiments, an ALK7 RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand duplexes of Tables 2, 7A, 7B, 8, 9A, or 10, and comprises a PK/PD modulator. In some embodiments, an ALK7 RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand duplexes of Tables 2, 7A, 7B, 8, 9A, or 10, and comprises one or more lipid moieties.

[0121] In some embodiments, an ALK7 RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand duplexes of Tables 2, 7A, 7B, 8, 9A, or 10, and comprises a lipid moiety. In some embodiments, an ALK7 RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand duplexes of Tables 2, 7A, 7B, 8, 9A, or 10, and comprises one or more lipid moieties.

[0122] In some embodiments, an ALK7 RNAi agent comprises an antisense strand and a sense strand having the modified nucleotide sequences of any of the antisense strand/sense strand duplexes of Tables 7A, 7B, 8, 9A, and 10.

[0123] In some embodiments, an ALK7 RNAi agent comprises an antisense strand and a sense strand having the modified nucleotide sequences of any of the antisense strand/sense strand duplexes of Tables 7A, 7B, 8, 9A, and 10, and comprises a lipid moiety.

[0124] In some embodiments, an ALK7 RNAi agent comprises, consists of, or consists essentially of any of the duplexes of Tables 7A, 7B, 8, 9A, and 10.

[0125] Table 7A. ALK7 RNAi Agent Duplexes with Corresponding Sense and Antisense Strand ID Numbers and Sequence ID numbers for the modified and unmodified nucleotide sequences. (Shown without Linking Agents or Conjugates)

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC004151	CA005139	265	554	CS005138-NL	360	595
AC004152	CA005141	266	555	CS005140-NL	361	596
AC004153	CA005143	267	556	CS005142-NL	362	597
AC004154	CA005145	268	557	CS005144-NL	363	598
AC004155	CA005147	269	558	CS005146-NL	364	599
AC004156	CA005149	270	559	CS005148-NL	365	600
AC004157	CA005151	271	560	CS005150-NL	366	601
AC004158	CA005153	272	561	CS005152-NL	367	602
AC004159	CA005155	273	562	CS005154-NL	368	603
AC004160	CA005157	274	563	CS005156-NL	369	604
AC004161	CA005159	275	564	CS005158-NL	370	605
AC004162	CA005161	276	565	CS005160-NL	371	606
AC004163	CA005163	277	566	CS005162-NL	372	607
AC004164	CA005165	278	567	CS005164-NL	373	608
AC004165	CA005167	279	568	CS005166-NL	374	609

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC004166	CA005169	280	569	CS005168-NL	375	610
AC004167	CA005171	281	570	CS005170-NL	376	611
AC004168	CA005173	282	571	CS005172-NL	377	612
AC004169	CA005175	283	572	CS005174-NL	378	613
AC004170	CA005177	284	573	CS005176-NL	379	614
AC004171	CA005179	285	574	CS005178-NL	380	615
AC004172	CA005181	286	575	CS005180-NL	381	616
AC004173	CA005183	287	576	CS005182-NL	382	617
AC004174	CA005185	288	577	CS005184-NL	383	618
AC004175	CA005187	289	578	CS005186-NL	384	619
AC004176	CA005189	290	579	CS005188-NL	385	620
AC004197	CA005139	265	554	CS005209-NL	386	595
AC004198	CA005141	266	555	CS005210-NL	387	596
AC004199	CA005143	267	556	CS005211-NL	388	597
AC004200	CA005145	268	557	CS005212-NL	389	598
AC004201	CA005147	269	558	CS005213-NL	390	599
AC004202	CA005149	270	559	CS005214-NL	391	600
AC004203	CA005151	271	560	CS005215-NL	392	601
AC004204	CA005153	272	561	CS005216-NL	393	602
AC004205	CA005155	273	562	CS005217-NL	394	603
AC004206	CA005157	274	563	CS005218-NL	395	604
AC004207	CA005159	275	564	CS005219-NL	396	605
AC004208	CA005161	276	565	CS005220-NL	397	606
AC004209	CA005163	277	566	CS005221-NL	398	607
AC004210	CA005165	278	567	CS005222-NL	399	608
AC004211	CA005167	279	568	CS005223-NL	400	609
AC004212	CA005169	280	569	CS005224-NL	401	610
AC004213	CA005171	281	570	CS005225-NL	402	611
AC004214	CA005173	282	571	CS005226-NL	403	612
AC004215	CA005175	283	572	CS005227-NL	404	613
AC004216	CA005177	284	573	CS005228-NL	405	614
AC004217	CA005179	285	574	CS005229-NL	406	615
AC004218	CA005181	286	575	CS005230-NL	407	616
AC004219	CA005183	287	576	CS005231-NL	408	617
AC004220	CA005185	288	577	CS005232-NL	409	618
AC004221	CA005187	289	578	CS005233-NL	410	619
AC004222	CA005189	290	579	CS005234-NL	411	620
AC005846	CA007125	292	557	CS005212-NL	389	598

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC005847	CA005145	268	557	CS007126-NL	412	598
AC005848	CA005145	268	557	CS007127-NL	413	598
AC005849	CA005145	268	557	CS007128-NL	414	598
AC005850	CA005145	268	557	CS007129-NL	415	598
AC005851	CA007130	293	557	CS005212-NL	389	598
AC005852	CA007131	294	557	CS005212-NL	389	598
AC005853	CA007132	295	557	CS005212-NL	389	598
AC005854	CA007134	297	557	CS005212-NL	389	598
AC005855	CA007133	296	557	CS005212-NL	389	598
AC005856	CA007135	298	558	CS005213-NL	390	599
AC005857	CA007137	299	580	CS007136-NL	416	621
AC005858	CA005147	269	558	CS007138-NL	417	599
AC005859	CA005147	269	558	CS007139-NL	418	599
AC005860	CA005147	269	558	CS007140-NL	419	599
AC005861	CA005147	269	558	CS007141-NL	420	599
AC005862	CA007142	300	558	CS005213-NL	390	599
AC005863	CA007143	301	558	CS005213-NL	390	599
AC005864	CA007144	302	558	CS005213-NL	390	599
AC005865	CA007145	303	558	CS005213-NL	390	599
AC005867	CA007147	304	559	CS005214-NL	391	600
AC005868	CA005149	270	559	CS007148-NL	421	600
AC005869	CA005149	270	559	CS007149-NL	422	600
AC005870	CA005149	270	559	CS007150-NL	423	600
AC005871	CA005149	270	559	CS007151-NL	424	600
AC005872	CA007152	305	559	CS005214-NL	391	600
AC005873	CA007153	306	559	CS005214-NL	391	600
AC005874	CA007154	307	559	CS005214-NL	391	600
AC005875	CA007155	308	559	CS005214-NL	391	600
AC005876	CA007156	309	559	CS005214-NL	391	600
AC006111	CA007379	310	581	CS005213-NL	390	599
AC006290	CA007563	312	569	CS005224-NL	401	610
AC006291	CA005169	280	569	CS007564-NL	425	610
AC006292	CA005169	280	569	CS007565-NL	426	610
AC006293	CA005169	280	569	CS007566-NL	427	610
AC006294	CA005169	280	569	CS007567-NL	428	610
AC006295	CA007568	313	569	CS005224-NL	401	610
AC006296	CA007569	314	569	CS005224-NL	401	610
AC006297	CA007570	315	569	CS005224-NL	401	610

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC006298	CA007571	316	569	CS005224-NL	401	610
AC006299	CA007572	317	569	CS005224-NL	401	610
AC006300	CA007573	318	569	CS005224-NL	401	610
AC006305	CA005173	282	571	CS007578-NL	429	612
AC006425	CA005175	283	572	CS007711-NL	430	622
AC006427	CA005175	283	572	CS007712-NL	431	613
AC006428	CA005175	283	572	CS007713-NL	432	613
AC006429	CA005175	283	572	CS007714-NL	433	613
AC006430	CA005175	283	572	CS007715-NL	434	613
AC006431	CA007591	327	572	CS005174-NL	378	613
AC006432	CA007592	328	572	CS005174-NL	378	613
AC006433	CA007593	329	572	CS005174-NL	378	613
AC006434	CA007594	330	572	CS005174-NL	378	613
AC006435	CA007595	331	572	CS005174-NL	378	613
AC006436	CA007596	332	572	CS005174-NL	378	613
AC006437	CA007574	319	571	CS005172-NL	377	612
AC006438	CA005173	282	571	CS007716-NL	435	612
AC006439	CA005173	282	571	CS007717-NL	436	612
AC006440	CA005173	282	571	CS007718-NL	437	612
AC006441	CA005173	282	571	CS007719-NL	438	612
AC006442	CA007579	320	571	CS005172-NL	377	612
AC006443	CA007580	321	571	CS005172-NL	377	612
AC006444	CA007581	322	571	CS005172-NL	377	612
AC006445	CA007582	323	571	CS005172-NL	377	612
AC006446	CA007583	324	571	CS005172-NL	377	612
AC006447	CA007584	325	571	CS005172-NL	377	612
AC006734	CA008042	333	571	CS007716-NL	435	612
AC006735	CA008047	334	571	CS007716-NL	435	612
AC006738	CA008050	335	581	CS005213-NL	390	599
AC006741	CA008052	336	559	CS005214-NL	391	600
AC006742	CA008052	336	559	CS007148-NL	421	600
AC006743	CA008053	337	559	CS007148-NL	421	600
AC006744	CA008054	338	569	CS005224-NL	401	610
AC006745	CA008054	338	569	CS007564-NL	425	610
AC006746	CA008055	339	569	CS007564-NL	425	610
AC006747	CA008050	335	581	CS007138-NL	417	599
AC006773	CA008087	340	581	CS007138-NL	417	599
AC007192	CA008542	341	582	CS007126-NL	412	598

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC007193	CA008544	342	583	CS008543-NL	439	623
AC007194	CA008546	343	584	CS008545-NL	440	624
AC007195	CA008547	344	585	CS008543-NL	439	623
AC007196	CA008548	345	586	CS008545-NL	440	624
AC007197	CA008550	346	587	CS008549-NL	441	625
AC007198	CA008551	347	588	CS008549-NL	441	625
AC007199	CA008553	348	580	CS008552-NL	442	621
AC007200	CA008554	349	581	CS007138-NL	417	599
AC007201	CA008555	350	581	CS007138-NL	417	599
AC007202	CA007137	299	580	CS008552-NL	442	621
AC007203	CA007380	311	581	CS007138-NL	417	599
AC007204	CA008556	351	581	CS007138-NL	417	599
AC008318	CA009908	352	557	CS007126-NL	412	598
AC008319	CA009909	353	589	CS007126-NL	412	598
AC008320	CA009910	354	589	CS007126-NL	412	598
AC008321	CA009911	355	590	CS008543-NL	439	623
AC008322	CA009912	356	591	CS007126-NL	412	598
AC008323	CA009913	357	592	CS008543-NL	439	623
AC008324	CA009914	358	593	CS007126-NL	412	598
AC008325	CA009915	359	594	CS007126-NL	412	598
AC008484	CA007380	311	581	CS005146-NL	364	599
AC009978	CA005173	282	571	CS011689-NL	443	612
AC009980	CA005173	282	571	CS011691-NL	444	612

[0126] Table 7B. ALK7 RNAi Agent Duplexes with Corresponding Sense and Antisense Strand ID Numbers and Sequence ID numbers for the modified and unmodified nucleotide sequences.

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC004151	CA005139	265	554	CS005138	445	626
AC004152	CA005141	266	555	CS005140	446	627
AC004153	CA005143	267	556	CS005142	447	628
AC004154	CA005145	268	557	CS005144	448	629
AC004155	CA005147	269	558	CS005146	449	630
AC004156	CA005149	270	559	CS005148	450	631
AC004157	CA005151	271	560	CS005150	451	632

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC004158	CA005153	272	561	CS005152	452	633
AC004159	CA005155	273	562	CS005154	453	634
AC004160	CA005157	274	563	CS005156	454	635
AC004161	CA005159	275	564	CS005158	455	636
AC004162	CA005161	276	565	CS005160	456	637
AC004163	CA005163	277	566	CS005162	457	638
AC004164	CA005165	278	567	CS005164	458	639
AC004165	CA005167	279	568	CS005166	459	640
AC004166	CA005169	280	569	CS005168	460	641
AC004167	CA005171	281	570	CS005170	461	642
AC004168	CA005173	282	571	CS005172	462	643
AC004169	CA005175	283	572	CS005174	463	644
AC004170	CA005177	284	573	CS005176	464	645
AC004171	CA005179	285	574	CS005178	465	646
AC004172	CA005181	286	575	CS005180	466	647
AC004173	CA005183	287	576	CS005182	467	648
AC004174	CA005185	288	577	CS005184	468	649
AC004175	CA005187	289	578	CS005186	469	650
AC004176	CA005189	290	579	CS005188	470	651
AC004197	CA005139	265	554	CS005209	471	595
AC004198	CA005141	266	555	CS005210	472	596
AC004199	CA005143	267	556	CS005211	473	597
AC004200	CA005145	268	557	CS005212	474	598
AC004201	CA005147	269	558	CS005213	475	599
AC004202	CA005149	270	559	CS005214	476	600
AC004203	CA005151	271	560	CS005215	477	601
AC004204	CA005153	272	561	CS005216	478	602
AC004205	CA005155	273	562	CS005217	479	603
AC004206	CA005157	274	563	CS005218	480	604
AC004207	CA005159	275	564	CS005219	481	605
AC004208	CA005161	276	565	CS005220	482	606
AC004209	CA005163	277	566	CS005221	483	607
AC004210	CA005165	278	567	CS005222	484	608
AC004211	CA005167	279	568	CS005223	485	609
AC004212	CA005169	280	569	CS005224	486	610
AC004213	CA005171	281	570	CS005225	487	611
AC004214	CA005173	282	571	CS005226	488	612
AC004215	CA005175	283	572	CS005227	489	613

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC004216	CA005177	284	573	CS005228	490	614
AC004217	CA005179	285	574	CS005229	491	615
AC004218	CA005181	286	575	CS005230	492	616
AC004219	CA005183	287	576	CS005231	493	617
AC004220	CA005185	288	577	CS005232	494	618
AC004221	CA005187	289	578	CS005233	495	619
AC004222	CA005189	290	579	CS005234	496	620
AC005846	CA007125	292	557	CS005212	474	598
AC005847	CA005145	268	557	CS007126	497	598
AC005848	CA005145	268	557	CS007127	498	598
AC005849	CA005145	268	557	CS007128	499	598
AC005850	CA005145	268	557	CS007129	500	598
AC005851	CA007130	293	557	CS005212	474	598
AC005852	CA007131	294	557	CS005212	474	598
AC005853	CA007132	295	557	CS005212	474	598
AC005854	CA007134	297	557	CS005212	474	598
AC005855	CA007133	296	557	CS005212	474	598
AC005856	CA007135	298	558	CS005213	475	599
AC005857	CA007137	299	580	CS007136	501	621
AC005858	CA005147	269	558	CS007138	502	599
AC005859	CA005147	269	558	CS007139	503	599
AC005860	CA005147	269	558	CS007140	504	599
AC005861	CA005147	269	558	CS007141	505	599
AC005862	CA007142	300	558	CS005213	475	599
AC005863	CA007143	301	558	CS005213	475	599
AC005864	CA007144	302	558	CS005213	475	599
AC005865	CA007145	303	558	CS005213	475	599
AC005867	CA007147	304	559	CS005214	476	600
AC005868	CA005149	270	559	CS007148	506	600
AC005869	CA005149	270	559	CS007149	507	600
AC005870	CA005149	270	559	CS007150	508	600
AC005871	CA005149	270	559	CS007151	509	600
AC005872	CA007152	305	559	CS005214	476	600
AC005873	CA007153	306	559	CS005214	476	600
AC005874	CA007154	307	559	CS005214	476	600
AC005875	CA007155	308	559	CS005214	476	600
AC005876	CA007156	309	559	CS005214	476	600
AC006111	CA007379	310	581	CS005213	475	599

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC006290	CA007563	312	569	CS005224	486	610
AC006291	CA005169	280	569	CS007564	510	610
AC006292	CA005169	280	569	CS007565	511	610
AC006293	CA005169	280	569	CS007566	512	610
AC006294	CA005169	280	569	CS007567	513	610
AC006295	CA007568	313	569	CS005224	486	610
AC006296	CA007569	314	569	CS005224	486	610
AC006297	CA007570	315	569	CS005224	486	610
AC006298	CA007571	316	569	CS005224	486	610
AC006299	CA007572	317	569	CS005224	486	610
AC006300	CA007573	318	569	CS005224	486	610
AC006305	CA005173	282	571	CS007578	514	612
AC006425	CA005175	283	572	CS007711	515	652
AC006427	CA005175	283	572	CS007712	516	644
AC006428	CA005175	283	572	CS007713	517	644
AC006429	CA005175	283	572	CS007714	518	644
AC006430	CA005175	283	572	CS007715	519	644
AC006431	CA007591	327	572	CS005174	463	644
AC006432	CA007592	328	572	CS005174	463	644
AC006433	CA007593	329	572	CS005174	463	644
AC006434	CA007594	330	572	CS005174	463	644
AC006435	CA007595	331	572	CS005174	463	644
AC006436	CA007596	332	572	CS005174	463	644
AC006437	CA007574	319	571	CS005172	462	643
AC006438	CA005173	282	571	CS007716	520	643
AC006439	CA005173	282	571	CS007717	521	643
AC006440	CA005173	282	571	CS007718	522	643
AC006441	CA005173	282	571	CS007719	523	643
AC006442	CA007579	320	571	CS005172	462	643
AC006443	CA007580	321	571	CS005172	462	643
AC006444	CA007581	322	571	CS005172	462	643
AC006445	CA007582	323	571	CS005172	462	643
AC006446	CA007583	324	571	CS005172	462	643
AC006447	CA007584	325	571	CS005172	462	643
AC006734	CA008042	333	571	CS007716	520	643
AC006735	CA008047	334	571	CS007716	520	643
AC006738	CA008050	335	581	CS005213	475	599
AC006741	CA008052	336	559	CS005214	476	600

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC006742	CA008052	336	559	CS007148	506	600
AC006743	CA008053	337	559	CS007148	506	600
AC006744	CA008054	338	569	CS005224	486	610
AC006745	CA008054	338	569	CS007564	510	610
AC006746	CA008055	339	569	CS007564	510	610
AC006747	CA008050	335	581	CS007138	502	599
AC006773	CA008087	340	581	CS007138	502	599
AC007192	CA008542	341	582	CS007126	497	598
AC007193	CA008544	342	583	CS008543	524	623
AC007194	CA008546	343	584	CS008545	525	624
AC007195	CA008547	344	585	CS008543	524	623
AC007196	CA008548	345	586	CS008545	525	624
AC007197	CA008550	346	587	CS008549	526	625
AC007198	CA008551	347	588	CS008549	526	625
AC007199	CA008553	348	580	CS008552	527	621
AC007200	CA008554	349	581	CS007138	502	599
AC007201	CA008555	350	581	CS007138	502	599
AC007202	CA007137	299	580	CS008552	527	621
AC007203	CA007380	311	581	CS007138	502	599
AC007204	CA008556	351	581	CS007138	502	599
AC008318	CA009908	352	557	CS007126	497	598
AC008319	CA009909	353	589	CS007126	497	598
AC008320	CA009910	354	589	CS007126	497	598
AC008321	CA009911	355	590	CS008543	524	623
AC008322	CA009912	356	591	CS007126	497	598
AC008323	CA009913	357	592	CS008543	524	623
AC008324	CA009914	358	593	CS007126	497	598
AC008325	CA009915	359	594	CS007126	497	598
AC008484	CA007380	311	581	CS005146	449	630
AC009978	CA005173	282	571	CS011689	528	643
AC009980	CA005173	282	571	CS011691	529	643

[0127] Table 8. ALK7 RNAi Agent Duplexes with Corresponding Sense and Antisense Strand ID Numbers and Sequence ID numbers for the modified and unmodified nucleotide sequences. (Shown with PK/PD modulators)

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC004390	CA005127	262	551	CS005429	530	653
AC004391	CA005129	263	552	CS005430	531	654
AC004392	CA005131	264	553	CS005431	532	655
AC005181	CA006102	291	551	CS005429	530	653
AC005823	CA005175	283	572	CS007081	533	613
AC005824	CA005173	282	571	CS007082	534	612
AC006188	CA005147	269	558	CS007437	535	599
AC006189	CA005149	270	559	CS007438	536	600
AC006643	CA007574	319	571	CS007082	534	612
AC006644	CA005173	282	571	CS007966	537	612
AC006645	CA005173	282	571	CS007967	538	612
AC006646	CA005173	282	571	CS007968	539	612
AC006647	CA005173	282	571	CS007969	540	612
AC006648	CA007579	320	571	CS007082	534	612
AC006649	CA007580	321	571	CS007082	534	612
AC006650	CA007581	322	571	CS007082	534	612
AC006651	CA007582	323	571	CS007082	534	612
AC006652	CA007583	324	571	CS007082	534	612
AC006653	CA007584	325	571	CS007082	534	612
AC006654	CA005175	283	572	CS007970	541	622
AC006655	CA007586	326	572	CS007081	533	613
AC006656	CA005175	283	572	CS007971	542	613
AC006657	CA005175	283	572	CS007972	543	613
AC006658	CA005175	283	572	CS007973	544	613
AC006659	CA005175	283	572	CS007974	545	613
AC006660	CA007591	327	572	CS007081	533	613
AC006661	CA007592	328	572	CS007081	533	613
AC006662	CA007593	329	572	CS007081	533	613
AC006663	CA007594	330	572	CS007081	533	613
AC006664	CA007595	331	572	CS007081	533	613
AC006665	CA007596	332	572	CS007081	533	613
AC007027	CA008042	333	571	CS007082	534	612
AC007028	CA008047	334	571	CS007082	534	612
AC007029	CA008042	333	571	CS007966	537	612
AC007030	CA008047	334	571	CS007966	537	612
AC007694	CA007125	292	557	CS009133	546	598
AC008492	CA007380	311	581	CS010070	547	630
AC008546	CA005147	269	558	CS010070	547	630

Duplex	AS ID	AS modified SEQ ID NO:	AS unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC009982	CA005173	282	571	CS011693	548	643
AC010357	CA005173	282	571	CS012040	549	612
AC013158	CA007586	326	572	CS015443	550	644

[0128] Table 9A. Conjugate Duplex ID Numbers Referencing Position Targeted On ALK7 Gene

Duplex	AS ID	SS ID	Targeted ALK7 Gene Position (Of SEQ ID NO:1)
AC004390	CA005127	CS005429	N/A
AC004391	CA005129	CS005430	N/A
AC004392	CA005131	CS005431	N/A
AC005181	CA006102	CS005429	N/A
AC005823	CA005175	CS007081	1334
AC005824	CA005173	CS007082	1331
AC006188	CA005147	CS007437	440
AC006189	CA005149	CS007438	1021
AC006643	CA007574	CS007082	1331
AC006644	CA005173	CS007966	1331
AC006645	CA005173	CS007967	1331
AC006646	CA005173	CS007968	1331
AC006647	CA005173	CS007969	1331
AC006648	CA007579	CS007082	1331
AC006649	CA007580	CS007082	1331
AC006650	CA007581	CS007082	1331
AC006651	CA007582	CS007082	1331
AC006652	CA007583	CS007082	1331
AC006653	CA007584	CS007082	1331
AC006654	CA005175	CS007970	1334
AC006655	CA007586	CS007081	1334
AC006656	CA005175	CS007971	1334
AC006657	CA005175	CS007972	1334
AC006658	CA005175	CS007973	1334
AC006659	CA005175	CS007974	1334
AC006660	CA007591	CS007081	1334
AC006661	CA007592	CS007081	1334

Duplex	AS ID	SS ID	Targeted ALK7 Gene Position (Of SEQ ID NO:1)
AC006662	CA007593	CS007081	1334
AC006663	CA007594	CS007081	1334
AC006664	CA007595	CS007081	1334
AC006665	CA007596	CS007081	1334
AC007027	CA008042	CS007082	1331
AC007028	CA008047	CS007082	1331
AC007029	CA008042	CS007966	1331
AC007030	CA008047	CS007966	1331
AC007694	CA007125	CS009133	437
AC008492	CA007380	CS010070	440
AC008546	CA005147	CS010070	440
AC009982	CA005173	CS011693	1331
AC010357	CA005173	CS012040	1331
AC013158	CA007586	CS015443	1334

[0129] ALK7 RNAi agent conjugate duplex IDs targeted to mouse ALK7 are denoted with “N/A” for their respective targeted ALK7 gene positions.

[0130] Table 10. Conjugate ID Numbers With Chemically Modified Antisense and Sense Strands (including Linkers and Conjugates)

AC ID Number	Sense Strand (Fully Modified with Conjugated PK/PD modulator) (5' → 3')	SEQ ID NO:	Antisense Strand (5' → 3')	SEQ ID NO:
AC004390	LP-379-a-L6-(NH-C6)s(invAb)sgugaagcaCfGfAfuucuaucuaas(invAb)-(C6-S)-LP-371-a	530	cPrpusAfsugauAfgaauCfgUfgCfuucasc	262
AC004391	LP-379-a-L6-(NH-C6)s(invAb)sgagacauaCfCfCfcagaauccuas(invAb)-(C6-S)-LP-371-a	531	cPrpusAfsaggauUfcuggGfgUfaUfgucusc	263
AC004392	LP-379-a-L6-(NH-C6)s(invAb)sagcgagcuGfAfCfaucauuucgas(invAb)-(C6-S)-LP-371-a	532	cPrpusCfsgaauAfgaugUfcAfgCfucgsu	264
AC005181	LP-379-a-L6-(NH-C6)s(invAb)sgugaagcaCfGfAfuucuaucuaas(invAb)-(C6-S)-LP-371-a	530	cPrpusAfsugauagaauCfgUfgCfuucasc	291
AC005823	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugcuUfGfAfugauaaca_2Nuas(invAb)-(C6-S)-LP-371-a	533	cPrpusAfsuuguAfucauCfaAfgCfauuusc	283
AC005824	LP-379-a-L6-(NH-C6)s(invAb)sccugaaauGfCfUfugaugauacas(invAb)-(C6-S)-LP-371-a	534	cPrpusGfsuaucAfucaaGfcAfuUfucagsg	282

AC ID Number	Sense Strand (Fully Modified with Conjugated PK/PD modulator) (5' → 3')	SEQ ID NO:	Antisense Strand (5' → 3')	SEQ ID NO:
AC006188	LP-379-a-L6-(NH-C6)s(invAb)sgucugucaUfAfGfuccaacaas(invAb)-(C6-S)-LP-371-a	535	cPrpasUfsuguuGfgaacUfaUfgAfcagasc	269
AC006189	LP-379-a-L6-(NH-C6)s(invAb)sgcugguauCfUfGfaauaucaugas(invAb)-(C6-S)-LP-371-a	536	cPrpusCfsaugaUfauucAfgAfuAfcagasc	270
AC006643	LP-379-a-L6-(NH-C6)s(invAb)sccugaaauGfCfUfugaugauacas(invAb)-(C6-S)-LP-371-a	534	cPrpusGfsuaucAfucaaGfcAfuUfucagsg	319
AC006644	LP-379-a-L6-(NH-C6)s(invAb)sccugaaauGfcUfuGfugaugauacas(invAb)-(C6-S)-LP-371-a	537	cPrpusGfsuaucAfucaaGfcAfuUfucagsg	282
AC006645	LP-379-a-L6-(NH-C6)s(invAb)sccugaaauGfCfuUfugaugauacas(invAb)-(C6-S)-LP-371-a	538	cPrpusGfsuaucAfucaaGfcAfuUfucagsg	282
AC006646	LP-379-a-L6-(NH-C6)s(invAb)sccugaaAfugCfUfugaugauacas(invAb)-(C6-S)-LP-371-a	539	cPrpusGfsuaucAfucaaGfcAfuUfucagsg	282

AC ID Number	Sense Strand (Fully Modified with Conjugated PK/PD modulator) (5' → 3')	SEQ ID NO:	Antisense Strand (5' → 3')	SEQ ID NO:
AC006647	LP-379-a-L6-(NH- C6)s(invAb)sccugaaAfuGfcUfugaugauacac(invAb)-(C6-S)- LP-371-a	540	cPrpusGfsuauAfucAAGfcAfuUfucagsg	282
AC006648	LP-379-a-L6-(NH- C6)s(invAb)sccugaaauGfCfUfugaugauacac(invAb)-(C6-S)- LP-371-a	534	cPrpusGfsuaucaucaaGfcAfuuuucagsg	320
AC006649	LP-379-a-L6-(NH- C6)s(invAb)sccugaaauGfCfUfugaugauacac(invAb)-(C6-S)- LP-371-a	534	cPrpusGfsUfaucacaaGfcAfuUfucagsg	321
AC006650	LP-379-a-L6-(NH- C6)s(invAb)sccugaaauGfCfUfugaugauacac(invAb)-(C6-S)- LP-371-a	534	cPrpusGfsuAfucacaaGfcAfuUfucagsg	322
AC006651	LP-379-a-L6-(NH- C6)s(invAb)sccugaaauGfCfUfugaugauacac(invAb)-(C6-S)- LP-371-a	534	cPrpusGfsuauCfaucaaGfcAfuUfucagsg	323
AC006652	LP-379-a-L6-(NH- C6)s(invAb)sccugaaauGfCfUfugaugauacac(invAb)-(C6-S)- LP-371-a	534	cPrpusGfsuauCfaUfcAfaGfcAfuUfucagsg	324

AC ID Number	Sense Strand (Fully Modified with Conjugated PK/PD modulator) (5' → 3')	SEQ ID NO:	Antisense Strand (5' → 3')	SEQ ID NO:
AC006653	LP-379-a-L6-(NH- C6)s(invAb)sccugaaauGfCfUfugaugauacac(invAb)-(C6-S)- LP-371-a	534	cPrpusGfsuAfuCfaUfcAfaGfcAfuUfucagsg	325
AC006654	LP-379-a-L6-(NH- C6)s(invAb)sgaaugcuUfGfAfugauacaaus(invAb)-(C6-S)- LP-371-a	541	cPrpusAfsuuguAfucanCfaAfgCfauuusc	283
AC006655	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugcuUfGfAfugauacaa_2Nuas(invAb)- (C6-S)-LP-371-a	533	cPrpusAfsuuguAfucanCfaAfgCfauuusc	326
AC006656	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugcuUfgAfuGfauacaa_2Nuas(invAb)- (C6-S)-LP-371-a	542	cPrpusAfsuuguAfucanCfaAfgCfauuusc	283
AC006657	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugcuUfGfAfugauacaa_2Nuas(invAb)- (C6-S)-LP-371-a	543	cPrpusAfsuuguAfucanCfaAfgCfauuusc	283
AC006658	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugCfuUfGfAfugauacaa_2Nuas(invAb)- (C6-S)-LP-371-a	544	cPrpusAfsuuguAfucanCfaAfgCfauuusc	283

AC ID Number	Sense Strand (Fully Modified with Conjugated PK/PD modulator) (5' → 3')	SEQ ID NO:	Antisense Strand (5' → 3')	SEQ ID NO:
AC006659	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugCfuUfgAfugauacaa_2Nuas(invAb)- (C6-S)-LP-371-a	545	cPrpusAfsuuguAfucauCfaAfgCfauuusc	283
AC006660	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugcuUfgAfugauacaa_2Nuas(invAb)- (C6-S)-LP-371-a	533	cPrpusAfsuuguaucauCfaAfgcauuusc	327
AC006661	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugcuUfgAfugauacaa_2Nuas(invAb)- (C6-S)-LP-371-a	533	cPrpusAfsUfugaucauCfaAfgCfauuusc	328
AC006662	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugcuUfgAfugauacaa_2Nuas(invAb)- (C6-S)-LP-371-a	533	cPrpusAfsuUfugaucauCfaAfgCfauuusc	329
AC006663	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugcuUfgAfugauacaa_2Nuas(invAb)- (C6-S)-LP-371-a	533	cPrpusAfsuugUfaucauCfaAfgCfauuusc	330
AC006664	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugcuUfgAfugauacaa_2Nuas(invAb)- (C6-S)-LP-371-a	533	cPrpusAfsuugUfaUfcAfuCfaAfgCfauuusc	331

AC ID Number	Sense Strand (Fully Modified with Conjugated PK/PD modulator) (5' → 3')	SEQ ID NO:	Antisense Strand (5' → 3')	SEQ ID NO:
AC006665	LP-379-a-L6-(NH-C6)s(invAb)sga_2NaaugcuUfGfAfugauacaa_2Nuas(invAb)-(C6-S)-LP-371-a	533	cPrpusAfsuUfgUfaUfcAfuCfaAfgCfauuusc	332
AC007027	LP-379-a-L6-(NH-C6)s(invAb)sccugaaaUfGfUfugauacaa(invAb)-(C6-S)-LP-371-a	534	cPrpusGfsuaUfcaucaaaGfcAfuUfucagsg	333
AC007028	LP-379-a-L6-(NH-C6)s(invAb)sccugaaaUfGfUfugauacaa(invAb)-(C6-S)-LP-371-a	534	cPrpusGfsuaUfcaucaaaGfcAfuUfucagsg	334
AC007029	LP-379-a-L6-(NH-C6)s(invAb)sccugaaaUfGfUfugauacaa(invAb)-(C6-S)-LP-371-a	537	cPrpusGfsuaUfcaucaaaGfcAfuUfucagsg	333
AC007030	LP-379-a-L6-(NH-C6)s(invAb)sccugaaaUfGfUfugauacaa(invAb)-(C6-S)-LP-371-a	537	cPrpusGfsuaUfcaucaaaGfcAfuUfucagsg	334
AC007694	LP-379-a-L6-(NH-C6)s(invAb)sgucuuucugUfCfAfuaugucaaaas(invAb)-(C6-S)-LP-371-a	546	cPrpusUfsuggaAfcuaUgfaCfaGfaagsasc	292

AC ID Number	Sense Strand (Fully Modified with Conjugated PK/PD modulator) (5' → 3')	SEQ ID NO:	Antisense Strand (5' → 3')	SEQ ID NO:
AC008492	LP-379-a-L6-(NH- C6)s(invAb)sgucugucaUfAfGfuuccaacaas(invAb)-(C6- SS)-MeC5-dT	547	cPrpisUfsuguuGfgaacUfaUfgAfcagasc	311
AC008546	LP-379-a-L6-(NH- C6)s(invAb)sgucugucaUfAfGfuuccaacaas(invAb)-(C6- SS)-MeC5-dT	547	cPrpasUfsuguuGfgaacUfaUfgAfcagasc	269
AC009982	LP-379-a-L6-(NH-C6)sccugaaAfuGfcUfugauguacac-(C6- SS)-MeC5-dT	548	cPrpusGfsuaucAfucaaGfcAfuUfucagsg	282
AC010357	LP-379-a-L6-(NH-C6)sccugaaAfuGfcUfugauguacac-(C6- S)-LP-371-a	549	cPrpusGfsuaucAfucaaGfcAfuUfucagsg	282
AC013158	LP-379-a-L6-(NH- C6)s(invAb)sga_2NaaugcuUfGfAfugauguacaa_2Nuas(invAb)- (C6-SS)-MeC5-dT	550	cPrpusAfsuuguAfucauCfaAfgCfaususc	326

[0131] In some embodiments, an ALK7 RNAi agent is prepared or provided as a salt, mixed salt, or a free-acid. In some embodiments, an ALK7 RNAi agent is prepared or provided as a pharmaceutically acceptable salt. In some embodiments, an ALK7 RNAi agent is prepared or provided as a pharmaceutically acceptable sodium or potassium salt. The RNAi agents described herein, upon delivery to a cell expressing an ALK7 gene, inhibit or knockdown expression of one or more ALK7 genes *in vivo* and/or *in vitro*.

Targeting Groups, Linking Groups, Lipid PK/PD moieties, and Delivery Vehicles

[0132] In some embodiments, an ALK7 RNAi agent contains or is conjugated to one or more non-nucleotide groups including, but not limited to, a targeting group, a linking group, a pharmacokinetic/pharmacodynamic (PK/PD) modulator, a delivery polymer, or a delivery vehicle. The non-nucleotide group can enhance targeting, delivery, or attachment of the RNAi agent. The non-nucleotide group can be covalently linked to the 3' and/or 5' end of either the sense strand and/or the antisense strand. In some embodiments, an ALK7 RNAi agent contains a non-nucleotide group linked to the 3' and/or 5' end of the sense strand. In some embodiments, a non-nucleotide group is linked to the 5' end of an ALK7 RNAi agent sense strand. A non-nucleotide group can be linked directly or indirectly to the RNAi agent via a linker/linking group. In some embodiments, a non-nucleotide group is linked to the RNAi agent via a labile, cleavable, or reversible bond or linker.

[0133] In some embodiments, a non-nucleotide group enhances the pharmacokinetic or biodistribution properties of an RNAi agent or conjugate to which it is attached to improve cell- or tissue-specific distribution and cell-specific uptake of the conjugate. In some embodiments, a non-nucleotide group enhances endocytosis of the RNAi agent.

[0134] Targeting groups or targeting moieties enhance the pharmacokinetic or biodistribution properties of a conjugate or RNAi agent to which they are attached to improve cell-specific (including, in some cases, organ specific) distribution and cell-specific (or organ specific) uptake of the conjugate or RNAi agent. A targeting group can be monovalent, divalent, trivalent, tetravalent, or have higher valency for the target to which it is directed. Representative targeting groups include, without limitation, compounds with affinity to cell surface molecule, cell receptor ligands, hapten, antibodies, monoclonal antibodies, antibody fragments, and antibody mimics with affinity to cell surface molecules. In some embodiments, a targeting group is linked to an RNAi agent using a linker, such as a PEG linker or one, two,

or three abasic and/or ribitol (abasic ribose) residues, which in some instances can serve as linkers.

[0135] A targeting group, with or without a linker, can be attached to the 5' or 3' end of any of the sense and/or antisense strands disclosed in Tables 2, 3, 4, 5, 6, and 10. A linker, with or without a targeting group, can be attached to the 5' or 3' end of any of the sense and/or antisense strands disclosed in Tables 2, 3, 4, 5, 6, and 10.

[0136] The ALK7 RNAi agents described herein can be synthesized having a reactive group, such as an amino group (also referred to herein as an amine), at the 5'-terminus and/or the 3'-terminus. The reactive group can be used subsequently to attach a targeting moiety using methods typical in the art.

[0137] For example, in some embodiments, the ALK7 RNAi agents disclosed herein are synthesized having an NH₂-C₆ group at the 5'-terminus of the sense strand of the RNAi agent. The terminal amino group subsequently can be reacted to form a conjugate with, for example, a group that includes a lipid moiety. In some embodiments, the ALK7 RNAi agents disclosed herein are synthesized having one or more alkyne groups at the 5'-terminus of the sense strand of the RNAi agent.

[0138] In some embodiments, targeting groups are linked to the ALK7 RNAi agents without the use of an additional linker. In some embodiments, the targeting group is designed having a linker readily present to facilitate the linkage to an ALK7 RNAi agent. In some embodiments, when two or more RNAi agents are included in a composition, the two or more RNAi agents can be linked to their respective targeting groups using the same linkers. In some embodiments, when two or more RNAi agents are included in a composition, the two or more RNAi agents are linked to their respective targeting groups using different linkers.

[0139] In some embodiments, a linking group is conjugated to the RNAi agent. The linking group facilitates covalent linkage of the agent to a targeting group, pharmacokinetic modulator, delivery polymer, or delivery vehicle. The linking group can be linked to the 3' and/or the 5' end of the RNAi agent sense strand or antisense strand. In some embodiments, the linking group is linked to the RNAi agent sense strand. In some embodiments, the linking group is conjugated to the 5' or 3' end of an RNAi agent sense strand. In some embodiments, a linking group is conjugated to the 5' end of an RNAi agent sense strand. Examples of linking groups, include but are not limited to: C₆-SS-C₆, 6-SS-6, reactive groups such as primary amines (e.g., NH₂-C₆) and alkynes, alkyl groups, abasic residues/nucleotides, amino acids, tri-alkyne

functionalized groups, ribitol, and/or PEG groups. Examples of certain linking groups are provided in Table 11.

[0140] A linker or linking group is a connection between two atoms that links one chemical group (such as an RNAi agent) or segment of interest to another chemical group (such as a targeting group, pharmacokinetic modulator, or delivery polymer) or segment of interest via one or more covalent bonds. A labile linkage contains a labile bond. A linkage can optionally include a spacer that increases the distance between the two joined atoms. A spacer may further add flexibility and/or length to the linkage. Spacers include, but are not limited to, alkyl groups, alkenyl groups, alkynyl groups, aryl groups, aralkyl groups, aralkenyl groups, and aralkynyl groups; each of which can contain one or more heteroatoms, heterocycles, amino acids, nucleotides, and saccharides. Spacer groups are well known in the art and the preceding list is not meant to limit the scope of the description. In some embodiments, an ALK7 RNAi agent is conjugated to a polyethylene glycol (PEG) moiety, or to a hydrophobic group having 12 or more carbon atoms, such as a cholesterol or palmitoyl group.

[0141] In some embodiments, an ALK7 RNAi agent is linked to one or more lipid PK/PD moieties (referred to herein as “lipid moieties” or “PK/PD modulators”.) Lipid PK/PD moieties may enhance the pharmacodynamic or pharmacokinetic properties of the RNAi agent. In some embodiments, the lipid moiety may be conjugated to a linker at the 3' or 5' end of a sense strand or an antisense strand of an RNAi agent described herein. In some embodiments, a lipid moiety may be linked at both the 3' or 5' end of either the sense strand or the antisense strand of an RNAi agent described herein.

[0142] In some embodiments, a lipid moiety may be conjugated to an ALK7 RNAi agent by reacting an ALK7 RNAi agent comprising an amine-comprising linker, for example, (NH₂-C₆) (see table 11). In some embodiments, the amine-comprising linker may be located on the 5' end of the sense strand or the antisense strand of an ALK7 RNAi agent. In some embodiments, the amine-comprising linker may be located on the 3' end of the sense strand or the antisense strand of an RNAi agent.

[0143] In some embodiments, an RNAi agent comprising an amine-comprising linker, such as (NH₂-C₆) or (NH₂-C₆)s, may be reacted with a lipid comprising an activated ester moiety.

[0144] In some embodiments, an RNAi agent comprising a disulfide linker, such as C₆-SS-C₆, may be reacted with a lipid comprising an aromatic sulfone, such as LP-371-p.

[0145] In some embodiments, an RNAi agent comprising an alkyne linker, such as L6-p, may be reacted with a lipid comprising an azide, such as LP-379-p. The lipid may be reacted with L6 to form a triazole before or after an amidation reaction with an amine such as NH₂-C6.

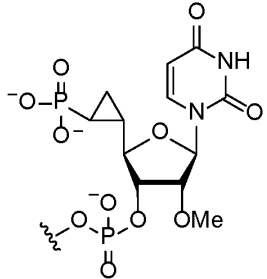
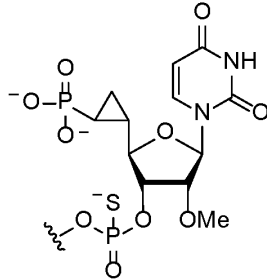
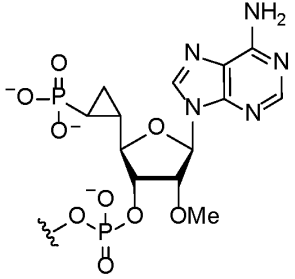
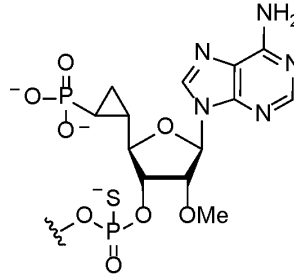
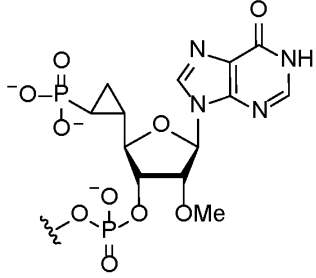
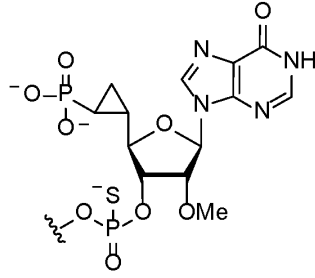
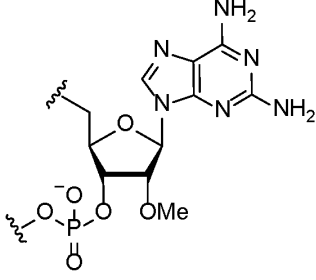
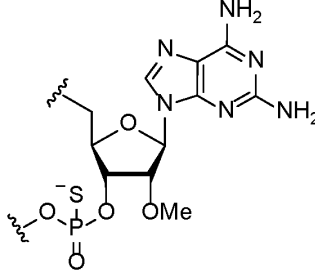
[0146] In some embodiments, an ALK7 RNAi agent may be conjugated to a lipid moiety using phosphoramidite synthesis. Synthesizing oligonucleotides using phosphoramidites is well-known in the art. In some embodiments, a lipid moiety may be conjugated to the 5' end of the sense strand or the antisense strand of an ALK7 RNAi agent using a phosphoramidite. In some embodiments, a lipid moiety may be conjugated to the 3' end of the sense strand or the antisense strand of an ALK7 RNAi agent using a phosphoramidite.

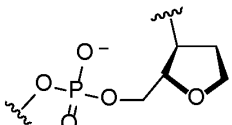
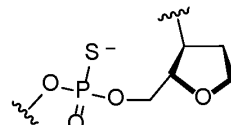
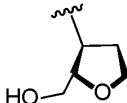
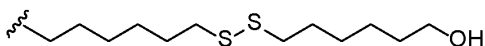
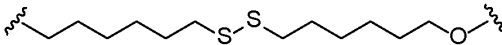
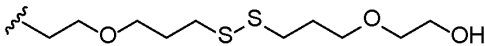
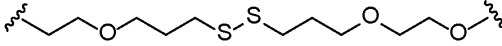
[0147] In some embodiments, ALK7 RNAi agents may comprise a lipid moiety on an internal nucleotide (i.e., not on the 3' or 5' terminal nucleotides.) In some embodiments, a lipid moiety on an internal nucleotide may be linked to the 2' position of ribose.

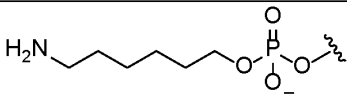
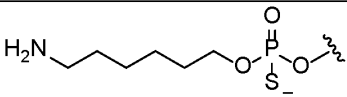
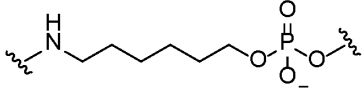
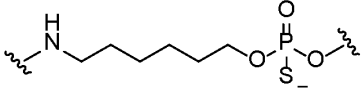
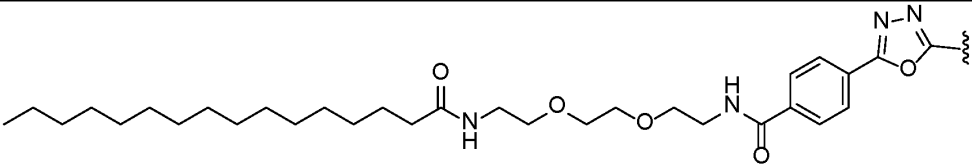
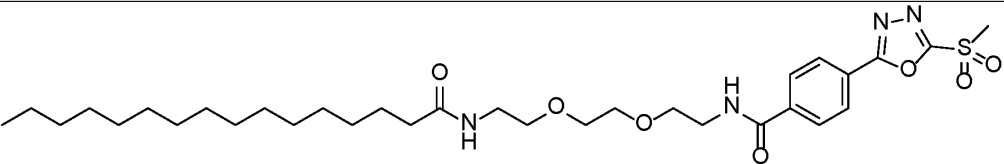
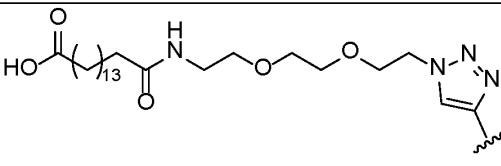
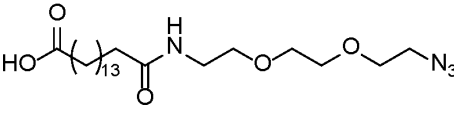
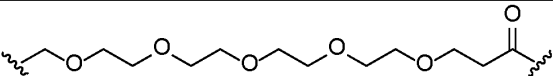
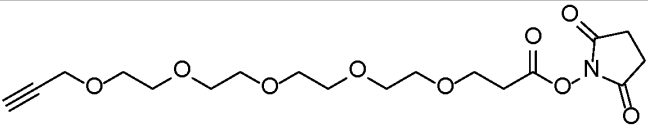
[0148] Any of the ALK7 RNAi agent nucleotide sequences listed in Tables 2, 3, 4, 5, 6, and 10, whether modified or unmodified, can contain 3' and/or 5' targeting group(s), linking group(s), and/or lipid PK/PD moieties. Any of the ALK7 RNAi agent sequences listed in Tables 3, 4, 5, 6, and 10, or are otherwise described herein, which contain a 3' or 5' targeting group, linking group, and/or lipid PK/PD moiety can alternatively contain no 3' or 5' targeting group, linking group, or lipid PK/PD moiety, or can contain a different 3' or 5' targeting group, linking group, or lipid PK/PD moiety including, but not limited to, those depicted in Table 11. Any of the ALK7 RNAi agent duplexes listed in Tables 7A, 7B, 8, 9A and 10, whether modified or unmodified, can further comprise a targeting group, linking group, or PK/PD moiety including, but not limited to, those depicted in Table 11, and the targeting group, linking group or PK/PD moiety can be attached to the 3' or 5' terminus of either the sense strand or the antisense strand of the ALK7 RNAi agent duplex.

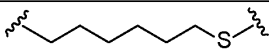
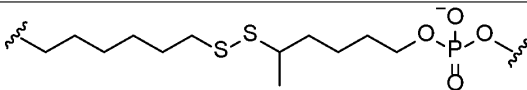
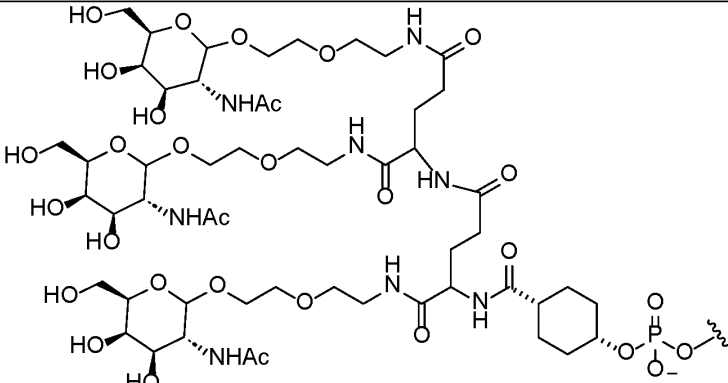
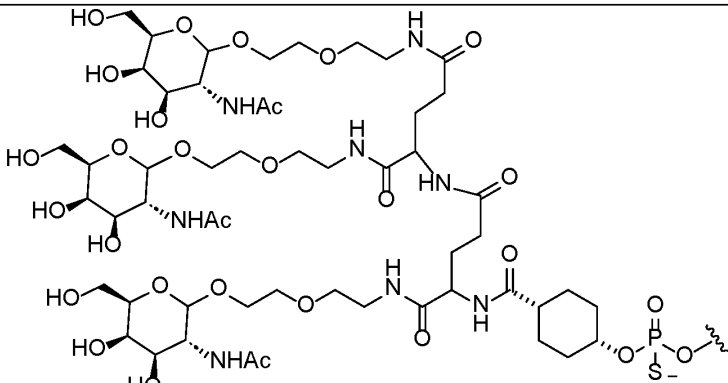
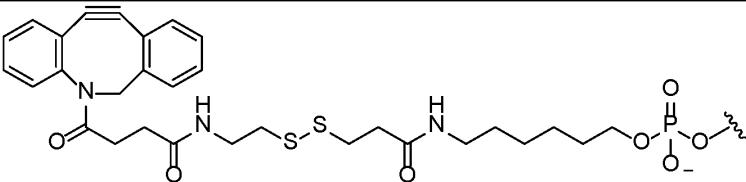
[0149] Examples of certain modified nucleotides, capping moieties, lipid moieties, and linking groups are provided in Table 11.

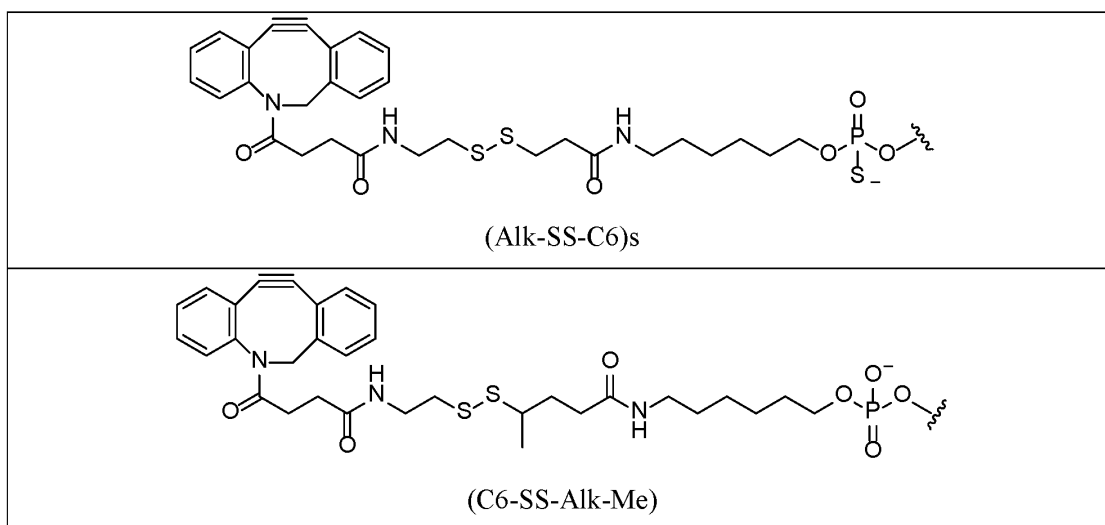
Table 11. Structures Representing Various Modified Nucleotides, Capping Moieties, lipid PK/PD moieties and Linking Groups (wherein $\sim$ indicates the point of connection)

 cPrpu	 cPrpus
 cPrpa	 cPrpas
 cPrpi	 cPrpis
 a₂N	 a₂Ns

When positioned internally: linkage towards 5' end  linkage towards 3' end (invAb) linkage towards 5' end  linkage towards 3' end (invAb)s	When positioned at the 3' terminal end: linkage towards 5' end  (invAb)
When position at the 3' terminal end: linkage towards 5' end  (C6-SS-C6)	When positioned internally: linkage towards 5' end  (C6-SS-C6) linkage towards 3' end
When position at the 3' terminal end: linkage towards 5' end  (6-SS-6)	When positioned internally: linkage towards 5' end  (6-SS-6) linkage towards 3' end

 (NH2-C6)	 (NH2-C6)s
 (NHC6)	 (NHC6)s
 LP-371-a	
 LP-371-p	
 LP-379-a	
 LP-379-p	
 L6	
 L6-p	

 (C6-S)
 (C6-SS-MeC5)
 (NAG37)
 (NAG37)s
 (Alk-SS-C6)



[0150] Alternatively, other linking groups known in the art may be used. In many instances, linking groups can be commercially acquired or alternatively, are incorporated into commercially available nucleotide phosphoramidites. (*See, e.g.*, International Patent Application Publication No. WO 2019/161213, which is incorporated herein by reference in its entirety).

[0151] To evaluate the activity of ALK7 RNAi agents in a ALK7-AAV model as described in the Examples below, certain ALK7 RNAi agents were conjugated to an N-acetyl-galactosamine (NAG) containing targeting ligand having the chemical structure referred to as NAG37 (*see* Table 11). NAG37 is known to have high affinity to bind to asialoglycoprotein receptors that are abundantly expressed on liver cells, including hepatocytes (*see, e.g.*, International Patent Application Publication No. WO2018044350A1). The use of NAG37-conjugated ALK7 RNAi agents was to evaluate the expression of AAV-ALK7 in the liver.

[0152] In some embodiments, an ALK7 RNAi agent is delivered without being conjugated to a targeting ligand or pharmacokinetic/pharmacodynamic (PK/PD) modulator (referred to as being “naked” or a “naked RNAi agent”).

[0153] In some embodiments, an ALK7 RNAi agent is conjugated to a targeting group, a linking group, a PK modulator, and/or another non-nucleotide group to facilitate delivery of the ALK7 RNAi agent to the cell or tissue of choice, for example, to adipose tissue *in vivo*. In some embodiments, an ALK7 RNAi agent is conjugated to a lipid moiety.

[0154] In some embodiments, a delivery vehicle may be used to deliver an RNAi agent to a cell or tissue. A delivery vehicle is a compound that improves delivery of the RNAi agent to a

cell or tissue. A delivery vehicle can include, or consist of, but is not limited to: a polymer, such as an amphipathic polymer, a membrane active polymer, a peptide, a melittin peptide, a melittin-like peptide (MLP), a lipid, a reversibly modified polymer or peptide, or a reversibly modified membrane active polyamine.

[0155] In some embodiments, the RNAi agents can be combined with lipids, nanoparticles, polymers, liposomes, micelles, DPCs or other delivery systems available in the art for nucleic acid delivery. The RNAi agents can also be chemically conjugated to targeting groups, lipids (including, but not limited to cholesteryl and cholesteryl derivatives), encapsulating in nanoparticles, liposomes, micelles, conjugating to polymers or DPCs (see, for example WO 2000/053722, WO 2008/022309, WO 2011/104169, and WO 2012/083185, WO 2013/032829, WO 2013/158141, each of which is incorporated herein by reference), by iontophoresis, or by incorporation into other delivery vehicles or systems available in the art such as hydrogels, cyclodextrins, biodegradable nanocapsules, bioadhesive microspheres, or proteinaceous vectors. In some embodiments the RNAi agents can be conjugated to antibodies having affinity for adipocyte cells. In some embodiments, the RNAi agents can be linked to targeting ligands that have affinity for cells in adipose tissue, for example adipocyte cells or receptors present on adipocyte cells.

Pharmaceutical Compositions and Formulations

[0156] The ALK7 RNAi agents disclosed herein can be prepared as pharmaceutical compositions or formulations (also referred to herein as “medicaments”). In some embodiments, pharmaceutical compositions include at least one ALK7 RNAi agent. These pharmaceutical compositions are particularly useful in the inhibition of the expression of ALK7 mRNA in a target cell, a group of cells, a tissue, or an organism. The pharmaceutical compositions can be used to treat a subject having a disease, disorder, or condition that would benefit from reduction in the level of the target mRNA, or inhibition in expression of the target gene. The pharmaceutical compositions can be used to treat a subject at risk of developing a disease or disorder that would benefit from reduction of the level of the target mRNA or an inhibition in expression the target gene. In one embodiment, the method includes administering an ALK7 RNAi agent linked to a PK/PD modulator as described herein, to a subject to be treated. In some embodiments, one or more pharmaceutically acceptable excipients (including vehicles, carriers, diluents, and/or delivery polymers) are added to the pharmaceutical

compositions that include an ALK7 RNAi agent, thereby forming a pharmaceutical formulation or medicament suitable for *in vivo* delivery to a subject, including a human.

[0157] The pharmaceutical compositions that include an ALK7 RNAi agent and methods disclosed herein decrease the level of the target mRNA in a cell, group of cells, tissue, organ, or subject, including by administering to the subject a therapeutically effective amount of a herein described ALK7 RNAi agent, thereby inhibiting the expression of ALK7 mRNA in the subject. In some embodiments, the subject has been previously identified or diagnosed as having a disease or disorder that can be mediated at least in part by a reduction in ALK7 gene expression. In some embodiments, the subject has been previously diagnosed with having one or more metabolic diseases such as obesity, diabetes, or insulin resistance.

[0158] In some embodiments the subject has been previously diagnosed with having obesity, diabetes, or insulin resistance.

[0159] Embodiments of the present disclosure include pharmaceutical compositions for delivering an ALK7 RNAi agent to adipose tissue *in vivo*. Such pharmaceutical compositions can include, for example, an ALK7 RNAi agent conjugated to a lipid moiety.

[0160] In some embodiments, the described pharmaceutical compositions including an ALK7 RNAi agent are used for treating or managing clinical presentations in a subject that would benefit from the inhibition of expression of ALK7. In some embodiments, a therapeutically or prophylactically effective amount of one or more of pharmaceutical compositions is administered to a subject in need of such treatment. In some embodiments, administration of any of the disclosed ALK7 RNAi agents can be used to decrease the number, severity, and/or frequency of symptoms of a disease in a subject.

[0161] In some embodiments, the described ALK7 RNAi agents are optionally combined with one or more additional (i.e., second, third, etc.) therapeutics. A second therapeutic can be another ALK7 RNAi agent (e.g., an ALK7 RNAi agent that targets a different sequence within an ALK7 gene). In some embodiments, a second therapeutic can be an RNAi agent that targets the ALK7 gene. An additional therapeutic can also be a small molecule drug, antibody, antibody fragment, and/or aptamer. The ALK7 RNAi agents, with or without the one or more additional therapeutics, can be combined with one or more excipients to form pharmaceutical compositions.

[0162] The described pharmaceutical compositions that include an ALK7 RNAi agent can be used to treat at least one symptom in a subject having a disease or disorder that would benefit from reduction or inhibition in expression of ALK7 mRNA. In some embodiments, the subject

is administered a therapeutically effective amount of one or more pharmaceutical compositions that include an ALK7 RNAi agent thereby treating the symptom. In other embodiments, the subject is administered a prophylactically effective amount of one or more ALK7 RNAi agents, thereby preventing or inhibiting the at least one symptom.

[0163] In some embodiments, one or more of the described ALK7 RNAi agents are administered to a mammal in a pharmaceutically acceptable carrier or diluent. In some embodiments, the mammal is a human.

[0164] The route of administration is the path by which an ALK7 RNAi agent is brought into contact with the body. In general, methods of administering drugs, oligonucleotides, and nucleic acids including the ALK7 RNAi agents described herein, for treatment of a mammal are well known in the art and can be applied to administration of the compositions described herein. The ALK7 RNAi agents disclosed herein can be administered via any suitable route in a preparation appropriately tailored to the particular route. Thus, in some embodiments, the herein described pharmaceutical compositions are administered via inhalation, intranasal administration, intratracheal administration, or oropharyngeal aspiration administration. In some embodiments, the pharmaceutical compositions can be administered by injection, for example, intravenously, intramuscularly, intracutaneously, subcutaneously, intracerebroventricularly, intraarticularly, intraocularly, or intraperitoneally, or topically.

[0165] The pharmaceutical compositions including an ALK7 RNAi agent described herein can be delivered to a cell, group of cells, tissue, or subject using oligonucleotide delivery technologies known in the art. In general, any suitable method recognized in the art for delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with the compositions described herein. For example, delivery can be by local administration, (e.g., direct injection, implantation, or topical administering), systemic administration, or subcutaneous, intravenous, intraperitoneal, or parenteral routes, including intracranial (e.g., intraventricular, intraparenchymal and intrathecal), intracerebroventricular, intramuscular, transdermal, airway (aerosol), nasal, oral, rectal, or topical (including buccal and sublingual) administration. In some embodiments, the compositions are administered via inhalation, intranasal administration, oropharyngeal aspiration administration, or intratracheal administration. For example, in some embodiments, it is desired that the ALK7 RNAi agents described herein inhibit the expression of an ALK7 gene in adipose tissue.

[0166] In some embodiments, the pharmaceutical compositions described herein comprise one or more pharmaceutically acceptable excipients. The pharmaceutical compositions described herein are formulated for administration to a subject.

[0167] As used herein, a pharmaceutical composition or medicament includes a pharmacologically effective amount of at least one of the described therapeutic compounds and one or more pharmaceutically acceptable excipients. Pharmaceutically acceptable excipients (excipients) are substances other than the Active Pharmaceutical Ingredient (API, therapeutic product, e.g., ALK7 RNAi agent) that are intentionally included in the drug delivery system. Excipients do not exert or are not intended to exert a therapeutic effect at the intended dosage. Excipients can act to a) aid in processing of the drug delivery system during manufacture, b) protect, support or enhance stability, bioavailability or patient acceptability of the API, c) assist in product identification, and/or d) enhance any other attribute of the overall safety, effectiveness, of delivery of the API during storage or use. A pharmaceutically acceptable excipient may or may not be an inert substance.

[0168] Excipients include, but are not limited to: absorption enhancers, anti-adherents, anti-foaming agents, anti-oxidants, binders, buffering agents, carriers, coating agents, colors, delivery enhancers, delivery polymers, detergents, dextran, dextrose, diluents, disintegrants, emulsifiers, extenders, fillers, flavors, glidants, humectants, lubricants, oils, polymers, preservatives, saline, salts, solvents, sugars, surfactants, suspending agents, sustained release matrices, sweeteners, thickening agents, tonicity agents, vehicles, water-repelling agents, and wetting agents.

[0169] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water-soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor® ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). It should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as

mannitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0170] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation include vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0171] Formulations suitable for intra-articular administration can be in the form of a sterile aqueous preparation of the drug that can be in microcrystalline form, for example, in the form of an aqueous microcrystalline suspension. Liposomal formulations or biodegradable polymer systems can also be used to present the drug for both intra-articular and ophthalmic administration.

[0172] The active compounds can be prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[0173] The ALK7 RNAi agents can be formulated in compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[0174] A pharmaceutical composition can contain other additional components commonly found in pharmaceutical compositions. Such additional components include, but are not limited to: anti-pruritics, astringents, local anesthetics, or anti-inflammatory agents (e.g., antihistamine, diphenhydramine, etc.). It is also envisioned that cells, tissues, or isolated organs that express or comprise the herein defined RNAi agents may be used as “pharmaceutical compositions.” As used herein, “pharmacologically effective amount,” “therapeutically effective amount,” or simply “effective amount” refers to that amount of an RNAi agent to produce a pharmacological, therapeutic, or preventive result.

[0175] In some embodiments, ALK7 RNAi agent pharmaceutical compositions may contain salts such as sodium chloride, calcium chloride, magnesium chloride, potassium chloride, sodium phosphate dibasic, sodium phosphate monobasic, or combinations thereof.

[0176] In some embodiments, the methods disclosed herein further comprise the step of administering a second therapeutic or treatment in addition to administering an RNAi agent disclosed herein. In some embodiments, the second therapeutic is another ALK7 RNAi agent (e.g., an ALK7 RNAi agent that targets a different sequence within the ALK7 target). In other embodiments, the second therapeutic can be a small molecule drug, an antibody, an antibody fragment, and/or an aptamer.

[0177] In some embodiments, described herein are compositions that include a combination or cocktail of at least two ALK7 RNAi agents having different sequences. In some embodiments, the two or more ALK7 RNAi agents are each separately and independently linked to lipids.

[0178] Described herein are compositions for delivery of ALK7 RNAi agents to adipose tissue. Furthermore, compositions for delivery of ALK7 RNAi agents to adipose tissue, *in vivo*, are generally described herein.

[0179] Generally, an effective amount of an ALK7 RNAi agent disclosed herein will be in the range of from about 0.0001 to about 20 mg/kg of body weight dose, e.g., from about 0.001 to about 5 mg/kg of body weight dose. In some embodiments, an effective amount of an ALK7 RNAi agent will be in the range of from about 0.01 mg/kg to about 3.0 mg/kg of body weight per dose. In some embodiments, an effective amount of an ALK7 RNAi agent will be in the range of from about 0.03 mg/kg to about 2.0 mg/kg of body weight per dose. In some embodiments, an effective amount of an ALK7 RNAi agent will be in the range of from about 0.01 to about 1.0 mg/kg. In some embodiments, an effective amount of an ALK7 RNAi agent will be in the range of from about 0.50 to about 1.0 mg/kg. In some embodiments, a fixed dose

of ALK7 RNAi agent is administered to the subject. In some embodiments the dose administered to the human subject is between about 1.0 mg and about 750 mg. In some embodiments, the dose of ALK7 RNAi agent administered to the human subject is between about 10 mg and about 450 mg. In some embodiments, the dose of ALK7 RNAi agent administered to the human subject is between about 25 mg and about 450 mg. In some embodiments, the dose of ALK7 RNAi agent administered to the human subject is about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, or about 450 mg. The amount administered will also likely depend on such variables as the overall health status of the patient, the relative biological efficacy of the compound delivered, the formulation of the drug, the presence and types of excipients in the formulation, and the route of administration. Also, it is to be understood that the initial dosage administered can be increased beyond the above upper level to rapidly achieve the desired blood-level or tissue level, or the initial dosage can be smaller than the optimum. In some embodiments, a dose is administered daily. In some embodiments, a dose is administered weekly. In further embodiments, a dose is administered bi-weekly, tri-weekly, once monthly, or once quarterly (i.e., once every three months).

[0180] For treatment of disease or for formation of a medicament or composition for treatment of a disease, the pharmaceutical compositions described herein including an ALK7 RNAi agent can be combined with an excipient or with a second therapeutic agent or treatment including, but not limited to: a second or other RNAi agent, a small molecule drug, an antibody, an antibody fragment, peptide, and/or an aptamer.

[0181] The described ALK7 RNAi agents, when added to pharmaceutically acceptable excipients or adjuvants, can be packaged into kits, containers, packs, or dispensers.

Methods of Treatment and Inhibition of ALK7 Gene Expression

[0182] The ALK7 RNAi agents disclosed herein can be used to treat a subject (e.g., a human or other mammal) having a disease or disorder that would benefit from administration of the RNAi agent. In some embodiments, the RNAi agents disclosed herein can be used to treat a subject (e.g., a human) that would benefit from a reduction and/or inhibition in expression of ALK7 mRNA and/or a reduction in ALK7 protein levels.

[0183] In some embodiments, the RNAi agents disclosed herein can be used to treat a subject (e.g., a human) having a disease or disorder for which the subject would benefit from reduction in ALK7 protein, including but not limited to, obesity, diabetes, or insulin resistance.

Treatment of a subject can include therapeutic and/or prophylactic treatment. The subject is administered a therapeutically effective amount of any one or more ALK7 RNAi agents described herein. The subject can be a human, patient, or human patient. The subject may be an adult, adolescent, child, or infant. Administration of a pharmaceutical composition described herein can be to a human being or animal.

[0184] ALK7 activity is known to play a role in metabolic disorders. In some embodiments, the described ALK7 RNAi agents are used to treat at least one symptom mediated at least in part by a reduction in ALK7 protein levels, in a subject. The subject is administered a therapeutically effective amount of any one or more of the described ALK7 RNAi agents. In some embodiments, the subject is administered a prophylactically effective amount of any one or more of the described RNAi agents, thereby treating the subject by preventing or inhibiting the at least one symptom.

[0185] In certain embodiments, the present disclosure provides methods for treatment of diseases, disorders, conditions, or pathological states mediated at least in part by ALK7 gene expression, in a patient in need thereof, wherein the methods include administering to the patient any of the ALK7 RNAi agents described herein.

[0186] In some embodiments, the ALK7 RNAi agents are used to treat or manage a clinical presentation or pathological state in a subject, wherein the clinical presentation or pathological state is mediated at least in part by a reduction in ALK7 gene expression. The subject is administered a therapeutically effective amount of one or more of the ALK7 RNAi agents or ALK7 RNAi agent-containing compositions described herein. In some embodiments, the method comprises administering a composition comprising an ALK7 RNAi agent described herein to a subject to be treated.

[0187] In a further aspect, the disclosure features methods of treatment (including prophylactic or preventative treatment) of diseases or symptoms that may be addressed by a reduction in ALK7 protein levels, the methods comprising administering to a subject in need thereof an ALK7 RNAi agent that includes an antisense strand comprising the sequence of any of the sequences in Table 2, Table 3, or Table 10. Also described herein are compositions for use in such methods.

[0188] The described ALK7 RNAi agents and/or compositions that include ALK7 RNAi agents can be used in methods for therapeutic treatment of disease or conditions caused by enhanced or elevated ALK7 protein levels. Such methods include administration of an ALK7 RNAi agent as described herein to a subject, e.g., a human or animal subject.

[0189] In another aspect, the disclosure provides methods for the treatment (including prophylactic treatment) of a pathological state (such as a condition or disease) mediated at least in part by ALK7 gene expression, wherein the methods include administering to a subject a therapeutically effective amount of an RNAi agent that includes an antisense strand comprising the sequence of any of the sequences in Table 2, Table 3, or Table 10.

[0190] In some embodiments, methods for inhibiting expression of an ALK7 gene are disclosed herein, wherein the methods include administering to a cell an RNAi agent that includes an antisense strand comprising the sequence of any of the sequences in Table 2, Table 3, or Table 10.

[0191] In some embodiments, methods for the treatment (including prophylactic treatment) of a pathological state mediated at least in part by ALK7 gene expression are disclosed herein, wherein the methods include administering to a subject a therapeutically effective amount of an RNAi agent that includes a sense strand comprising the sequence of any of the sequences in Table 2, Table 4, Table 5, Table 6, Table 6a, or Table 10.

[0192] In some embodiments, methods for inhibiting expression of an ALK7 gene are disclosed herein, wherein the methods comprise administering to a cell an RNAi agent that includes a sense strand comprising the sequence of any of the sequences in Table 2, Table 4, Table 5, Table 6, Table 6a, or Table 10.

[0193] In some embodiments, methods for the treatment (including prophylactic treatment) of a pathological state mediated at least in part by ALK7 gene expression are disclosed herein, wherein the methods include administering to a subject a therapeutically effective amount of an RNAi agent that includes a sense strand comprising the sequence of any of the sequences in Table 4, Table 5, Table 6, Table 6a, or Table 10, and an antisense strand comprising the sequence of any of the sequences in Table 3 or Table 10.

[0194] In some embodiments, methods for inhibiting expression of an ALK7 gene are disclosed herein, wherein the methods include administering to a cell an RNAi agent that includes a sense strand comprising the sequence of any of the sequences in Table 4, Table 5, Table 6, Table 6a, or Table 10, and an antisense strand comprising the sequence of any of the sequences in Table 3 or Table 10.

[0195] In some embodiments, methods of inhibiting expression of an ALK7 gene are disclosed herein, wherein the methods include administering to a subject an ALK7 RNAi agent that includes a sense strand consisting of the nucleobase sequence of any of the sequences in Table 4, Table 5, Table 6, Table 6a, or Table 10, and the antisense strand consisting of the nucleobase

sequence of any of the sequences in Table 3 or Table 10. In other embodiments, disclosed herein are methods of inhibiting expression of an ALK7 gene, wherein the methods include administering to a subject an ALK7 RNAi agent that includes a sense strand consisting of the modified sequence of any of the modified sequences in Table 4, Table 5, Table 6, Table 6a, or Table 10, and the antisense strand consisting of the modified sequence of any of the modified sequences in Table 3 or Table 10.

[0196] In some embodiments, methods for inhibiting expression of an ALK7 gene in a cell are disclosed herein, wherein the methods include administering one or more ALK7 RNAi agents comprising a duplex structure of one of the duplexes set forth in Tables 7A, 7B, 8, 9A, and 10.

[0197] In some embodiments, the gene expression level and/or mRNA level of an ALK7 gene in certain adipose tissues of subject to whom a described ALK7 RNAi agent is administered is reduced by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater than 99%, relative to the subject prior to being administered the ALK7 RNAi agent or to a subject not receiving the ALK7 RNAi agent. In some embodiments, the ALK7 protein levels in certain adipose tissues of a subject to whom a described ALK7 RNAi agent is administered is reduced by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater than 99%, relative to the subject prior to being administered the ALK7 RNAi agent or to a subject not receiving the ALK7 RNAi agent. The gene expression level, protein level, and/or mRNA level in the subject may be reduced in a cell, group of cells, and/or tissue of the subject. In some embodiments, the ALK7 mRNA levels in certain adipose tissues subject to whom a described ALK7 RNAi agent has been administered is reduced by at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98% relative to the subject prior to being administered the ALK7 RNAi agent or to a subject not receiving the ALK7 RNAi agent.

[0198] A reduction in gene expression, mRNA, and protein levels can be assessed by any methods known in the art. Reduction or decrease in ALK7 protein levels are collectively referred to herein as a decrease in, reduction of, or inhibition of ALK7 expression. The Examples set forth herein illustrate known methods for assessing inhibition of ALK7 gene expression, including but not limited to determining ALK7 protein levels.

Cells, Tissues, Organs, and Non-Human Organisms

[0199] Cells, tissues, organs, and non-human organisms that include at least one of the ALK7 RNAi agents described herein are contemplated. The cell, tissue, organ, or non-human organism is made by delivering the RNAi agent to the cell, tissue, organ, or non-human organism.

Additional Illustrative Embodiments

[0200] Provided here are certain additional illustrative embodiments of the disclosed technology. These embodiments are illustrative only and do not limit the scope of the present disclosure or of the claims attached hereto.

Embodiment 1. An RNAi agent for inhibiting expression of a Activin Receptor-Like Kinase 7 (ALK7) gene, comprising:

- an antisense strand comprising at least 17 contiguous nucleotides differing by 0 or 1 nucleotides from any one of the sequences provided in Table 2 or Table 3; and
- a sense strand comprising a nucleotide sequence that is at least partially complementary to the antisense strand.

Embodiment 2. The RNAi agent of embodiment 1, wherein the antisense strand comprises nucleotides 2-18 of any one of the sequences provided in Table 2 or Table 3.

Embodiment 3. The RNAi agent of embodiment 1 or embodiment 2, wherein the sense strand comprises a nucleotide sequence of at least 17 contiguous nucleotides differing by 0 or 1 nucleotides from any one of the sequences provided in Table 2 or Table 4, and wherein the sense strand has a region of at least 85% complementarity over the 17 contiguous nucleotides to the antisense strand.

Embodiment 4. The RNAi agent of any one of embodiments 1-3, wherein at least one nucleotide of the ALK7 RNAi agent is a modified nucleotide or includes a modified internucleoside linkage.

Embodiment 5. The RNAi agent of any one of embodiments 1-4, wherein all or substantially all of the nucleotides are modified nucleotides.

Embodiment 6. The RNAi agent of any one of embodiments 4-5, wherein the modified nucleotide is selected from the group consisting of: 2'-O-methyl nucleotide, 2'-fluoro nucleotide, 2'-deoxy nucleotide, 2',3'-seco nucleotide mimic, locked nucleotide, 2'-F-arabino nucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted 2'-O-methyl nucleotide, inverted 2'-deoxy nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-

modified nucleotide, morpholino nucleotide, vinyl phosphonate-containing nucleotide, cyclopropyl phosphonate-containing nucleotide, and 3'-O-methyl nucleotide.

Embodiment 7. The RNAi agent of embodiment 5, wherein all or substantially all of the nucleotides are modified with 2'-O-methyl nucleotides, 2'-fluoro nucleotides, or combinations thereof.

Embodiment 8. The RNAi agent of any one of embodiments 1-7, wherein the antisense strand comprises the nucleotide sequence of any one of the modified sequences provided in Table 3.

Embodiment 9. The RNAi agent of any one of embodiments 1-8, wherein the sense strand comprises the nucleotide sequence of any one of the modified sequences provided in Table 4.

Embodiment 10. The RNAi agent of embodiment 1, wherein the antisense strand comprises the nucleotide sequence of any one of the modified sequences provided in Table 3 and the sense strand comprises the nucleotide sequence of any one of the modified sequences provided in Table 4.

Embodiment 11. The RNAi agent of any one of embodiments 1-10, wherein the sense strand is between 18 and 30 nucleotides in length, and the antisense strand is between 18 and 30 nucleotides in length.

Embodiment 12. The RNAi agent of embodiment 11, wherein the sense strand and the antisense strand are each between 18 and 27 nucleotides in length.

Embodiment 13. The RNAi agent of embodiment 12, wherein the sense strand and the antisense strand are each between 18 and 24 nucleotides in length.

Embodiment 14. The RNAi agent of embodiment 13, wherein the sense strand and the antisense strand are each 21 nucleotides in length.

Embodiment 15. The RNAi agent of embodiment 14, wherein the RNAi agent has two blunt ends.

Embodiment 16. The RNAi agent of any one of embodiments 1-15, wherein the sense strand comprises one or two terminal caps.

Embodiment 17. The RNAi agent of any one of embodiments 1-16, wherein the sense strand comprises one or two inverted abasic residues.

Embodiment 18. The RNAi agent of embodiment 1, wherein the RNAi agent is comprised of a sense strand and an antisense strand that form a duplex having the structure of any one of the duplexes in Table 7A, Table 7B, Table 8, Table 9A, or Table 10.

Embodiment 19. The RNAi agent of embodiment 18, wherein all or substantially all of the nucleotides are modified nucleotides.

Embodiment 20. The RNAi agent of embodiment 1, comprising an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5' → 3'):

AUUGUUGGAACUAUGACAGAC (SEQ ID NO: 558); or
UUCGAUAGUGUUCAGUAUUGC (SEQ ID NO: 569); or
UGUAUCAUCAAGCAUUUCAGG (SEQ ID NO: 571).

Embodiment 21. The RNAi agent of embodiment 20, wherein the sense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5' → 3'):

GUCUGUCAUAGUCCAACAAU (SEQ ID NO: 599); or
GCAAUACUGAACACUAUCGAA (SEQ ID NO: 610); or
CCUGAAAUGCUUGAUGAUACA (SEQ ID NO: 612).

Embodiment 22. The RNAi agent of embodiment 20 or 21, wherein all or substantially all of the nucleotides are modified nucleotides.

Embodiment 23. The RNAi agent of embodiment 1, comprising an antisense strand that comprises, consists of, or consists essentially of a modified nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5' → 3'):

cPrpasUfsuguuGfgaacUfaUfgAfcagasc (SEQ ID NO: 269);
cPrpusUfscgAfuaguguUfcAfgUfauugssc (SEQ ID NO: 339); or
cPrpusGfsuaucafucaaGfcAfuUfucagsg (SEQ ID NO: 282).

wherein a represents 2'-O-methyl adenosine, c represents 2'-O-methyl cytidine, g represents 2'-O-methyl guanosine, and u represent 2'-O-methyl uridine; Af represents 2'-fluoro adenosine, Cf represents 2'-fluoro cytidine, Gf represents 2'-fluoro guanosine, and Uf represents 2'-fluoro uridine; cPrpu represents a 5'-cyclopropyl phosphonate-2'-O-methyl uridine, cPrpa represents a 5'-cyclopropyl phosphonate-2'-O-methyl adenosine; s represents a phosphorothioate linkage, ss represents a phosphorodithioate linkage; and wherein all or substantially all of the nucleotides on the sense strand are modified nucleotides.

Embodiment 24. The RNAi agent of embodiment 1, wherein the sense strand comprises, consists of, or consists essentially of a modified nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5' → 3'):

gucugucaUfAfGfuuccaacaau (SEQ ID NO: 364);
 gcaauacuGfaAfcAfcuaucgaa (SEQ ID NO: 425); or
 ccugaaAfuGfcUfugaugauaca (SEQ ID NO: 429).

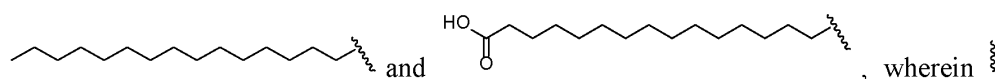
wherein a represents 2'-O-methyl adenosine, c represents 2'-O-methyl cytidine, g represents 2'-O-methyl guanosine, and u represent 2'-O-methyl uridine; Af represents 2'-fluoro adenosine, Cf represents 2'-fluoro cytidine, Gf represents 2'-fluoro guanosine, and Uf represents 2'-fluoro uridine; and s represents a phosphorothioate linkage; and wherein all or substantially all of the nucleotides on the antisense strand are modified nucleotides.

Embodiment 25. The RNAi agent of any one of embodiments 20-24, wherein the sense strand further includes inverted abasic residues at the 3' terminal end of the nucleotide sequence, at the 5' end of the nucleotide sequence, or at both.

Embodiment 26. The RNAi agent of any one of embodiments 1-25, wherein the RNAi agent is linked to a lipid moiety.

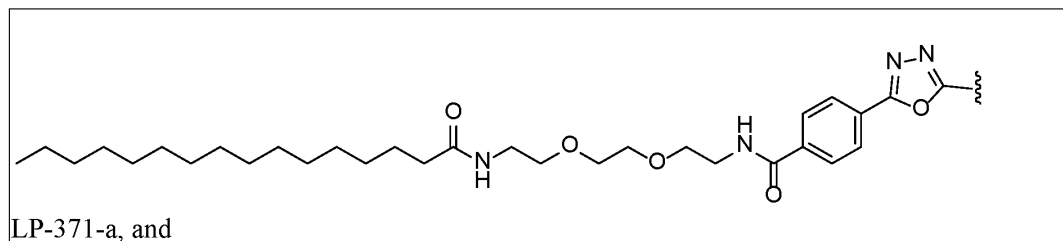
Embodiment 27. The RNAi agent of embodiment 26, wherein the RNAi agent is linked to two or more lipid moieties.

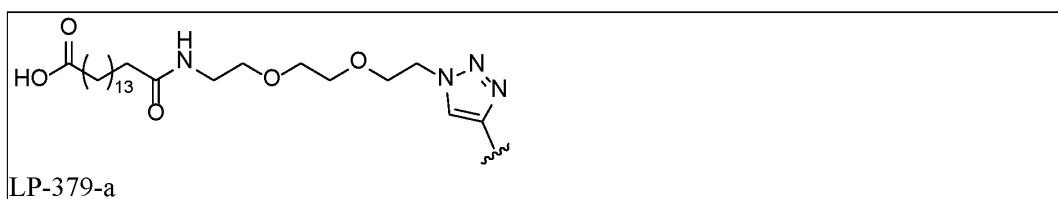
Embodiment 28. The RNAi agent of embodiment 27, wherein each of the lipid moieties is independently selected from the group consisting of:



indicates the point of connection to the RNAi agent.

Embodiment 29. The RNAi agent of any one of embodiments 26-28, wherein each of the lipid moieties is independently selected from the group consisting of:





wherein  indicates the point of connection to the RNAi agent.

Embodiment 30. The RNAi agent of embodiment 26 or embodiment 27, wherein the lipid moiety is conjugated to the sense strand.

Embodiment 31. The RNAi agent of embodiment 26, wherein the RNAi agent is linked to LP-379-a on the 5' terminus of the sense strand, and linked to LP-371-a on the 3' terminus of the sense strand.

Embodiment 32. A composition comprising the RNAi agent of any one of embodiments 1-31, wherein the composition further comprises a pharmaceutically acceptable excipient.

Embodiment 33. The RNAi agent of embodiment 1, wherein the RNAi agent has a duplex structure of any one of the duplexes of Table 10.

Embodiment 34. The RNAi agent of embodiment 32, wherein the duplex structure is selected from the group consisting of: AC006188, AC006189, AC006648, AC006661, or AC006663.

Embodiment 35. The RNAi agent of embodiment 1, wherein the RNAi agent has the structure of any one of Figures 4A-4D to 13A-13D.

Embodiment 36. The composition of embodiment 35, further comprising a second RNAi agent capable of inhibiting the expression of Activin Receptor-Like Kinase 7 gene expression.

Embodiment 37. The composition of any one of embodiments 35-36, further comprising one or more additional therapeutics.

Embodiment 38. The composition of any of embodiments 35-37, wherein the RNAi agent is a sodium salt.

Embodiment 39. The composition of any of embodiments 35-37, wherein the pharmaceutically acceptable excipient is water for injection.

Embodiment 40. The composition of any of embodiments 35-38, wherein the pharmaceutically acceptable excipient is a buffered saline solution.

Embodiment 41. The composition of any of embodiments 35-38, wherein the pharmaceutically acceptable excipient is sodium chloride.

Embodiment 42. A method for inhibiting expression of an ALK7 gene in a cell, the method comprising introducing into a cell an effective amount of an RNAi agent of any one of embodiments 1-34 or the composition of any one of embodiments 35-41.

Embodiment 43. The method of embodiment 42, wherein the cell is within a subject.

Embodiment 44. The method of embodiment 43, wherein the subject is a human subject.

Embodiment 45. The method of any one of embodiments 42-44, wherein following the administration of the RNAi agent the Activin Receptor-Like Kinase 7 (ALK7) gene expression is inhibited by at least about 30%.

Embodiment 46. A method of treating one or more symptoms or diseases associated with enhanced or elevated ALK7 activity levels, the method comprising administering to a human subject in need thereof a therapeutically effective amount of the composition of any one of embodiments 35-41.

Embodiment 47. The method of embodiment 46, wherein the disease is a metabolic disease.

Embodiment 48. The method of embodiment 47, wherein the metabolic disease is obesity, diabetes, or insulin resistance.

Embodiment 49. The method of embodiment 46, wherein the disease is obesity, diabetes, or insulin resistance.

Embodiment 50. The method of embodiment 49, wherein the disease is obesity.

Embodiment 51. The method of any one of embodiments 42-50, wherein the RNAi agent is administered at a dose of about 0.01 mg/kg to about 5.0 mg/kg of body weight of the subject.

Embodiment 52. The method of any one of embodiments 42-51, wherein the RNAi agent is administered at a dose of about 0.03 mg/kg to about 2.0 mg/kg of body weight of the subject.

Embodiment 53. The method of any one of embodiments 42-50, wherein the RNAi agent is administered at a fixed dose of about 25 mg to about 450 mg.

Embodiment 54. The method of embodiment 53, wherein the RNAi agent is administered at a dose of about 25 mg, about 50 mg, about 150 mg, or about 450 mg.

Embodiment 55. The method of any of embodiments 42-54, wherein the RNAi agent is administered in two or more doses.

Embodiment 56. The method of any one of embodiments 43-55, wherein the subject is administered a second therapeutic.

Embodiment 57. The method of embodiment 56, wherein the second therapeutic is a glucagon-like peptide 1 (GLP1) agonist.

Embodiment 58. The method of embodiment 57, wherein the GLP1 agonist is tirzepatide.

Embodiment 59. Use of the RNAi agent of any one of embodiments 1-34, for the treatment of a disease, disorder, or symptom that is mediated at least in part by ALK7 activity and/or ALK7 gene expression.

Embodiment 60. Use of the composition according to any one of embodiments 35-41, for the treatment of a disease, disorder, or symptom that is mediated at least in part by Activin Receptor-Like Kinase 7 (ALK7) activity and/or Activin Receptor-Like Kinase 7 (ALK7) gene expression.

Embodiment 61. Use of the composition according to any one of embodiments 35-41, for the manufacture of a medicament for treatment of a disease, disorder, or symptom that is mediated at least in part by Activin Receptor-Like Kinase 7 (ALK7) and/or Activin Receptor-Like Kinase 7 (ALK7) gene expression.

Embodiment 62. The use of any one of embodiments 59-61, wherein the disease is a metabolic disease.

Embodiment 63. A method of making an RNAi agent of any one of embodiments 1-34, comprising annealing a sense strand and an antisense strand to form a double-stranded ribonucleic acid molecule.

Embodiment 64. The method of embodiment 63, wherein the sense strand comprises a lipid moiety.

Embodiment 65. The method of embodiment 64, comprising conjugating a lipid moiety to the sense strand.

EXAMPLES

Example 1. Synthesis of ALK7 RNAi Agents.

[0201] ALK7 RNAi agent duplexes disclosed herein were synthesized in accordance with the following:

[0202] A. *Synthesis.* The sense and antisense strands of the ALK7 RNAi agents were synthesized according to phosphoramidite technology on solid phase used in oligonucleotide synthesis. Depending on the scale, a MerMade96E® (Bioautomation), a MerMade12® (Bioautomation), or an OP Pilot 100 (GE Healthcare) was used. Syntheses were performed on a solid support made of controlled pore glass (CPG, 500 Å or 600Å, obtained from Prime Synthesis, Aston, PA, USA). All RNA and 2'-modified RNA phosphoramidites were purchased from Thermo Fisher Scientific (Milwaukee, WI, USA). Specifically, the 2'-O-

methyl phosphoramidites that were used included the following: (5'-O-dimethoxytrityl-N⁶-(benzoyl)-2'-O-methyl-adenosine-3'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite, 5'-O-dimethoxy-trityl-N⁴-(acetyl)-2'-O-methyl-cytidine-3'-O-(2-cyanoethyl-N,N-diisopropyl-amino) phosphoramidite, (5'-O-dimethoxytrityl-N²-(isobutyryl)-2'-O-methyl-guanosine-3'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite, and 5'-O-dimethoxytrityl-2'-O-methyl-uridine-3'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite. The 2'-deoxy-2'-fluoro-phosphoramidites carried the same protecting groups as the 2'-O-methyl RNA amidites. 5'-dimethoxytrityl-2'-O-methyl-inosine-3'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidites were purchased from Glen Research (Virginia). The inverted abasic (3'-O-dimethoxytrityl-2'-deoxyribose-5'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidites were purchased from ChemGenes (Wilmington, MA, USA). The following UNA phosphoramidites were used: 5'-(4,4'-Dimethoxytrityl)-N⁶-(benzoyl)-2',3'-seco-adenosine, 2'-benzoyl-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-(4,4'-Dimethoxytrityl)-N-acetyl-2',3'-seco-cytosine, 2'-benzoyl-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5'-(4,4'-Dimethoxytrityl)-N-isobutyryl-2',3'-seco-guanosine, 2'-benzoyl-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, and 5'-(4,4'-Dimethoxy-trityl)-2',3'-seco-uridine, 2'-benzoyl-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite. TFA aminolink phosphoramidites were also commercially purchased (ThermoFisher). Linker L6 was purchased as propargyl-PEG5-NHS from BroadPharm (catalog # BP-20907) and coupled to the NH₂-C₆ group from an aminolink phosphoramidite to form -L6-(NHC6)-, using standard coupling conditions. The linker Alk-cyHex was similarly commercially purchased from Lumiprobe (alkyne phosphoramidite, 5'-terminal) as a propargyl-containing compound phosphoramidite compound to form the linker -Alk-cyHex-. In each case, phosphorothioate or phosphorodithioate linkages were introduced as specified using the conditions set forth herein. The cyclopropyl phosphonate phosphoramidites were synthesized in accordance with International Patent Application Publication No. WO 2017/214112 (*see also* Altenhofer et. al., Chem. Communications (Royal Soc. Chem.), 57(55):6808-6811 (July 2021)).

[0203] Tri-alkyne-containing phosphoramidites were dissolved in anhydrous dichloromethane or anhydrous acetonitrile (50 mM), while all other amidites were dissolved in anhydrous acetonitrile (50 mM) and molecular sieves (3Å) were added. 5-Benzylthio-1H-tetrazole (BTT, 250 mM in acetonitrile) or 5-Ethylthio-1H-tetrazole (ETT, 250 mM in acetonitrile) was used as activator solution. Coupling times were 10 minutes (RNA), 90 seconds (2' O-Me), and 60 seconds (2' F). In order to introduce phosphorothioate linkages, a 100 mM solution of 3-phenyl

1,2,4-dithiazoline-5-one (POS, obtained from PolyOrg, Inc., Leominster, MA, USA) in anhydrous acetonitrile was employed.

[0204] Alternatively, tri-alkyne moieties were introduced post-synthetically (see section E, below). For this route, the sense strand was functionalized with a 5' and/or 3' terminal nucleotide containing a primary amine. TFA aminolink phosphoramidite was dissolved in anhydrous acetonitrile (50 mM) and molecular sieves (3Å) were added. 5-Benzylthio-1H-tetrazole (BTT, 250 mM in acetonitrile) or 5-Ethylthio-1H-tetrazole (ETT, 250 mM in acetonitrile) was used as activator solution. Coupling times were 10 minutes (RNA), 90 seconds (2' O-Me), and 60 seconds (2' F). In order to introduce phosphorothioate linkages, a 100 mM solution of 3-phenyl 1,2,4-dithiazoline-5-one (POS, obtained from PolyOrg, Inc., Leominster, MA, USA) in anhydrous acetonitrile was employed.

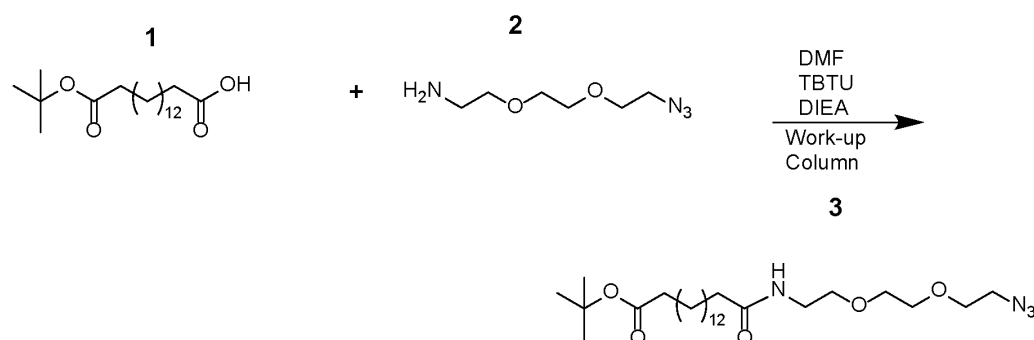
[0205] B. *Cleavage and deprotection of support bound oligomer.* After finalization of the solid phase synthesis, the dried solid support was treated with a 1:1 volume solution of 40 wt. % methylamine in water and 28% to 31% ammonium hydroxide solution (Aldrich) for 1.5 hours at 30°C. The solution was evaporated and the solid residue was reconstituted in water (see below).

[0206] C. *Purification.* Crude oligomers were purified by anionic exchange HPLC using a TSKgel SuperQ-5PW 13µm column and Shimadzu LC-8 system. Buffer A was 20 mM Tris, 5 mM EDTA, pH 9.0 and contained 20% Acetonitrile and buffer B was the same as buffer A with the addition of 1.5 M sodium chloride. UV traces at 260 nm were recorded. Appropriate fractions were pooled then run on size exclusion HPLC using a GE Healthcare XK 16/40 column packed with Sephadex G-25 fine with a running buffer of 100mM ammonium bicarbonate, pH 6.7 and 20% Acetonitrile or filtered water. Alternatively, pooled fractions were desalted and exchanged into an appropriate buffer or solvent system via tangential flow filtration.

[0207] D. *Annealing.* Complementary strands were mixed by combining equimolar RNA solutions (sense and antisense) in 1× PBS (Phosphate-Buffered Saline, 1×, Corning, Cellgro) to form the RNAi agents. Some RNAi agents were lyophilized and stored at -15 to -25°C. Duplex concentration was determined by measuring the solution absorbance on a UV-Vis spectrometer in 1× PBS. The solution absorbance at 260 nm was then multiplied by a conversion factor (0.050 mg/(mL·cm)) and the dilution factor to determine the duplex concentration.

[0208] E. *Synthesis of Lipids*

[0209] Synthesis of LP-379-p

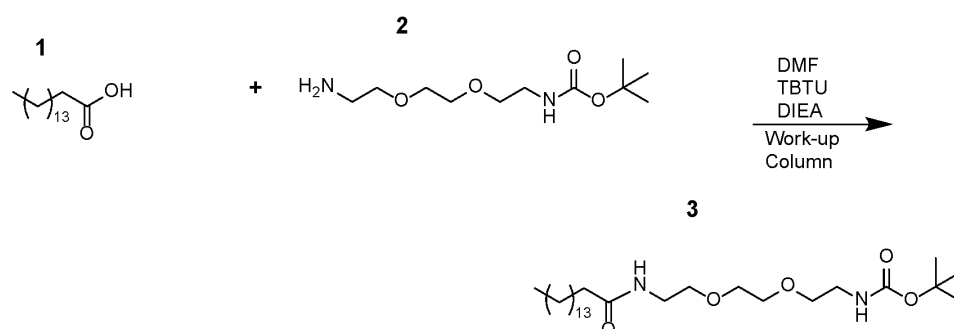


[0210] Compound 1 (Asta Tech® #89929, 3.00 g) was dissolved in 50 mL DMF. Then TBTU (3.09 g) and DIPEA (6.2 mL) were added and the mixture was stirred for 10 minutes. Compound 2 (1.68 g in DMF) was then added. The mixture was allowed to stir for 1 hour. Then the mixture was diluted with 300 mL EtOAc and washed with 3% citric acid (3x60 mL), H₂O (2x60 mL), and NaCl (1x60 mL), dried over Na₂SO₄, filtered and concentrated on rotary evaporator and placed under high vacuum. The crude product was loaded onto 80 g column in 14 mL DCM with 2 drops MeOH and purified with flash chromatography (MeOH/DCM, 0-4% over 40 min.) Yield 4.033 g. LC-MS: calculated [M+H] 498.71, found 499.85.

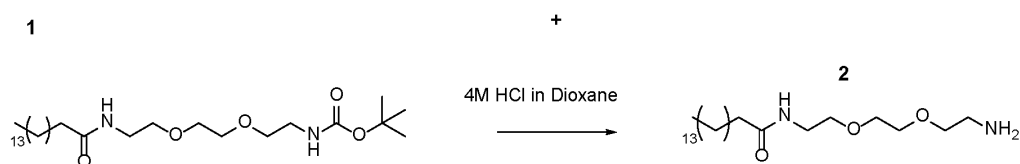


[0211] Compound 1 (4.033 g) was dissolved in 50 mL 1:1 DCM:TFA. The mixture was stirred for 1 hour. The product was concentrated on rotary evaporator and placed under high vacuum. The product was then dissolved in ACN, concentrated, then dissolved in DCM and concentrated again. Yield 3.839 g. LC-MS: calculated [M+H] 442.60, found 443.81.

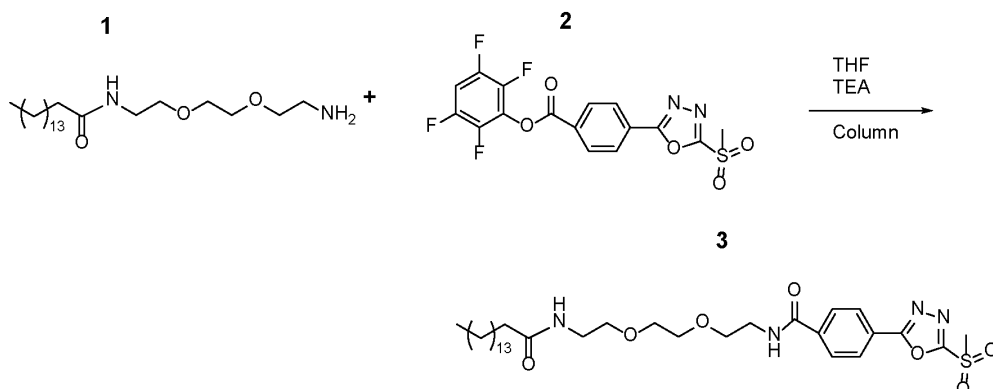
[0212] Synthesis of LP-371-p



[0213] Compound 1 (palmitic acid, 2.50 g) was dissolved in 60 mL DMF. Then TBTU (3.44 g) and DIPEA (6.9 mL) were added. The reaction was stirred for 10 minutes then compound 2 (2.66 g in DMF) was added. The reaction was complete after 1 hour. The mixture was diluted with 300 mL EtOAc and washed with 3% citric acid (3x60 mL), H₂O (2x60 mL), and NaCl (1x60 mL), then dried over Na₂SO₄. The product was filtered and concentrated on rotary evaporator and high vacuum. The product was purified using column chromatography (loaded in DCM (15 mL) with a drop of MeOH onto a 80G RediSep Gold Rf column, mobile phase MeOH/DCM, 0-5% over 30 minutes. Yield 4.094 g. LC-MS: calculated [M+H] 486.74, found 488.11.



[0214] Compound 1 (4.094 g) was dissolved in 4M HCl in dioxane (28 mL) at 0°C for 10 minutes. The reaction was allowed to warm to room temperature then stirred for 2 hours. The product was concentrated on rotary evaporator and high vacuum. Yield 3.485 g. LC-MS: calculated [M+H] 386.57, found 388.02.



[0215] Compound 1 (2.3 g) was dissolved in 80 mL THF. Then TEA (4.975 mL) and compound 2 (3.10 g) were added. The reaction was stirred for 1 hour. The reaction was dry loaded with Celite 545, the mixture was concentrated in a 28°C water bath and placed on high vacuum to fully dry. The product was purified with flash chromatography (MeOH/DCM, 0-6% over 40 min.) Yield 2.905 g. LC-MS: calculated [M+H] 636.85, found 637.95.

[0216] Linkers

[0217] Linker L6 was purchased as propargyl-PEG5-NHS from BroadPharm (catalog # BP-20907) and coupled to the NH₂-C₆ group from an aminolink phosphoramidite to form -L6-C₆-, using standard coupling conditions.

[0218] Conjugation of lipid PK/PD modulator precursors

[0219] Either prior to or after annealing, one or more lipid PK/PD modulator precursors can be linked to the RNAi agents disclosed herein. The following describes the general conjugation process used to link lipid PK/PD modulator precursors to the constructs set forth in the Examples depicted herein.

[0001] A. Conjugation of a sulfone-containing lipid PK/PD modulator precursor

[0002] In a vial, functionalized sense strand was dissolved at 50mg/mL in sterilized water. Then 20 equivalents of each of 0.1M Hepes pH 8.5 buffer and dithiothreitol are added. The mixture was allowed to react for one hour, then the conjugate was precipitated in acetonitrile and PBS, and the solids were centrifuged into a pellet.

[0003] The pellet was brought up in a 70/30 mixture of DMSO/water at a solids concentration of 30 mg/mL. Then, the sulfone-containing lipid PK/PD modulator precursor was added at 1.5 equivalents. The vial was purged with N₂, and heated to 40°C while stirring. The mixture was allowed to react for one hour. The product was purified on an AEX-HPLC (mobile phase A: 25 mM TRIS pH=7.2, 1 mM EDTA, 50% acetonitrile; mobile phase B: 25 mM TRIS pH=7.2, 1 mM EDTA, 500 mM NaBr, 50% acetonitrile; solid phase TSKgel-30; 1.5 cmx10 cm.) The solvent was removed by rotary evaporator, and desalted with a 3K spin column using 2x10 mL exchanges with sterilized water. The solid product was dried using lyophilization and stored for later use.

[0004] B. Conjugation of an azide-containing lipid PK/PD modulator precursor

[0005] One molar equivalent of TG-TBTA resin loaded with Cu(I) was weighed into a glass vial. The vial was purged with N₂ for 15 minutes. Then, functionalized sense strand was dissolved in a separate vial in sterilized water at a concentration of 100 mg/mL. Then two equivalents of the azide-containing lipid PK/PD modulator precursor (50 mg/mL in DMF) is added to the vial. Then TEA, DMF and water are added until the final reaction conditions are 33 mM TEA, 60% DMF, and 20 mg/mL of the conjugated product. The solution was then transferred to the vial with resin via a syringe. The N₂ purge was removed and the vial was sealed and moved to a stir plate at 40°C. The mixture was allowed to react for 16 hours. The resin was filtered off using a 0.45 µm filter.

[0006] The product was purified using AEX purification (mobile phase A: 25 mM TRIS pH=7.2, 1mM EDTA, 50% acetonitrile; mobile phase B: 25mM TRIS pH=7.2, 1mM EDTA, 500mM NaBr, 50% acetonitrile solid phase TSKgel-30; 1.5 cmx10 cm.) The acetonitrile was removed using a rotary evaporator, and desalted with a 3K spin column using 2x10 mL exchanges with sterilized water. The solid product was dried using lyophilization and stored for later use.

Example 2. *In Vivo Administration of ALK7 RNAi agents in Mice.*

[0220] ALK7 RNAi agents were evaluated *in vivo* in mice. On Day 1, five (n=5) female C57bl/6 mice in each group were given a single subcutaneous (SQ) injection of 250 µl per 25g body weight containing either 1.0 mg/kg (mpk), 3.0 mg/kg (mpk) of an ALK7 RNAi agent, or saline. Dosing was in accordance with Table 12 below.

[0221] **Table 12.** Dosing groups for the mice of Example 2.

Group ID	Dose (RNAi Agent)	# of Animals
1. Saline	Day 1: Single SQ Injection	n = 5
2. 1.0 mg/kg AC004391	Day 1: Single SQ Injection	n = 5
3. 3.0 mg/kg AC004391	Day 1: Single SQ Injection	n = 5
4. 1.0 mg/kg AC004390	Day 1: Single SQ Injection	n = 5
5. 3.0 mg/kg AC004390	Day 1: Single SQ Injection	n = 5
6. 1.0 mg/kg AC004392	Day 1: Single SQ Injection	n = 5
7. 3.0 mg/kg AC004392	Day 1: Single SQ Injection	n = 5
8. 1.0 mg/kg AC005181	Day 1: Single SQ Injection	n = 5
9. 3.0 mg/kg AC005181	Day 1: Single SQ Injection	n = 5
10. 1.0 mg/kg AC005824	Day 1: Single SQ Injection	n = 5
11. 3.0 mg/kg AC005824	Day 1: Single SQ Injection	n = 5
12. 1.0 mg/kg AC005823	Day 1: Single SQ Injection	n = 5
13. 3.0 mg/kg AC005823	Day 1: Single SQ Injection	n = 5

[0222] ALK7 RNAi agents AC004391, AC004390, AC004392, and AC005181 target and initiate RNAi and RNA-induced silencing complex (RISC) of mouse ALK7. ALK7 RNAi agents AC005824 and AC005823 target and initiate RNAi and RNA-induced silencing complex (RISC) of human ALK7.

[0223] Five (n=5) mice were dosed in each group. Mice were injected subcutaneously (SQ) on day 1. On day 15, mice were euthanized, and ~50 mg adipose tissues (inguinal white adipose tissue iWAT, perigonadal white adipose tissue pgWAT) were collected for analysis. Samples were analyzed by qPCR for mALK7 mRNA knockdown, using mARL1 as the endogenous control gene. Each group was normalized to group 1 (saline). Average results for each group are shown in Table 13 below.

[0224] **Table 13.** Relative expression of ALK7 mRNA in various tissues analyzed by qPCR for each of the dosing groups of Example 2.

Group ID	iWAT			pgWAT		
	Group Average (n=5)			Group Average (n=5)		
	Rel. Exp.	Error (Low)	Error (High)	Rel. Exp.	Error (Low)	Error (High)
1. Saline	1.000	0.111	0.124	1.000	0.144	0.168
2. 1.0 mg/kg AC004391	0.501	0.101	0.127	0.566	0.135	0.178
3. 3.0 mg/kg AC004391	0.504	0.085	0.103	0.503	0.097	0.121
4. 1.0 mg/kg AC004390	0.454	0.066	0.078	0.496	0.062	0.071
5. 3.0 mg/kg AC004390	0.339	0.097	0.137	0.394	0.142	0.223
6. 1.0 mg/kg AC004392	0.542	0.109	0.137	0.520	0.124	0.163
7. 3.0 mg/kg AC004392	0.376	0.095	0.127	0.542	0.125	0.163
8. 1.0 mg/kg AC005181	0.399	0.057	0.067	0.349	0.091	0.124
9. 3.0 mg/kg AC005181	0.337	0.069	0.087	0.187	0.063	0.096
10. 1.0 mg/kg AC005824	0.505	0.130	0.175	0.461	0.103	0.133
11. 3.0 mg/kg AC005824	0.294	0.039	0.044	0.347	0.062	0.075
12. 1.0 mg/kg AC005823	0.422	0.122	0.171	0.407	0.114	0.158
13. 3.0 mg/kg AC005823	0.345	0.100	0.141	0.322	0.104	0.154

[0225] As shown in Table 13, Groups 2-13 showed reductions in ALK7 in comparison to Group 1 dosed with no ALK7 RNAi agent, in both iWAT and pgWAT. Particularly, ALK7 RNAi agent AC005824 at 3.0 mg/kg dose achieved ~70% ALK7 inhibition (0.294) in iWAT on Day 15. Dose response was observed in Groups 4&5, 6&7, 8&9, 10&11, and 12&13 in iWAT, and also observed in Groups 2&3, 4&5, 8&9, 10&11, and 12&13 in pgWAT.

Example 3. ALK7-SEAP Mouse Model.

[0226] To evaluate ALK7 RNAi agents *in vivo*, an ALK7-SEAP mouse model was used. C57bl6/Albino female mice were transiently transfected *in vivo* with plasmid by hydrodynamic tail vein (HTV) injection. Mice were injected, via hydrodynamic tail vein

(HTV) injection, with plasmid pMIR1066 containing the nucleobases 220-3200 of the ALK7 cDNA sequence (NCBI Reference Sequence: NM_145259.3 (Seq ID No. 1)) inserted into the 3' UTR of the SEAP (secreted human placental alkaline phosphatase) reporter gene. 20 µg of the plasmid containing the ALK7 genome in Ringer's solution in a total volume of 10% of the animal's body weight was injected, via HTV, to create ALK7-SEAP model mice. Following transfection with ALK7-SEAP, the mice were subsequently administered ALK7 RNAi agents. Inhibition of ALK7 gene expression by ALK7 RNAi agent results in concomitant inhibition of SEAP expression. SEAP expression levels were measured by Phospha-Light™ SEAP Reporter Gene Assay System (ThermoFisher Cat #T1016). Prior to treatment, SEAP expression levels in serum were measured and the mice were grouped according to average SEAP levels.

[0227] Analyses: SEAP levels may be measured at various times, both before and after administration of ALK7 RNAi agents.

[0228] i) Serum collection: Mice were anesthetized with 2-3% isoflurane and blood samples were collected from the submandibular area into serum separation tubes (Sarstedt AG & Co., Nümbrecht, Germany). Blood was allowed to coagulate at ambient temperature for 20 min. The tubes were centrifuged at 8,000 ×g for 3 min to separate the serum and stored at 4°C.

[0229] ii) Serum SEAP levels: Serum was collected and measured by the Phospha-Light™ SEAP Reporter Gene Assay System (ThermoFisher) according to the manufacturer's instructions. Serum SEAP levels for each animal was normalized to the control group of mice injected with saline in order to account for the non-treatment related decline in ALK7 sequence expression with this model. First, the SEAP level for each animal at a time point was divided by the pre-treatment level of expression in that animal ("pre-treatment") in order to determine the ratio of expression "normalized to pre-treatment". Expression at a specific time point was then normalized to the control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the average "normalized to pre-treatment" ratio of all mice in the normal saline control group. Alternatively, in some Examples set forth herein, the serum SEAP levels for each animal were assessed by normalizing to pre-treatment levels only.

Example 4. In vivo administration of ALK7 RNAi agents in ALK7-SEAP mice.

[0230] The ALK7-SEAP model described in Example 3, above, was used. On Day -21, four (n=4) female C57bl/6 albino mice in each group were dosed with plasmid containing the nucleobases 220-3200 of the ALK7 cDNA sequence, via HTV injection. On Day 1, the mice

were dosed with either isotonic saline or ALK7 RNAi agents formulated in saline (at 3 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g (10 mL/kg) body weight injection volume. The dosing regimen is in accordance with Table 14 below.

[0231] Table 14. Dosing for mice of Example 4.

Group	Dose (RNAi Agent)	Dosing Route	# of Animals (n=)
1	Saline	Day 1 SQ Injection	n = 4
2	3 mg/kg AC004197	Day 1 SQ Injection	n = 4
3	3 mg/kg AC004198	Day 1 SQ Injection	n = 4
4	3 mg/kg AC004199	Day 1 SQ Injection	n = 4
5	3 mg/kg AC004200	Day 1 SQ Injection	n = 4
6	3 mg/kg AC004201	Day 1 SQ Injection	n = 4
7	3 mg/kg AC004202	Day 1 SQ Injection	n = 4
8	3 mg/kg AC004204	Day 1 SQ Injection	n = 4
9	3 mg/kg AC004205	Day 1 SQ Injection	n = 4
10	3 mg/kg AC004206	Day 1 SQ Injection	n = 4
11	3 mg/kg AC004207	Day 1 SQ Injection	n = 4
12	3 mg/kg AC004208	Day 1 SQ Injection	n = 4

[0232] Serum was collected on Day -7, 1, 8, 15, and 22. SEAP expression levels were determined pursuant to the procedure set forth in Example 3, above. Data from the experiment are shown in the following Table 15, with average SEAP reflecting the normalized average value of SEAP.

[0233] Table 15. Average SEAP normalized to pre-treatment and saline control in ALK7-SEAP mice of Example 4.

Group ID	Day 8		Day 15		Day 22	
	Avg SEAP	Std Dev	Avg SEAP	Std Dev	Avg SEAP	Std Dev
1. Saline	1.000	0.091	1.000	0.299	1.000	0.231
2. 3 mg/kg AC004197	0.334	0.151	0.219	0.122	0.249	0.176
3. 3 mg/kg AC004198	0.701	0.165	0.740	0.244	0.544	0.328
4. 3 mg/kg AC004199	0.452	0.105	0.473	0.213	0.457	0.143
5. 3 mg/kg AC004200	0.086	0.063	0.039	0.025	0.023	0.027
6. 3 mg/kg AC004201	0.141	0.083	0.097	0.063	0.081	0.063
7. 3 mg/kg AC004202	0.164	0.018	0.076	0.053	0.053	0.042

8. 3 mg/kg AC004204	0.466	0.120	0.484	0.112	0.539	0.087
9. 3 mg/kg AC004205	0.428	0.127	0.525	0.186	0.561	0.239
10. 3 mg/kg AC004206	0.267	0.126	0.170	0.073	0.208	0.067
11. 3 mg/kg AC004207	0.212	0.102	0.180	0.071	0.173	0.053
12. 3 mg/kg AC004208	0.344	0.078	0.316	0.062	0.343	0.174

[0234] Groups 2-12 showed reduction in SEAP-ALK7 at all time points compared to the saline control Group 1. In particular, AC004200 achieved substantial inhibition (0.023, ~98% knockdown) after a single subcutaneous administration of 3 mg/kg at Day 22.

Example 5. In vivo administration of ALK7 RNAi agents in ALK7-SEAP mice.

[0235] The ALK7-SEAP model described in Example 3, above, was used. On Day -21, four (n=4) female C57bl/6 albino mice in each group were dosed with plasmid containing the nucleobases 220-3200 of the ALK7 cDNA sequence, via HTV injection. On Day 1, the mice were dosed with either isotonic saline or ALK7 RNAi agents formulated in saline (at 3 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g (10 mL/kg) body weight injection volume. The dosing regimen is in accordance with Table 16 below.

[0236] **Table 16.** Dosing for mice of Example 5.

Group	Dose (RNAi Agent)	Dosing Route	# of Animals (n=)
1	Saline	Day 1 SQ Injection	n = 4
2	3 mg/kg AC004210	Day 1 SQ Injection	n = 4
3	3 mg/kg AC004211	Day 1 SQ Injection	n = 4
4	3 mg/kg AC004212	Day 1 SQ Injection	n = 4
5	3 mg/kg AC004213	Day 1 SQ Injection	n = 4
6	3 mg/kg AC004214	Day 1 SQ Injection	n = 4
7	3 mg/kg AC004215	Day 1 SQ Injection	n = 4
8	3 mg/kg AC004216	Day 1 SQ Injection	n = 4
9	3 mg/kg AC004217	Day 1 SQ Injection	n = 4
10	3 mg/kg AC004218	Day 1 SQ Injection	n = 4
11	3 mg/kg AC004219	Day 1 SQ Injection	n = 4
12	3 mg/kg AC004220	Day 1 SQ Injection	n = 4
13	3 mg/kg AC004221	Day 1 SQ Injection	n = 4

14	3 mg/kg AC004222	Day 1 SQ Injection	n = 4
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[0237] Serum was collected on Day -6, 1, 8, 15, and 22. SEAP expression levels were determined pursuant to the procedure set forth in Example 3, above. Data from the experiment are shown in the following Table 17, with average SEAP reflecting the normalized average value of SEAP.

[0238] **Table 17.** Average SEAP normalized to pre-treatment and saline control in ALK7-SEAP mice of Example 5.

Group ID	Day 8		Day 15		Day 22	
	Avg SEAP	Std Dev	Avg SEAP	Std Dev	Avg SEAP	Std Dev
1. Saline	1.000	0.151	1.000	0.269	1.000	0.359
2. 3 mg/kg AC004210	0.388	0.154	0.225	0.203	0.221	0.197
3. 3 mg/kg AC004211	0.251	0.126	0.279	0.219	0.680	1.002
4. 3 mg/kg AC004212	0.129	0.043	0.057	0.060	0.063	0.060
5. 3 mg/kg AC004213	0.375	0.119	0.223	0.150	0.140	0.122
6. 3 mg/kg AC004214	0.156	0.108	0.036	0.033	0.140	0.193
7. 3 mg/kg AC004215	0.121	0.065	0.099	0.102	0.136	0.161
8. 3 mg/kg AC004216	0.235	0.127	0.082	0.084	0.177	0.163
9. 3 mg/kg AC004217	0.370	0.121	0.246	0.061	0.264	0.098
10. 3 mg/kg AC004218	0.344	0.106	0.357	0.064	0.537	0.314
11. 3 mg/kg AC004219	0.487	0.136	0.290	0.079	0.316	0.117
12. 3 mg/kg AC004220	0.383	0.153	0.219	0.086	0.263	0.200
13. 3 mg/kg AC004221	0.324	0.132	0.233	0.067	0.164	0.061
14. 3 mg/kg AC004222	0.139	0.062	0.091	0.032	0.044	0.016

[0239] Groups 2-14 showed reduction in SEAP-ALK7 at all time points compared to the saline control Group 1. In particular, on AC004214 achieved substantial inhibition (0.036, ~96% knockdown) after a single subcutaneous administration of 3 mg/kg at Day 15.

Example 6. ALK7-GLuc AAV Mouse Model.

[0240] To evaluate certain ALK7 RNAi agents, an ALK7-GLuc (Gaussia Luciferase) AAV (Adeno-associated virus) mouse model was used. Six- to eight-week-old male C57BL/6 mice were transduced with ALK7-GLuc AAV serotype 8, administered at least 14 days prior to administration of an ALK7 RNAi agent or control. The genome of the ALK7-GLuc AAV

contains the 220-3200 region of the human ALK7 cDNA sequence (GenBank NM_145259.3 (Seq ID No. 1)) inserted into the 3' UTR of the GLuc reporter gene sequence. 5E12 to 1E13 GC/kg (genome copies per kg animal body weight) of the respective virus in PBS in a total volume of 10 mL/kg animal's body weight was injected into mice via the tail vein to create ALK7-GLuc AAV model mice. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured. Prior to administration of a treatment (between day -7 and day 1 pre-dose), GLuc expression levels in serum were measured by the Pierce™ Gaussia Luciferase Glow Assay Kit (Thermo Fisher Scientific), and the mice were grouped according to average GLuc levels.

[0241] Mice were anesthetized with 2-3% isoflurane and blood samples were collected from the submandibular area into serum separation tubes (Sarstedt AG & Co., Nümbrecht, Germany). Blood was allowed to coagulate at ambient temperature for 20 min. The tubes were centrifuged at $8,000 \times g$ for 3 min to separate the serum and stored at 4°C. Serum was collected and measured by the Pierce™ Gaussia Luciferase Glow Assay Kit according to the manufacturer's instructions. Serum GLuc levels for each animal can be normalized to the control group of mice injected with vehicle control in order to account for the non-treatment related shift in ALK7 expression with this model. To do so, first, the GLuc level for each animal at a time point was divided by the pre-treatment level of expression in that animal (Day 1) in order to determine the ratio of expression "normalized to pre-treatment". Expression at a specific time point was then normalized to the control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the average "normalized to pre-treatment" ratio of all mice in the normal vehicle control group. Alternatively, the serum GLuc levels for each animal was assessed by normalizing to pre-treatment levels only.

Example 7. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0242] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day -21, four (n=4) male C57bl/6 mice in each group were dosed with $\sim 5 \times 10^{12}$ GC/kg ALK7-GLuc AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline

(at 3.0 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 18 below.

[0243] Table 18. Dosing Groups of Example 7.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
1	Saline	N/A	Day 1 SQ Injection	n = 4
2	AC004197	3.0 mg/kg	Day 1 SQ Injection	n = 4
3	AC004198	3.0 mg/kg	Day 1 SQ Injection	n = 4
4	AC004199	3.0 mg/kg	Day 1 SQ Injection	n = 4
5	AC004200	3.0 mg/kg	Day 1 SQ Injection	n = 4
6	AC004201	3.0 mg/kg	Day 1 SQ Injection	n = 4
7	AC004202	3.0 mg/kg	Day 1 SQ Injection	n = 4
8	AC004203	3.0 mg/kg	Day 1 SQ Injection	n = 4
9	AC004204	3.0 mg/kg	Day 1 SQ Injection	n = 4
10	AC004205	3.0 mg/kg	Day 1 SQ Injection	n = 4
11	AC004206	3.0 mg/kg	Day 1 SQ Injection	n = 4
12	AC004207	3.0 mg/kg	Day 1 SQ Injection	n = 4
13	AC004208	3.0 mg/kg	Day 1 SQ Injection	n = 4
14	AC004209	3.0 mg/kg	Day 1 SQ Injection	n = 4

[0244] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0245] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and

structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0246] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 19, with average GLuc reflecting the normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0247] **Table 19.** Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 7.

Group ID	Day 8		Day 15		Day 22	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. Saline	1.000	0.148	1.000	0.038	1.000	0.054
2. 3.0 mg/kg AC004197	0.165	0.012	0.132	0.016	0.171	0.032
3. 3.0 mg/kg AC004198	0.695	0.048	0.736	0.060	0.874	0.032
4. 3.0 mg/kg AC004199	0.172	0.026	0.173	0.013	0.234	0.023
5. 3.0 mg/kg AC004200	0.085	0.010	0.084	0.010	0.081	0.012
6. 3.0 mg/kg AC004201	0.112	0.012	0.123	0.011	0.120	0.022
7. 3.0 mg/kg AC004202	0.098	0.023	0.112	0.027	0.105	0.007
8. 3.0 mg/kg AC004203	0.254	0.050	0.305	0.053	0.268	0.068
9. 3.0 mg/kg AC004204	0.249	0.064	0.295	0.063	0.283	0.091
10. 3.0 mg/kg AC004205	0.295	0.043	0.399	0.073	0.351	0.053
11. 3.0 mg/kg AC004206	0.216	0.035	0.250	0.038	0.226	0.027
12. 3.0 mg/kg AC004207	0.164	0.009	0.173	0.029	0.148	0.014
13. 3.0 mg/kg AC004208	0.242	0.032	0.237	0.042	0.228	0.033
14. 3.0 mg/kg AC004209	0.657	0.121	0.778	0.113	0.734	0.088

[0248] Groups 2-14 showed reduction in ALK7-GLuc at all time points compared to the saline control Group 1. Particularly, AC004200 achieved substantial inhibition (0.081, ~92% knockdown) at 3.0 mg/kg on Day 22.

Example 8. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0249] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day -21, four (n=4) male C57bl/6 mice in each group were dosed with $\sim 5 \times 10^{12}$ GC/kg ALK7-

GLuc AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline (at 3.0 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 20 below.

[0250] Table 20. Dosing Groups of Example 8.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
1	Saline	N/A	Day 1 SQ Injection	n = 4
2	AC004210	3.0 mg/kg	Day 1 SQ Injection	n = 4
3	AC004211	3.0 mg/kg	Day 1 SQ Injection	n = 4
4	AC004212	3.0 mg/kg	Day 1 SQ Injection	n = 4
5	AC004213	3.0 mg/kg	Day 1 SQ Injection	n = 4
6	AC004214	3.0 mg/kg	Day 1 SQ Injection	n = 4
7	AC004215	3.0 mg/kg	Day 1 SQ Injection	n = 4
8	AC004216	3.0 mg/kg	Day 1 SQ Injection	n = 4
9	AC004217	3.0 mg/kg	Day 1 SQ Injection	n = 4
10	AC004218	3.0 mg/kg	Day 1 SQ Injection	n = 4
11	AC004219	3.0 mg/kg	Day 1 SQ Injection	n = 4
12	AC004220	3.0 mg/kg	Day 1 SQ Injection	n = 4
13	AC004221	3.0 mg/kg	Day 1 SQ Injection	n = 4
14	AC004222	3.0 mg/kg	Day 1 SQ Injection	n = 4

[0251] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0252] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and

structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0253] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 21, with average GLuc reflecting the normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0254] **Table 21.** Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 8.

Group ID	Day 8		Day 15		Day 22	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. Saline	1.000	0.079	1.000	0.157	1.000	0.161
2. 3.0 mg/kg AC004210	0.370	0.094	0.491	0.056	0.525	0.062
3. 3.0 mg/kg AC004211	0.181	0.017	0.294	0.035	0.287	0.045
4. 3.0 mg/kg AC004212	0.141	0.028	0.273	0.018	0.267	0.027
5. 3.0 mg/kg AC004213	0.392	0.051	0.489	0.033	0.500	0.091
6. 3.0 mg/kg AC004214	0.149	0.018	0.263	0.029	0.275	0.027
7. 3.0 mg/kg AC004215	0.125	0.020	0.245	0.033	0.264	0.022
8. 3.0 mg/kg AC004216	0.175	0.043	0.288	0.064	0.316	0.057
9. 3.0 mg/kg AC004217	0.394	0.023	0.478	0.071	0.568	0.062
10. 3.0 mg/kg AC004218	0.492	0.127	0.573	0.186	0.624	0.172
11. 3.0 mg/kg AC004219	0.397	0.066	0.485	0.058	0.566	0.087
12. 3.0 mg/kg AC004220	0.386	0.061	0.422	0.040	0.429	0.040
13. 3.0 mg/kg AC004221	0.175	0.016	0.283	0.027	0.366	0.068
14. 3.0 mg/kg AC004222	0.150	0.021	0.234	0.053	0.260	0.049

[0255] Groups 2-14 showed reduction in ALK7-GLuc at all time points compared to the saline control Group 1. In particular, AC004215 achieved ~87% inhibition (0.125) at 3.0 mg/kg on Day 8.

Example 9. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0256] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day - 21, six (n=6) male C57bl/6 mice in each group were dosed with $\sim 5 \times 10^{12}$ GC/kg ALK7-GLuc

AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline (at 0.75 or 1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 22 below.

[0257] **Table 22.** Dosing Groups of Example 9.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
1	Saline	N/A	Day 1 SQ Injection	n = 6
2	AC004200	0.75 mg/kg	Day 1 SQ Injection	n = 6
3	AC004200	1.5 mg/kg	Day 1 SQ Injection	n = 6
4	AC004201	0.75 mg/kg	Day 1 SQ Injection	n = 6
5	AC004201	1.5 mg/kg	Day 1 SQ Injection	n = 6
6	AC004202	0.75 mg/kg	Day 1 SQ Injection	n = 6
7	AC004202	1.5 mg/kg	Day 1 SQ Injection	n = 6
8	AC004212	0.75 mg/kg	Day 1 SQ Injection	n = 6
9	AC004212	1.5 mg/kg	Day 1 SQ Injection	n = 6
10	AC004214	0.75 mg/kg	Day 1 SQ Injection	n = 6
11	AC004214	1.5 mg/kg	Day 1 SQ Injection	n = 6
12	AC004215	0.75 mg/kg	Day 1 SQ Injection	n = 6
13	AC004215	1.5 mg/kg	Day 1 SQ Injection	n = 6

[0258] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0259] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and

structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0260] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 23, with average GLuc reflecting the normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0261] **Table 23.** Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 9.

Group ID	Day 8		Day 15		Day 22	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. Saline	1.000	0.168	1.000	0.174	1.000	0.111
2. 0.75 mg/kg AC004200	0.198	0.054	0.187	0.034	0.194	0.047
3. 1.5 mg/kg AC004200	0.098	0.016	0.097	0.023	0.100	0.032
4. 0.75 mg/kg AC004201	0.342	0.072	0.339	0.055	0.386	0.139
5. 1.5 mg/kg AC004201	0.156	0.048	0.191	0.037	0.178	0.032
6. 0.75 mg/kg AC004202	0.218	0.048	0.242	0.046	0.285	0.051
7. 1.5 mg/kg AC004202	0.138	0.016	0.128	0.024	0.170	0.033
8. 0.75 mg/kg AC004212	0.300	0.047	0.279	0.032	0.319	0.061
9. 1.5 mg/kg AC004212	0.149	0.034	0.140	0.024	0.154	0.026
10. 0.75 mg/kg AC004214	0.253	0.032	0.220	0.013	0.208	0.021
11. 1.5 mg/kg AC004214	0.145	0.022	0.165	0.018	0.155	0.015
12. 0.75 mg/kg AC004215	0.303	0.056	0.355	0.064	0.365	0.088
13. 1.5 mg/kg AC004215	0.212	0.013	0.211	0.039	0.197	0.031

[0262] Groups 2-13 showed reduction in ALK7-GLuc at all time points compared to the saline control Group 1. Particularly, AC004200 achieved ~90% inhibition (0.097) at 1.5 mg/kg on Day 15. Furthermore, dose response was observed for each of the ALK7 RNAi agent tested, at all time points.

Example 10. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0263] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day - 21, six (n=6) male C57bl/6 mice in each group were dosed with $\sim 5 \times 10^{12}$ GC/kg ALK7-GLuc

AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline (at 1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 24 below.

[0264] Table 24. Dosing Groups of Example 10.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
1	Saline	N/A	Day 1 SQ Injection	n = 6
2	AC004200	1.5 mg/kg	Day 1 SQ Injection	n = 6
3	AC005846	1.5 mg/kg	Day 1 SQ Injection	n = 6
4	AC005847	1.5 mg/kg	Day 1 SQ Injection	n = 6
5	AC005848	1.5 mg/kg	Day 1 SQ Injection	n = 6
6	AC005849	1.5 mg/kg	Day 1 SQ Injection	n = 6
7	AC005850	1.5 mg/kg	Day 1 SQ Injection	n = 6
8	AC005851	1.5 mg/kg	Day 1 SQ Injection	n = 6
9	AC005852	1.5 mg/kg	Day 1 SQ Injection	n = 6
10	AC005853	1.5 mg/kg	Day 1 SQ Injection	n = 6
11	AC005855	1.5 mg/kg	Day 1 SQ Injection	n = 6
12	AC005854	1.5 mg/kg	Day 1 SQ Injection	n = 6

[0265] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0266] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0267] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 25, with average GLuc reflecting the

normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0268] Table 25. Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 10.

Group ID	Day 8		Day 15		Day 22	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. Saline	1.000	0.115	1.000	0.163	1.000	0.170
2. 1.5 mg/kg AC004200	0.192	0.046	0.132	0.034	0.131	0.017
3. 1.5 mg/kg AC005846	0.177	0.043	0.107	0.012	0.111	0.014
4. 1.5 mg/kg AC005847	0.187	0.025	0.105	0.017	0.113	0.015
5. 1.5 mg/kg AC005848	0.225	0.064	0.121	0.019	0.145	0.019
6. 1.5 mg/kg AC005849	0.286	0.071	0.140	0.038	0.204	0.047
7. 1.5 mg/kg AC005850	0.267	0.058	0.138	0.027	0.163	0.033
8. 1.5 mg/kg AC005851	0.238	0.065	0.123	0.020	0.131	0.020
9. 1.5 mg/kg AC005852	0.326	0.043	0.175	0.019	0.231	0.049
10. 1.5 mg/kg AC005853	0.251	0.091	0.150	0.051	0.179	0.049
11. 1.5 mg/kg AC005855	0.239	0.050	0.141	0.035	0.189	0.052
12. 1.5 mg/kg AC005854	0.156	0.035	0.098	0.030	0.139	0.028

[0269] Groups 2-12 showed reduction in ALK7-GLuc at all time points compared to the saline control Group 1. Particularly, AC005854 achieved ~90% inhibition (0.098) at 1.5 mg/kg on Day 15.

Example 11. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0270] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day -21, six (n=6) male C57bl/6 mice in each group were dosed with $\sim 5 \times 10^{12}$ GC/kg ALK7-GLuc AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline (at

1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 26 below.

[0271] **Table 26.** Dosing Groups of Example 11.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
1	Saline	N/A	Day 1 SQ Injection	n = 6
2	AC004202	1.5 mg/kg	Day 1 SQ Injection	n = 6
3	AC005867	1.5 mg/kg	Day 1 SQ Injection	n = 6
4	AC005868	1.5 mg/kg	Day 1 SQ Injection	n = 6
5	AC005869	1.5 mg/kg	Day 1 SQ Injection	n = 6
6	AC005870	1.5 mg/kg	Day 1 SQ Injection	n = 6
7	AC005871	1.5 mg/kg	Day 1 SQ Injection	n = 6
8	AC005872	1.5 mg/kg	Day 1 SQ Injection	n = 6
9	AC005873	1.5 mg/kg	Day 1 SQ Injection	n = 6
10	AC005874	1.5 mg/kg	Day 1 SQ Injection	n = 6
11	AC005875	1.5 mg/kg	Day 1 SQ Injection	n = 6
12	AC005876	1.5 mg/kg	Day 1 SQ Injection	n = 6

[0272] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0273] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0274] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 27, with average GLuc reflecting the

normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0275] Table 27. Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 11.

Group ID	Day 8		Day 15		Day 22	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. Saline	1.000	0.074	1.000	0.186	1.000	0.189
2. 1.5 mg/kg AC004202	0.154	0.020	0.187	0.026	0.230	0.038
3. 1.5 mg/kg AC005867	0.253	0.068	0.304	0.057	0.335	0.078
4. 1.5 mg/kg AC005868	0.170	0.030	0.228	0.032	0.271	0.054
5. 1.5 mg/kg AC005869	0.214	0.046	0.276	0.082	0.305	0.076
6. 1.5 mg/kg AC005870	0.307	0.064	0.353	0.080	0.385	0.067
7. 1.5 mg/kg AC005871	0.368	0.352	0.466	0.509	0.458	0.400
8. 1.5 mg/kg AC005872	0.250	0.037	0.323	0.037	0.434	0.137
9. 1.5 mg/kg AC005873	0.217	0.020	0.268	0.058	0.310	0.037
10. 1.5 mg/kg AC005874	0.244	0.021	0.288	0.030	0.321	0.055
11. 1.5 mg/kg AC005875	0.217	0.043	0.252	0.057	0.340	0.073
12. 1.5 mg/kg AC005876	0.254	0.073	0.297	0.086	0.316	0.088

[0276] Groups 2-12 showed reduction in ALK7-GLuc at all time points compared to the saline control Group 1. Particularly, AC004202 achieved ~85% inhibition (0.154) at 1.5 mg/kg on Day 8.

Example 12. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0277] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day -21, five (n=5) male C57bl/6 mice in each group were dosed with $\sim 5 \times 10^{12}$ GC/kg ALK7-GLuc AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline

(at 1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 28 below.

[0278] Table 28. Dosing Groups of Example 12.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
1	Saline	N/A	Day 1 SQ Injection	n = 5
2	AC004201	1.5 mg/kg	Day 1 SQ Injection	n = 5
3	AC005856	1.5 mg/kg	Day 1 SQ Injection	n = 5
4	AC005857	1.5 mg/kg	Day 1 SQ Injection	n = 5
5	AC006111	1.5 mg/kg	Day 1 SQ Injection	n = 5
6	AC005858	1.5 mg/kg	Day 1 SQ Injection	n = 5
7	AC005859	1.5 mg/kg	Day 1 SQ Injection	n = 5
8	AC005860	1.5 mg/kg	Day 1 SQ Injection	n = 5
9	AC005861	1.5 mg/kg	Day 1 SQ Injection	n = 5
10	AC005862	1.5 mg/kg	Day 1 SQ Injection	n = 5
11	AC005863	1.5 mg/kg	Day 1 SQ Injection	n = 5
12	AC005864	1.5 mg/kg	Day 1 SQ Injection	n = 5
13	AC005865	1.5 mg/kg	Day 1 SQ Injection	n = 5

[0279] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0280] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0281] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 29, with average GLuc reflecting the

normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0282] Table 29. Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 12.

Group ID	Day 8		Day 15		Day 22	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. 1.5 mg/kg Saline	1.000	0.324	1.000	0.181	1.000	0.322
2. 1.5 mg/kg AC004201	0.228	0.039	0.204	0.040	0.228	0.057
3. 1.5 mg/kg AC005856	0.311	0.082	0.292	0.086	0.307	0.066
4. 1.5 mg/kg AC005857	0.193	0.047	0.143	0.038	0.183	0.037
5. 1.5 mg/kg AC006111	0.172	0.036	0.172	0.026	0.206	0.051
6. 1.5 mg/kg AC005858	0.177	0.078	0.143	0.055	0.197	0.095
7. 1.5 mg/kg AC005859	0.322	0.087	0.256	0.097	0.321	0.115
8. 1.5 mg/kg AC005860	0.589	0.158	0.514	0.064	0.579	0.073
9. 1.5 mg/kg AC005861	0.275	0.087	0.217	0.048	0.257	0.078
10. 1.5 mg/kg AC005862	0.480	0.196	0.412	0.186	0.581	0.206
11. 1.5 mg/kg AC005863	0.390	0.080	0.339	0.062	0.469	0.101
12. 1.5 mg/kg AC005864	0.127	0.051	0.147	0.091	0.187	0.108
13. 1.5 mg/kg AC005865	0.541	0.063	0.437	0.114	0.598	0.120

[0283] Groups 2-13 showed reduction in ALK7-GLuc at all time points compared to the saline control Group 1. Particularly, AC005864 achieved ~87% inhibition (0.127) at 1.5 mg/kg on Day 8.

Example 13. In vivo administration of ALK7 RNAi agents in Cynomolgus Monkeys.

[0284] ALK7 RNAi agents were tested in Cynomolgus monkeys for inhibition of ALK7. On Day 1 and Day 29, three (n=3) male Cynomolgus monkeys for each test group were dosed with ALK7 RNAi agents formulated in saline (at 3.0 mg/kg), via subcutaneous (SQ) injection with syringe and needle in the mid-scapular region, at 0.3 mL/kg dose volume. Adipose biopsies were collected from all test animals on Day -7 (pre-dose), 15, 29, 57, and 85. The dosing regimen was in accordance with Table 30 below.

[0285] Table 30. Dosing for test animals of Example 13.

Group	Dose (RNAi Agent)	# of Animals	Dosing Route	Dose Volume
1	3.0 mg/kg AC006188	n = 3	Day 1 & 29 SQ Injection	0.3 mL/kg
2	3.0 mg/kg AC006189	n = 3	Day 1 & 29 SQ Injection	0.3 mL/kg

[0286] All animals were fasted for at least 12 hours and less than 24 hours for scheduled blood collections and biopsy procedures. Blood collection site was femoral vein. A saphenous vein (not used for dose administration) may be used as an alternative collection site.

[0287] Adipose biopsies were collected as a sedated procedure. Sedation was accomplished using Ketamine HCl (10 mg/kg) or Telazol (5-8mg/kg), administered as an intramuscular (IM) injection and supplemented with Ketamine (5 mg/kg) as needed.

[0288] An approximate 3-5 cm skin incision was made followed by collection of adipose tissue (optimally 50-150 mg). The skin will then be closed in a routine manner using suture (or alternate) materials maintaining aseptic technique.

[0289] Each biopsy site was separated by at least 1-2 cm. Biopsies were separated into 2 pieces (one piece of ~25 to 75 mg and second piece of ~25-75 mg) for each collection time point. Analgesics may be administered at veterinarian's discretion.

[0290] Blood was collected into tubes containing no anticoagulant (serum separator tubes). Blood was allowed to clot at ambient temperature prior to centrifugation to obtain serum.

[0291] Blood was collected on Day -7, Day 1, Day 15, Day 29, Day 57, and Day 85, prior to biopsy sample collections or dose administration (when applicable), and from any animals found in moribund condition or sacrificed at an unscheduled interval. Samples may be further collected at additional timepoints. Should there be any additional collected samples at other timepoints, their corresponding relevant data are shown below.

[0292] Individual doses of ALK7 RNAi agents were calculated based on the body weights recorded on each day of dosing.

[0293] The adipose biopsies and serum collected from the test animals were used for analysis for ALK7 expression and additional biological parameters. ALK7 mRNA expression levels were quantified via qPCR, using cARL1 as the endogenous control gene, normalized to Day -7. The data is shown in the following Table 31.

[0294] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end and the 3' terminal end of the sense strand to a lipid moiety having the

modified sequences as set forth in the duplex structures herein (see Tables 3, 6, 6a, 8, 9, and 10, for specific modifications and structure information related to the ALK7 RNAi agents; see Table 11 for structures of LP-379-a and LP-371-a).

[0295] **Table 31.** ALK7 expression in adipose tissues of Cynomolgus monkeys of Example 13.

	Day -7			Day 15		
Group ID	Rel. Exp.	Error Low	Error High	Rel. Exp.	Error Low	Error High
1. 3.0 mg/kg AC006188	1.000	0.212	0.269	0.171	0.073	0.128
2. 3.0 mg/kg AC006189	1.000	0.161	0.191	0.105	0.054	0.110
	Day 29			Day 57		
Group ID	Rel. Exp.	Error Low	Error High	Rel. Exp.	Error Low	Error High
1. 3.0 mg/kg AC006188	0.119	0.051	0.090	0.205	0.028	0.032
2. 3.0 mg/kg AC006189	0.139	0.032	0.041	0.212	0.044	0.056
	Day 85			Day 113		
Group ID	Rel. Exp.	Error Low	Error High	Rel. Exp.	Error Low	Error High
1. 3.0 mg/kg AC006188	0.229	0.075	0.111	0.197	0.055	0.075
2. 3.0 mg/kg AC006189	0.285	0.125	0.221	0.321	0.172	0.372

[0296] Both Groups 1 and 2, AC006188 and AC006189 showed significant inhibition of ALK7 in cynomolgus monkeys' adipose tissues. Most notably, at nadir, AC006189 achieved ~89% ALK7 inhibition (0.105) at 3.0 mg/kg on Day 15. Both AC006188 and AC006189 showed ALK7 inhibition out to at least Day 113, with AC006188 achieving ~80% ALK7 inhibition (0.197) at 3.0 mg/kg (2x dose on Day 1 and Day 29) on Day 113.

Example 14. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0297] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day -21, five (n=5) male C57bl/6 mice in each group were dosed with $\sim 5 \times 10^{12}$ GC/kg ALK7-GLuc AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline

(at 1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 32 below.

[0298] Table 32. Dosing Groups of Example 14.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
1	Saline	N/A	Day 1 SQ Injection	n = 5
2	AC006111	1.5 mg/kg	Day 1 SQ Injection	n = 5
3	AC006738	1.5 mg/kg	Day 1 SQ Injection	n = 5
4	AC006747	1.5 mg/kg	Day 1 SQ Injection	n = 5
5	AC006773	1.5 mg/kg	Day 1 SQ Injection	n = 5
6	AC004202	1.5 mg/kg	Day 1 SQ Injection	n = 5
7	AC006741	1.5 mg/kg	Day 1 SQ Injection	n = 5
8	AC006742	1.5 mg/kg	Day 1 SQ Injection	n = 5
9	AC006743	1.5 mg/kg	Day 1 SQ Injection	n = 5
10	AC004212	1.5 mg/kg	Day 1 SQ Injection	n = 5
11	AC006744	1.5 mg/kg	Day 1 SQ Injection	n = 5
12	AC006745	1.5 mg/kg	Day 1 SQ Injection	n = 5
13	AC006746	1.5 mg/kg	Day 1 SQ Injection	n = 5

[0299] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0300] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0301] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 33, with average GLuc reflecting the

normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0302] Table 33. Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 14.

Group ID	Day 8		Day 15		Day 22	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. Saline	1.000	0.149	1.000	0.160	1.000	0.100
2. 1.5 mg/kg AC006111	0.200	0.059	0.151	0.040	0.388	0.056
3. 1.5 mg/kg AC006738	0.582	0.168	0.401	0.066	0.692	0.146
4. 1.5 mg/kg AC006747	0.346	0.058	0.345	0.090	0.545	0.086
5. 1.5 mg/kg AC006773	0.342	0.059	0.271	0.053	0.568	0.091
6. 1.5 mg/kg AC004202	0.184	0.046	0.160	0.045	0.385	0.088
7. 1.5 mg/kg AC006741	0.300	0.056	0.172	0.017	0.415	0.078
8. 1.5 mg/kg AC006742	0.283	0.090	0.171	0.037	0.447	0.081
9. 1.5 mg/kg AC006743	0.236	0.068	0.241	0.107	0.347	0.162
10. 1.5 mg/kg AC004212	0.271	0.064	0.237	0.018	0.445	0.085
11. 1.5 mg/kg AC006744	0.239	0.049	0.182	0.020	0.343	0.032
12. 1.5 mg/kg AC006745	0.223	0.023	0.165	0.039	0.315	0.068
13. 1.5 mg/kg AC006746	0.198	0.036	0.140	0.028	0.314	0.084

[0303] Groups 2-13 showed reduction in ALK7-GLuc at all time points compared to the saline control Group 1. Particularly, AC006746 achieved ~86% inhibition (0.140) at 1.5 mg/kg on Day 15.

Example 15. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0304] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day -21, six (n=6) male C57bl/6 mice in each group were dosed with $\sim 4 \times 10^{12}$ GC/kg ALK7-GLuc AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline (at

1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 34 below.

[0305] Table 34. Dosing Groups of Example 15.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
1	Saline	N/A	Day 1 SQ Injection	n = 6
2	AC005847	1.5 mg/kg	Day 1 SQ Injection	n = 6
3	AC007192	1.5 mg/kg	Day 1 SQ Injection	n = 6
4	AC007193	1.5 mg/kg	Day 1 SQ Injection	n = 6
5	AC007194	1.5 mg/kg	Day 1 SQ Injection	n = 6
6	AC007195	1.5 mg/kg	Day 1 SQ Injection	n = 6
7	AC007196	1.5 mg/kg	Day 1 SQ Injection	n = 6
8	AC007197	1.5 mg/kg	Day 1 SQ Injection	n = 6
9	AC007198	1.5 mg/kg	Day 1 SQ Injection	n = 6

[0306] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0307] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0308] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 35, with average GLuc reflecting the

normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0309] Table 35. Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 15.

Group ID	Day 8		Day 15		Day 22	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. Saline	1.000	0.055	1.000	0.085	1.000	0.103
2. 1.5 mg/kg AC005847	0.197	0.068	0.112	0.058	0.089	0.035
3. 1.5 mg/kg AC007192	0.193	0.040	0.182	0.028	0.172	0.044
4. 1.5 mg/kg AC007193	0.183	0.033	0.156	0.030	0.124	0.022
5. 1.5 mg/kg AC007194	0.217	0.030	0.193	0.048	0.171	0.028
6. 1.5 mg/kg AC007195	0.327	0.052	0.245	0.034	0.225	0.062
7. 1.5 mg/kg AC007196	0.149	0.014	0.146	0.044	0.159	0.030
8. 1.5 mg/kg AC007197	0.799	0.273	1.104	0.239	0.840	0.246
9. 1.5 mg/kg AC007198	1.053	0.183	1.238	0.377	0.960	0.347

[0310] Groups 2-7 showed reduction in ALK7-GLuc at all time points compared to the saline control Group 1. Groups 8 and 9 showed little to negligible reduction in ALK7-GLuc at all time points compared to the saline control Group 1. Particularly, AC005847 achieved ~91% inhibition (0.089) at 1.5 mg/kg on Day 22.

Example 16. In vivo administration of ALK7 RNAi agents in ALK7-SEAP mice.

[0311] The ALK7-SEAP model described in Example 3, above, was used. On Day -21, six (n=6) female C57bl/6 albino mice in each group were dosed with plasmid containing the nucleobases 220-3200 of the ALK7 cDNA sequence, via HTV injection. On Day 1, the mice were dosed with either isotonic saline or ALK7 RNAi agents formulated in saline (at 1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g (10 mL/kg) body weight injection volume. The dosing regimen is in accordance with Table 36 below.

[0312] Table 36. Dosing for mice of Example 16.

Group	Dose (RNAi Agent)	Dosing Route	# of Animals (n=)
1	Saline	Day 1 SQ Injection	n = 6

2	1.5 mg/kg AC004212	Day 1 SQ Injection	n = 6
3	1.5 mg/kg AC006290	Day 1 SQ Injection	n = 6
4	1.5 mg/kg AC006291	Day 1 SQ Injection	n = 6
5	1.5 mg/kg AC006292	Day 1 SQ Injection	n = 6
6	1.5 mg/kg AC006293	Day 1 SQ Injection	n = 6
7	1.5 mg/kg AC006294	Day 1 SQ Injection	n = 6
8	1.5 mg/kg AC006295	Day 1 SQ Injection	n = 6
9	1.5 mg/kg AC006296	Day 1 SQ Injection	n = 6
10	1.5 mg/kg AC006297	Day 1 SQ Injection	n = 6
11	1.5 mg/kg AC006298	Day 1 SQ Injection	n = 6
12	1.5 mg/kg AC006299	Day 1 SQ Injection	n = 6
13	1.5 mg/kg AC006300	Day 1 SQ Injection	n = 6

[0313] Serum was collected on Day -6, 1, 8, 15, and 22. SEAP expression levels were determined pursuant to the procedure set forth in Example 3, above. Data from the experiment are shown in the following Table 37, with average SEAP reflecting the normalized average value of SEAP.

[0314] Table 37. Average SEAP normalized to pre-treatment and saline control in ALK7-SEAP mice of Example 16.

Group ID	Day 8		Day 15		Day 22	
	Avg SEAP	Std Dev	Avg SEAP	Std Dev	Avg SEAP	Std Dev
1. Saline	1.000	0.120	1.000	0.149	1.000	0.226
2. 1.5 mg/kg AC004212	0.267	0.053	0.201	0.050	0.257	0.066
3. 1.5 mg/kg AC006290	0.316	0.081	0.260	0.133	0.371	0.178
4. 1.5 mg/kg AC006291	0.391	0.034	0.281	0.036	0.368	0.040
5. 1.5 mg/kg AC006292	0.425	0.060	0.347	0.072	0.378	0.083
6. 1.5 mg/kg AC006293	0.535	0.100	0.378	0.096	0.475	0.146
7. 1.5 mg/kg AC006294	0.328	0.029	0.240	0.050	0.313	0.055
8. 1.5 mg/kg AC006295	0.669	0.096	0.699	0.107	0.895	0.180
9. 1.5 mg/kg AC006296	0.545	0.074	0.615	0.111	0.787	0.211
10. 1.5 mg/kg AC006297	0.443	0.084	0.456	0.126	0.581	0.171
11. 1.5 mg/kg AC006298	0.424	0.065	0.413	0.103	0.589	0.144

12. 1.5 mg/kg AC006299	0.298	0.035	0.253	0.053	0.339	0.099
13. 1.5 mg/kg AC006300	0.268	0.051	0.188	0.057	0.251	0.068

[0315] Groups 2-13 showed reduction in SEAP-ALK7 at all time points compared to the saline control Group 1. In particular, on AC006300 achieved substantial inhibition (0.188, ~81% knockdown) after a single subcutaneous administration of 1.5 mg/kg at Day 15.

Example 17. *In vivo* administration of ALK7 RNAi agents in mice.

[0316] ALK7 RNAi agents were tested *in vivo* in mice. On Day 1, five (n=5) female C57bl/6 mice in each group were dosed with either isotonic saline or ALK7 RNAi agents formulated in saline (at 1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen is in accordance with Table 38 below.

[0317] **Table 38.** Dosing for mice of Example 17.

Group	Dose (RNAi Agent)	Dosing Route	# of Animals (n=)
1	Saline	Day 1 SQ Injection	n = 5
2	1.5 mg/kg AC005824	Day 1 SQ Injection	n = 5
3	1.5 mg/kg AC006643	Day 1 SQ Injection	n = 5
4	1.5 mg/kg AC006644	Day 1 SQ Injection	n = 5
5	1.5 mg/kg AC006645	Day 1 SQ Injection	n = 5
6	1.5 mg/kg AC006646	Day 1 SQ Injection	n = 5
7	1.5 mg/kg AC006647	Day 1 SQ Injection	n = 5
8	1.5 mg/kg AC006648	Day 1 SQ Injection	n = 5
9	1.5 mg/kg AC006649	Day 1 SQ Injection	n = 5
10	1.5 mg/kg AC006650	Day 1 SQ Injection	n = 5
11	1.5 mg/kg AC006651	Day 1 SQ Injection	n = 5
12	1.5 mg/kg AC006652	Day 1 SQ Injection	n = 5
13	1.5 mg/kg AC006653	Day 1 SQ Injection	n = 5

[0318] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight.

[0319] On Day 15, adipose tissues were harvested from the mice test animals. Inguinal white adipose tissue (iWAT) and perigonadal white adipose tissue (pgWAT) were collected for analysis.

[0320] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end and the 3' terminal end of the sense strand to a lipid moiety having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 6, 6a, 8, 9, and 10, for specific modifications and structure information related to the ALK7 RNAi agents; *see* Table 11 for structures of LP-379-a and LP-371-a).

[0321] The ALK7 RNAi agents tested in this Example were fully cross-reactive across both human and mouse ALK7.

[0322] The adipose biopsies collected from the test animals were used for analysis for ALK7 expression and additional biological parameters. ALK7 mRNA expression levels were quantified via qPCR, using mARL1 as the endogenous control gene, normalized to saline control Group 1. The data are shown in the following Table 39.

[0323] **Table 39.** ALK7 expression in mice adipose tissues of Example 17.

Group ID	Day 15					
	iWAT			pgWAT		
	Rel Exp ALK7	Error Low	Error High	Rel Exp ALK7	Error Low	Error High
1. Saline	1.000	0.277	0.383	1.000	0.194	0.241
2. 1.5 mg/kg AC005824	0.266	0.055	0.069	0.277	0.050	0.062
3. 1.5 mg/kg AC006643	0.348	0.034	0.037	0.296	0.063	0.081
4. 1.5 mg/kg AC006644	0.355	0.051	0.059	0.317	0.040	0.046
5. 1.5 mg/kg AC006645	0.366	0.058	0.069	0.321	0.064	0.079
6. 1.5 mg/kg AC006646	0.365	0.076	0.096	0.344	0.034	0.038
7. 1.5 mg/kg AC006647	0.222	0.057	0.076	0.175	0.059	0.088
8. 1.5 mg/kg AC006648	0.246	0.038	0.045	0.170	0.046	0.063
9. 1.5 mg/kg AC006649	0.246	0.060	0.079	0.223	0.034	0.040
10. 1.5 mg/kg AC006650	0.313	0.070	0.091	0.318	0.086	0.119
11. 1.5 mg/kg AC006651	0.317	0.048	0.057	0.343	0.075	0.096
12. 1.5 mg/kg AC006652	0.387	0.076	0.094	0.367	0.054	0.063
13. 1.5 mg/kg AC006653	0.318	0.043	0.049	0.364	0.063	0.076

[0324] Groups 2-13 showed reduction in ALK7 in mice iWAT and pgWAT at all time points compared to the saline control Group 1. In particular, AC006647 achieved substantial inhibition (0.222, ~78% knockdown) on Day 15 in iWAT after a single subcutaneous administration of 1.5 mg/kg. Additionally, AC006648 achieved substantial inhibition (0.170, ~83% knockdown) on Day 15 in pgWAT after a single subcutaneous administration of 1.5 mg/kg.

Example 18. *In vivo* administration of ALK7 RNAi agents in mice.

[0325] ALK7 RNAi agents were tested *in vivo* in mice. On Day 1, five (n=5) female C57bl/6 mice in each group were dosed with either isotonic saline or ALK7 RNAi agents formulated in saline (at 1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen is in accordance with Table 40 below.

[0326] **Table 40.** Dosing for mice of Example 18.

Group	Dose (RNAi Agent)	Dosing Route	# of Animals (n=)
1	Saline	Day 1 SQ Injection	n = 5
2	1.5 mg/kg AC005824	Day 1 SQ Injection	n = 5
3	1.5 mg/kg AC007027	Day 1 SQ Injection	n = 5
4	1.5 mg/kg AC007028	Day 1 SQ Injection	n = 5
5	1.5 mg/kg AC007029	Day 1 SQ Injection	n = 5
6	1.5 mg/kg AC007030	Day 1 SQ Injection	n = 5

[0327] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight.

[0328] On Day 15, adipose tissues were harvested from the mice test animals. Specifically, inguinal white adipose tissue (iWAT) and perigonadal white adipose tissue (pgWAT) were collected for analysis.

[0329] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end and the 3' terminal end of the sense strand to a lipid moiety having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 6, 6a, 8, 9, and 10, for specific modifications and structure information related to the ALK7 RNAi agents; *see* Table 11 for structures of LP-379-a and LP-371-a).

[0330] The ALK7 RNAi agents tested in this Example were fully cross-reactive across both human and mouse ALK7.

[0331] The adipose biopsies collected from the test animals were used for analysis for ALK7 expression and additional biological parameters. ALK7 mRNA expression levels were quantified via qPCR, using mARL1 as the endogenous control gene, normalized to saline control Group 1. The data are shown in the following Table 41.

[0332] **Table 41.** ALK7 expression in mice adipose tissues of Example 18.

Group ID	Day 15					
	iWAT			pgWAT		
	Rel Exp ALK7	Error Low	Error High	Rel Exp ALK7	Error Low	Error High
1. Saline	1.000	0.190	0.234	1.000	0.201	0.252
2. 1.5 mg/kg AC005824	0.395	0.094	0.124	0.250	0.062	0.083
3. 1.5 mg/kg AC007027	0.394	0.090	0.117	0.293	0.084	0.118
4. 1.5 mg/kg AC007028	0.377	0.107	0.149	0.342	0.089	0.121
5. 1.5 mg/kg AC007029	0.326	0.077	0.101	0.321	0.096	0.136
6. 1.5 mg/kg AC007030	0.262	0.043	0.051	0.299	0.102	0.154

[0333] Groups 2-6 showed reduction in ALK7 in mice iWAT and pgWAT at all time points compared to the saline control Group 1. In particular, AC007030 achieved substantial inhibition (0.262, ~74% knockdown) on Day 15 in iWAT after a single subcutaneous administration of 1.5 mg/kg. Additionally, AC005824 achieved substantial inhibition (0.250, ~75% knockdown) on Day 15 in pgWAT after a single subcutaneous administration of 1.5 mg/kg.

Example 19. *In vivo* administration of ALK7 RNAi agents in mice.

[0334] ALK7 RNAi agents were tested *in vivo* in mice. On Day 1, five (n=5) female C57bl/6 mice in each group were dosed with either isotonic saline or ALK7 RNAi agents formulated in saline (at 1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen is in accordance with Table 42 below.

[0335] **Table 42.** Dosing for mice of Example 19.

Group	Dose (RNAi Agent)	Dosing Route	# of Animals (n=)
1	Saline	Day 1 SQ Injection	n = 5

2	1.5 mg/kg AC005823	Day 1 SQ Injection	n = 5
3	1.5 mg/kg AC006654	Day 1 SQ Injection	n = 5
4	1.5 mg/kg AC006655	Day 1 SQ Injection	n = 5
5	1.5 mg/kg AC006656	Day 1 SQ Injection	n = 5
6	1.5 mg/kg AC006657	Day 1 SQ Injection	n = 5
7	1.5 mg/kg AC006658	Day 1 SQ Injection	n = 5
8	1.5 mg/kg AC006659	Day 1 SQ Injection	n = 5
9	1.5 mg/kg AC006660	Day 1 SQ Injection	n = 5
10	1.5 mg/kg AC006661	Day 1 SQ Injection	n = 5
11	1.5 mg/kg AC006662	Day 1 SQ Injection	n = 5
12	1.5 mg/kg AC006663	Day 1 SQ Injection	n = 5
13	1.5 mg/kg AC006664	Day 1 SQ Injection	n = 5
14	1.5 mg/kg AC006665	Day 1 SQ Injection	n = 5

[0336] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight.

[0337] On Day 15, adipose tissues were harvested from the mice test animals. Specifically, inguinal white adipose tissue (iWAT) and perigonadal white adipose tissue (pgWAT) were collected for analysis.

[0338] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end and the 3' terminal end of the sense strand to a lipid moiety having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 6, 6a, 8, 9, and 10, for specific modifications and structure information related to the ALK7 RNAi agents; *see* Table 11 for structures of LP-379-a and LP-371-a).

[0339] The ALK7 RNAi agents tested in this Example were fully cross-reactive across both human and mouse ALK7 genes.

[0340] The adipose biopsies collected from the test animals were used for analysis for ALK7 expression and additional biological parameters. ALK7 mRNA expression levels were quantified via qPCR, using mARL1 as the endogenous control gene, normalized to saline control Group 1. The data are shown in the following Table 43.

[0341] Table 43. ALK7 expression in mice adipose tissues of Example 19.

Group ID	Day 15					
	iWAT			pgWAT		
	Rel Exp ALK7	Error Low	Error High	Rel Exp ALK7	Error Low	Error High
1. Saline	1.000	0.215	0.273	1.000	0.206	0.259
2. 1.5 mg/kg AC005823	0.379	0.053	0.061	0.235	0.036	0.043
3. 1.5 mg/kg AC006654	0.359	0.086	0.113	0.279	0.033	0.037
4. 1.5 mg/kg AC006655	0.266	0.067	0.090	0.238	0.073	0.106
5. 1.5 mg/kg AC006656	0.300	0.044	0.052	0.329	0.056	0.067
6. 1.5 mg/kg AC006657	0.297	0.048	0.057	0.282	0.028	0.031
7. 1.5 mg/kg AC006658	0.301	0.053	0.064	0.291	0.062	0.079
8. 1.5 mg/kg AC006659	0.273	0.052	0.064	0.299	0.182	0.463
9. 1.5 mg/kg AC006660	0.292	0.041	0.048	0.260	0.059	0.077
10. 1.5 mg/kg AC006661	0.272	0.073	0.100	0.223	0.057	0.077
11. 1.5 mg/kg AC006662	0.289	0.046	0.055	0.269	0.073	0.100
12. 1.5 mg/kg AC006663	0.220	0.047	0.059	0.243	0.069	0.097
13. 1.5 mg/kg AC006664	0.278	0.070	0.093	0.420	0.090	0.115
14. 1.5 mg/kg AC006665	0.344	0.056	0.068	0.405	0.041	0.045

[0342] Groups 2-14 showed reduction in ALK7 in mice iWAT and pgWAT at all time points compared to the saline control Group 1. In particular, AC006663 achieved substantial inhibition (0.220, ~78% knockdown) on Day 15 in iWAT after a single subcutaneous administration of 1.5 mg/kg. Additionally, AC006661 achieved substantial inhibition (0.223, ~78% knockdown) on Day 15 in pgWAT after a single subcutaneous administration of 1.5 mg/kg.

Example 20. In vivo administration of ALK7 RNAi agents in DIO mice with GLP-1 Agonist.

[0343] ALK7 RNAi agents, GLP-1 agonists and combinations thereof were tested in an obese mouse DIO mouse model. Mice were dosed according to the dosing groups shown in Table 44, below.

[0344] **Table 44.** Dosing groups for mice of Example 20.

Group	Dose (RNAi Agent)	Dosing Route	# of Animals (n=)
1	Saline	Weekly SQ administration	n = 10

2	3 mg/kg AC005181	Weekly SQ administration	n = 10
3	3 mg/kg AC005181, 0.21 mpk tirzepatide	Weekly SQ administration of AC005181, daily administration of tirzepatide	n = 10
4	3 mg/kg AC005181, 0.14 mpk tirzepatide	Weekly SQ administration of AC005181, daily administration of tirzepatide	n = 10
5	3 mg/kg AC005181, 0.07 mpk tirzepatide	Weekly SQ administration of AC005181, daily administration of tirzepatide	n = 10
6	0.21 mpk tirzepatide	Daily administration	n = 10
7	0.14 mpk tirzepatide	Daily administration	n = 10
8	0.07 mpk tirzepatide	Daily administration	n = 10

[0345] AC005181 is an RNAi agent specific to mouse ALK7. On study day -22, body weights for each animal were measured, and body weight for each animal was measured weekly thereafter. On study days -22, 29, 57, 85, 113, and 141, animals were fasted for 6 hours before being bled to collect serum. Animals were harvested on study day 152. Average body weights for groups 2, 5, 6 and 8 are shown in FIG 1A. Body composition for groups 2, 5, 6 and 8 at day 152 are shown in FIG 1B.

[0346] These data demonstrate that co-administration of an ALK7 RNAi agent with a GLP-1 agonist such as tirzepatide may be more effective than either therapeutic alone in reducing body weight. Furthermore, a smaller dose of a GLP-1 agonist in combination with an ALK7 RNAi agent may be effective at retaining more lean mass (muscle) while reducing fat mass.

Example 21. In vivo administration of an ALK7 RNAi agent in cynomolgus monkeys.

[0347] ALK7 RNAi agents were tested in Cynomolgus monkeys for inhibition of ALK7. On Day 1, four (n=4) male Cynomolgus monkeys for each test group were dosed with ALK7 RNAi agents formulated in saline (at 0.75, 1.5, or 3.0 mg/kg), via subcutaneous (SQ) injection with syringe and needle in the mid-scapular region, at 0.3 mL/kg dose volume. Adipose biopsies were collected from all test animals on Day -12 (pre-dose), 15, 29, 57, 85, 113, 141, and 169. The dosing regimen was in accordance with Table 45 below.

[0348] **Table 45.** Dosing for test animals of Example 21.

Group	Study Material	# of Animals	Dosing Route	Dose Volume
1	Saline	n = 4	Day 1 SQ Injection	0.3 mL/kg
2	0.75 mg/kg AC006188	n = 4	Day 1 SQ Injection	0.3 mL/kg
3	1.5 mg/kg AC006188	n = 4	Day 1 SQ Injection	0.3 mL/kg
4	3.0 mg/kg AC006188	n = 4	Day 1 SQ Injection	0.3 mL/kg

[0349] All animals were fasted for at least 12 hours but less than 18 hours for scheduled blood collections and biopsy procedures. Blood collection site was femoral vein. A saphenous vein (not used for dose administration) may be used as an alternative collection site.

[0350] Adipose biopsies were collected as a sedated procedure. Sedation was accomplished using Telazol (4-8mg/kg), administered as an intramuscular (IM) injection and supplemented with Ketamine (~5 mg/kg) as needed.

[0351] An approximate 3-5 cm skin incision was made followed by collection of adipose tissue (optimally 50-150 mg). The skin will then be closed in a routine manner using suture (or alternate) materials maintaining aseptic technique.

[0352] Each biopsy site was separated by at least 1-2 cm. Biopsies were separated into 2 pieces (one piece of ~25 to 75 mg and second piece of ~25-75 mg) for each collection time point. Analgesics may be administered at veterinarian's discretion.

[0353] Blood was collected into tubes containing no anticoagulant (serum separator tubes). Blood was allowed to clot at ambient temperature prior to centrifugation to obtain serum.

[0354] Blood was collected on Day -12, 1, 15, 29, 57, 85, 113, 141, and 169 prior to biopsy sample collections or dose administration (when applicable), and from any animals found in moribund condition or sacrificed at an unscheduled interval. Samples may be further collected at

additional timepoints. Should there be any additional collected samples at other timepoints, their corresponding relevant data are shown below.

[0355] Individual doses of ALK7 RNAi agents were calculated based on the body weights recorded on each day of dosing.

[0356] The adipose biopsies and serum collected from the test animals were used for analysis for ALK7 expression and additional biological parameters. ALK7 mRNA expression levels in WAT (from biopsy) were quantified via qPCR, using cARL1 as the endogenous control gene, normalized to Day -12 pre-dose. Results from the WAT tissue biopsy knockdown are shown in Table 46, below.

[0357] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end and the 3' terminal end of the sense strand to a lipid moiety having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 6, 6a, 8, 9, and 10, for specific modifications and structure information related to the ALK7 RNAi agent; *see* Table 11 for structures of LP-379-a and LP-371-a).

[0358] **Table 46.** Average expression of ALK7 in cynomolgus monkeys in WAT relative to saline control, of Example 21.

Group ID	Day 15			Day 29		
	Rel. Exp. cALK7	Error Low	Error High	Rel. Exp. cALK7	Error Low	Error High
1. Saline	1.423	0.606	1.057	0.981	0.726	2.795
2. 0.75 mg/kg AC006188	0.207	0.095	0.175	0.161	0.066	0.111
3. 1.5 mg/kg AC006188	0.095	0.054	0.124	0.150	0.048	0.071
4. 3.0 mg/kg AC006188	0.154	0.070	0.130	0.111	0.034	0.049
Group ID	Day 57			Day 85		
	Rel. Exp. cALK7	Error Low	Error High	Rel. Exp. cALK7	Error Low	Error High
1. Saline	2.031	0.762	1.220	1.731	0.614	0.952
2. 0.75 mg/kg AC006188	0.224	0.061	0.083	0.205	0.048	0.063
3. 1.5 mg/kg AC006188	0.221	0.067	0.095	0.251	0.099	0.165
4. 3.0 mg/kg AC006188	0.189	0.026	0.030	0.214	0.067	0.097
Group ID	Day 113			Day 134		
	Rel. Exp. cALK7	Error Low	Error High	Rel. Exp. cALK7	Error Low	Error High
1. Saline	2.511	0.862	1.312	1.902	0.727	1.177
2. 0.75 mg/kg AC006188	0.236	0.072	0.104	ND*	ND*	ND*
3. 1.5 mg/kg AC006188	0.350	0.037	0.042	ND*	ND*	ND*

4. 3.0 mg/kg AC006188	0.312	0.135	0.239	0.328	0.134	0.226
	Day 141			Day 169		
Group ID	Rel. Exp. cALK7	Error Low	Error High	Rel. Exp. cALK7	Error Low	Error High
1. Saline	ND*	ND*	ND*	ND*	ND*	ND*
2. 0.75 mg/kg AC006188	0.898	0.455	0.922	0.331	0.107	0.158
3. 1.5 mg/kg AC006188	0.552	0.224	0.378	0.397	0.082	0.104
4. 3.0 mg/kg AC006188	ND*	ND*	ND*	ND*	ND*	ND*

*Groups 1 and 4 were terminated on Day 134. Groups 2 and 3 continued with the planned collection days.

[0359] ALK7 RNAi agent AC006188 achieved inhibition of ALK7 at all time points. A dose-response was observed at Day 29, 57, and 141. These data show a generally dose-dependent knockdown of ALK7 with AC006188, with expression of ALK7 being inhibited at all dose levels even as long as Day 169 from a single dose.

Example 22. Phase 1/2A Clinical Study of ALK7 RNAi Agents in Adult Volunteers with Obesity with and without Type 2 Diabetes Mellitus

[0360] ALK7 RNAi agents will be tested in human clinical trials.

[0361] **Proposed Study Design:** A Phase 1/2a dose-escalating study to evaluate the safety, tolerability, PK, and PD of single and multiple doses of an ALK7 RNAi agent in adult volunteers with obesity (in Part 1) and the safety, tolerability, and PD of repeat doses of an ALK7 RNAi agent in adult volunteers with obesity with and without type 2 diabetes mellitus receiving tirzepatide (in Part 2). The duration of study participation will be approximately 24-32 weeks, from the beginning of the 56-day Screening period to the end of study (Day 113 or 169 for Part 1, and Day 169 for Part 2). The Study Schema for the proposed study are set forth in Figure 3 (Part 1) and Figure 4 (Part 2).

Summary of Proposed Part 1

[0362] Proposed Part 1A of the study will evaluate single ascending doses (SAD) of ALK7 RNAi agent in volunteers with obesity in Cohorts 1a, 2a, 3a, and 4a, to enroll 6 subjects in each cohort to be randomized with 4 subjects administered the ALK7 RNAi agent and 2 subjects administered placebo (PBO). Proposed Part 1B will evaluate multiple ascending doses (MAD) of ALK7 RNAi agent in volunteers with obesity in Cohorts 2b, 3b, and 4b, to also enroll 6 subjects in each cohort to be randomized with 4 subjects administered the ALK7 RNAi agent and 2 subjects administered placebo (PBO). Subcutaneous adipose tissue biopsies

will be obtained at several timepoints for assessment of PD. Eligible subjects for Part 1 of the proposed study will include adult non-pregnant, non-lactating subjects, between 18 – 65 years old, with obesity (BMI 30 – 50 kg/m²), without evidence of Type 2 Diabetes at Screening (confirmed by laboratory assessment), stable weight at the time of Screening (no increase or decrease in weight >5% in the preceding 3 months), and at least one self-reported unsuccessful attempt at weight loss with lifestyle modification.

Summary of Proposed Part 2

[0363] Proposed Part 2 of the study will evaluate repeat doses of ALK7 RNAi agent in subjects with obesity with and without Type 2 Diabetes Mellitus also receiving tirzepatide. Each of Cohorts 5A and 5B of proposed Part 2 of the study will enroll and randomize 12 subjects with obesity without Type 2 Diabetes Mellitus, with 8 subjects administered the ALK7 RNAi agent and 4 subjects administered placebo (PBO). Cohort 5C will enroll and randomize 12 subjects with obesity with Type 2 Diabetes Mellitus, with 8 subjects administered the ALK7 RNAi agent and 4 subjects administered placebo (PBO). As shown in Figure 4, eligible subjects enrolled in Cohort 5A, Cohort 5B, and Cohort 5C will be randomized (2:1) to combined therapy with tirzepatide and an ALK7 RNAi agent (intervention arm) or tirzepatide monotherapy (control/PBO arm). Tirzepatide in Cohort 5A and Cohort 5C will be initiated on Day 1 at a dose of 2.5mg SC weekly for four weeks, then escalated to a dose of 5mg SC weekly. Tirzepatide in Cohort 5B will be initiated in all subjects on Day 1 at a dose of 2.5mg SC weekly for four weeks; subjects assigned to the control arm will then have dose escalation to 5mg SC weekly, while subjects assigned to the intervention group will continue tirzepatide at 2.5mg SC weekly. ALK7 RNAi agent (or matched volume of PBO) will be administered as subcutaneous injections on Day 1 and Day 29, at a dose level to be determined based on safety and pharmacodynamic data from Part 1 of the study. Subcutaneous adipose tissue biopsies will be obtained at several timepoints for assessment of PD. Subjects in Cohorts 5A, 5B, and 5C will be followed until Day 169 (end of study).

[0364] Eligible subjects for Part 2 of the study, subject to certain additional exclusion criteria, will include adult non-pregnant, non-lactating subjects, between 18 – 65 years old, with obesity (BMI 30 – 50 kg/m²), either with [Cohort 5C] or without [Cohorts 5A, 5B] Type 2 Diabetes Mellitus (T2DM), stable weight at the time of screening (no increase or decrease in weight >5%

in the preceding 3 months), and at least one self-reported unsuccessful attempt at weight loss with lifestyle modification.

Example 23. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0365] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day -21, six (n=6) male C57bl/6 mice in each group were dosed with $\sim 4 \times 10^{12}$ GC/kg ALK7-GLuc AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline (at 1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 47 below.

[0366] Table 47. Dosing Groups of Example 23.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
1	Saline	N/A	Day 1 SQ Injection	n = 6
2	AC005864	1.5 mg/kg	Day 1 SQ Injection	n = 6
3	AC007199	1.5 mg/kg	Day 1 SQ Injection	n = 6
4	AC007200	1.5 mg/kg	Day 1 SQ Injection	n = 6
5	AC007201	1.5 mg/kg	Day 1 SQ Injection	n = 6
6	AC004201	1.5 mg/kg	Day 1 SQ Injection	n = 6
7	AC007202	1.5 mg/kg	Day 1 SQ Injection	n = 6
8	AC007203	1.5 mg/kg	Day 1 SQ Injection	n = 6
9	AC007204	1.5 mg/kg	Day 1 SQ Injection	n = 6

[0367] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0368] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and

structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0369] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 48, with average GLuc reflecting the normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0370] **Table 48.** Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 23.

Group ID	Day 1		Day 8		Day 15	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. Saline	1.000	0.000	1.000	0.225	1.000	0.224
2. 1.5 mg/kg AC005864	1.000	0.000	0.311	0.041	0.243	0.054
3. 1.5 mg/kg AC007199	1.000	0.000	0.285	0.077	0.336	0.068
4. 1.5 mg/kg AC007200	1.000	0.000	0.238	0.069	0.160	0.037
5. 1.5 mg/kg AC007201	1.000	0.000	0.395	0.142	0.337	0.120
6. 1.5 mg/kg AC004201	1.000	0.000	0.324	0.090	0.255	0.057
7. 1.5 mg/kg AC007202	1.000	0.000	0.286	0.109	0.236	0.092
8. 1.5 mg/kg AC007203	1.000	0.000	0.187	0.032	0.191	0.039
9. 1.5 mg/kg AC007204	1.000	0.000	0.199	0.069	0.191	0.041

[0371] Groups 2-9 showed reduction in ALK7-GLuc at all time points (Day 8 and Day 15) compared to the saline control Group 1. In particular, AC007200 achieved ~84% inhibition (0.160) at 1.5 mg/kg on Day 15.

Example 24. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0372] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day -21, six (n=6) male C57bl/6 mice in each group were dosed with $\sim 4 \times 10^{12}$ GC/kg ALK7-GLuc AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline (at

1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 49 below.

[0373] Table 49. Dosing Groups of Example 24.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
1	Saline	N/A	Day 1 SQ Injection	n = 6
2	AC005847	1.5 mg/kg	Day 1 SQ Injection	n = 6
3	AC008318	1.5 mg/kg	Day 1 SQ Injection	n = 6
4	AC008319	1.5 mg/kg	Day 1 SQ Injection	n = 6
5	AC008320	1.5 mg/kg	Day 1 SQ Injection	n = 6
6	AC008321	1.5 mg/kg	Day 1 SQ Injection	n = 6
7	AC008322	1.5 mg/kg	Day 1 SQ Injection	n = 6
8	AC008323	1.5 mg/kg	Day 1 SQ Injection	n = 6
9	AC008324	1.5 mg/kg	Day 1 SQ Injection	n = 6
10	AC008325	1.5 mg/kg	Day 1 SQ Injection	n = 6

[0374] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0375] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0376] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 50, with average GLuc reflecting the

normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0377] Table 50. Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 24.

Group ID	Day 1		Day 8		Day 15	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. Saline	1.000	0.000	1.000	0.111	1.000	0.167
2. 1.5 mg/kg AC005847	1.000	0.000	0.135	0.050	0.079	0.029
3. 1.5 mg/kg AC008318	1.000	0.000	0.170	0.048	0.146	0.055
4. 1.5 mg/kg AC008319	1.000	0.000	0.235	0.046	0.146	0.037
5. 1.5 mg/kg AC008320	1.000	0.000	0.180	0.046	0.144	0.052
6. 1.5 mg/kg AC008321	1.000	0.000	0.301	0.049	0.248	0.027
7. 1.5 mg/kg AC008322	1.000	0.000	0.247	0.082	0.230	0.078
8. 1.5 mg/kg AC008323	1.000	0.000	0.476	0.097	0.524	0.080
9. 1.5 mg/kg AC008324	1.000	0.000	0.224	0.046	0.161	0.023
10. 1.5 mg/kg AC008325	1.000	0.000	0.144	0.044	0.145	0.042

[0378] Groups 2-10 showed reduction in ALK7-GLuc at all time points (Day 8 and Day 15) compared to the saline control Group 1. In particular, AC005847 achieved ~92% inhibition (0.079) at 1.5 mg/kg on Day 15.

Example 25. In vivo administration of ALK7 RNAi agents in ALK7-Gluc-AAV mice.

[0379] The ALK7-GLuc-AAV model as described in Example 6, above, was used. On Day -21, five (n=5) male C57bl/6 mice in each group were dosed with $\sim 4 \times 10^{12}$ GC/kg ALK7-GLuc AAV8, via intravenous IV injection, at 250 μ L per 25 g body weight injection volume. On Day 1, the mice were dosed with either saline or ALK7 RNAi agents formulated in saline (at 0.25, 0.5, or 1.5 mg/kg), via subcutaneous (SQ) injection, at 250 μ L per 25 g body weight injection volume. The dosing regimen was in accordance with Table 51 below.

[0380] Table 51. Dosing Groups of Example 25.

Group ID	RNAi Agent	Dose	Dosing Regimen	# of Animals (n =)
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1	Saline	N/A	Day 1 SQ Injection	n = 5
2	AC004201	0.25 mg/kg	Day 1 SQ Injection	n = 5
3	AC004201	0.5 mg/kg	Day 1 SQ Injection	n = 5
4	AC004201	1.5 mg/kg	Day 1 SQ Injection	n = 5
5	AC006746	0.25 mg/kg	Day 1 SQ Injection	n = 5
6	AC006746	0.5 mg/kg	Day 1 SQ Injection	n = 5
7	AC006746	1.5 mg/kg	Day 1 SQ Injection	n = 5
8	AC006305	0.25 mg/kg	Day 1 SQ Injection	n = 5
9	AC006305	0.5 mg/kg	Day 1 SQ Injection	n = 5
10	AC006305	1.5 mg/kg	Day 1 SQ Injection	n = 5

[0381] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[0382] Each of the ALK7 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included three N-acetyl-galactosamine groups (tridentate ligand) having the modified sequences as set forth in the duplex structures herein (*see* Tables 3, 4, 5, 6, 7A, 7B, and 8 for specific modifications and structure information related to the ALK7 RNAi agents; *see* Table 11 for structure of (NAG37) and (NAG37)s ligand).

[0383] GLuc levels were determined pursuant to the procedure set forth in Example 6, above. Data from the experiment are shown in the following Table 52, with average GLuc reflecting the normalized average value of GLuc. Inhibition of ALK7 expression by an ALK7 RNAi agent results in concomitant inhibition of GLuc expression, which is measured.

[0384] Table 52. Average GLuc normalized to pre-treatment and saline control in ALK7-Gluc AAV mice of Example 25.

Group ID	Day 8		Day 15		Day 22	
	Avg GLuc	Std Dev	Avg GLuc	Std Dev	Avg GLuc	Std Dev
1. Saline	1.000	0.151	1.000	0.220	1.000	0.112
2. 0.25 mg/kg AC004201	0.882	0.266	0.751	0.371	0.701	0.262

3. 0.5 mg/kg AC004201	0.502	0.190	0.433	0.146	0.405	0.195
4. 1.5 mg/kg AC004201	0.344	0.072	0.291	0.078	0.293	0.080
5. 0.25 mg/kg AC006746	0.849	0.205	0.781	0.215	0.721	0.162
6. 0.5 mg/kg AC006746	0.558	0.220	0.485	0.110	0.428	0.166
7. 1.5 mg/kg AC006746	0.326	0.063	0.307	0.072	0.319	0.047
8. 0.25 mg/kg AC006305	0.566	0.064	0.677	0.134	0.639	0.099
9. 0.5 mg/kg AC006305	0.421	0.086	0.435	0.095	0.438	0.122
10. 1.5 mg/kg AC006305	0.271	0.034	0.231	0.043	0.236	0.010

[0385] Groups 2-10 showed reduction in ALK7-GLuc at all time points (Day 8, Day 15, and Day 22) compared to the saline control Group 1. In particular, AC006305 achieved ~77% inhibition (0.231) at 1.5 mg/kg on Day 15. A dose-response was observed for AC004201, AC006746, and AC006305 at all time points (Day 8, Day 15, and Day 22).

OTHER EMBODIMENTS

[0386] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

Claims:

1. An RNAi agent for inhibiting expression of a Activin Receptor-Like Kinase 7 (ALK7) gene, comprising:
 - an antisense strand comprising at least 17 contiguous nucleotides differing by 0 or 1 nucleotides from any one of the sequences provided in Table 2 or Table 3; and
 - a sense strand comprising a nucleotide sequence that is at least partially complementary to the antisense strand.
2. The RNAi agent of claim 1, wherein the antisense strand comprises nucleotides 2-18 of any one of the sequences provided in Table 2 or Table 3.
3. The RNAi agent of claim 1 or claim 2, wherein the sense strand comprises a nucleotide sequence of at least 17 contiguous nucleotides differing by 0 or 1 nucleotides from any one of the sequences provided in Table 2 or Table 4, and wherein the sense strand has a region of at least 85% complementarity over the 17 contiguous nucleotides to the antisense strand.
4. The RNAi agent of any one of claims 1-3, wherein at least one nucleotide of the ALK7 RNAi agent is a modified nucleotide or includes a modified internucleoside linkage.
5. The RNAi agent of any one of claims 1-4, wherein all or substantially all of the nucleotides are modified nucleotides.
6. The RNAi agent of any one of claims 4-5, wherein the modified nucleotide is selected from the group consisting of: 2'-O-methyl nucleotide, 2'-fluoro nucleotide, 2'-deoxy nucleotide, 2',3'-seco nucleotide mimic, locked nucleotide, 2'-F-arabino nucleotide, 2'-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted 2'-O-methyl nucleotide, inverted 2'-deoxy nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, vinyl phosphonate-containing nucleotide, cyclopropyl phosphonate-containing nucleotide, and 3'-O-methyl nucleotide.
7. The RNAi agent of claim 5, wherein all or substantially all of the nucleotides are modified with 2'-O-methyl nucleotides, 2'-fluoro nucleotides, or combinations thereof.
8. The RNAi agent of any one of claims 1-7, wherein the antisense strand comprises the nucleotide sequence of any one of the modified sequences provided in Table 3.
9. The RNAi agent of any one of claims 1-8, wherein the sense strand comprises the nucleotide sequence of any one of the modified sequences provided in Table 4.

10. The RNAi agent of claim 1, wherein the antisense strand comprises the nucleotide sequence of any one of the modified sequences provided in Table 3 and the sense strand comprises the nucleotide sequence of any one of the modified sequences provided in Table 4.
11. The RNAi agent of any one of claims 1-10, wherein the sense strand is between 18 and 30 nucleotides in length, and the antisense strand is between 18 and 30 nucleotides in length.
12. The RNAi agent of claim 11, wherein the sense strand and the antisense strand are each between 18 and 27 nucleotides in length.
13. The RNAi agent of claim 12, wherein the sense strand and the antisense strand are each between 18 and 24 nucleotides in length.
14. The RNAi agent of claim 13, wherein the sense strand and the antisense strand are each 21 nucleotides in length.
15. The RNAi agent of claim 14, wherein the RNAi agent has two blunt ends.
16. The RNAi agent of any one of claims 1-15, wherein the sense strand comprises one or two terminal caps.
17. The RNAi agent of any one of claims 1-16, wherein the sense strand comprises one or two inverted abasic residues.
18. The RNAi agent of claim 1, wherein the RNAi agent is comprised of a sense strand and an antisense strand that form a duplex having the structure of any one of the duplexes in Table 7A, Table 7B, Table 8, Table 9A, or Table 10.
19. The RNAi agent of claim 18, wherein all or substantially all of the nucleotides are modified nucleotides.
20. The RNAi agent of claim 1, comprising an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5' → 3'):
AUUGUUGGAACUAUGACAGAC (SEQ ID NO: 558); or
UUCGAUAGUGUUCAGUAUUGC (SEQ ID NO: 569); or
UGUAUCAUCAAGCAUUUCAGG (SEQ ID NO: 571).
21. The RNAi agent of claim 20, wherein the sense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5' → 3'):

GUCUGUCAUAGUCCAAACAAU (SEQ ID NO: 599); or
GCAAUACUGAACACUAUCGAA (SEQ ID NO: 610); or
CCUGAAAUGCUUGAUGAUACA (SEQ ID NO: 612).

22. The RNAi agent of claim 20 or 21, wherein all or substantially all of the nucleotides are modified nucleotides.

23. The RNAi agent of claim 1, comprising an antisense strand that comprises, consists of, or consists essentially of a modified nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5' → 3'):

cPrpasUfsuguuGfgaacUfaUfgAfcagasc (SEQ ID NO: 269);
cPrpusUfscgAfuaguguUfcAfgUfauugssc (SEQ ID NO: 339); or
cPrpusGfsuaucafucaaGfcAfuUfucagsg (SEQ ID NO: 282).

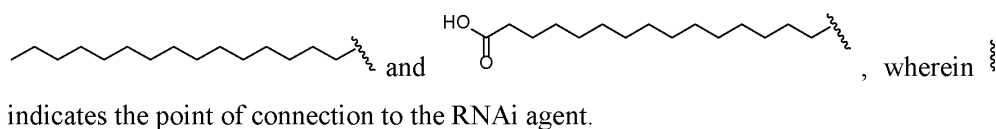
wherein a represents 2'-O-methyl adenosine, c represents 2'-O-methyl cytidine, g represents 2'-O-methyl guanosine, and u represent 2'-O-methyl uridine; Af represents 2'-fluoro adenosine, Cf represents 2'-fluoro cytidine, Gf represents 2'-fluoro guanosine, and Uf represents 2'-fluoro uridine; cPrpu represents a 5'-cyclopropyl phosphonate-2'-O-methyl uridine, cPrpa represents a 5'-cyclopropyl phosphonate-2'-O-methyl adenosine; s represents a phosphorothioate linkage, ss represents a phosphorodithioate linkage; and wherein all or substantially all of the nucleotides on the sense strand are modified nucleotides.

24. The RNAi agent of claim 1, wherein the sense strand comprises, consists of, or consists essentially of a modified nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5' → 3'):

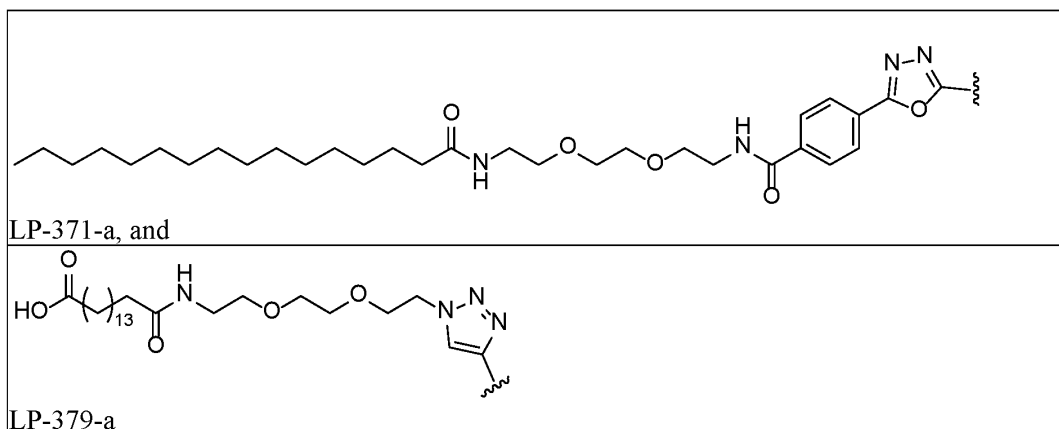
gucugucaUfAfGfuuccaacaau (SEQ ID NO: 364);
gcaauacuGfaAfcAfcuaucgaa (SEQ ID NO: 425); or
ccugaaAfuGfcUfugaugauaca (SEQ ID NO: 429).

wherein a represents 2'-O-methyl adenosine, c represents 2'-O-methyl cytidine, g represents 2'-O-methyl guanosine, and u represent 2'-O-methyl uridine; Af represents 2'-fluoro adenosine, Cf represents 2'-fluoro cytidine, Gf represents 2'-fluoro guanosine, and Uf represents 2'-fluoro uridine; and s represents a phosphorothioate linkage; and wherein all or substantially all of the nucleotides on the antisense strand are modified nucleotides.

25. The RNAi agent of any one of claims 20-24, wherein the sense strand further includes inverted abasic residues at the 3' terminal end of the nucleotide sequence, at the 5' end of the nucleotide sequence, or at both.
26. The RNAi agent of any one of claims 1-25, wherein the RNAi agent is linked to a lipid moiety.
27. The RNAi agent of claim 26, wherein the RNAi agent is linked to two or more lipid moieties.
28. The RNAi agent of claim 27, wherein each of the lipid moieties is independently selected from the group consisting of:



29. The RNAi agent of any one of claims 26-28, wherein each of the lipid moieties is independently selected from the group consisting of:



wherein  indicates the point of connection to the RNAi agent.

30. The RNAi agent of claim 26 or claim 27, wherein the lipid moiety is conjugated to the sense strand.
31. The RNAi agent of claim 26, wherein the RNAi agent is linked to LP-379-a on the 5' terminus of the sense strand, and linked to LP-371-a on the 3' terminus of the sense strand.
32. The RNAi agent of claim 1, wherein the RNAi agent has a duplex structure of any one of the duplexes of Table 10.

33. The RNAi agent of claim 32, wherein the duplex structure is selected from the group consisting of: AC006188, AC006189, AC006648, AC006661, or AC006663.
34. The RNAi agent of claim 1, wherein the RNAi agent has the structure of any one of Figures 4A-4D to 13A-13D.
35. A composition comprising the RNAi agent of any one of claims 1-34, wherein the composition further comprises a pharmaceutically acceptable excipient.
36. The composition of claim 35, further comprising a second RNAi agent capable of inhibiting the expression of Activin Receptor-Like Kinase 7 gene expression.
37. The composition of any one of claims 35-36, further comprising one or more additional therapeutics.
38. The composition of any of claims 35-37, wherein the RNAi agent is a sodium salt.
39. The composition of any of claims 35-37, wherein the pharmaceutically acceptable excipient is water for injection.
40. The composition of any of claims 35-38, wherein the pharmaceutically acceptable excipient is a buffered saline solution.
41. The composition of any of claims 35-38, wherein the pharmaceutically acceptable excipient is sodium chloride.
42. A method for inhibiting expression of an ALK7 gene in a cell, the method comprising introducing into a cell an effective amount of an RNAi agent of any one of claims 1-34 or the composition of any one of claims 35-41.
43. The method of claim 42, wherein the cell is within a subject.
44. The method of claim 43, wherein the subject is a human subject.
45. The method of any one of claims 42-44, wherein following the administration of the RNAi agent the Activin Receptor-Like Kinase 7 (ALK7) gene expression is inhibited by at least about 30%.
46. A method of treating one or more symptoms or diseases associated with enhanced or elevated ALK7 activity levels, the method comprising administering to a human subject in need thereof a therapeutically effective amount of the composition of any one of claims 35-41.
47. The method of claim 46, wherein the disease is a metabolic disease.
48. The method of claim 47, wherein the metabolic disease is obesity, diabetes, or insulin resistance.
49. The method of claim 46, wherein the disease is obesity, diabetes, or insulin resistance.

50. The method of claim 49, wherein the disease is obesity.
51. The method of any one of claims 42-50, wherein the RNAi agent is administered at a dose of about 0.01 mg/kg to about 5.0 mg/kg of body weight of the subject.
52. The method of any one of claims 42-51, wherein the RNAi agent is administered at a dose of about 0.03 mg/kg to about 2.0 mg/kg of body weight of the subject.
53. The method of any one of claims 42-50, wherein the RNAi agent is administered at a fixed dose of about 25 mg to about 450 mg.
54. The method of claim 53, wherein the RNAi agent is administered at a dose of about 25 mg, about 50 mg, about 150 mg, or about 450 mg.
55. The method of any of claims 42-54, wherein the RNAi agent is administered in two or more doses.
56. The method of any one of claims 43-55, wherein the subject is administered a second therapeutic.
57. The method of claim 56, wherein the second therapeutic is a glucagon-like peptide 1 (GLP1) agonist.
58. The method of claim 57, wherein the GLP1 agonist is tirzepatide.
59. Use of the RNAi agent of any one of claims 1-34, for the treatment of a disease, disorder, or symptom that is mediated at least in part by ALK7 activity and/or ALK7 gene expression.
60. Use of the composition according to any one of claims 35-41, for the treatment of a disease, disorder, or symptom that is mediated at least in part by Activin Receptor-Like Kinase 7 (ALK7) activity and/or Activin Receptor-Like Kinase 7 (ALK7) gene expression.
61. Use of the composition according to any one of claims 35-41, for the manufacture of a medicament for treatment of a disease, disorder, or symptom that is mediated at least in part by Activin Receptor-Like Kinase 7 (ALK7) and/or Activin Receptor-Like Kinase 7 (ALK7) gene expression.
62. The use of any one of claims 59-61, wherein the disease is a metabolic disease.
63. A method of making an RNAi agent of any one of claims 1-34, comprising annealing a sense strand and an antisense strand to form a double-stranded ribonucleic acid molecule.
64. The method of claim 63, wherein the sense strand comprises a lipid moiety.
65. The method of claim 64, comprising conjugating a lipid moiety to the sense strand.