SUPEROXIDE DISMUTASE 1 (SOD1) iRNA COMPOSITIONS AND METHODS OF USE THEREOF FOR TREATING OR PREVENTING SUPEROXIDE DISMUTASE 1- (SOD1-) ASSOCIATED NEURODEGENERATIVE DISEASES

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 63/148,991, filed on February 12, 2021, and U.S. Provisional Application No. 63/270,176, filed on October 21, 2021. The entire contents of each of the foregoing applications are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 8, 2022, is named 121301_12720_SL.txt and is 365,213 bytes in size.

BACKGROUND OF THE INVENTION

Superoxide dismutase 1 (SOD1), also known as Cu/Zn superoxide dismutase (Cu/ZnSOD), is a ubiquitously expressed antioxidant enzyme that metabolizes superoxide radicals produced during cellular metabolism to molecular oxygen and hydrogen peroxide, and plays an important role in protecting cells from oxidative damage (McCord JM and Fridovich I. (1969) *J Bio Chem.* 244:6049-6055; Rosen DR (1993) *Nature.* 364:362; Trist B, *et al.* (2020) *Angew Chem Int Ed Engl.* Accepted Author Manuscript). The human SOD1 gene is located on chromosome 21q22.11 and it produces a 16 kDa protein that normally forms a 32 kDa homodimer. In mammalian cells, SOD1 is widely distributed in the cytosol, nucleus, lysosomes, peroxisomes, and intermembrane space (IMS) of mitochondria (Fukai T and Ushio-Fukai M. (2011) *Antioxid Redox Signal*. 15(6):1583-1606; Zhang S, *et al.* (2015) *Free Radic Biol Med.* 85:33-44; Huai J and Zhang Z. (2019) *Front. Neurol.* 10:527). The high cytosolic abundance of SOD1 distinguishes it from two other mammalian superoxide dismutases also involved in metabolizing superoxide radicals: the mitochondrial tetrameric manganese superoxide dismutase (SOD2) and the extracellular tetrameric Cu/Zn superoxide dismutase (SOD3). However, the activity of SOD1 accounts for approximately 50 – 80% of the total SOD activity in mammalian cells (Mindola P, *et al.* (2016) *Front. Physiol.* 7:594).

The structure of each SOD1 subunit consists of a β-barrel core and seven loops at the edge which are held together by an intramolecular disulfide bond, a binuclear metal binding site holding a copper and a zinc ions responsible for the catalyzing activity of SOD1, and a global hydrogen bond network (Huai J and Zhang Z. (2019) *Front. Neurol.* 10:527). Previous studies have shown that the stability, structure, and function of SOD1 is controlled by its posttranslational modification, metal ion binding, and disulfide bond status. Loss of the metal cofactors and/or disruption of the disulfide bonds,

often associated with mutations in the SOD1 gene, can result in pathogenic misfolding, aggregation, and/or dysfunction of SOD1 protein (Huai J and Zhang Z. (2019) *Front. Neurol.* 10:527).

Approximately 200 mutations have been documented throughout coding and non-coding regions of the SOD1 gene. Mutant SOD1 proteins are susceptible to misfolding which can compromise protective antioxidant function and result in the formation of abnormal molecular interactions (*e.g.,* between multiple misfolded SOD1 units (aggregation) and between misfolded SOD1 and other cellular constituents), thereby contributing to pathology through both loss-of-function actions (*e.g.,* diminished antioxidant activity, impaired nuclear translocation and promoter binding, and disrupted SOD1 redox signaling) and gain-of-function actions (*e.g.,* neurotoxicity) (Trist B, *et al.* (2020) *Angew Chem Int Ed Engl.* Accepted Author Manuscript). SOD1 has been implicated in contributing to the pathology of many different diseases, including, for example, heart failure, cancer (*e.g.,* lung adenocarcinoma, non-small-cell lung cancer, and breast cancer), diabetes, Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease, and Down's syndrome (DS) (Banks CJ and Anderson JL. (2019) *Redox Biol.* 26:101270; Trist B, *et al.* (2020) *Angew Chem Int Ed Engl.* Accepted Author Manuscript). In the context of neurodegenerative disease, SOD1 has been implicated in the initiation and/or acceleration of damaging pathways in both neurons and surrounding glia, including: disruption of proteasome function, degradation of microtubules and microfilaments, endoplasmic reticulum stress, and redox dyshomeostasis. More specifically, mutant SOD1 has been implicated in a familial form of amyotrophic lateral sclerosis (fALS) where various SOD1 mutations can increase the propensity of SOD1 to aggregate, which is understood to induce motor neuron death. Additionally, wild-type SOD1 misfolding and dysfunction has been implicated in the death of spinal cord motor neurons in sporadic ALS (aALS), in the death of substantia nigra pars compacta (SNc) dopamine neurons in Parkinson's disease, and the death of neurons within the frontal cortex and hippocampus in Alzheimer's disease (Trist B, *et al.* (2020) *Angew Chem Int Ed Engl.* Accepted Author Manuscript). In addition, SOD1 has been implicated in neuron death by exacerbating other detrimental cellular pathologies, including Aβ plaque formation in Alzheimer's disease and α-synuclein deposition in Parkinson's disease.

Effective treatments for superoxide dismutase 1-associated neurodegenerative diseases are currently not available and any treatments that are available are palliative. Thus, there remains a need for an agent that can selectively and efficiently silence the SOD1 gene using the cell's own RNAi machinery that has both high biological activity and *in vivo* stability, and that can effectively inhibit expression of a target SOD1 gene.

BRIEF SUMMARY OF THE INVENTION

The present disclosure provides RNAi agent compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a superoxide dismutase 1 (SOD1) gene. The SOD1 gene may be within a cell, *e.g.,* a cell within a subject, such as a human. The present disclosure also provides methods of using the RNAi agent compositions of the disclosure for inhibiting the expression of a SOD1 gene and/or for treating a subject who would benefit from inhibiting or

reducing the expression of a SOD1 gene, *e.g.,* a subject suffering or prone to suffering from a SOD1-associated neurodegenerative disease, *e.g.,* Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

Accordingly, in one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent, or a pharmaceutically acceptable salt thereof, comprising a sense strand and an antisense strand forming a double stranded region, wherein

a) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusdCsugdGadTagagdGaUfuaaagugsasg-3' (SEQ ID NO: 12), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC, dG, and dT are 2′-deoxy C, G, and T; and Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U;

b) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA and dT are 2′-deoxy A and T; and Af and Cf are 2′-deoxy-2'-fluoro (2'-F) A and C;

c) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC is 2′-deoxy C; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

d) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA is 2′-deoxy A; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U;

e) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20), wherein VP is a 5'-vinyl

phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (G2p) is guanosine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; dA is 2'-deoxy A; and Af, Cf, Gf, and Uf are 2'-deoxy-2'-fluoro (2'-F) A, C, G, and U;

f) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22), wherein VP is a 5'-vinyl phosphonate; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af, Cf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, and U;

g) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

h) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Agn) is adenosine-glycol nucleic acid (GNA), S-isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af and Uf are 2′-deoxy-2'-fluoro (2'-F) A and U;

i) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U; or

j) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a

phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2’-fluoro (2’-F) A, C, G, and U.

In one embodiment,

a) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5’-csascuu(Uhd)aaUfCfCfucuauccasgsa-3’ (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5’-VPusdCsugdGadTagagdGaUfuaaagugsasg-3’ (SEQ ID NO: 12), wherein VP is a 5’-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC, dG, and dT are 2′-deoxy C, G, and T; and Cf and Uf are 2′-deoxy-2’-fluoro (2’-F) C and U;

b) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5’-csasggu(Chd)cuCfAfCfuuuaauccsusa-3’ (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5’-VPusdAsggdAudTaaagdTgAfggaccugscsg-3’ (SEQ ID NO: 14), wherein VP is a 5’-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA and dT are 2′-deoxy A and T; and Af and Cf are 2′-deoxy-2’-fluoro (2’-F) A and C;

c) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5’-ususcgag(Chd)aGfAfAfggaaaguasasa-3’ (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5’-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3’ (SEQ ID NO: 16), wherein VP is a 5’-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC is 2′-deoxy C; and Af, Cf, Gf, and Uf are 2′-deoxy-2’-fluoro (2’-F) A, C, G, and U;

d) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5’-gsasaag(Uhd)aaUfGfGfaccagugasasa-3’ (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5’-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3’ (SEQ ID NO: 18), wherein VP is a 5’-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA is 2′-deoxy A; and Af, Gf, and Uf are 2′-deoxy-2’-fluoro (2’-F) A, G, and U;

e) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5’-asgsga(Uhd)gaaGfAfGfaggcaugususa-3’ (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5’-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3’ (SEQ ID NO: 20), wherein VP is a 5’-vinyl phosphonate; (Uhd) is 2’-O-hexadecyl-uridine-3’-phosphate; (G2p) is guanosine-2’-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2’-O-methyl (2’-OMe) A, G, C, and U; dA is 2’-deoxy A; and Af, Cf, Gf, and Uf are 2’-deoxy-2’-fluoro (2’-F) A, C, G, and U; f) the nucleotide sequence of the

sense strand differs by no more than 3 bases from the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22), wherein VP is a 5'-vinyl phosphonate; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af, Cf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, and U;

g) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

h) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Agn) is adenosine-glycol nucleic acid (GNA), S-isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af and Uf are 2′-deoxy-2'-fluoro (2'-F) A and U;

i) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U; or

j) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U.

In one embodiment,

a) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusdCsugdGadTagagdGaUfuaaagugsasg-3' (SEQ ID NO: 12), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC, dG, and dT are 2′-deoxy C, G, and T; and Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U;

b) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA and dT are 2′-deoxy A and T; and Af and Cf are 2′-deoxy-2'-fluoro (2'-F) A and C;

c) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC is 2′-deoxy C; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

d) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA is 2′-deoxy A; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U;

e) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (G2p) is guanosine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; dA is 2'-deoxy A; and Af, Cf, Gf, and Uf are 2'-deoxy-2'-fluoro (2'-F) A, C, G, and U;

f) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide

sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22), wherein VP is a 5'-vinyl phosphonate; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af, Cf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, and U;

g) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

h) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Agn) is adenosine-glycol nucleic acid (GNA), S-isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af and Uf are 2′-deoxy-2'-fluoro (2'-F) A and U;

i) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U; or

j) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U.

In one embodiment,

a) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-

VPusdCsugdGadTagagdGaUfuaaagugsasg-3' (SEQ ID NO: 12), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC, dG, and dT are 2′-deoxy C, G, and T; and Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U;

b) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA and dT are 2′-deoxy A and T; and Af and Cf are 2′-deoxy-2'-fluoro (2'-F) A and C;

c) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC is 2′-deoxy C; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

d) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer;
s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA is 2′-deoxy A; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U;

e) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (G2p) is guanosine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; dA is 2'-deoxy A; and Af, Cf, Gf, and Uf are 2'-deoxy-2'-fluoro (2'-F) A, C, G, and U;

f) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22), wherein VP is a 5'-vinyl phosphonate; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid

(GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af, Cf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, and U;

g) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

h) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Agn) is adenosine-glycol nucleic acid (GNA), S-isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af and Uf are 2′-deoxy-2'-fluoro (2'-F) A and U;

i) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U; or

j) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U.

In one embodiment,

a) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusdCsugdGadTagagdGaUfuaaagugsasg-3' (SEQ ID NO: 12), wherein

VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC, dG, and dT are 2′-deoxy C, G, and T; and Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U;

b) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA and dT are 2′-deoxy A and T; and Af and Cf are 2′-deoxy-2'-fluoro (2'-F) A and C;

c) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC is 2′-deoxy C; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

d) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA is 2′-deoxy A; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U;

e) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (G2p) is guanosine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; dA is 2'-deoxy A; and Af, Cf, Gf, and Uf are 2'-deoxy-2'-fluoro (2'-F) A, C, G, and U;

f) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22), wherein VP is a 5'-vinyl phosphonate; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af, Cf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, and U;

g) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense

strand comprises from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

h) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Agn) is adenosine-glycol nucleic acid (GNA), S-isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af and Uf are 2′-deoxy-2'-fluoro (2'-F) A and U;

i) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuusug-3' (SEQ ID NO: 28), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U; or

j) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U.

In one embodiment,

a) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusdCsugdGadTagagdGaUfuaaagugsasg-3' (SEQ ID NO: 12), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC, dG, and dT are 2′-deoxy C, G, and T; and Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U;

b) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA and dT are 2′-deoxy A and T; and Af and Cf are 2′-deoxy-2'-fluoro (2'-F) A and C;

c) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense

strand consists of the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dC is 2′-deoxy C; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

d) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA is 2′-deoxy A; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U;

e) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (G2p) is guanosine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; dA is 2'-deoxy A; and Af, Cf, Gf, and Uf are 2'-deoxy-2'-fluoro (2'-F) A, C, G, and U;

f) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22), wherein VP is a 5'-vinyl phosphonate; (Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate; (Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af, Cf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, and U;

g) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand consists of from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U;

h) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (Agn) is adenosine-glycol nucleic acid (GNA), S-isomer; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dA, dG, and dT are 2′-deoxy A, G, and T; and Af and Uf are 2′-deoxy-2'-fluoro (2'-F) A and U;

i) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28), wherein VP is a 5'-vinyl phosphonate; (Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, G, and U; or

j) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30), wherein VP is a 5'-vinyl phosphonate; (Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate; (C2p) is cytidine-2'-phosphate; s is a phosphorothioate linkage; a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; dT is 2′-deoxy T; and Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U.

In one embodiment, the dsRNA agent is in the form of a sodium salt.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of superoxide dismutase 1 (SOD1), wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides from any one of the nucleotide sequences of nucleotides 201-223, 204-226, 207-229, 216-238, 219-241, 328-350, 333-355, 336-358, 372-394, or 373-395 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2, wherein (i) the dsRNA agent comprises at least one modified nucleotide, (ii) the double stranded region is 15-30 nucleotide pairs in length, and (iii) the sense strand or the antisense strand is conjugated to one or more lipophilic moieties.

In one embodiment, the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides from any one of the nucleotide sequences of nucleotides 207-229, 219-241, 328-350, or 336-358 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

In another embodiment, the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21 contiguous nucleotides from any one of the nucleotide sequences of nucleotides 207-229, 328-350, or 336-358 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

In one embodiment, the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides from nucleotides 336-358 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

In another embodiment, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides from any one of the antisense strand nucleotide sequences of a

duplex selected from the group consisting of AD-1395762, AD-1395756, AD-1395731, AD-1395743, AD-1395771, AD-1395738, AD-1395718, AD-1395760, AD-1395764, or AD-1395724.

In one embodiment, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1395762, AD-1395756, AD-1395731, and AD-1395743.

In one embodiment, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1395762, AD-1395756, and AD-1395731.

In another embodiment, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides from the antisense strand nucleotide sequence of duplex AD-1395762.

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

In one embodiment, at least one of the modified nucleotides is selected from the group a deoxy-nucleotide, a 3'-terminal deoxythimidine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a 2'-5'-linked ribonucleotide (3'-RNA), a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide comprising a 5' phosphate or 5' phosphate mimic, a nucleotide comprising vinyl phosphonate, a glycol nucleic acid (GNA), a glycol nucleic acid S-Isomer (S-GNA), a nucleotide comprising 2-hydroxymethyl-tetrahydrofuran-5-phosphate, a nucleotide comprising 2'-deoxythymidine-3'phosphate, a nucleotide comprising 2'-deoxyguanosine-3'-phosphate, and a terminal nucleotide linked to a cholesteryl derivative and a dodecanoic acid bisdecylamide group; and combinations thereof.

In one embodiment, the modified nucleotide is selected from the group consisting of a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, 3'-terminal deoxythimidine nucleotides (dT), a locked nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

In another embodiment, the modifications on the nucleotides are independently selected from the group consisting of 2'-deoxy, 2'-O-methyl, 3'-RNA, GNA, S-GNA, and 2'-deoxy-2'-fluoro modifications.

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

In one embodiment, the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages.

In one embodiment, at least one strand comprises a 3' overhang of at least 1 nucleotide.

In another embodiment, at least one strand comprises a 3' overhang of 2 nucleotides.

In one embodiment, the double stranded region is 17-23 nucleotide pairs in length.

In another embodiment, the double stranded region is 19-21 nucleotide pairs in length.

In one embodiment, the double stranded region is 21-23 nucleotide pairs in length.

In one embodiment, each strand has 19-30 nucleotides.

In one embodiment, the one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand.

In one embodiment, one lipophilic moiety is conjugated an internal position selected from the group consisting of positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.

In one embodiment, the internal position is selected from the group consisting of positions 5, 6, 7, 15, and 17 on the sense strand, counting from the 5'-end of the strand.

In another embodiment, the internal position is selected from the group consisting of positions 15 and 17 on the antisense strand, counting from the 5'-end of the strand.

In one embodiment, the internal position is selected from the group consisting of positions 6 and 7 of the sense strand.

In one embodiment, the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound.

In one embodiment, the lipophilic moiety contains a saturated or unsaturated C4-C30 hydrocarbon chain, and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

In one embodiment, the lipophilic moiety contains a saturated or unsaturated C6-C18 hydrocarbon chain.

In one embodiment, the lipophilic moiety contains a saturated or unsaturated C16 hydrocarbon chain.

In one embodiment, the saturated or unsaturated C16 hydrocarbon chain is conjugated to position 6 or 7 of the sense strand, counting from the 5'-end of the sense strand.

In one embodiment, the dsRNA agent further comprises a phosphate or phosphate mimic at the 5'-end of the antisense strand.

In one embodiment, the phosphate mimic is a 5'-vinyl phosphonate (VP).

In another embodiment, the phosphate mimic is a 5'-*E*-vinyl phosphonate (VP).

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

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

The present invention also provides cells and pharmaceutical compositions comprising a pharmaceutically acceptable diluent comprising the dsRNA agents disclosed herein.

In one aspect the present invention provides a method of inhibiting expression of a SOD1 gene in a cell. The method includes contacting the cell with a dsRNA agent or a pharmaceutical composition of the invention; and maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the SOD1 gene, thereby inhibiting expression of the SOD1 gene in the cell.

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

In one embodiment, the subject meets at least one diagnostic criterion for a SOD1-associated neurodegenerative disease or has been diagnosed with a SOD1-associated neurodegenerative disease.

In one embodiment, the SOD1-associated neurodegenerative disease is selected from the group consisting of Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

In one aspect, the preent invention provides a method of treating a subject diagnosed with a SOD1-associated neurodegenerative disease, the method comprising administering to the subject a therapeutically effective amount of a dsRNA agent or a pharmaceutical composition of the invention, thereby treating the subject.

In one embodiment, treating comprises amelioration of at least one sign or symptom of the disease.

In another embodiment, treating comprises prevention of progression of the disease.

In one embodiment, the SOD1-associated neurodegenerative disease is is selected from the group consisting of Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

In another aspect, the present invention provides a method of preventing development of a SOD1-associated neurodegenerative disease in a subject meeting at least one diagnostic criterion for a SOD1-associated neurodegenerative disease, the method comprising administering to the subject a therapeutically effective amount of a dsRNA agent or a pharmaceutical composition of the invention, thereby preventing the development of a SOD1-associated neurodegenerative disease in the subject meeting at least one diagnostic criterion for a SOD1-associated neurodegenerative disease.

In one embodiment, the subject is human.

In one embodiment, the subject has been diagnosed with a SOD1-associated neurodegenerative disease.

In one embodiment, the SOD1-associated neurodegenerative disease is selected from the group consisting of Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

In one embodiment, the dsRNA agent is administered to the subject intrathecally or intracerebroventricularly.

In one aspect, the instant disclosure provides a double stranded ribonucleic acid (RNAi) agent for inhibiting expression of a superoxide dismutase 1 (SOD1) gene, wherein the RNAi agent includes a sense strand and an antisense strand, and wherein the antisense strand includes a region of complementarity which includes at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous

nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the antisense sequences listed in any one of Tables 2-7, 12, 13, and 18-20. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides of any one of the antisense sequences listed in any one of Tables 2-7, 12, 13, and 18-20. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 19, *e.g.,* 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the antisense sequences listed in any one of Tables 2-7, 12, 13, and 18-20. In certain embodiments, the antisense strand includes a region of complementarity which includes at least 19 *e.g.,* 19, 20, 21, 22, or 23, contiguous nucleotides of any one of the antisense sequences listed in any one of Tables 2-7, 12, 13, and 18-20. In certain embodiments, thymine-to-uracil or uracil-to-thymine differences between aligned (compared) sequences are not counted as nucleotides that differ between the aligned (compared) sequences.

In some embodiments, the sense strand or the antiense strand is conjugated to one or more lipophilic moieties.

In some embodiments, the agents include one or more lipophilic moieties conjugated to one or more internal nucleotide positions, optionally via a linker or carrier.

In other embodiments, the agent further comprises a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

In yet other embodiments, the agents further comprise one or more lipophilic moieties conjugated to one or more internal nucleotide positions, optionally via a linker or carrier and a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a superoxide dismutase 1 (SOD1) gene, wherein the dsRNA agent includes a sense strand and an antisense strand, wherein the sense strand includes at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the sense strand sequences presented in Tables 2-7, 12, 13, and 18-20; and wherein the antisense strand includes at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 1, nucleotides from any one of antisense strand nucleotide sequences presented in Tables 2-7, 12, 13, and 18-20. In certain embodiments, the sense strand includes at least 15 *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides of any one of the sense strand sequences presented in Tables 2-7, 12, 13, and 18-20; and the antisense strand includes at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides of any one of the antisense strand nucleotide sequences presented in Tables 2-7, 12, 13, and 18-20. In certain embodiments, the sense strand includes at least 19 *e.g.,* 19, 20, or 21, contiguous nucleotides of any one of the sense strand sequences presented in Tables 2-7, 12, 13, and 18-20; and the antisense strand includes at least 19 *e.g.,*

19, 20, 21, 22, or 23, contiguous nucleotides of any one of the antisense strand nucleotide sequences presented in Tables 2-7, 12, 13, and 18-20.

In some embodiments, the agents include one or more lipophilic moieties conjugated to one or more internal nucleotide positions, optionally via a linker or carrier.

In other embodiments, the agent further comprises a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

In yet other embodiments, the agents further comprise one or more lipophilic moieties conjugated to one or more internal nucleotide positions, optionally via a linker or carrier and a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

An additional aspect of the disclosure provides a double stranded RNAi agent for inhibiting expression of an superoxide dismutase 1 (SOD1) gene, wherein the dsRNA agent includes a sense strand and an antisense strand, wherein the sense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 1, 3, 5, 7, or 9, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 1, 3, 5, 7, or 9, wherein a substitution of a uracil for any thymine of SEQ ID NOs: 1, 3, 5, 7, and 9 (when comparing aligned sequences) does not count as a difference that contributes to the differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 1, 3, 5, 7, or 9, or the nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 1, 3, 5, 7, or 9; and wherein the antisense strand includes at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NOs: 2, 4, 6, 8, or 10, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, 8, or 10, wherein a substitution of a uracil for any thymine of SEQ ID NOs: 2, 4, 6, 8, or 10 (when comparing aligned sequences) does not count as a difference that contributes to the differing by no more than 3 nucleotides from any one of the nucleotide sequences of SEQ ID NOs: 2, 4, 6, 8, or 10, or the nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, 8, or 10, wherein at least one of the sense strand and the antisense strand includes one or more lipophilic moieties conjugated to one or more internal nucleotide positions, optionally via a linker or carrier.

In one embodiment, the double stranded RNAi agent targeted to SOD1 comprises a sense strand which includes at least 15 *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from the nucleotide sequence of the sense strand nucleotide sequence of a duplex in Tables 2-7, 12, 13, and 18-20.

In one embodiment, the double stranded RNAi agent targeted to SOD1 comprises an antisense strand which includes at least 15 *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from the antisense nucleotide sequence of a duplex in one of Tables 2-7, 12, 13, and 18-20.

In some embodiments, the agent further comprises a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier.

Optionally, the double stranded RNAi agent includes at least one modified nucleotide.

In certain embodiments, the lipophilicity of the lipophilic moiety, measured by $\log K_{ow}$, exceeds 0.

In some embodiments, the hydrophobicity of the double-stranded RNAi agent, measured by the unbound fraction in a plasma protein binding assay of the double-stranded RNAi agent, exceeds 0.2. In a related embodiment, the plasma protein binding assay is an electrophoretic mobility shift assay using human serum albumin protein.

In certain embodiments, substantially all of the nucleotides of the sense strand are modified nucleotides. Optionally, all of the nucleotides of the sense strand are modified nucleotides.

In some embodiments, substantially all of the nucleotides of the antisense strand are modified nucleotides. Optionally, all of the nucleotides of the antisense strand are modified nucleotides.

Optionally, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

In one embodiment, at least one of the modified nucleotides is a deoxy-nucleotide, a 3'-terminal deoxythimidine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide comprising a 5' phosphate or 5' phosphate mimic, a nucleotide comprising vinyl phosphonate, a nucleotide comprising adenosine-glycol nucleic acid (GNA), a nucleotide comprising thymidine-glycol nucleic acid (GNA) S-Isomer, a nucleotide comprising 2-hydroxymethyl-tetrahydrofurane-5-phosphate, a nucleotide comprising 2'-deoxythymidine-3'phosphate, a nucleotide comprising 2'-deoxyguanosine-3'-phosphate, or a terminal nucleotide linked to a cholesteryl derivative or a dodecanoic acid bisdecylamide group.

In a related embodiment, the modified nucleotide is a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, 3'-terminal deoxythimidine nucleotides (dT), a locked nucleotide, an

abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide.

In one embodiment, the modified nucleotide includes a short sequence of 3'-terminal deoxythimidine nucleotides (dT).

In another embodiment, the modifications on the nucleotides are 2'-O-methyl, 2'fluoro, and GNA modifications.

In an additional embodiment, the double stranded RNAi agent includes at least one phosphorothioate internucleotide linkage. Optionally, the double stranded RNAi agent includes 6-8 (*e.g.,* 6, 7, or 8) phosphorothioate internucleotide linkages.

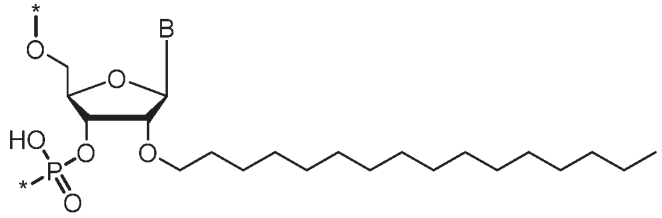
In certain embodiments, the region of complementarity is at least 17 nucleotides in length. Optionally, the region of complementarity is 19-23 nucleotides in length. Optionally, the region of complementarity is 19 nucleotides in length.

In one embodiment, each strand is no more than 30 nucleotides in length.

In another embodiment, at least one strand includes a 3' overhang of at least 1 nucleotide. Optionally, at least one strand includes a 3' overhang of at least 2 nucleotides.

In certain embodiments, the double stranded RNAi agent further includes a lipophilic ligand, *e.g.*, a C16 ligand, conjugated to the 3' end of the sense strand through a monovalent or branched bivalent or trivalent linker. In certain embodiments, the double stranded RNAi agent further includes a lipophilic ligand, e.g., a C16 ligand, conjugated to an internal nucleotide positon, e.g., through a monovalent or branched bivalent or trivalent linker.

In certain embodiments, the ligand is a C16 ligand. In one embodiment, the ligand is conjugated at the 2'-position of a nucleotide or modified nucleotide within the sense or antisense strand. For example, a C16 ligand may be conjugated as shown in the following structure:



where * denotes a bond to an adjacent nucleotide, and B is a nucleobase or a nucleobase analog, optionally where B is adenine, guanine, cytosine, thymine or uracil.

In other embodiments, the agent further comprises a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent via a linker or carrier. In certain embodiments, one of the modified strands in Tables 3, 5, 7, 13, 18, and 20 are conjugated to a targeting ligand that targets a liver tissue. In certain embodiments, the targeting ligand is an L96 ligand, *e.g.*, one or more GalNAc derivatives, optionally conjugated to the double stranded RNAi agent *via* a linker or carrier. In certain embodiments, the L96 ligand is conjugated to the

end of one of the strands. In certain embodiments the L96 ligand is conjugated to the 3' end of the sense strand.

In yet other embodiments, the agents further comprise a lipophilic ligand, *e.g.*, a C16 ligand, conjugated to an internal nucleotide position, *e.g.*, through a monovalent or branched bivalent or trivalent linker, and a targeting ligand that targets a liver tissue, *e.g.*, one or more GalNAc derivatives conjugated to the 3' end of the sense strand through a monovalent or branched bivalent or trivalent linker.

In yet other embodiments, the agents further comprise a lipophilic ligand, *e.g.*, a C16 ligand, conjugated to the 3' end of the sense strand through a monovalent or branched bivalent or trivalent linker and a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives conjugated to the 3' end of the sense strand through a monovalent or branched bivalent or trivalent linker.

In another embodiment, the region of complementarity to SOD1 includes any one of the antisense sequences in any one of Tables 2-7, 12, 13, and 18-20.

In an additional embodiment, the region of complementarity to SOD1 is that of any one of the antisense sequences in any one of Tables 2-7, 12, 13, and 18-20. In some embodiments, the internal nucleotide positions include all positions except the terminal two positions from each end of the strand.

In a related embodiment, the internal positions include all positions except terminal three positions from each end of the strand. Optionally, the internal positions exclude the cleavage site region of the sense strand.

In some embodiments, the internal positions exclude positions 9-12, counting from the 5'-end of the sense strand. In certain emodiments, the sense strand is 21 nucleotides in length.

In other embodiments, the internal positions exclude positions 11-13, counting from the 3'-end of the sense strand. Optionally, the internal positions exclude the cleavage site region of the antisense strand. In certain emodiments, the sense strand is 21 nucleotides in length.

In some embodiments, the internal positions exclude positions 12-14, counting from the 5'-end of the antisense strand. In certain emodiments, the antisense strand is 23 nucleotides in length.

In another embodiment, the internal positions exclude positions 11-13 on the sense strand, counting from the 3'-end, and positions 12-14 on the antisense strand, counting from the 5'-end. In certain emodiments, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

In an additional embodiment, one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand. Optionally, one or more lipophilic moieties are conjugated to one or more of the following internal positions: positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand. In certain emodiments, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

In certain embodiments, the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound. Optionally, the lipophilic moiety is lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O(hexadecyl)glycerol, geranyloxyhexyanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine.

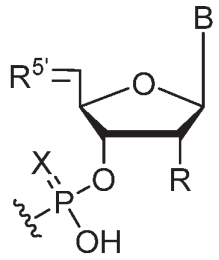
In some embodiments, the lipophilic moiety contains a saturated or unsaturated C_4-C_{30} hydrocarbon chain, and an optional functional group selected that is hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, or alkyne.

In certain embodiments, the lipophilic moiety contains a saturated or unsaturated C_6-C_{18} hydrocarbon chain. Optionally, the lipophilic moiety contains a saturated or unsaturated C_{16} hydrocarbon chain. In a related embodiment, the lipophilic moiety is conjugated via a carrier that replaces one or more nucleotide(s) in the internal position(s). In certain embodiments, the carrier is a cyclic group that is pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, or decalinyl; or is an acyclic moiety based on a serinol backbone or a diethanolamine backbone.

In some embodiments, the lipophilic moiety is conjugated to the double-stranded RNAi agent via a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction, or carbamate.

In one embodiment, the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic linkage.

In another embodiment, the double-stranded RNAi agent further includes a phosphate or phosphate mimic at the 5'-end of the antisense strand. Optionally, the phosphate mimic is a 5'-vinyl phosphonate (VP). When the phosphate mimic is a 5'-vinyl phosphonate (VP), the 5'-terminal nucleotide may have the following structure,



wherein X is O or S;

R is hydrogen, hydroxy, fluoro, or $C_{1\text{-}20}$alkoxy (e.g., methoxy or n-hexadecyloxy);

$R^{5'}$ is $=C(H)\text{-}P(O)(OH)_2$ and the double bond between the C5' carbon and R5' is in the E or Z orientation (e.g., E orientation); and

B is a nucleobase or a modified nucleobase, optionally where B is adenine, guanine, cytosine, thymine, or uracil.

In certain embodiments, the double-stranded RNAi agent further includes a targeting ligand that targets a receptor which mediates delivery to a CNS tissue, *e.g.*, a hydrophilic ligand. In certain embodiments, the targeting ligand is a C16 ligand.

In some embodiments, the double-stranded RNAi agent further includes a targeting ligand that targets a brain tissue, *e.g.,* striatum.

In some embodiments, the double-stranded RNAi agent further includes a targeting ligand that targets a liver tissue or cell type, *e.g.,* hepatocytes.

In one embodiment, the lipophilic moeity or targeting ligand is conjugated via a bio-cleavable linker that is DNA, RNA, disulfide, amide, functionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, or a combination thereof.

In a related embodiment, the 3' end of the sense strand is protected via an end cap which is a cyclic group having an amine, the cyclic group being pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, or decalinyl.

In one embodiment, the RNAi agent includes at least one modified nucleotide that is a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a nucleotide that includes a glycol nucleic acid (GNA) or a nucleotide that includes a vinyl phosphonate. Optionally, the RNAi agent includes at least one of each of the following modifications: 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a nucleotide comprising a glycol nucleic acid (GNA) and a nucleotide comprising vinyl phosphonate.

In another embodiment, the RNAi agent includes a pattern of modified nucleotides as provided below in Tables 2-7, 12, 13, and 18-20 where locations of 2'-C16, 2'-O-methyl, GNA, phosphorothioate, and 2'-fluoro modifications, irrespective of the individual nucleotide base sequences of the displayed RNAi agents.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a SOD1 gene, wherein the double stranded RNAi agent includes a sense strand complementary to an antisense strand, wherein the antisense strand includes a region complementary to part of an mRNA encoding SOD1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (III)

wherein:

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

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

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

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

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

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

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

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

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

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

In certain embodiments, XXX is complementary to X′X′X′, YYY is complementary to Y′Y′Y′, and ZZZ is complementary to Z′Z′Z′.

In another embodiment, the YYY motif occurs at or near the cleavage site of the sense strand.

In an additional embodiment, the Y′Y′Y′ motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end. Optionally, the Y′ is 2′-O-methyl.

In some embodiments, formula (III) is represented by formula (IIIa):

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

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

In another embodiment, formula (III) is represented by formula (IIIb):

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

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

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

In an additional embodiment, formula (III) is represented by formula (IIIc):

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

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

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

In certain embodiments, formula (III) is represented by formula (IIId):

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

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

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

In another embodiment, the double stranded region is 15-30 nucleotide pairs in length. Optionally, the double stranded region is 17-23 nucleotide pairs in length.

In certain embodiments, the double stranded region is 17-25 nucleotide pairs in length. Optionally, the double stranded region is 23-27 nucleotide pairs in length.

In some embodiments, the double stranded region is 19-21 nucleotide pairs in length. Optionally, the double stranded region is 21-23 nucleotide pairs in length.

In certain embodiments, each strand independently has 15-30 nucleotides. Optionally, each strand independently has 19-30 nucleotides. Optionally, each strand independently has 19-23 nucleotides.

In certain embodiments, the double stranded region is 19-21 nucleotide pairs in length and each strand has 19-23 nucleotides.

In another embodiment, the modifications on the nucleotides of the RNAi agent are LNA, glycol nucleic acid (GNA), hexitol nucleic acid (HNA), a cyclohexene nucleic acid (CeNA), 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy or 2'-hydroxyl, and combinations thereof. Optionally, the modifications on nucleotides include 2'-O-methyl, 2'-fluoro, or GNA, and combinations thereof. In a related embodiment, the modifications on the nucleotides are 2′-O-methyl or 2′-fluoro modifications.

In one embodiment the RNAi agent includes a ligand that is or includes one or more lipophilic, *e.g.,* C16, moieties attached through a bivalent or trivalent branched linker.

In other embodiments, the agent further comprises a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives.

In yet other embodiments, the agents further comprise a lipophilic ligand, *e.g.*, a C16 ligand, conjugated to the 3' end of the sense strand through a monovalent or branched bivalent or trivalent linker and a targeting ligand that targets a liver tissue, *e.g.,* one or more GalNAc derivatives conjugated to the 3' end of the sense strand through a monovalent or branched bivalent or trivalent linker.

In certain embodiments, the ligand is attached to the 3′ end of the sense strand.

In some embodiments, the RNAi agent further includes at least one phosphorothioate or methylphosphonate internucleotide linkage. In a related embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand. In a related embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand.

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

In an additional embodiment, the base pair at the 1 position of the 5′-end of the antisense strand of the RNAi agent duplex is an A:U base pair.

In certain embodiments, the Y nucleotides contain a 2′-fluoro modification.

In some embodiments, the Y′ nucleotides contain a 2′-O-methyl modification.

In certain embodiments, p'>0. Optionally, p'=2.

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

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

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

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

In certain embodiments, the SOD1 RNAi agent of the instant disclosure is one of those listed in Tables 2-7, 12, 13, and 18-20. In some embodiments, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand include a modification.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a SOD1 gene in a cell, wherein the double stranded RNAi agent includes a sense strand complementary to an antisense strand, wherein the antisense strand includes a region complementary to part of an mRNA encoding a SOD1 gene, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

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

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

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

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

wherein the sense strand is conjugated to at least one ligand, optionally wherein the ligand is one or more lipophilic, *e.g.*, C16, ligands, or one or more GalNAc derivatives.

An additional aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a SOD1 gene in a cell, wherein the double stranded RNAi agent includes a

sense strand complementary to an antisense strand, wherein the antisense strand includes a region complementary to part of an mRNA encoding SOD1, wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

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

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

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

XXX, YYY, ZZZ, X′X′X′, Y′Y′Y′, and Z′Z′Z′ each independently represent one motif of three identical modifications on three consecutive nucleotides, and wherein the modifications are 2′-O-methyl, glycol nucleic acid (GNA) or 2′-fluoro modifications;

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

wherein the sense strand is conjugated to at least one ligand, optionally where the ligand is one or more lipophilic, *e.g.*, C16, ligands, or one or more GalNAc derivatives.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a SOD1 gene in a cell, wherein the double stranded RNAi agent includes a sense strand complementary to an antisense strand, wherein the antisense strand includes a region complementary to part of an mRNA encoding SOD1 (SEQ ID NO: 1, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity, to the entire nucleotide sequence of SEQ ID NO:1), wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

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

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

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

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

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

wherein the sense strand is conjugated to at least one ligand, optionally wherein the ligand is one or more lipophilic, *e.g.*, C16, ligands, or one or more GalNAc derivatives.

An additional aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a SOD1 gene in a cell, wherein the double stranded RNAi agent includes a sense strand complementary to an antisense strand, wherein the antisense strand includes a region complementary to part of an mRNA encoding SOD1 (SEQ ID NO: 1, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity, to the entire nucleotide sequence of SEQ ID NO: 1), wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

antisense: $3'\ n_p'\text{-}N_a'\text{-}(X'X'X')_k\text{-}N_b'\text{-}Y'Y'Y'\text{-}N_b'\text{-}(Z'Z'Z')_l\text{-}N_a'\text{-}n_q'\ 5'$ (III)

wherein:

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

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

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

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

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

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

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

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

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

wherein the sense strand is conjugated to at least one ligand, optionally wherein the ligand is one or more lipophilic, *e.g.*, C16, ligands or one or more GalNAc derivatives.

Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a SOD1 gene in a cell, wherein the double stranded RNAi agent includes a sense strand complementary to an antisense strand, wherein the antisense strand includes a region complementary to part of an mRNA encoding SOD1 (SEQ ID NO: 1, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity, to the entire nucleotide sequence of SEQ ID NO: 1), wherein each strand is about 14 to about 30 nucleotides in length, wherein the double stranded RNAi agent is represented by formula (III):

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

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

wherein:

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

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

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

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

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

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

wherein the sense strand is conjugated to at least one ligand, optionally wherein the ligand is one or more lipophilic, *e.g.*, C16 ligands, or one or more GalNAc derivatives.

An additional aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a SOD1 gene, wherein the double stranded RNAi agent targeted to SOD1 includes a sense strand and an antisense strand forming a double stranded region, wherein the sense strand includes at least 15 *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 1, 3, 5, 7, and 9, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 1, 3, 5, 7, or 9, and the antisense strand includes at least 15 *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 2, 4, 6, 8, and 10, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, 8, and 10; wherein a substitution of a uracil for any thymine in the sequences provided in the SEQ ID

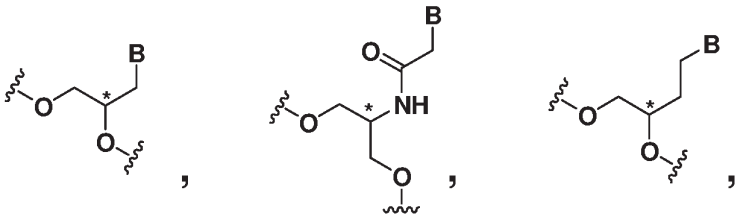
NOs: 1-6 (when comparing aligned sequences) does not count as a difference that contributes to the differing by no more than 3 nucleotides from any one of the nucleotide sequences provided in SEQ ID NOs: 1-6, wherein substantially all of the nucleotides of the sense strand include a modification that is a 2'-O-methyl modification, a GNA, or a 2'-fluoro modification, wherein the sense strand includes two phosphorothioate internucleotide linkages at the 5'-terminus, wherein substantially all of the nucleotides of the antisense strand include a modification selected from the group consisting of a 2'-O-methyl modification and a 2'-fluoro modification, wherein the antisense strand includes two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and wherein the sense strand is conjugated to one or more lipophilic, *e.g.*, C16, ligands, optionally, further comprising a liver targeting ligand, *e.g.,* a ligand comprising one or more GalNAc derivatives.

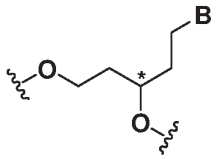
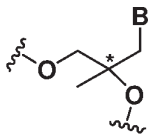
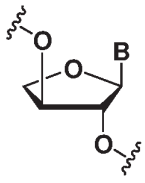
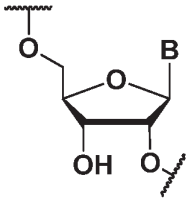
Another aspect of the instant disclosure provides a double stranded RNAi agent for inhibiting expression of a SOD1 gene, wherein the double stranded RNAi agent targeted to SOD1 includes a sense strand and an antisense strand forming a double stranded region, wherein the sense strand includes at least 15 *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 1, 3, 5, 7, and 9, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 1, 3, 5, 7, or 9, and the antisense strand includes at least 15 *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides) from any one of the nucleotide sequences of SEQ ID NOs: 2, 4, 6, 8, and 10, or a nucleotide sequence having at least 90% nucleotide sequence identity, *e.g.* 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity, to the entire nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, 8, and 10, wherein a substitution of a uracil for any thymine in the sequences provided in the SEQ ID NOs: 1-10 (when comparing aligned sequences) does not count as a difference that contributes to the differing by no more than 3 nucleotides from any one of the nucleotide sequences provided in SEQ ID NOs:1-10; wherein the sense strand includes at least one 3'-terminal deoxythimidine nucleotide (dT), and wherein the antisense strand includes at least one 3'-terminal deoxythimidine nucleotide (dT).

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

In another embodiment, each strand has 19-30 nucleotides.

In certain embodiments, the antisense strand of the RNAi agent includes at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions of the 5′ region or a precursor thereof. Optionally, the thermally destabilizing modification of the duplex is one or more of



wherein B is nucleobase.

Another aspect of the instant disclosure provides a cell containing a double stranded RNAi agent of the instant disclosure.

An additional aspect of the instant disclosure provides a pharmaceutical composition for inhibiting expression of a SOD1 gene that includes a double stranded RNAi agent of the instant disclosure.

In one embodiment, the double stranded RNAi agent is administered in an unbuffered solution. Optionally, the unbuffered solution is saline or water.

In another embodiment, the double stranded RNAi agent is administered with a buffer solution. Optionally, the buffer solution includes acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof. In another embodiment, the buffer solution is phosphate buffered saline (PBS).

Another aspect of the disclosure provides a pharmaceutical composition that includes a double stranded RNAi agent of the instant disclosure and a lipid formulation.

In one embodiment, the lipid formulation includes a lipid nanoparticle (LNP).

An additional aspect of the disclosure provides a method of inhibiting expression of a SOD1 gene in a cell, the method involving: (a) contacting the cell with a double stranded RNAi agent of the instant disclosure or a pharmaceutical composition of of the instant disclosure; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a SOD1 gene, thereby inhibiting expression of the SOD1 gene in the cell.

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

In certain embodiments, the subject is a rhesus monkey, a cynomolgous monkey (also known as a crab-eating macaque), a mouse, dog, or a rat.

In certain embodiments, the human subject suffers from a SOD1-associated neurodegenerative disease, *e.g.,* Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

In certain embodiments, the method further involves administering an additional therapeutic agent or therapy to the subject. Exemplary additional therapeutics and treatments include, for example,

sedatives, antidepressants, clonazepam, sodium valproate, opiates, antiepileptic drugs, cholinesterase inhibitors, memantine, benzodiazepines, levodopa, COMT inhibitors (*e.g.*, tolcapone and entacapone), dopamine agonists (*e.g.*, bromocriptine, pergolide, pramipexole, ropinirole, piribedil, cabergoline, apomorphine and lisuride), MAO-B inhibitors (*e.g.*, safinamide, selegiline and rasagiline), amantadine, an anticholinergic, modafinil, pimavanserin, doxepin, rasagline, an antipsychotic, an atypical antipsychotic (*e.g.*, amisulpride, olanzapine, risperidone, and clozapine), riluzole, edaravone , deep brain stimulation, non-invasive ventilation (NIV), invasive ventilation physical therapy, occupational therapy, speech therapy, dietary changes and swallowing technique a feeding tube, a PEG tube, probiotics, and psychological therapy.

In certain embodiments, the double stranded RNAi agent is administered at a dose of about 0.01 mg/kg to about 50 mg/kg.

In some embodiments, the double stranded RNAi agent is administered to the subject intrathecally.

In one embodiment, the method reduces the expression of a SOD1 gene in a brain (*e.g.,* striatum) or spine tissue. Optionally, the brain or spine tissue is striatum, frontal cortex, temporal cortex, cerebellum, hippocampus, cervical spine, lumbar spine, or thoracic spine.

In one embodiment, the method reduces the expression of a SOD1 gene in an eye (with or without lens), heart, kidney, liver, lung and/or muscle tissue or cell.

In some embodiments, the double stranded RNAi agent is administered to the subject subcutaneously.

In some embodiments, the double stranded RNAi agent is administered to the subject intracerebroventricularly.

In some embodiments, the double stranded RNAi agent is administered to the subject intrathecally.

In one embodiment, the method reduces the expression of a SOD1 gene in the liver.

In other embodiments, the method reduces the expression of a SOD1 gene in the liver and the brain.

Another aspect of the instant disclosure provides a method of inhibiting the expression of SOD1 in a subject, the method involving: administering to the subject a therapeutically effective amount of a double stranded RNAi agent of the disclosure or a pharmaceutical composition of the disclosure, thereby inhibiting the expression of SOD1 in the subject.

An additional aspect of the disclosure provides a method for treating or preventing a disorder or SOD1-associated neurodegenerative disease or disorder in a subject, the method involving administering to the subject a therapeutically effective amount of a double stranded RNAi agent of the disclosure or a pharmaceutical composition of the disclosure, thereby treating or preventing a SOD1-associated neurodegenerative disease or disorder in the subject.

In certain embodiments, the SOD1-associated neurodegenerative disease or disorder is selected from the group consisting of Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

Another aspect of the instant disclosure provides a kit for performing a method of the instant disclosure, the kit including: a) a double stranded RNAi agent of the instant disclosure, and b) instructions for use, and c) optionally, a device for administering the double stranded RNAi agent to the subject.

An additional aspect of the instant disclosure provides a double stranded ribonucleic acid (RNAi) agent for inhibiting expression of a SOD1 gene, wherein the RNAi agent possesses a sense strand and an antisense strand, and wherein the antisense strand includes a region of complementarity which includes at least 15 *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3 nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides), *e.g.*, at least 15 *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides), at least 19, *e.g.,* 19, 20, 21, 22, or 23, nucleotides (*i.e.,* differing by 3, 2, 1, or 0 nucleotides), from any one of the antisense strand nucleobase sequences of Tables 2-7, 12, 13, and 18-20. In one embodiment, the RNAi agent includes one or more of the following modifications: a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-C-alkyl-modified nucleotide, a nucleotide comprising a glycol nucleic acid (GNA), a phosphorothioate (PS) and a vinyl phosphonate (VP). Optionally, the RNAi agent includes at least one of each of the following modifications: a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-C-alkyl-modified nucleotide, a nucleotide comprising a glycol nucleic acid (GNA), a phosphorothioate and a vinyl phosphonate (VP).

In another embodiment, the RNAi agent includes four or more PS modifications, optionally six to ten PS modifications, optionally eight PS modifications.

In an additional embodiment, each of the sense strand and the antisense strand of the RNAi agent possesses a 5'-terminus and a 3'-terminus, and the RNAi agent includes eight PS modifications positioned at each of the penultimate and ultimate internucleotide linkages from the respective 3'- and 5'-termini of each of the sense and antisense strands of the RNAi agent.

In another embodiment, each of the sense strand and the antisense strand of the RNAi agent includes a 5'-terminus and a 3'-terminus, and the RNAi agent includes only one nucleotide including a GNA. Optionally, the nucleotide including a GNA is positioned on the antisense strand at the seventh nucleobase residue from the 5'-terminus of the antisense strand.

In an additional embodiment, each of the sense strand and the antisense strand of the RNAi agent includes a 5'-terminus and a 3'-terminus, and the RNAi agent includes one to four 2'-C-alkyl-modified nucleotides. Optionally, the 2'-C-alkyl-modified nucleotide is a 2'-C16-modified nucleotide. Optionally, the RNAi agent includes a single 2'- C-alkyl, *e.g.*, C16-modified nucleotide. Optionally, the single 2'- C-alkyl, *e.g.*, C16-modified nucleotide is located on the sense strand at the sixth nucleobase position from the 5'-terminus of the sense strand.

In another embodiment, each of the sense strand and the antisense strand of the RNAi agent includes a 5'-terminus and a 3'-terminus, and the RNAi agent includes two or more 2'-fluoro modified nucleotides. Optionally, each of the sense strand and the antisense strand of the RNAi agent includes two or more 2'-fluoro modified nucleotides. Optionally, the 2'-fluoro modified nucleotides are located on the sense strand at nucleobase positions 7, 9, 10, and 11 from the 5'-terminus of the sense strand and on the antisense strand at nucleobase positions 2, 14, and 16 from the 5'-terminus of the antisense strand.

In an additional embodiment, each of the sense strand and the antisense strand of the RNAi agent includes a 5'-terminus and a 3'-terminus, and the RNAi agent includes one or more VP modifications. Optionally, the RNAi agent includes a single VP modification at the 5'-terminus of the antisense strand.

In another embodiment, each of the sense strand and the antisense strand of the RNAi agent includes a 5'-terminus and a 3'-terminus, and the RNAi agent includes two or more 2'-O-methyl modified nucleotides. Optionally, the RNAi agent includes 2'-O-methyl modified nucleotides at all nucleobase locations not modified by a 2'-fluoro, a 2'-C-alkyl or a glycol nucleic acid (GNA). Optionally, the two or more 2'-O-methyl modified nucleotides are located on the sense strand at positions 1, 2, 3, 4, 5, 8, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21 from the 5'-terminus of the sense strand and on the antisense strand at positions 1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 15, 17, 18, 19, 20, 21, 22 and 23 from the 5'-terminus of the antisense strand.

In another embodiment, the RNAi agent is a pharmaceutically acceptable salt thereof. "Pharmaceutically acceptable salts" of each of RNAi agents herein include, but are not limited to, a sodium salt, a calcium salt, a lithium salt, a potassium salt, an ammonium salt, a magnesium salt, an mixtures thereof. One skilled in the art will appreciate that the RNAi agent, when provided as a polycationic salt having one cation per free acid group of the optionally modified phosophodiester backbone and/or any other acidic modifications (e.g., 5'-terminal phosphonate groups). For example, an oligonucleotide of "n" nucleotides in length contains n-1 optionally modified phosophodiesters, so that an oligonucleotide of 21 nt in length may be provided as a salt having up to 20 cations (e.g, 20 sodium cations). Similarly, an RNAi agentshaving a sense strand of 21 nt in length and an antisense strand of 23 nt in length may be provided as a salt having up to 42 cations (e.g, 42 sodium cations). In the preceding example, where the RNAi agent also includes a 5'-terminal phosphate or a 5'-terminal vinylphosphonate group, the RNAi agent may be provided as a salt having up to 44 cations (e.g, 44 sodium cations).

In another embodiment, an antisense oligonucleotide is provided having a nucleotide sequence that is any one of the antisense nucleotide sequences listed in any one of Tables 3, 5, 7, 13, 18, and 20 but lacking the 3'-terminal nucleotide (3'N-1 AS), or a pharmaceutically acceptable salt thereof (see, for example, Figure 5). In another embodiment, a dsRNA duplex is provided comprising an antisense strand having a nucleotide sequence that is any one of the antisense nucleotide sequences listed in any one of Tables 3, 5, 7, 13, 18, and 20 but lacking the 3'-terminal nucleotide (3'N-1 AS), and a sense

strand that is substantially complementary to the antisense oligonucleotide, or a pharmaceutically acceptable salt thereof. In another embodiment, a dsRNA duplex is provided comprising any one of the duplexes in any one of Tables 3, 5, 7, 13, 18, or 20, where the antisense nucleotide sequence is replaced with an antisense oligonucleotide sequence lacking the 3'-terminal nucleotide (3'N-1 AS), or a pharmaceutically acceptable salt thereof.

In one embodiment, an antisense oligonucleotide is provided having the nucleotide sequence of any one of SEQ ID NO. 1369 – 1378 (see Table 23), or a pharmaceutically acceptable salt thereof. In one embodiment, an antisense oligonucleotide is provided having the nucleotide sequence of SEQ ID NO. 1369, or a pharmaceutically acceptable salt thereof. In one embodiment, an antisense oligonucleotide is provided having the nucleotide sequence of SEQ ID NO. 1370, or a pharmaceutically acceptable salt thereof. In one embodiment, an antisense oligonucleotide is provided having the nucleotide sequence of SEQ ID NO. 1371, or a pharmaceutically acceptable salt thereof.

In another embodiment, a dsRNA duplex is provided comprising an antisense strand having a nucleotide sequence of SEQ ID NO. 1369, and a sense strand that is substantially complementary to the antisense oligonucleotide, or a pharmaceutically acceptable salt thereof. In another embodiment, a dsRNA duplex is provided comprising an antisense strand having a nucleotide sequence of SEQ ID NO. 1370, and a sense strand that is substantially complementary to the antisense oligonucleotide, or a pharmaceutically acceptable salt thereof. In another embodiment, a dsRNA duplex is provided comprising an antisense strand having a nucleotide sequence of SEQ ID NO. 1371, and a sense strand that is substantially complementary to the antisense oligonucleotide, or a pharmaceutically acceptable salt thereof.

In another embodiment, a dsRNA duplex is provided comprising an antisense strand having a nucleotide sequence of SEQ ID NO. 1369, and a sense strand having a sequence of SEQ ID NO. 11, or a pharmaceutically acceptable salt thereof. In another embodiment, a dsRNA duplex is provided comprising an antisense strand having a nucleotide sequence of SEQ ID NO. 1370, and a sense strand having a nucleotide sequence of SEQ ID NO. 1371, or a pharmaceutically acceptable salt thereof. In another embodiment, a dsRNA duplex is provided comprising an antisense strand having a nucleotide sequence of SEQ ID NO. 1371, and a sense strand having a nucleotide sequence of SEQ ID NO. 15, or a pharmaceutically acceptable salt thereof.

The present invention is further illustrated by the following detailed description and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph depicting human SOD1 mRNA levels in mice subcutaneously administered a single 3 mg/kg dose of the indicated dsRNA duplexes. Human SOD1 mRNA levels are shown relative to control levels detected with PBS treatment.

Figure 2 is a graph depicting human SOD1 mRNA levels in the indicated areas of the brain and spinal cord of mice following intracerebroventricular injection (ICV) of a single 25 μg, 50 μg, 100 μg, 150 μg , 200 μg, or 300 μg dose of AD-401824, or artificial CSF (aCSF).

Figure 3A is a graph depicting the effect of a single 50 nM, 10 nM, 1 nM, or 0.1 nM dose of the indicated duplexes on hSOD1 mRNA expression in BE(2)c cells on Day 14 post-dose.

Figure 3B is a graph depicting the effect of a single 0.9 mg intrathecally administered dose of the indicated duplexes on hSOD1 mRNA expression in the lumbar, thoracic, and cervical spinal cord regions of G93A rats on Day 14 post-dose.

Figure 4A are graphs depicting the concentration of the indicated duplexes in the cervical, thoracic or lumbar spinal cords of rats administered a single dose of the indicated duplexes.

Figure 4B are graphs depicting the concentration of the indicated duplexes in the cerebral cortices or brainstems of rats administered a single dose of the indicated duplexes.

Figure 4C is a Table depicting the retention times of the indicated duplexes in the cervical spinal cords, thoracic spinal cords, lumbar spinal cords, cerebral cortices or brainstems of rats administered a single dose of the indicated duplexes.

Figure 5 is a Table depicting the metabolite profiles in the cerebral cortices and lumbar spinal cords of rats administered a single dose of the indicated duplexes. Figure 5 discloses SEQ ID NOS 1366, 1366, 1366, 1366-1367, 1367, 1367-1368 and 1368, respectively, in order of appearance.

Figure 6 is a Table summarizing the tissue exposure and metabolite profiling of duplexes AD-1395762, AD-1395756, and AD-1395731.

Figure 7 is a Table depicting the study protocol assessing the effect of the indicated uplexes on SOD1 mRNA and protein expression in non-human primates.

Figure 8A is a Table depicting the numbers of non-human primates considered to have received "bad exposure" (duplex levels in a CSF sample <1,000 ng/mL at 24 hours), "good exposure" (duplex levels in a CSF sample >3,000 ng/mL at 24 hours), or "partial exposure" (duplex levels in a CSF sample 1,000-3,000 ng/mL at 24 hours) to a single 70 mg intrathecally administered dose of AD-1395762, AD-1395756, or AD-1395731.

Figure 8B are graphs depicting the level of SOD1 mRNA in lumbar spinal cord (L3), thoracic spinal cord (T1-T5), cervical spinal cord (C7), frontal cortex (FC), brainstem (BS), or pons samples in non-human primates following intrathecal administration of a single 70 mg dose of the indicated duplexes at Days 31 and 85 post-dose.

Figure 8C are graphs depicting the level of SOD1 mRNA in lumbar spinal cord (L3), thoracic spinal cord (T1-T5), cervical spinal cord (C7), frontal cortex (FC), brainstem (BS), or pons samples in non-human primates following intrathecal administration of a single 70 mg dose of the indicated duplexes at Day 85 post-dose. Figure 8C discloses SEQ ID NOS 1281, 1284, 71 and 1366-1368, respectively, in order of appearance.

Figure 9A are graphs depicting the level of SOD1 protein levels in CSF samples from individual non-human primates following intrathecal administration of a single 70 mg dose of the indicated duplexes at Days 0, 8, 14, 29, 57, and 85 post-dose.

Figure 9A are graphs depicting the level of SOD1 protein levels in CSF samples from individual non-human primates following intrathecal administration of a single 70 mg dose of the indicated duplexes at Days 0, 8, 14, 29, 57, and 85 post-dose.

Figure 9B are graphs depicting the level of SOD1 protein levels in CSF samples from individual non-human primates following intrathecal administration of a single 70 mg dose of the indicated duplexes out to Day 85 post-dose (top) and the level of SOD1 protein level in CSF samples from three individual non-human primates following intrathecal administration of a single 70 mg dose of the indicated duplexes in CSF samples from individual non-human primates following intrathecal administration of a single 70 mg dose of the indicated duplexes out to Day 85 post-dose extended out to Day 141 post-dose.

Figure 9C is a graph depicting the level of SOD1 protein levels in prefrontal, thoracic spinal cord (T9-12) and CSF samples in non-human primates following intrathecal administration of a single 70 mg dose of the indicated duplexes at Day 85 post-dose.

Figure 10 are graphs depicting that the observed reductions in mRNA levels and protein levels in prefrontal cortex samples and thoracic spinal cord (T9-12) samples are highly and significantly correlated in NHP following intrathecal administration of a single 70 mg dose of the indicated duplexes.

Figure 11A are graphs depicting the mRNA remaining versus siRNA exposure in prefrontal, brainstem, pons, cervical spinal cord (SC_C5-C6), cervical spinal cord (SC_C7), thoracic spinal cord (SC_T1-T5), thoracic spinal cord (SC_T9-T12), and lumbar spinal cord (SC_L3) tissues following intrathecal administration of a single 70 mg dose of the indicated duplexes.

Figure 11B are graphs depicting that there is a strong correlation between both mRNA and protein reduction to the amount of siRNA exposure in both prefrontal cortex and thoracic spinal cord (SC_T0-T12) samples following a single intrathecally administered 70 mg dose of the indicated duplexes.

Figures 12A – 12H are stacked bar graphs illustrating the *in vitro* SOD1 knockdown for each of the duplexes of Tables 14 and 15 in PCH or Be(2)C cells as mapped against NM_000454.4 for duplexes having target sequences starting at positions 199-225 (Fig. 12A, PCH; Fig. 12B, Be(2)C); positions 319-337 (Fig. 12C, PCH; Fig. 12D, Be(2)C); positions 364-382 (Fig. 12E, PCH; Fig. 12F, Be(2)C); and positions 516-540 (Fig. 12G, PCH; Fig. 12H, Be(2)C).

Figure 13 is a graph showing a substantial absence of pharmacodynamic effect of the indicated dsRNA agents administered in the kidney and liver of NHP at Days 31 and 85 post-intrathecal administration.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure provides RNAi compositions, which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a SOD1 gene. The SOD1 gene may be within a cell, *e.g.,* a cell within a subject, such as a human. The present disclosure also provides methods of using the RNAi compositions of the disclosure for inhibiting the expression of a SOD1 gene or for

treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a SOD1 gene, *e.g.,* a SOD1-associated neurodegenerative disesase, *e.g.,* Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

The RNAi agents of the disclosure include an RNA strand (the antisense strand) having a region which is about 30 nucleotides or less in length, *e.g.*, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a SOD1 gene. In certain embodiments, the RNAi agents of the disclosure include an RNA strand (the antisense strand) having a region which is about 21-23 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a SOD1 gene.

In certain embodiments, the RNAi agents of the disclosure include an RNA strand (the antisense strand) which can include longer lengths, for example up to 66 nucleotides, *e.g.*, 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of a SOD1 gene. These RNAi agents with the longer length antisense strands preferably include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

The use of these RNAi agents enables the targeted degradation of mRNAs of a SOD1 gene in mammals. Thus, methods and compositions including these RNAi agents are useful for treating a subject who would benefit by a reduction in the levels or activity of a SOD1 protein, such as a subject having a SOD1-associated neurodegenerative disease, *e.g.,* Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

The following detailed description discloses how to make and use compositions containing RNAi agents to inhibit the expression of a SOD1 gene, as well as compositions and methods for treating subjects having diseases and disorders that would benefit from inhibition or reduction of the expression of the genes.

I. Definitions

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

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

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

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

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

The term “at least”, “no less than”, or “or more” prior to a number or series of numbers is understood to include the number adjacent to the term “at least”, and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, “at least 18 nucleotides of a 21 nucleotide nucleic acid molecule” means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that “at least” can modify each of the numbers in the series or range.

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

As used herein, methods of detection can include determination that the amount of analyte present is below the level of detection of the method.

In the event of a conflict between an indicated target site and the nucleotide sequence for a sense or antisense strand, the indicated sequence takes precedence.

In the event of a conflict between a chemical structure and a chemical name, the chemical structure takes precedence.

As used herein, the term “Superoxide dismutase 1,” used interchangeably with the term “SOD1,” refers to the well-known gene and polypeptide, also known in the art as Superoxide Dismutase [Cu-Zn], Cu/Zn Superoxide Dismutase, Epididymis Secretory Protein Li 44, EC 1.15.1.1, and Indophenoloxidase A. The term “SOD1” includes human SOD1, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. NM_000454.4 (GI:48762945; SEQ ID NO:1); mouse SOD1, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. NM_011434.1 (GI:45597446; SEQ ID NO:3); Macaca fascicularis (crab-eating macaque, also known as cynomolgus monkey) SOD1, the amino acid and nucleotide sequence of which

may be found in, for example, GenBank Accession No. NM_001285406.1 (GI:549432988; SEQ ID NO:5); dog SOD1, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. NM_001003035.1 (GI:50978673; SEQ ID NO:7); and rat SOD1, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. NM_017050.1 (GI:8394327; SEQ ID NO:9).

Additional examples of SOD1 mRNA sequences are readily available using, *e.g.*, GenBank, UniProt, OMIM, and the Macaca genome project web site.

Exemplary SOD1 nucleotide sequences may also be found in SEQ ID NOs:1-10. SEQ ID NOs: 2, 4, 6, 8, and 10 are the reverse complement sequences of SEQ ID NOs: 1, 3, 5, 7, and 9, respectively.

Further information on SOD1 is provided, for example in the NCBI Gene database at www.ncbi.nlm.nih.gov/gene/6647.

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

The terms "Superoxide dismutase 1" and "SOD1," as used herein, also refers to naturally occurring DNA sequence variations of the SOD1 gene. Numerous seuqnce variations within the SOD1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp?LinkName=gene_snp&from_uid=6647, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a SOD1 gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodment, the target portion of the sequence will be at least long enough to serve as a substrate for RNAi-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a SOD1 gene. In one embodiment, the target sequence is within the protein coding region of the SOD1 gene. In another embodiment, the target sequence is within the 3' UTR of the SOD1 gene.

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

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

"G," "C," "A," "T", and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively in the context of a modified or unmodified nucleotide. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.,* Table 1). The skilled person is well aware that guanine, cytosine, adenine, thymidine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the disclosure by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the disclosure.

The terms "iRNA", "RNAi agent," "iRNA agent," "RNA interference agent" as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. RNA interference (RNAi) is a process that directs the sequence-specific degradation of mRNA. RNAi modulates, *e.g.,* inhibits, the expression of SOD1 in a cell, *e.g.,* a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the disclosure includes a single stranded RNAi that interacts with a target RNA sequence, *e.g.,* a SOD1 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into double-stranded short interfering RNAs (siRNAs) comprising a sense strand and an antisense strand by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev*. 15:485). Dicer, a ribonuclease-III-like enzyme, processes these dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al*., (2001) *Nature* 409:363). These siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al*., (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al*., (2001) *Genes Dev*. 15:188). Thus, in one aspect the disclosure relates to a single stranded RNA (ssRNA) (the antisense strand of a siRNA duplex) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.,* a SOD1 gene. Accordingly, the term "siRNA" is also used herein to refer to an RNAi as described above.

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

In another embodiment, a "RNAi agent" for use in the compositions and methods of the disclosure is a double stranded RNA and is referred to herein as a "double stranded RNAi agent," "double stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA" refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.,* a SOD1 gene. In some embodiments of the disclosure, a double stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.*, an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, a dsRNA molecule can include ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide, a modified nucleotide. In addition, as used in this specification, an "RNAi agent" may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides.

As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, or a modified nucleobase. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.,* a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the disclosure include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "RNAi agent" for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide – which is acknowledged as a naturally occurring form of nucleotide – if present within a RNAi agent can be considered to constitute a modified nucleotide.

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

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

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired nucleotides or nucleotides not directed to the target site of the dsRNA. In some embodiments, the hairpin loop can be 10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

In certain embodiment, the two strands of double-stranded oligomeric compound can be linked together. The two strands can be linked to each other at both ends, or at one end only. By linking at one end is meant that 5'-end of first strand is linked to the 3'-end of the second strand or 3'-end of first strand is linked to 5'-end of the second strand. When the two strands are linked to each other at both ends, 5'-end of first strand is linked to 3'-end of second strand and 3'-end of first strand is linked to 5'-end of second strand. The two strands can be linked together by an oligonucleotide linker including, but not limited to, (N)n; wherein N is independently a modified or unmodified nucleotide and n is 3-23. In some embodiemtns, n is 3-10, *e.g.*, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the oligonucleotide linker is selected from the group consisting of GNRA, (G)4, (U)4, and (dT)4, wherein N is a modified or unmodified nucleotide and R is a modified or unmodified purine nucleotide. Some of the nucleotides in the linker can be involved in base-pair interactions with other nucleotides in the linker. The two strands can also be linked together by a non-nucleosidic linker, *e.g.* a linker described herein. It will be appreciated by one of skill in the art that any oligonucleotide chemical modifications or variations describe herein can be used in the oligonucleotide linker.

Hairpin and dumbbell type oligomeric compounds will have a duplex region equal to or at least 14, 15, 15, 16, 17, 18, 19, 29, 21, 22, 23, 24, or 25 nucleotide pairs. The duplex region can be equal to or less than 200, 100, or 50, in length. In some embodiments, ranges for the duplex region are 15-30, 17 to 23, 19 to 23, and 19 to 21 nucleotides pairs in length.

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

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of

one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs.

In one embodiment, an RNAi agent of the invention is a dsRNA, each strand of which is 24-30 nucleotides in length, that interacts with a target RNA sequence, *e.g.*, a SOD1 target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev*. 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev*. 15:188).

In one embodiment, an RNAi agent of the invention is a dsRNA agent, each strand of which comprises 19-23 nucleotides that interacts with a SOD1 RNA sequence to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev*. 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev*. 15:188). In one embodiment, an RNAi agent of the invention is a dsRNA of 24-30 nucleotides that interacts with a SOD1 RNA sequence to direct the cleavage of the target RNA.

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

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

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, 0-3, 1-3, 2-4, 2-5, 4-10, 5-10, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

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

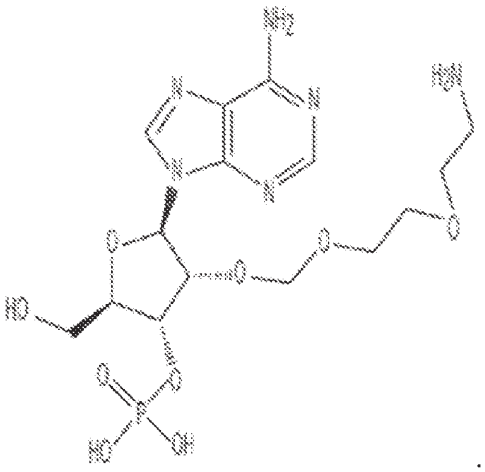
In certain embodiments, at least one end of at least one strand is extended beyond a duplex targeting region, including structures where one of the strands includes a thermodynamically -stabilizing tetraloop structure (see, *e.g.*, U.S. Patent Nos. 8,513,207 and 8,927,705, as well as W02010033225, the entire contents of each of which are incorporated by reference herein). Such structures may include single-stranded extensions (on one or both sides of the molecule)as well as double-stranded extensions.

In certain embodiments, the 3' end of the sense strand and the 5' end of the antisense strand are joined by a polynucleotide sequence comprising ribonucleotides, deoxyribonucleotides or both, optionally wherein the polynucleotide sequence comprises a tetraloop sequence. In certain embodiments, the sense strand is 25-35 nucleotides in length.

A tetraloop may contain ribonucleotides, deoxyribonucleotides, modified nucleotides, and combinations thereof. Typically, a tetraloop has 4 to 5 nucleotides. In some embodiments, the loop comprises a sequence set forth as GAAA. In some embodiments, at least one of the nucleotide of the loop (GAAA) comprises a nucleotide modification. In some embodiments, the modified nucleotide comprises a 2'-modification. In some embodiments, the 2 '-modification is a modification selected from the group consisting of 2'-aminoethyl, 2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl, 2'-aminodiethoxymethanol, 2'- adem, and 2'-deoxy-2'-fhioro- -d-arabinonucleic acid. In some

embodiments, all of the nucleotides of the loop are modified. In some embodiments, the G in the GAAA sequence comprises a 2'-OH. In some embodiments, each of the nucleotides in the GAAA sequence comprises a 2'-O-methyl modification. In some embodiments, each of the A in the GAAA sequence comprises a 2'-OH and the G in the GAAA sequence comprises a 2'-O-methyl modification. In preferred embodiments, In some embodiments, each of the A in the GAAA sequence comprises a 2'-O-methoxyethyl (MOE) modification and the G in the GAAA sequence comprises a 2'-O-methyl modification; or each of the A in the GAAA sequence comprises a 2'- adem modification and the G in the GAAA sequence comprises a 2'-O-methyl modification. See, *e.g.,* PCT Publication No. WO 2020/206350, the entire contents of which are incorporated herein by reference.

An exemplary 2'adem modified nucleotide is shown below:



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

In one embodiment, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, 0-3, 1-3, 2-4, 2-5, 4-10, 5-10, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, *e.g.*, 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the

sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate. In certain embodiments, the overhang includes a self-complementary portion such that the overhang is capable of forming a hairpin structure that is stable under physiological conditions.

The terms "blunt" or "blunt ended" as used herein in reference to a dsRNA mean that there are no unpaired nucleotides or nucleotide analogs at a given terminal end of a dsRNA, *i.e.*, no nucleotide overhang. One or both ends of a dsRNA can be blunt. Where both ends of a dsRNA are blunt, the dsRNA is said to be blunt ended. To be clear, a "blunt ended" dsRNA is a dsRNA that is blunt at both ends, *i.e.*, no nucleotide overhang at either end of the molecule. Most often such a molecule will be double stranded over its entire length.

The term "antisense strand" or "guide strand" refers to the strand of a RNAi agent, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.,* a SOD1 mRNA.

As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, *e.g.,* a SOD1 nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, 3, or 2 nucleotides of the 5'- or 3'-terminus of the RNAi agent.

In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the antisense strand. In some embodiments, the antisense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the target mRNA, *e.g.*, the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the target mRNA. In some embodiments, the antisense strand double stranded RNA agent of the invention includes no more than 4 mismatches with the sense strand, *e.g.*, the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the sense strand. In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the sense strand. In some embodiments, the sense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the antisense strand, *e.g.*, the sense strand includes 4, 3, 2, 1, or 0 mismatches with the antisense strand. In some embodiments, the nucleotide mismatch is, for example, within 5, 4, 3 nucleotides from the 3'-end of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide of the iRNA agent. In some embodiments, the mismatch(s) is not in the seed region.

Thus, an RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, a RNAi agent as described herein contains no more than 3 mismatches (*i.e.,* 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more

than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments, if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of a SOD1 gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of a SOD1 gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a SOD1 gene is important, especially if the particular region of complementarity in a SOD1 gene is known to have polymorphic sequence variation within the population.

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

As used herein, "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

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

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

Complementary sequences within a RNAi agent, *e.g.*, within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to

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

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

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

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding SOD1). For example, a polynucleotide is complementary to at least a part of a SOD1 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding SOD1.

Accordingly, in some embodiments, the antisense strand polynucleotides disclosed herein are fully complementary to the target SOD1 sequence.

In other embodiments, the antisense strand polynucleotides disclosed herein are substantially complementary to the target SOD1 sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 1, 3, 5, 7, or 9 for SOD1, or a fragment of SEQ ID NOs: 1, 3, 5, 7, or 9, such as about 85%, about 90%, or about 95% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target SOD1 sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 2-7, 12, 13, and 18-20, or a fragment of any one of the sense strand

nucleotide sequences in any one of Tables 2-7, 12, 13, and 18-20, such as about 85%, about 90%, or about 95% complementary.

In one embodiment, an RNAi agent of the disclosure includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is the same as a target SOD1 sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 2, 4, 6, 8, or 10, or a fragment of any one of SEQ ID NOs: 2, 4, 6, 8, or 10, such as about 85%, about 90%, or about 95% complementary.

In some embodiments, an iRNA of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target SOD1 sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in any one of any one of Tables 2-7, 12, 13, and 18-20, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2-7, 12, 13, and 18-20, such as about 85%, about 90%, or about 95% complementary.

In some embodiments, the double-stranded region of a double-stranded iRNA agent is equal to or at least, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, 30 or more nucleotide pairs in length.

In some embodiments, the antisense strand of a double-stranded iRNA agent is equal to or at least 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

In some embodiments, the sense strand of a double-stranded iRNA agent is equal to or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each independently 15 to 30 nucleotides in length.

In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each independently 19 to 25 or 19 to 30 nucleotides in length.

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

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

In one aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense nucleic acid molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense RNA molecule is complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al.*, (2002) *Mol Cancer Ther* 1:347-355. The single-stranded antisense RNA molecule may be about 15 to about 30 nucleotides in length and have a sequence that is complementary

to a target sequence. For example, the single-stranded antisense RNA molecule may comprise a sequence that is at least about 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

In one embodiment, at least partial suppression of the expression of a SOD1 gene, is assessed by a reduction of the amount of SOD1 mRNA which can be isolated from or detected in a first cell or group of cells in which a SOD1 gene is transcribed and which has or have been treated such that the expression of a SOD1 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition may be expressed in terms of:

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

In one embodiment, inhibition of expression is determined by the dual luciferase method in Example 1 wherein the RNAi agent is present at 10 nM.

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

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the RNAi agent. Contacting a cell *in vivo* may be done, for example, by injecting the RNAi agent into or near the tissue where the cell is located, or by injecting the RNAi agent into another area, *e.g.*, the central nervous system (CNS), optionally *via* intrathecal, intracerebroventricular or other injection, or to the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the RNAi agent may contain or be coupled to a ligand, *e.g.*, a lipophilic moiety or moieties as described below and further detailed, *e.g.*, in PCT Publication No. WO 2019/217459, which is incorporated herein by reference, that directs or otherwise stabilizes the RNAi agent at a site of interest, *e.g.*, the CNS. In some embodiments, the RNAi agent may contain or be coupled to a ligand, *e.g.*, one or more GalNAc derivatives as described below, that directs or otherwise stabilizes the RNAi agent at a site of interest, *e.g.*, the liver. In other embodiments, the RNAi agent may contain or be coupled to a lipophilic moiety or moieties and one or more GalNAc derivatives. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. For example, a cell may also be contacted *in vitro* with an RNAi agent and subsequently transplanted into a subject.

In one embodiment, contacting a cell with an RNAi agent includes "introducing" or "delivering the RNAi agent into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of a RNAi agent can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. Introducing a RNAi agent into a cell may be *in vitro* or *in vivo*. For example, for *in vivo* introduction, a RNAi agent can be injected into a tissue site or administered

systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or are known in the art.

The term "lipophile" or "lipophilic moiety" broadly refers to any compound or chemical moiety having an affinity for lipids. One way to characterize the lipophilicity of the lipophilic moiety is by the octanol-water partition coefficient, $\log K_{ow}$, where K_{ow} is the ratio of a chemical's concentration in the octanol-phase to its concentration in the aqueous phase of a two-phase system at equilibrium. The octanol-water partition coefficient is a laboratory-measured property of a substance. However, it may also be predicted by using coefficients attributed to the structural components of a chemical which are calculated using first-principle or empirical methods (see, for example, Tetko *et al.*, *J. Chem. Inf. Comput. Sci.* 41:1407-21 (2001), which is incorporated herein by reference in its entirety). It provides a thermodynamic measure of the tendency of the substance to prefer a non-aqueous or oily milieu rather than water (*i.e.* its hydrophilic/lipophilic balance). In principle, a chemical substance is lipophilic in character when its $\log K_{ow}$ exceeds 0. Typically, the lipophilic moiety possesses a $\log K_{ow}$ exceeding 1, exceeding 1.5, exceeding 2, exceeding 3, exceeding 4, exceeding 5, or exceeding 10. For instance, the $\log K_{ow}$ of 6-amino hexanol, for instance, is predicted to be approximately 0.7. Using the same method, the $\log K_{ow}$ of cholesteryl N-(hexan-6-ol) carbamate is predicted to be 10.7.

The lipophilicity of a molecule can change with respect to the functional group it carries. For instance, adding a hydroxyl group or amine group to the end of a lipophilic moiety can increase or decrease the partition coefficient (*e.g.*, $\log K_{ow}$) value of the lipophilic moiety.

Alternatively, the hydrophobicity of the double-stranded RNAi agent, conjugated to one or more lipophilic moieties, can be measured by its protein binding characteristics. For instance, in certain embodiments, the unbound fraction in the plasma protein binding assay of the double-stranded RNAi agent could be determined to positively correlate to the relative hydrophobicity of the double-stranded RNAi agent, which could then positively correlate to the silencing activity of the double-stranded RNAi agent.

In one embodiment, the plasma protein binding assay determined is an electrophoretic mobility shift assay (EMSA) using human serum albumin protein. An exemplary protocol of this binding assay is illustrated in detail in, *e.g.*, PCT Publication No. WO 2019/217459. The hydrophobicity of the double-stranded RNAi agent, measured by fraction of unbound siRNA in the binding assay, exceeds 0.15, exceeds 0.2, exceeds 0.25, exceeds 0.3, exceeds 0.35, exceeds 0.4, exceeds 0.45, or exceeds 0.5 for an enhanced *in vivo* delivery of siRNA.

Accordingly, conjugating the lipophilic moieties to the internal position(s) of the double-stranded RNAi agent provides optimal hydrophobicity for the enhanced *in vivo* delivery of siRNA.

The term "lipid nanoparticle" or "LNP" is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, *e.g.,* a RNAi agent or a plasmid from which a RNAi agent is transcribed. LNPs are described in, for example, U.S. Patent Nos. 6,858,225, 6,815,432, 8,158,601, and 8,058,069, the entire contents of which are hereby incorporated herein by reference.

As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), or a non-primate (such as a a rat, or a mouse). In a preferred embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder, or condition that would benefit from reduction in SOD1 expression; a human at risk for a disease, disorder, or condition that would benefit from reduction in SOD1 expression; a human having a disease, disorder, or condition that would benefit from reduction in SOD1 expression; or human being treated for a disease, disorder, or condition that would benefit from reduction in SOD1 expression as described herein.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more signs or symptoms associated with SOD1 gene expression or SOD1 protein production, *e.g.,* SOD1-associated neurodegenerative disease, *e.g.,* Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS), decreased expression, deposition, and/or activity of SOD1 in regions of increased neuronal death in subjcts having such neurodegenerative diseases. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

The term "lower" in the context of the level of SOD1 in a subject or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, 15%, 20%, 25%, 30%, %, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more. In certain embodiments, a decrease is at least 20%. In certain embodiments, the decrease is at least 50% in a disease marker, *e.g.*, protein or gene expression level. "Lower" in the context of the level of SOD1 in a subject is preferably down to a level accepted as within the range of normal for an individual without such disorder. In certain embodiments, "lower" is the decrease in the difference between the level of a marker or symptom for a subject suffering from a disease and a level accepted within the range of normal for an individual, *e.g.*, the level of decrease in bodyweight between an obese individual and an individual having a weight accepted within the range of normal.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder, or condition thereof, that would benefit from a reduction in expression of a SOD1 gene or production of a SOD1 protein, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, *e.g.,* a symptom of a SOD1-associated neurodegenerative disease. The failure to develop a disease, disorder, or condition, or the reduction in the development of a symptom associated with such a disease, disorder, or condition, *e.g.,* neuroinflammation (*e.g.*, by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms delayed (*e.g.*, by days, weeks, months or years) is considered effective prevention.

As used herein, the term "SOD1-associated neurodegenerative disease" or "SOD1-associated neurodegenerative disorder" is understood as any disease or disorder that would benefit from reduction in the expression or activity of SOD1. Such SOD1-neurodegenerative diseases are characterized by SOD1 protein misfolding , *e.g.,* increased SOD1 deposition in areas of the brain associated with neuronal cell death in such diseases (see, *e.g.,* Trist B, *et al.* (2020) *Angew Chem Int Ed Engl.* Accepted

Author Manuscript), *e.g.,* Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

In one embodiment, a SOD1-associated neurodegenerative disease is "Amyotrophic Lateral Sclerosis" ("ALS"), also known as Lou Gehrig's disease.

Amyotrophic lateral sclerosis (ALS) is a progressive disease that affects motor neurons in the spinal cord and the brain. In ALS, motor neurons die (atrophy) over time, leading to muscle weakness, a loss of muscle mass, and an inability to control movement.

Mutations in the SOD1 gene cause approximately 20% of inherited familial amyotrophic lateral sclerosis (fALS) cases and up to approximately 5% of sporadic ALS (sALS) cases. The resulting mutations, including, *e.g.,* amino-acid substitutions, insertions, deletions, and/or genetic polymorphisms, destabilize SOD1's protein structure, leading to its misfolding and self-assembly into neurotoxic oligomers and aggregates, a process that contributes to the characteristic motor neuron degeneration in affected individuals.

People with sporadic ALS usually first develop features of the condition in their late fifties or early sixties. Sporadic and familial ALS present similar pathological and clinical profiles.

The earliest symptoms of ALS include muscle twitching, cramping, stiffness, or weakness. Affected individuals may develop slurred speech (dysarthria) and, later, difficulty chewing or swallowing (dysphagia). Many people with ALS experience malnutrition because of reduced food intake due to dysphagia and an increase in their body's energy demands (metabolism) due to prolonged illness. Muscles become weaker as the disease progresses, and arms and legs begin to look thinner as muscle tissue atrophies. Individuals with ALS eventually lose muscle strength and the ability to walk. Affected individuals eventually become wheelchair-dependent and increasingly require help with personal care and other activities of daily living. Over time, muscle weakness causes affected individuals to lose the use of their hands and arms. Breathing becomes difficult because the muscles of the respiratory system weaken. Most people with ALS die from respiratory failure within 2 to 10 years after the signs and symptoms of ALS first appear; however, disease progression varies widely among affected individuals.

SOD1 accumulation has been found in the motor neurons, spinal cord, and associated cells of postmortem mutant SOD1 transgenic mice and human ALS patient tissues. Furthermore, SOD1 transgenic mice, an art-recognized model of ALS, which express mutated SOD1 forms in similar or elevated levels as to the endogenous mouse protein have been shown to recapitulate the ALS-phenotype. These mice develop severe degeneration of motor neurons which leads to progressive paralysis of the hindlimbs and forelimbs and death, as seen in ALS human patients. These mice also present pathology found in postmortem tissues from human patients, such as SOD1 cytoplasmic inclusions, gliosis, glutamate excitotoxicity, vacuolization of mitochondria, and disrupted axonal transport (Mina M, *et al.* (2018) *J Transl Neurosci.* 3:9).

In one embodiment, a SOD1-associated neurodegenerative disease is "Alzheimer's disease" ("AD"). AD is a chronic neurodegenerative disease that usually starts slowly and gradually worsens

over time. The most common early symptom is difficulty in remembering recent events. As the disease advances, symptoms can include problems with language, disorientation (including easily getting lost), mood swings, loss of motivation, not managing self-care, and behavioral issues. As a person's condition declines, they often withdraw from family and society. Gradually, bodily functions are lost, ultimately leading to death.

Neuropathologically, AD is characterised by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions, including degeneration in the temporal lobe and parietal lobe, and parts of the frontal cortex and cingulate gyrus. Degeneration is also present in brainstem nuclei like the locus coeruleus. Studies using MRI and PET have documented reductions in the size of specific brain regions in people with AD as they progressed from mild cognitive impairment to Alzheimer's disease, and in comparison with similar images from healthy older adults.

Both amyloid plaques and neurofibrillary tangles are clearly visible by microscopy in brains of those afflicted by AD. Plaques are dense, mostly insoluble deposits of beta-amyloid peptide and cellular material outside and around neurons. Tangles (neurofibrillary tangles) are aggregates of the microtubule-associated protein tau which has become hyperphosphorylated and accumulate inside the cells themselves. Although many older individuals develop some plaques and tangles as a consequence of ageing, the brains of people with AD have a greater number of them in specific brain regions such as the temporal lobe. Lewy bodies are not rare in the brains of people with AD.

The post-mortem and *in vivo* examinations of indiviauls with AD have also showed an accumulation of products of free radicals damage in the central nervous system and in the peripheral tissues. Additionally, SOD1 aggregates have been identified in AD brains and we shown to be associated with amyloid senile plaques and neurofibrillary tangles (Choi J, *et al.* (2005) *JBC*. 280:11648-11655).

In one embodiment, SOD1-associated neurodegenerative disease is “Parkinson’s disease” (“PD”). Parkinson disease is a progressive disorder of the nervous system. The disorder affects several regions of the brain, especially an area called the substantia nigra that controls balance and movement. PD leads to loss of dopaminergic neurons within the basal ganglia.

Often the first symptom of Parkinson disease is trembling or shaking (tremor) of a limb, especially when the body is at rest. Typically, the tremor begins on one side of the body, usually in one hand. Tremors can also affect the arms, legs, feet, and face. Other characteristic symptoms of Parkinson disease include rigidity or stiffness of the limbs and torso, slow movement (bradykinesia) or an inability to move (akinesia), and impaired balance and coordination (postural instability). These symptoms worsen slowly over time.

Parkinson disease can also affect emotions and thinking ability (cognition). Some affected individuals develop psychiatric conditions such as depression and visual hallucinations. People with Parkinson disease also have an increased risk of developing dementia, which is a decline in intellectual functions including judgment and memory.

Previous studies have demonstrated that the total level of SOD1 protein is significantly increased the brains of PD patients(Choi J, *et al.* (2005) *JBC*. 280:11648-11655). Additionally, deposition of SOD1 in Lewy bodies in the brains of PD patients has beed detected (Nishiyama L, *et al.* (1995) *Acta Neuropathologica,* 89:471-474).

In one embodiment, a SOD1-associated neurodegenerative disease is "Down's syndrome (DS)". Down syndrome ("DS") is caused by trisomy of human chromosome 21 (Hsa21) and the increased expression, due to dosage, of some subset of the encoded genes. DS patients present different morphological characteristics, for example, short height, obesity and bilateral epicanthic eyefolds. Additionally, muscular hypotonia and neurodegeneration with age may be noted during life. The syndrome is associated with mental retardation, congenital heart disease, immune system disorders, digestive problems, endocrine system deficits, and different biochemical disorders. Evidence from *in vivo*, *in vitro*, and animal models studies have shown that oxidative stress is involved in DS. Thus, it has been proposed that the increased oxidative stress observed in these subjects is mainly caused to an excessive activity of SOD1, an enzyme coded on HSA21 (21q22.1). Patients with DS have also shown elevated levels of SOD1 and appear to have increased lipid peroxidation and oxidative damage to DNA as well as elevated glutathione peroxidase activity (Campos C and Casado A. (2015) *Indian J Med Res*. 142(2):113-119).

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a SOD1-associated neurodegenerative disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating, or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of a RNAi agent that, when administered to a subject having a SOD1-associated neurodegenerative disorder, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylacticaly effective amount" also includes an amount of a RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. A RNAi agent employed in the methods of the present disclosure may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds (including salts), materials, compositions, or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human subjects and animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium state, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

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

II. RNAi Agents of the Disclosure

Described herein are RNAi agents which inhibit the expression of a SOD1 gene. In one embodiment, the RNAi agent includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a SOD1 gene in a cell, such as a cell within a subject, *e.g.,* a mammal, such as a human having a SOD1-associated neurodegenerative disease, *e.g.*, Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS). The dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of a SOD1 gene. The region of complementarity is about 15-30 nucleotides or less in length. Upon contact with a cell expressing the SOD1 gene, the RNAi agent inhibits the expression of the SOD1 gene (*e.g.,* a human gene, a primate gene, a non-primate gene) by at least 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting or flowcytometric techniques. In preferred embodiments, inhibition of expression is by at least 50% as assayed by the Dual-Glo lucifierase assay in Example 1where the siRNA is at a 10 nM concentration.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of a SOD1 gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

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

Similarly, the region of complementarity to the target sequence is 15 to 30 nucleotides in length, *e.g.,* 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length,

for example 19-23 nucleotides in length or 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

In some embodiments, the dsRNA is 15 to 23 nucleotides in length, 25 to 30 nucleotides in length, 20 to 30 nucleotides in length or 19 to 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well known in the art that dsRNAs longer than about 21-23 nucleotides can serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 15 to 36 base pairs, *e.g.*, 15-36, 15-35, 15-34, 15-33, 15-32, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs, for example, 19-21 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, a RNAi agent useful to target SOD1 expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.,* 1, 2, 3, or 4 nucleotides. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA. In certain embodiments, longer, extended overhangs are possible.

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

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

An siRNA can be produced, *e.g.*, in bulk, by a variety of methods. Exemplary methods include: organic synthesis and RNA cleavage, *e.g.*, *in vitro* cleavage.

An siRNA can be made by separately synthesizing a single stranded RNA molecule, or each respective strand of a double-stranded RNA molecule, after which the component strands can then be annealed.

A large bioreactor, *e.g.*, the OligoPilot II from Pharmacia Biotec AB (Uppsala Sweden), can be used to produce a large amount of a particular RNA strand for a given siRNA. The OligoPilotII reactor can efficiently couple a nucleotide using only a 1.5 molar excess of a phosphoramidite nucleotide. To make an RNA strand, ribonucleotides amidites are used. Standard cycles of monomer addition can be used to synthesize the 21 to 23 nucleotide strand for the siRNA. Typically, the two complementary strands are produced separately and then annealed, *e.g.*, after release from the solid support and deprotection.

Organic synthesis can be used to produce a discrete siRNA species. The complementary of the species to a SOD1 gene can be precisely specified. For example, the species may be complementary to a region that includes a polymorphism, *e.g.*, a single nucleotide polymorphism. Further the location of the polymorphism can be precisely defined. In some embodiments, the polymorphism is located in an internal region, *e.g.*, at least 4, 5, 7, or 9 nucleotides from one or both of the termini.

In one embodiment, RNA generated is carefully purified to remove endsiRNA is cleaved *in vitro* into siRNAs, for example, using a Dicer or comparable RNAse III-based activity. For example, the dsiRNA can be incubated in an *in vitro* extract from *Drosophila* or using purified components, *e.g.*, a purified RNAse or RISC complex (RNA-induced silencing complex). See, *e.g.*, Ketting *et al. Genes Dev* 2001 Oct 15;15(20):2654-9 and Hammond *Science* 2001 Aug 10;293(5532):1146-50.

dsiRNA cleavage generally produces a plurality of siRNA species, each being a particular 21 to 23 nt fragment of a source dsiRNA molecule. For example, siRNAs that include sequences complementary to overlapping regions and adjacent regions of a source dsiRNA molecule may be present.

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

In one aspect, a dsRNA of the disclosure includes at least two nucleotide sequences, a sense sequence and an antisense sequence. The sense strand sequence for SOD1 may be selected from the group of sequences provided in any one of Tables 2-7, 12, 13, and 18-20, and the corresponding nucleotide sequence of the antisense strand of the sense strand may be selected from the group of sequences of any one of Tables 2-7, 12, 13, and 18-20. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a SOD1 gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense

strand (passenger strand) in any one of Tables 2-7, 12, 13 and 18-20, and the second oligonucleotide is described as the corresponding antisense strand (guide strand) of the sense strand in any one of Tables 2-7, 12, 13, and 18-20 for SOD1.

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

It will be understood that, although the sequences provided herein are described as modified or conjugated sequences, the RNA of the RNAi agent of the disclosure *e.g.*, a dsRNA of the disclosure, may comprise any one of the sequences set forth in any one of Tables 2-7, 12, 13, and 18-20 that is un-modified, un-conjugated, or modified or conjugated differently than described therein. One or more lipophilic ligands or one or more GalNAc ligands can be included in any of the positions of the RNAi agents provided in the instant application.

The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 23 base pairs, *e.g.,* 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al*., (2001) *EMBO J.*, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided herein, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences provided herein, and differing in their ability to inhibit the expression of a SOD1 gene by not more than 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence using the *in vitro* assay with Cos7 and a 10 nM concentration of the RNA agent and the PCR assay as provided in the examples herein, are contemplated to be within the scope of the present disclosure.

In addition, the RNAs described herein identify a site(s) in a SOD1 transcript that is susceptible to RISC-mediated cleavage. As such, the present disclosure further features RNAi agents that target within this site(s). As used herein, a RNAi agent is said to target within a particular site of an RNA transcript if the RNAi agent promotes cleavage of the transcript anywhere within that particular site. Such a RNAi agent will generally include at least about 15 contiguous nucleotides, preferably at least 19 nucleotides, from one of the sequences provided herein coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a SOD1 gene.

An RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, an RNAi agent as described herein contains no more than 3 mismatches (*i.e.,* 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments,

if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of a SOD1 gene generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of a SOD1 gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a SOD1 gene is important, especially if the particular region of complementarity in a SOD1 gene is known to have polymorphic sequence variation within the population.

III. Modified RNAi Agents of the Disclosure

In one embodiment, the RNA of the RNAi agent of the disclosure *e.g.*, a dsRNA, is un-modified, and does not comprise, *e.g.,* chemical modifications or conjugations known in the art and described herein. In preferred embodiments, the RNA of an RNAi agent of the disclosure, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the disclosure, substantially all of the nucleotides of an RNAi agent of the disclosure are modified. In other embodiments of the disclosure, all of the nucleotides of an RNAi agent of the disclosure are modified. RNAi agents of the disclosure in which "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides. In still other embodiments of the disclosure, RNAi agents of the disclosure can include not more than 5, 4, 3, 2 or 1 modified nucleotides.

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

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

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

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

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

In other embodiments, suitable RNA mimetics are contemplated for use in RNAi agents, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an RNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the

preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the RNAi agents of the disclosure are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the disclosure include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--CH_2-, --CH_2--N(CH_3)--O--CH_2--[known as a methylene (methylimino) or MMI backbone], --CH_2--O--N(CH_3)--CH_2--, --CH_2--N(CH_3)--N(CH_3)--CH_2-- and --N(CH_3)--CH_2--CH_2-- of the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced US5,034,506. The native phosphodiester backbone can be represented as O-P(O)(OH)-OCH2-.

Modified RNAs can also contain one or more substituted sugar moieties. The RNAi agents, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include O[$(CH_2)_n$O] $_m$$CH_3$, O($CH_2$).$_nOCH_3$, O$(CH_2)_n$$NH_2$, O($CH_2$) $_n$$CH_3$, O$(CH_2)_nONH_2$, and O$(CH_2)_n$ON[$(CH_2)_n$$CH_3$)]$_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of a RNAi agent, or a group for improving the pharmacodynamic properties of a RNAi agent, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.*, a O$(CH_2)_2$O$N(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--N$(CH_2)_2$. Further exemplary modifications include: 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$), 2'-*O*-hexadecyl, and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of a RNAi agent, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. RNAi agents can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873;

5,670,633; and 5,700,920, certain of which are commonly owned with the instant application. The entire contents of each of the foregoing are hereby incorporated herein by reference.

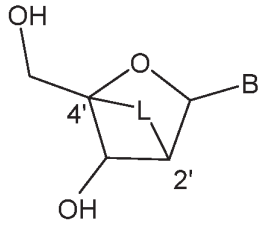
An RNAi agent of the disclosure can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and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

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

An RNAi agent of the disclosure can also be modified to include one or more bicyclic sugar moities. A "bicyclic sugar" is a furanosyl ring modified by a ring formed by the bridging of two carbons, whether adjacent or non-adjacent. A "bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a ring formed by bridging two carbons, whether adjacent or non-adjacent, of

the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4′-carbon and the 2′-carbon of the sugar ring, optionally, via the 2'-acyclic oxygen atom. Thus, in some embodiments an agent of the disclosure may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH2-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the disclosure include without limitation nucleosides comprising a bridge between the 4′ and the 2′ ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the disclosure include one or more bicyclic nucleosides comprising a 4′ to 2′ bridge.

A locked nucleoside can be represented by the structure (omitting stereochemistry),



wherein B is a nucleobase or modified nucleobase and L is the linking group that joins the 2'-carbon to the 4'-carbon of the ribose ring. Examples of such 4′ to 2′ bridged bicyclic nucleosides, include but are not limited to 4′-(CH_2)—O-2′ (LNA); 4′-(CH_2)—S-2′; 4′-(CH_2)2—O-2′ (ENA); 4′-$CH(CH_3)$—O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′-$CH(CH_2OCH_3)$—O-2′ (and analogs thereof; see, *e.g.*, U.S. Pat. No. 7,399,845); 4′-$C(CH_3)(CH_3)$—O-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,283); 4′-CH_2—$N(OCH_3)$-2′ (and analogs thereof; see *e.g.*, US Patent No. 8,278,425); 4′-CH_2—O—$N(CH_3)$-2′ (see, *e.g.*,U.S. Patent Publication No. 2004/0171570); 4′-CH2—N(R)—O-2′, wherein R is H, C1-C12 alkyl, or a nitrogen protecting group (see, *e.g.*, U.S. Pat. No. 7,427,672; *Greene's Protective Groups in Organic Synthesis, Fourth Edition*, 2006, eds. John Wiley & Sons, Inc.); 4′-CH_2—$C(H)(CH_3)$-2′ (see, *e.g.*, Chattopadhyaya *et al., J. Org. Chem.*, 2009, 74, 118-134); and 4′-CH_2—$C(=CH_2)$-2′ (and analogs thereof; see, *e.g.*, US Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

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

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

An RNAi agent of the disclosure can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH_3)-O-2' bridge (*i.e.*, L in the preceding structure). In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

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

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US 2013/0190383; and WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

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

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, US8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3'- phosphate, inverted 2'-deoxy-modified ribonucleotide, such as inverted dT(idT), inverted dA (idA), and inverted abasic 2'-deoxyribonucleotide (iAb) and others. Disclosure of this modification can be found in WO 2011/005861.

In one example, the 3' or 5' terminal end of a oligonucleotide is linked to an inverted 2'-deoxy-modified ribonucleotide, such as inverted dT(idT), inverted dA (idA), or a inverted abasic 2'-deoxyribonucleotide (iAb). In one particular example, the inverted 2'-deoxy-modified ribonucleotide is linked to the 3'end of an oligonucleotide, such as the 3'-end of a sense strand described herein, where the linking is via a 3'-3' phosphodiester linkage or a 3'-3'-phosphorothioate linkage.

In another example, the 3'-end of a sense strand is linked via a 3'-3'-phosphorothioate linkage to an inverted abasic ribonucleotide (iAb). In another example, the 3'-end of a sense strand is linked via a 3'-3'-phosphorothioate linkage to an inverted dA (idA).

In one particular example, the inverted 2'-deoxy-modified ribonucleotide is linked to the 3'end of an oligonucleotide, such as the 3'-end of a sense strand described herein, where the linking is via a 3'-3' phosphodiester linkage or a 3'-3'-phosphorothioate linkage.

In another example, the 3'-terminal nucleotides of a sense strand is an inverted dA (idA) and is linked to the preceding nucleotide via a 3'-3'- linkage (e.g., 3'-3'-phosphorothioate linkage).

Other modifications of a RNAi agent of the disclosure include a 5' phosphate or 5' phosphate mimic, *e.g.,* a 5'-terminal phosphate or phosphate mimic on the antisense strand of a RNAi agent. Suitable phosphate mimics are disclosed in, for example US 2012/0157511, the entire contents of which are incorporated herein by reference.

A. Modified RNAi agents Comprising Motifs of the Disclosure

In certain aspects of the disclosure, the double-stranded RNAi agents of the disclosure include agents with chemical modifications as disclosed, for example, in WO 2013/075035, the entire contents of which are incorporated herein by reference. As shown herein and in WO 2013/075035, a superior result may be obtained by introducing one or more motifs of three identical modifications on three consecutive nucleotides into a sense strand or antisense strand of an RNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the RNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The RNAi agent may be optionally conjugated with a lipophilic ligand, *e.g.*, a C16 ligand, for instance on the sense strand. The RNAi agent may be optionally modified with a (*S*)-glycol nucleic acid (GNA) modification, for instance on one or more residues of the antisense strand.

Accordingly, the disclosure provides double stranded RNAi agents capable of inhibiting the expression of a target gene (*i.e.*, a SOD1 gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be 15-30 nucleotides in length. For example, each strand may be 16-30 nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length. In certain embodiments, each strand is 19-23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as an "RNAi agent." The duplex region of an RNAi agent may be 15-30 nucleotide pairs in length. For example, the duplex region can be 16-30 nucleotide pairs in length, 17-30 nucleotide pairs in length, 27-30 nucleotide pairs in length, 17-23 nucleotide pairs in length, 17-21 nucleotide pairs in length, 17-19 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23

nucleotide pairs in length, 19- 21 nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length. In preferred embodiments, the duplex region is 19-21 nucleotide pairs in length.

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

In one embodiment, the nucleotides in the overhang region of the RNAi agent can each independently be a modified or unmodified nucleotide including, but no limited to 2'-sugar modified, such as, 2-F, 2'-O-methyl, thymidine (T), and any combinations thereof.

For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

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

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

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

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

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

In one embodiment, the RNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand. When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In one embodiment, every nucleotide in the sense strand and the antisense strand of the RNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In one embodiment each residue is independently modified with a 2'-O-methyl or 2'-fluoro, *e.g.,* in an alternating motif. Optionally, the RNAi agent further comprises a ligand (*e.g.*, a lipophilic ligand, optionally a C16 ligand).

In one embodiment, the RNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; the antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1- 23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is

introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

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

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

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

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

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

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

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

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

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

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

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

In one embodiment, the RNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mistmatch may occur in the overhang region or the duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described

elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

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

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

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

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

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

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

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

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

each n_p and n_q independently represent an overhang nucleotide;

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

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

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

In one embodiment, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8, 7, 8, 9, 8, 9, 10, 9, 10, 11, 10, 11,12 or 11, 12, 13) of - the sense strand, the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'-end.

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

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

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

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

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

Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

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

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

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

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

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

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

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

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

wherein:

k and l are each independently 0 or 1;

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

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

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

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

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

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

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

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

from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end. Preferably, the Y′Y′Y′ motif occurs at positions 11, 12, 13.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(III)

wherein:

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

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

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

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

wherein

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

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

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

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

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

3' n_p'-N_a'-Y′Y′Y′ -N_a'n_q' 5'

(IIIa)

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

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

(IIIb)

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

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

(IIIc)

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

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

(IIId)

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

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

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

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

In one embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications. In another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications and n_p′ >0 and at least one n_p′ is linked to a neighboring nucleotide a via phosphorothioate linkage. In yet another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , n_p′ >0 and at least one n_p′ is linked to a neighboring nucleotide via phosphorothioate linkage, and the sense strand is conjugated to one or more C16 (or related) moieties attached through a bivalent or trivalent branched linker (described below). In another embodiment, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , n_p′ >0 and at least one n_p′ is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more lipophilic, *e.g.*, C16 (or related) moieties, optionally attached through a bivalent or trivalent branched linker.

In one embodiment, when the RNAi agent is represented by formula (IIIa), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , n_p′ >0 and at least one n_p′ is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more lipophilic, *e.g.*, C16 (or related) moieties attached through a bivalent or trivalent branched linker.

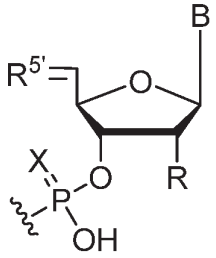
In one embodiment, the RNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of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

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

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

Various publications describe multimeric RNAi agents that can be used in the methods of the disclosure. Such publications include WO2007/091269, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520; and US 7858769, the entire contents of each of which are hereby incorporated herein by reference.

In certain embodiments, the compositions and methods of the disclosure include a vinyl phosphonate (VP) modification of an RNAi agent as described herein. In exemplary embodiments, a 5'-vinyl phosphonate modified nucleotide of the disclosure has the structure:



wherein X is O or S;

R is hydrogen, hydroxy, fluoro, or C_{1-20}alkoxy (e.g., methoxy or n-hexadecyloxy);

$R^{5'}$ is $=C(H)-P(O)(OH)_2$ and the double bond between the C5' carbon and R5' is in the E or Z orientation (e.g., E orientation); and

B is a nucleobase or a modified nucleobase, optionally where B is adenine, guanine, cytosine, thymine, or uracil.

In one embodiment, $R^{5'}$ is $=C(H)-P(O)(OH)_2$ and the double bond between the C5' carbon and R5' is in the E orientation. In another embodiment, R is methoxy and $R^{5'}$ is $=C(H)-P(O)(OH)_2$ and the double bond between the C5' carbon and R5' is in the E orientation. In another embodiment, X is S, R is methoxy, and $R^{5'}$ is $=C(H)-P(O)(OH)_2$ and the double bond between the C5' carbon and R5' is in the E orientation.

A vinyl phosphonate of the instant disclosure may be attached to either the antisense or the sense strand of a dsRNA of the disclosure. In certain preferred embodiments, a vinyl phosphonate of the instant disclosure is attached to the antisense strand of a dsRNA, optionally at the 5' end of the antisense strand of the dsRNA.

Vinyl phosphate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphate structure includes the preceding structure, where $R^{5'}$ is $=C(H)-OP(O)(OH)_2$ and the double bond between the C5' carbon and $R^{5'}$ is in the E or Z orientation (e.g., E orientation).

E. *Thermally Destabilizing Modifications*

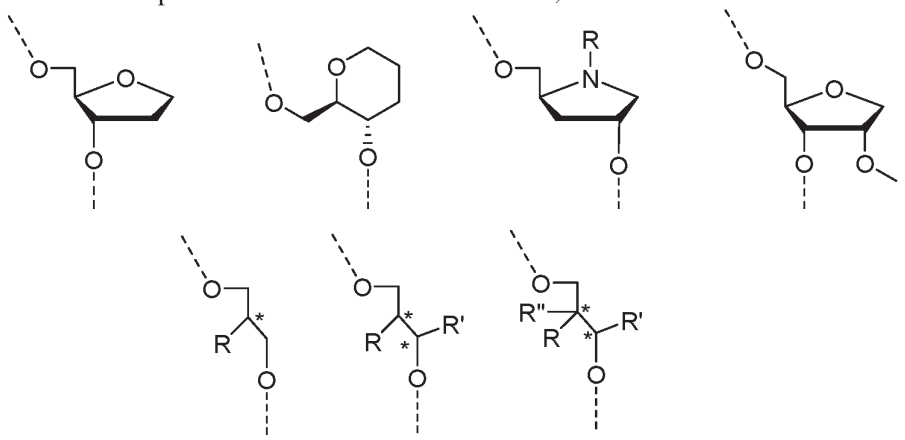
In certain embodiments, a dsRNA molecule can be optimized for RNA interference by incorporating thermally destabilizing modifications in the seed region of the antisense strand. As used herein "seed region" means at positions 2-9 of the 5'-end of the referenced strand. For example, thermally destabilizing modifications can be incorporated in the seed region of the antisense strand to reduce or inhibit off-target gene silencing.

The term "thermally destabilizing modification(s)" includes modification(s) that would result with a dsRNA with a lower overall melting temperature (Tm) than the Tm of the dsRNA without having such modification(s). For example, the thermally destabilizing modification(s) can decrease the Tm of the dsRNA by 1 – 4 °C, such as one, two, three or four degrees Celcius. And, the term "thermally destabilizing nucleotide" refers to a nucleotide containing one or more thermally destabilizing modifications.

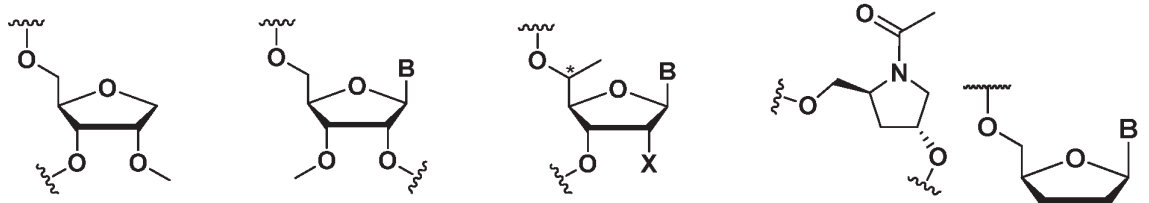
It has been discovered that dsRNAs with an antisense strand comprising at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5' end, of the antisense strand have reduced off-target gene silencing activity. Accordingly, in some embodiments, the antisense strand comprises at least one (*e.g.*, one, two, three, four, five or more) thermally destabilizing modification of the duplex within the first 9 nucleotide positions of the 5' region of the antisense strand. In some embodiments, one or more thermally destabilizing modification(s) of the duplex is/are located in positions 2-9, or preferably positions 4-8, from the 5'-end of the antisense strand. In some further embodiments, the thermally destabilizing modification(s) of the duplex is/are located at position 6, 7 or 8 from the 5'-end of the antisense strand. In still some further embodiments, the thermally destabilizing modification of the duplex is located at position 7 from the 5'-end of the antisense strand. In some embodiments, the thermally destabilizing modification of the duplex is located at position 2, 3, 4, 5 or 9 from the 5'-end of the antisense strand.

The thermally destabilizing modifications can include, but are not limited to, abasic modification; mismatch with the opposing nucleotide in the opposing strand; and sugar modification such as 2'-deoxy modification, acyclic nucleotide, *e.g.*, unlocked nucleic acids (UNA) or glycol nucleic acid (GNA); and 2'-5'-linked ribonucleotides ("3'-RNA").

Exemplified abasic modifications include, but are not limited to the following:

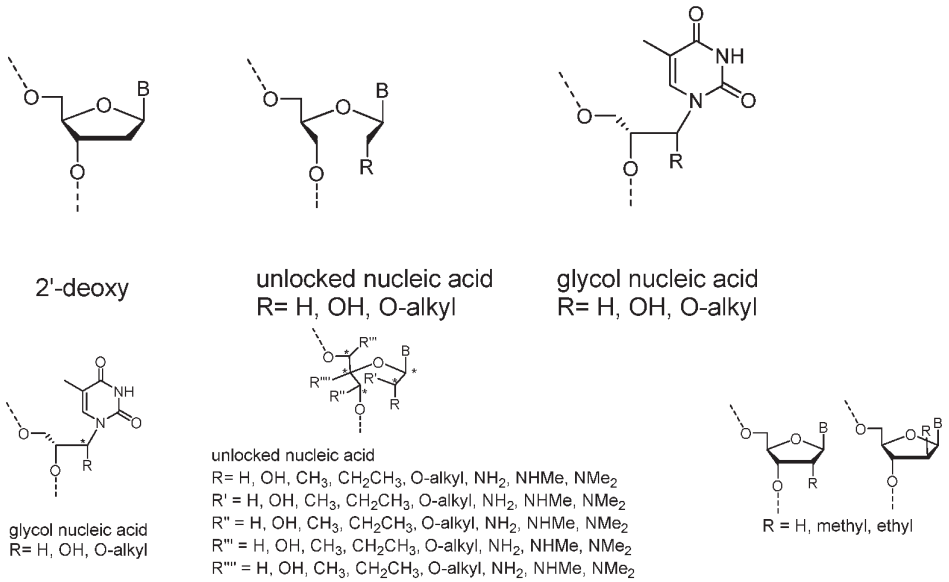


Wherein R = H, Me, Et or OMe; R' = H, Me, Et or OMe; R" = H, Me, Et or OMe



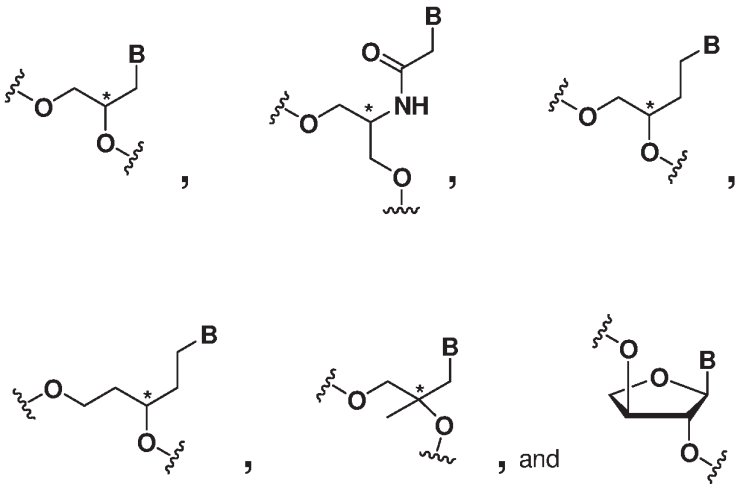
wherein B is a modified or unmodified nucleobase.

Exemplified sugar modifications include, but are not limited to the following:



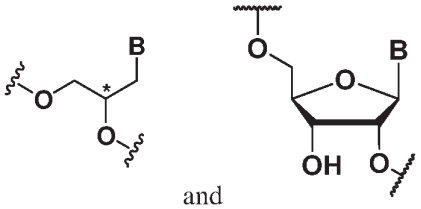
wherein B is a modified or unmodified nucleobase.

In some embodiments the thermally destabilizing modification of the duplex is selected from the group consisting of:



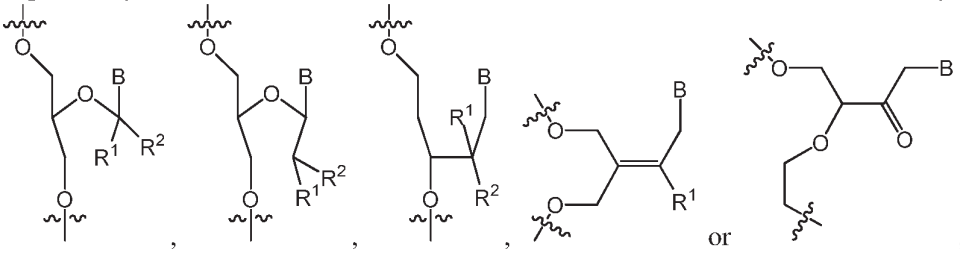
wherein B is a modified or unmodified nucleobase and the asterisk on each structure represents either *R*, *S* or *racemic*.

In some embodiments the thermally destabilizing modification of the duplex is selected from the group consisting of:



wherein B is a modified or unmodified nucleobase and the asterisk represents either *R*, *S* or *racemic* (e.g. S).

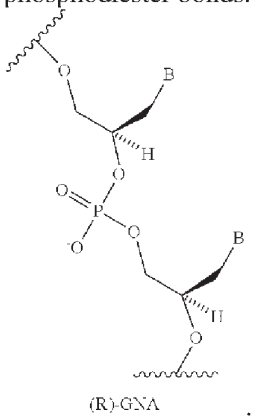
The term "acyclic nucleotide" refers to any nucleotide having an acyclic ribose sugar, for example, where any of bonds between the ribose carbons (*e.g.*, C1'-C2', C2'-C3', C3'-C4', C4'-O4', or C1'-O4') is absent or at least one of ribose carbons or oxygen (*e.g.*, C1', C2', C3', C4', or O4') are independently or in combination absent from the nucleotide. In some embodiments, acyclic nucleotide



is , , , or , wherein B is a modified or unmodified nucleobase, R^1 and R^2 independently are H, halogen, OR_3, or alkyl; and R_3 is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar). The term "UNA" refers to unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In

one example, UNA also encompasses monomers with bonds between C1'-C4' being removed (*i.e.* the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar is removed (see Mikhailov et. al., Tetrahedron Letters, 26 (17): 2059 (1985); and Fluiter et al., Mol. Biosyst., 10: 1039 (2009), which are hereby incorporated by reference in their entirety). The acyclic derivative provides greater backbone flexibility without affecting the Watson-Crick pairings. The acyclic nucleotide can be linked via 2'-5' or 3'-5' linkage.

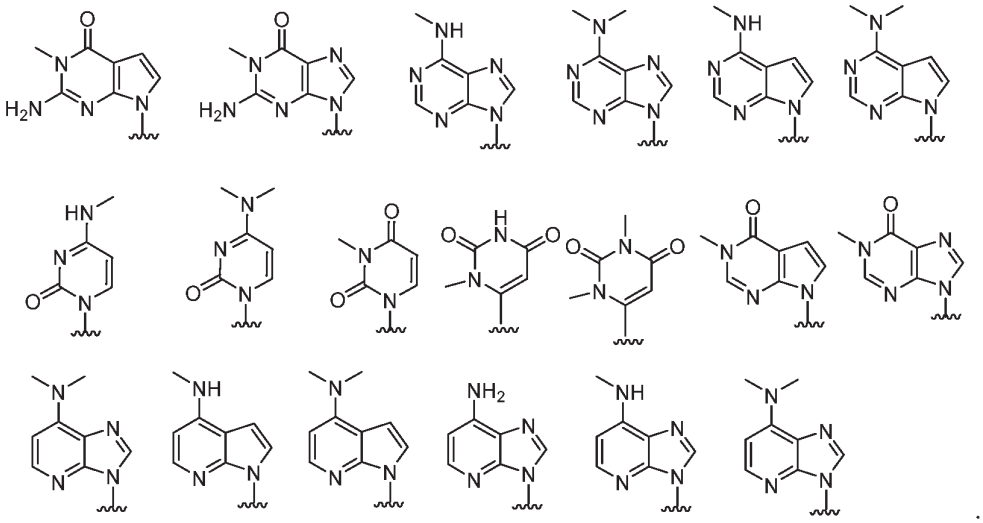
The term 'GNA' refers to glycol nucleic acid which is a polymer similar to DNA or RNA but differing in the composition of its "backbone" in that is composed of repeating glycerol units linked by phosphodiester bonds:



(R)-GNA .

The thermally destabilizing modification of the duplex can be mismatches (*i.e.*, noncomplementary base pairs) between the thermally destabilizing nucleotide and the opposing nucleotide in the opposite strand within the dsRNA duplex. Exemplary mismatch base pairs include G:G, G:A, G:U, G:T, A:A, A:C, C:C, C:U, C:T, U:U, T:T, U:T, or a combination thereof. Other mismatch base pairings known in the art are also amenable to the present invention. A mismatch can occur between nucleotides that are either naturally occurring nucleotides or modified nucleotides, *i.e.*, the mismatch base pairing can occur between the nucleobases from respective nucleotides independent of the modifications on the ribose sugars of the nucleotides. In certain embodiments, the dsRNA molecule contains at least one nucleobase in the mismatch pairing that is a 2'-deoxy nucleobase; *e.g.*, the 2'-deoxy nucleobase is in the sense strand.

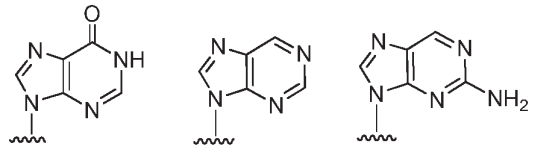
In some embodiments, the thermally destabilizing modification of the duplex in the seed region of the antisense strand includes nucleotides with impaired Watson-Crick hydrogen-bonding to the complementary base on the target mRNA, such as modified nucleobases:



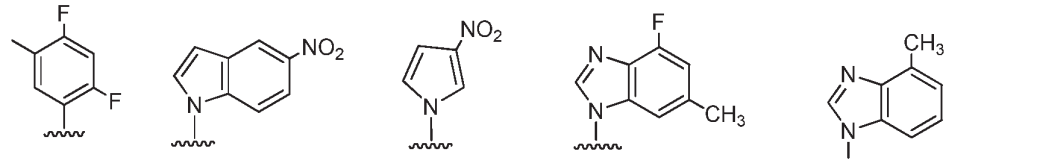
More examples of abasic nucleotide, acyclic nucleotide modifications (including UNA and GNA), and mismatch modifications have been described in detail in WO 2011/133876, which is herein incorporated by reference in its entirety.

The thermally destabilizing modifications may also include universal base with reduced or abolished capability to form hydrogen bonds with the opposing bases, and phosphate modifications.

In some embodiments, the thermally destabilizing modification of the duplex includes nucleotides with non-canonical bases such as, but not limited to, nucleobase modifications with impaired or completely abolished capability to form hydrogen bonds with bases in the opposite strand. These nucleobase modifications have been evaluated for destabilization of the central region of the dsRNA duplex as described in WO 2010/0011895, which is herein incorporated by reference in its entirety. Exemplary nucleobase modifications are:

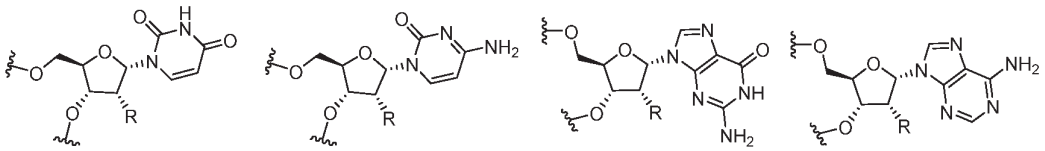


inosine nebularine 2-aminopurine



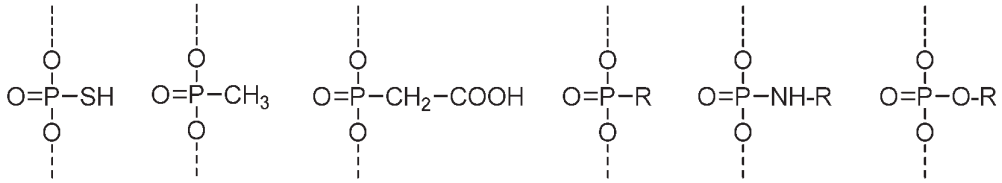
2,4-difluorotoluene 5-nitroindole 3-nitropyrrole 4-Fluoro-6-methylbenzimidazole 4-Methylbenzimidazole

In some embodiments, the thermally destabilizing modification of the duplex in the seed region of the antisense strand includes one or more α-nucleotide complementary to the base on the target mRNA, such as:



wherein R is H, OH, OCH_3, F, NH_2, NHMe, NMe_2 or O-alkyl.

Exemplary phosphate modifications known to decrease the thermal stability of dsRNA duplexes compared to natural phosphodiester linkages are:



R = alkyl

The alkyl for the R group can be a C_1-C_6alkyl. Specific alkyls for the R group include, but are not limited to methyl, ethyl, propyl, isopropyl, butyl, pentyl and hexyl.

As the skilled artisan will recognize, in view of the functional role of nucleobases is defining specificity of a RNAi agent of the disclosure, while nucleobase modifications can be performed in the various manners as described herein, *e.g.*, to introduce destabilizing modifications into a RNAi agent of the disclosure, *e.g.*, for purpose of enhancing on-target effect relative to off-target effect, the range of modifications available and, in general, present upon RNAi agents of the disclosure tends to be much greater for non-nucleobase modifications, *e.g.*, modifications to sugar groups or phosphate backbones of polyribonucleotides. Such modifications are described in greater detail in other sections of the instant disclosure and are expressly contemplated for RNAi agents of the disclosure, either possessing native nucleobases or modified nucleobases as described above or elsewhere herein.

In addition to the antisense strand comprising a thermally destabilizing modification, the dsRNA can also comprise one or more stabilizing modifications. For example, the dsRNA can comprise at least two (*e.g.*, two, three, four, five, six, seven, eight, nine, ten or more) stabilizing modifications. Without limitations, the stabilizing modifications all can be present in one strand. In some embodiments, both the sense and the antisense strands comprise at least two stabilizing modifications. The stabilizing modification can occur on any nucleotide of the sense strand or antisense strand. For instance, the stabilizing modification can occur on every nucleotide on the sense strand or antisense strand; each stabilizing modification can occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand comprises both stabilizing modification in an alternating pattern. The alternating pattern of the stabilizing modifications on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the stabilizing modifications on the sense strand can have a shift relative to the alternating pattern of the stabilizing modifications on the antisense strand.

In some embodiments, the antisense strand comprises at least two (*e.g.*, two, three, four, five, six, seven, eight, nine, ten or more) stabilizing modifications. Without limitations, a stabilizing modification in the antisense strand can be present at any positions. In some embodiments, the antisense

comprises stabilizing modifications at positions 2, 6, 8, 9, 14, and 16 from the 5'-end. In some other embodiments, the antisense comprises stabilizing modifications at positions 2, 6, 14, and 16 from the 5'-end. In still some other embodiments, the antisense comprises stabilizing modifications at positions 2, 14, and 16 from the 5'-end.

In some embodiments, the antisense strand comprises at least one stabilizing modification adjacent to the destabilizing modification. For example, the stabilizing modification can be the nucleotide at the 5'-end or the 3'-end of the destabilizing modification, *i.e.*, at position -1 or +1 from the position of the destabilizing modification. In some embodiments, the antisense strand comprises a stabilizing modification at each of the 5'-end and the 3'-end of the destabilizing modification, *i.e.*, positions -1 and +1 from the position of the destabilizing modification.

In some embodiments, the antisense strand comprises at least two stabilizing modifications at the 3'-end of the destabilizing modification, *i.e.*, at positions +1 and +2 from the position of the destabilizing modification.

In some embodiments, the sense strand comprises at least two (*e.g.*, two, three, four, five, six, seven, eight, nine, ten or more) stabilizing modifications. Without limitations, a stabilizing modification in the sense strand can be present at any positions. In some embodiments, the sense strand comprises stabilizing modifications at positions 7, 10, and 11 from the 5'-end. In some other embodiments, the sense strand comprises stabilizing modifications at positions 7, 9, 10, and 11 from the 5'-end. In some embodiments, the sense strand comprises stabilizing modifications at positions opposite or complimentary to positions 11, 12, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some other embodiments, the sense strand comprises stabilizing modifications at positions opposite or complimentary to positions 11, 12, 13, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some embodiments, the sense strand comprises a block of two, three, or four stabilizing modifications.

In some embodiments, the sense strand does not comprise a stabilizing modification in position opposite or complimentary to the thermally destabilizing modification of the duplex in the antisense strand.

Exemplary thermally stabilizing modifications include, but are not limited to, 2'-fluoro modifications. Other thermally stabilizing modifications include, but are not limited to, LNA.

In some embodiments, the dsRNA of the disclosure comprises at least four (*e.g.*, four, five, six, seven, eight, nine, ten, or more) 2'-fluoro nucleotides. Without limitations, the 2'-fluoro nucleotides all can be present in one strand. In some embodiments, both the sense and the antisense strands comprise at least two 2'-fluoro nucleotides. The 2'-fluoro modification can occur on any nucleotide of the sense strand or antisense strand. For instance, the 2'-fluoro modification can occur on every nucleotide on the sense strand or antisense strand; each 2'-fluoro modification can occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand comprises both 2'-fluoro modifications in an alternating pattern. The alternating pattern of the 2'-fluoro modifications on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the 2'-

fluoro modifications on the sense strand can have a shift relative to the alternating pattern of the 2'-fluoro modifications on the antisense strand.

In some embodiments, the antisense strand comprises at least two (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, or more) 2'-fluoro nucleotides. Without limitations, a 2'-fluoro modification in the antisense strand can be present at any positions. In some embodiments, the antisense comprises 2'-fluoro nucleotides at positions 2, 6, 8, 9, 14, and 16 from the 5'-end. In some other embodiments, the antisense comprises 2'-fluoro nucleotides at positions 2, 6, 14, and 16 from the 5'-end. In still some other embodiments, the antisense comprises 2'-fluoro nucleotides at positions 2, 14, and 16 from the 5'-end.

In some embodiments, the antisense strand comprises at least one 2'-fluoro nucleotide adjacent to the destabilizing modification. For example, the 2'-fluoro nucleotide can be the nucleotide at the 5'-end or the 3'-end of the destabilizing modification, *i.e.*, at position -1 or +1 from the position of the destabilizing modification. In some embodiments, the antisense strand comprises a 2'-fluoro nucleotide at each of the 5'-end and the 3'-end of the destabilizing modification, *i.e.*, positions -1 and +1 from the position of the destabilizing modification.

In some embodiments, the antisense strand comprises at least two 2'-fluoro nucleotides at the 3'-end of the destabilizing modification, *i.e.*, at positions +1 and +2 from the position of the destabilizing modification.

In some embodiments, the sense strand comprises at least two (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, or more) 2'-fluoro nucleotides. Without limitations, a 2'-fluoro modification in the sense strand can be present at any positions. In some embodiments, the antisense comprises 2'-fluoro nucleotides at positions 7, 10, and 11 from the 5'-end. In some other embodiments, the sense strand comprises 2'-fluoro nucleotides at positions 7, 9, 10, and 11 from the 5'-end. In some embodiments, the sense strand comprises 2'-fluoro nucleotides at positions opposite or complimentary to positions 11, 12, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some other embodiments, the sense strand comprises 2'-fluoro nucleotides at positions opposite or complimentary to positions 11, 12, 13, and 15 of the antisense strand, counting from the 5'-end of the antisense strand. In some embodiments, the sense strand comprises a block of two, three or four 2'-fluoro nucleotides.

In some embodiments, the sense strand does not comprise a 2'-fluoro nucleotide in position opposite or complimentary to the thermally destabilizing modification of the duplex in the antisense strand.

In some embodiments, the dsRNA molecule of the disclosure comprises a 21 nucleotides (nt) sense strand and a 23 nucleotides (nt) antisense, wherein the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide occurs in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein one end of the dsRNA is blunt, while the other end is comprises a 2 nt overhang, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the

following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4, or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA comprises a blunt end at 5'-end of the antisense strand. Preferably, the 2 nt overhang is at the 3'-end of the antisense.

In some embodiments, the dsRNA molecule of the disclosure comprising a sense and antisense strands, wherein: the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1), positions 1 to 23 of said sense strand comprise at least 8 ribonucleotides; antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, at least 8 ribonucleotides in the positions paired with positions 1- 23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when said double stranded nucleic acid is introduced into a mammalian cell; and wherein the antisense strand contains at least one thermally destabilizing nucleotide, where at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.* at position 2-9 of the 5'-end of the antisense strand). For example, the thermally destabilizing nucleotide occurs between positions opposite or complimentary to positions 14-17 of the 5'-end of the sense strand, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4, or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; and (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA comprises a duplex region of 12-30 nucleotide pairs in length.

In some embodiments, the dsRNA molecule of the disclosure comprises a sense and antisense strands, wherein said dsRNA molecule comprises a sense strand having a length which is at least 25 and at most 29 nucleotides and an antisense strand having a length which is at most 30 nucleotides with the sense strand comprises a modified nucleotide that is susceptible to enzymatic degradation at position 11 from the 5'end, wherein the 3' end of said sense strand and the 5' end of said antisense strand form a blunt end and said antisense strand is 1-4 nucleotides longer at its 3' end than the sense strand, wherein the duplex region which is at least 25 nucleotides in length, and said antisense strand is sufficiently

complementary to a target mRNA along at least 19 nt of said antisense strand length to reduce target gene expression when said dsRNA molecule is introduced into a mammalian cell, and wherein dicer cleavage of said dsRNA preferentially results in an siRNA comprising said 3' end of said antisense strand, thereby reducing expression of the target gene in the mammal, wherein the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.* at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4, or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; and (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA has a duplex region of 12-29 nucleotide pairs in length.

In some embodiments, every nucleotide in the sense strand and antisense strand of the dsRNA molecule may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, *e.g.*, of the 2′ hydroxyl on the ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or modification of the ribose-phosphate backbone.

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

It may be possible, *e.g.*, to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, *e.g.*, in a 5' or 3' overhang, or in both. *E.g.*, it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3' or 5' overhang may be modified, *e.g.*, with a modification described herein. Modifications can include, *e.g.*, the use of modifications at the 2' position of the ribose sugar with modifications that are known in the art, *e.g.*, the use of deoxyribonucleotides, 2'-deoxy-2'-

fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase, and modifications in the phosphate group, *e.g.*, phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

In some embodiments, each residue of the sense strand and antisense strand is independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-deoxy, or 2'-fluoro. The strands can contain more than one modification. In some embodiments, each residue of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. It is to be understood that these modifications are in addition to the at least one thermally destabilizing modification of the duplex present in the antisense strand.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'-deoxy, 2'- O-methyl or 2'-fluoro modifications, acyclic nucleotides or others. In some embodiments, the sense strand and antisense strand each comprises two differently modified nucleotides selected from 2'-O-methyl or 2'-deoxy. In some embodiments, each residue of the sense strand and antisense strand is independently modified with 2'-O-methyl nucleotide, 2'-deoxy nucleotide, 2´-deoxy-2'-fluoro nucleotide, 2'-O-N-methylacetamido (2'-O-NMA) nucleotide, a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE) nucleotide, 2'-O-aminopropyl (2'-O-AP) nucleotide, or 2'-ara-F nucleotide. Again, it is to be understood that these modifications are in addition to the at least one thermally destabilizing modification of the duplex present in the antisense strand.

In some embodiments, the dsRNA molecule of the disclosure comprises modifications of an alternating pattern. The term "alternating motif" or "alternative pattern" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABABAB…," "AABBAABBAABB…," "AABAABAABAAB…," "AAABAAABAAAB…," "AAABBBAAABBB…," or "ABCABCABCABC…," etc.

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

In some embodiments, the dsRNA molecule of the disclosure comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and vice versa. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 3'-5'of the

strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BBAABBAA" from 3'-5'of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5'-3' of the strand, where each A is an unmodified ribonucleotide and each B is a 2'-Omethyl modified nucleotide.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5'-3' of the strand, where each A is an 2'-deoxy-2'-fluoro modified nucleotide and each B is a 2'-Omethyl modified nucleotide.

In another particular example, the alternating motif in the antisense strand is "BABABA" from 3'-5'of the strand, where each A is a 2'-deoxy-2'-fluoro modified nucleotide and each B is a 2'-Omethyl modified nucleotide.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5'-3' of the strand and the alternating motif in the antisense strand is "BABABA" from 3'-5'of the strand, where each A is an unmodified ribonucleotide and each B is a 2'-Omethyl modified nucleotide.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5'-3' of the strand and the alternating motif in the antisense strand is "BABABA" from 3'-5'of the strand, where each A is a 2'-deoxy-2'-fluoro modified nucleotide and each B is a 2'-Omethyl modified nucleotide.

The dsRNA molecule of the disclosure may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand or antisense strand or both in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand comprises both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand.

In some embodiments, the dsRNA molecule comprises the phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region comprises two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal paired nucleotides within duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or

methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three nucleotides are overhang nucleotides, and the third is a paired nucleotide next to the overhang nucleotide. Preferably, these terminal three nucleotides may be at the 3'-end of the antisense strand.

In some embodiments, the sense strand of the dsRNA molecule comprises 1-10 blocks of two to ten phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said sense strand is paired with an antisense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of two phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of three phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of four phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of five phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of

phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of six phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of seven phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, or 8 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of eight phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, 4, 5, or 6 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the antisense strand of the dsRNA molecule comprises two blocks of nine phosphorothioate or methylphosphonate internucleotide linkages separated by 1, 2, 3, or 4 phosphate internucleotide linkages, wherein one of the phosphorothioate or methylphosphonate internucleotide linkages is placed at any position in the oligonucleotide sequence and the said antisense strand is paired with a sense strand comprising any combination of phosphorothioate, methylphosphonate and phosphate internucleotide linkages or an antisense strand comprising either phosphorothioate or methylphosphonate or phosphate linkage.

In some embodiments, the dsRNA molecule of the disclosure further comprises one or more phosphorothioate or methylphosphonate internucleotide linkage modification within 1-10 nucleotides of the termini position(s) of the sense or antisense strand. For example, at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage at one end or both ends of the sense or antisense strand.

In some embodiments, the dsRNA molecule of the disclosure further comprises one or more phosphorothioate or methylphosphonate internucleotide linkage modification within 1-10 of the internal region of the duplex of each of the sense or antisense strand. For example, at least nucleotides 2, 3, 4,

5, 6, 7, 8, 9, or 10 nucleotides may be linked through phosphorothioate methylphosphonate internucleotide linkage at position 8-16 of the duplex region counting from the 5'-end of the sense strand; the dsRNA molecule can optionally further comprise one or more phosphorothioate or methylphosphonate internucleotide linkage modification within 1-10 of the termini position(s).

In some embodiments, the dsRNA molecule of the disclosure further comprises one to five phosphorothioate or methylphosphonate internucleotide linkage modification(s) within position 1-5 and one to five phosphorothioate or methylphosphonate internucleotide linkage modification(s) within position 18-23 of the sense strand (counting from the 5'-end), and one to five phosphorothioate or methylphosphonate internucleotide linkage modification at positions 1 and 2 and one to five within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification within position 1-5 and one phosphorothioate or methylphosphonate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 or 2 and two phosphorothioate or methylphosphonate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and one phosphorothioate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 or 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and two phosphorothioate internucleotide linkage modifications within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 or 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and two phosphorothioate internucleotide linkage modifications within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 or 2 and one phosphorothioate internucleotide linkage modification within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification within position 1-5 and one phosphorothioate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two

phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification within position 1-5 and one within position 18-23 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modification at positions 1 and 2 and one phosphorothioate internucleotide linkage modification within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification within position 1-5 (counting from the 5'-end) of the sense strand, and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and one phosphorothioate internucleotide linkage modification within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 (counting from the 5'-end) of the sense strand, and one phosphorothioate internucleotide linkage modification at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and one within position 18-23 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and one phosphorothioate internucleotide linkage modification within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and one phosphorothioate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications within position 1-5 and one phosphorothioate internucleotide linkage modification within position 18-23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications at position 1 and 2, and two phosphorothioate internucleotide linkage modifications at position 20 and 21 of the sense strand (counting from the 5'-

end), and one phosphorothioate internucleotide linkage modification at positions 1 and one at position 21 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification at position 1, and one phosphorothioate internucleotide linkage modification at position 21 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications at positions 20 and 21 the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications at position 1 and 2, and two phosphorothioate internucleotide linkage modifications at position 21 and 22 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and one phosphorothioate internucleotide linkage modification at position 21 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification at position 1, and one phosphorothioate internucleotide linkage modification at position 21 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications at positions 21 and 22 the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises two phosphorothioate internucleotide linkage modifications at position 1 and 2, and two phosphorothioate internucleotide linkage modifications at position 22 and 23 of the sense strand (counting from the 5'-end), and one phosphorothioate internucleotide linkage modification at positions 1 and one phosphorothioate internucleotide linkage modification at position 21 of the antisense strand (counting from the 5'-end).

In some embodiments, the dsRNA molecule of the disclosure further comprises one phosphorothioate internucleotide linkage modification at position 1, and one phosphorothioate internucleotide linkage modification at position 21 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications at positions 23 and 23 the antisense strand (counting from the 5'-end).

In some embodiments, compound of the disclosure comprises a pattern of backbone chiral centers. In some embodiments, a common pattern of backbone chiral centers comprises at least 5 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 6 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 7 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers

comprises at least 8 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 9 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 10 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 11 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 12 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 13 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 14 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 15 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 16 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 17 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 18 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 19 internucleotidic linkages in the Sp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 8 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 7 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 6 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 5 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 4 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 3 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 2 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 1 internucleotidic linkages in the Rp configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 8 internucleotidic linkages which are not chiral (as a non-limiting example, a phosphodiester). In some embodiments, a common pattern of backbone chiral centers comprises no more than 7 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 5 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 4 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 3 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 2 internucleotidic linkages which are not chiral. In some

embodiments, a common pattern of backbone chiral centers comprises no more than 1 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 10 internucleotidic linkages in the Sp configuration, and no more than 8 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 11 internucleotidic linkages in the Sp configuration, and no more than 7 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 12 internucleotidic linkages in the Sp configuration, and no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 13 internucleotidic linkages in the Sp configuration, and no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 14 internucleotidic linkages in the Sp configuration, and no more than 5 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 15 internucleotidic linkages in the Sp configuration, and no more than 4 internucleotidic linkages which are not chiral. In some embodiments, the internucleotidic linkages in the Sp configuration are optionally contiguous or not contiguous. In some embodiments, the internucleotidic linkages in the Rp configuration are optionally contiguous or not contiguous. In some embodiments, the internucleotidic linkages which are not chiral are optionally contiguous or not contiguous.

In some embodiments, compound of the disclosure comprises a block that is a stereochemistry block. In some embodiments, a block is an Rp block in that each internucleotidic linkage of the block is Rp. In some embodiments, a 5'-block is an Rp block. In some embodiments, a 3'-block is an Rp block. In some embodiments, a block is an Sp block in that each internucleotidic linkage of the block is Sp. In some embodiments, a 5'-block is an Sp block. In some embodiments, a 3'-block is an Sp block. In some embodiments, provided oligonucleotides comprise both Rp and Sp blocks. In some embodiments, provided oligonucleotides comprise one or more Rp but no Sp blocks. In some embodiments, provided oligonucleotides comprise one or more Sp but no Rp blocks. In some embodiments, provided oligonucleotides comprise one or more PO blocks wherein each internucleotidic linkage in a natural phosphate linkage.

In some embodiments, compound of the disclosure comprises a 5'-block that is an Sp block wherein each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block is an Sp block wherein each of internucleotidic linkage is a modified internucleotidic linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block is an Sp block wherein each of internucleotidic linkage is a phosphorothioate linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block comprises 4 or more nucleoside units. In some embodiments, a 5'-block comprises 5 or more nucleoside units. In some embodiments, a 5'-block comprises 6 or more nucleoside units. In some embodiments, a 5'-block comprises 7 or more nucleoside units. In some embodiments, a 3'-block is an Sp block wherein each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block is an Sp block wherein each of internucleotidic linkage

is a modified internucleotidic linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block is an Sp block wherein each of internucleotidic linkage is a phosphorothioate linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block comprises 4 or more nucleoside units. In some embodiments, a 3'-block comprises 5 or more nucleoside units. In some embodiments, a 3'-block comprises 6 or more nucleoside units. In some embodiments, a 3'-block comprises 7 or more nucleoside units.

In some embodiments, compound of the disclosure comprises a type of nucleoside in a region or an oligonucleotide is followed by a specific type of internucleotidic linkage, *e.g.*, natural phosphate linkage, modified internucleotidic linkage, Rp chiral internucleotidic linkage, Sp chiral internucleotidic linkage, etc. In some embodiments, A is followed by Sp. In some embodiments, A is followed by Rp. In some embodiments, A is followed by natural phosphate linkage (PO). In some embodiments, U is followed by Sp. In some embodiments, U is followed by Rp. In some embodiments, U is followed by natural phosphate linkage (PO). In some embodiments, C is followed by Sp. In some embodiments, C is followed by Rp. In some embodiments, C is followed by natural phosphate linkage (PO). In some embodiments, G is followed by Sp. In some embodiments, G is followed by Rp. In some embodiments, G is followed by natural phosphate linkage (PO). In some embodiments, C and U are followed by Sp. In some embodiments, C and U are followed by Rp. In some embodiments, C and U are followed by natural phosphate linkage (PO). In some embodiments, A and G are followed by Sp. In some embodiments, A and G are followed by Rp.

In some embodiments, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.
In some embodiments, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the sense strand is conjugated with a ligand; (iii) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (iv) the

sense strand comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (v) the dsRNA comprises at least four 2'-fluoro modifications; (vi) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In some embodiments, the sense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5, or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 3, 4 or 5 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In some embodiments, the sense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the sense strand is conjugated with a ligand; (iii) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (iv) the sense strand comprises 3, 4 or 5 phosphorothioate internucleotide linkages; (v) the dsRNA comprises at least four 2'-fluoro modifications; (vi) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (vii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In some embodiments, the dsRNA molecule of the disclosure comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch can occur in the overhang region or the duplex region. The base pair can be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

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

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

It was found that introducing 4'-modified or 5'-modified nucleotide to the 3'-end of a phosphodiester (PO), phosphorothioate (PS), or phosphorodithioate (PS2) linkage of a nucleotide at any position of single stranded or double stranded oligonucleotide can exert steric effect to the internucleotide linkage and, hence, protecting or stabilizing it against nucleases.

In some embodiments, 5'-modified nucleotide is introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. For instance, a 5'-alkylated nucleoside may be introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. The alkyl group at the 5' position of the ribose sugar can be racemic or chirally pure *R* or *S* isomer. An exemplary 5'-alkylated nucleoside is 5'-methyl nucleoside. The 5'-methyl can be either racemic or chirally pure *R* or *S* isomer.

In some embodiments, 4'-modified nucleoside is introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. For instance, a 4'-alkylated nucleoside may be introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. The alkyl group at the 4' position of the ribose sugar can be racemic or chirally pure *R* or *S* isomer. An exemplary 4'-alkylated nucleoside is 4'-methyl nucleoside. The 4'-methyl can be either racemic or chirally pure *R* or *S* isomer. Alternatively, a 4'-*O*-alkylated nucleoside may be introduced at the 3'-end of a dinucleotide at any position of single stranded or double stranded siRNA. The 4'-*O*-alkyl of the ribose sugar can be racemic or chirally pure *R* or *S* isomer. An exemplary 4'-*O*-alkylated nucleoside is 4'-*O*-methyl nucleoside. The 4'-*O*-methyl can be either racemic or chirally pure *R* or *S* isomer.

In some embodiments, 5'-alkylated nucleoside is introduced at any position on the sense strand or antisense strand of a dsRNA, and such modification maintains or improves potency of the dsRNA. The 5'-alkyl can be either racemic or chirally pure *R* or *S* isomer. An exemplary 5'-alkylated nucleoside is 5'-methyl nucleoside. The 5'-methyl can be either racemic or chirally pure *R* or *S* isomer.

In some embodiments, 4'-alkylated nucleoside is introduced at any position on the sense strand or antisense strand of a dsRNA, and such modification maintains or improves potency of the dsRNA. The 4'-alkyl can be either racemic or chirally pure *R* or *S* isomer. An exemplary 4'-alkylated nucleoside is 4'-methyl nucleoside. The 4'-methyl can be either racemic or chirally pure *R* or *S* isomer.

In some embodiments, 4'-*O*-alkylated nucleoside is introduced at any position on the sense strand or antisense strand of a dsRNA, and such modification maintains or improves potency of the dsRNA. The 5'-alkyl can be either racemic or chirally pure *R* or *S* isomer. An exemplary 4'-*O*-alkylated nucleoside is 4'-*O*-methyl nucleoside. The 4'-*O*-methyl can be either racemic or chirally pure *R* or *S* isomer.

In some embodiments, the dsRNA molecule of the disclosure can comprise 2'-5' linkages (with 2'-H, 2'-OH and 2'-OMe and with P=O or P=S). For example, the 2'-5' linkages modifications can be used to promote nuclease resistance or to inhibit binding of the sense to the antisense strand, or can be used at the 5' end of the sense strand to avoid sense strand activation by RISC.

In another embodiment, the dsRNA molecule of the disclosure can comprise L sugars (*e.g.*, L ribose, L-arabinose with 2'-H, 2'-OH and 2'-OMe). For example, these L sugars modifications can be used to promote nuclease resistance or to inhibit binding of the sense to the antisense strand, or can be used at the 5' end of the sense strand to avoid sense strand activation by RISC.

Various publications describe multimeric siRNA which can all be used with the dsRNA of the disclosure. Such publications include WO2007/091269, US 7858769, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520 which are hereby incorporated by their entirely.

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

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

carrier. Thus, the cyclic carrier will often include a functional group, *e.g.*, an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, *e.g.*, a ligand to the constituent ring.

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

In certain specific embodiments, the RNAi agent for use in the methods of the disclosure is an agent selected from the group of agents listed in any one of Tables 2-7, 12, 13, and 18-20. These agents may further comprise a ligand, such as one or more lipophilic moieties, one or more GalNAc derivatives, or both of one of more lipophilic moieties and one or more GalNAc derivatives.

IV. iRNAs Conjugated to Ligands

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

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

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan,

chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand may also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an α helical peptide.

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

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

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a cancer cell, endothelial cell, or bone cell. Ligands may also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

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

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

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

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

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

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

standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

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

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

In certain embodiments, the lipid-based ligand binds HSA. For example, the ligand can bind HSA with a sufficient affinity such that distribution of the conjugate to a non-kidney tissue is enhanced. However, the affinity is typically not so strong that the HSA-ligand binding cannot be reversed.

In certain embodiments, the lipid-based ligand binds HSA weakly or not at all, such that distribution of the conjugate to the kidney is enhanced. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid-based ligand.

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

B. Cell Permeation Agents

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

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar

to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp, or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 31). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO: 32)) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO: 33)) and the *Drosophila Antennapedia* protein (RQIKIWFQNRRMKWKK (SEQ ID NO: 34)) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, *Nature*, 354:82-84, 1991). Typically, the peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit is a cell targeting peptide such as an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

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

An RGD peptide moiety can be used to target a particular cell type, *e.g.*, a tumor cell, such as an endothelial tumor cell or a breast cancer tumor cell (Zitzmann *et al.*, *Cancer Res.*, 62:5139-43, 2002). An RGD peptide can facilitate targeting of an dsRNA agent to tumors of a variety of other tissues, including the lung, kidney, spleen, or liver (Aoki *et al.*, *Cancer Gene Therapy* 8:783-787, 2001). Typically, the RGD peptide will facilitate targeting of an iRNA agent to the kidney. The RGD peptide can be linear or cyclic, and can be modified, *e.g.,* glycosylated or methylated to facilitate targeting to specific tissues. For example, a glycosylated RGD peptide can deliver an iRNA agent to a tumor cell expressing $\alpha_V\beta_3$ (Haubner *et al.*, *Jour. Nucl. Med.*, 42:326-336, 2001).

A "cell permeation peptide" is capable of permeating a cell, *e.g.,* a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide

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

C. Carbohydrate Conjugates

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

In certain embodiments, a carbohydrate conjugate comprises a monosaccharide.

In certain embodiments, the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc conjugates, which comprise one or more N-acetylgalactosamine (GalNAc) derivatives, are described, for example, in US 8,106,022, the entire content of which is hereby incorporated herein by reference. In some embodiments, the GalNAc conjugate serves as a ligand that targets the iRNA to particular cells. In some embodiments, the GalNAc conjugate targets the iRNA to liver cells, *e.g.*, by serving as a ligand for the asialoglycoprotein receptor of liver cells (*e.g.*, hepatocytes).

In some embodiments, the carbohydrate conjugate comprises one or more GalNAc derivatives. The GalNAc derivatives may be attached via a linker, *e.g.*, a bivalent or trivalent branched linker. In some embodiments the GalNAc conjugate is conjugated to the 3' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 3' end of the sense strand) via a linker, *e.g.*, a linker as described herein. In some embodiments the GalNAc conjugate is conjugated to the 5' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 5' end of the sense strand) via a linker, *e.g.*, a linker as described herein.

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

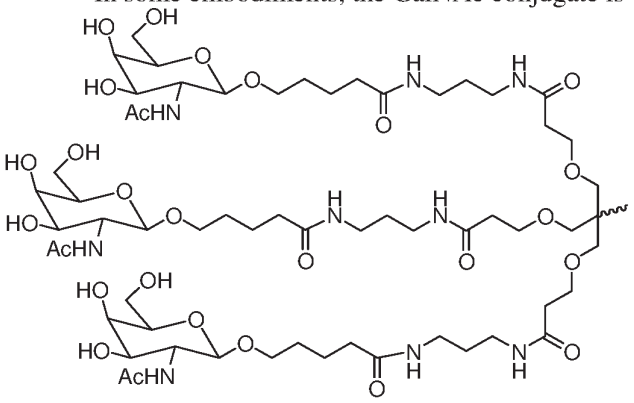
derivative is attached to an iRNA agent of the invention via a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a trivalent linker. In other embodiments of the invention, the GalNAc or GalNAc derivative is attached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

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

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

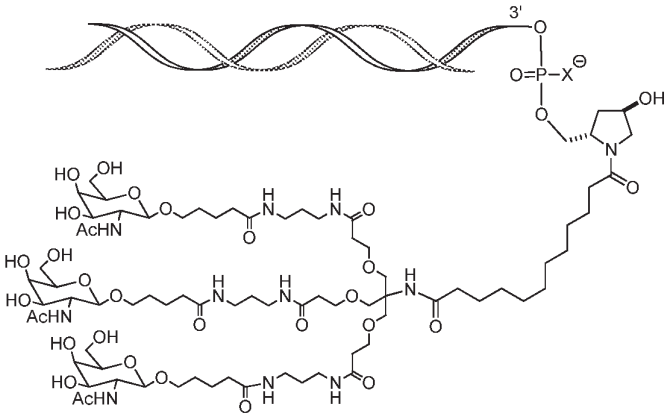
In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of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

In some embodiments, the GalNAc conjugate is

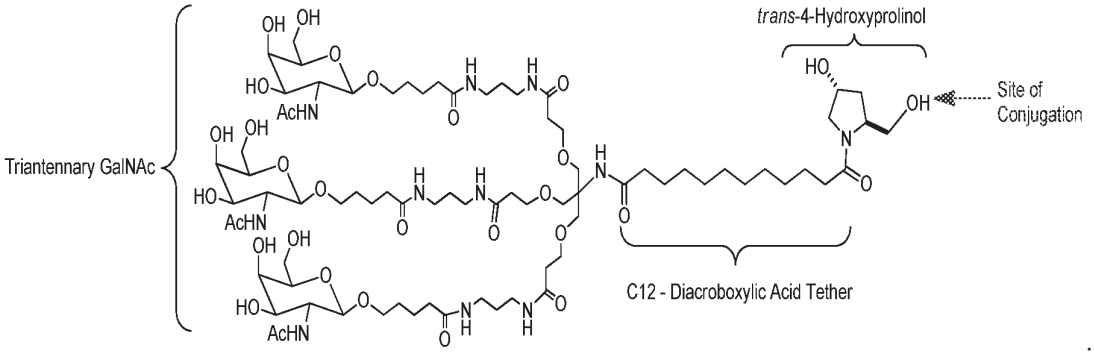


Formula II.

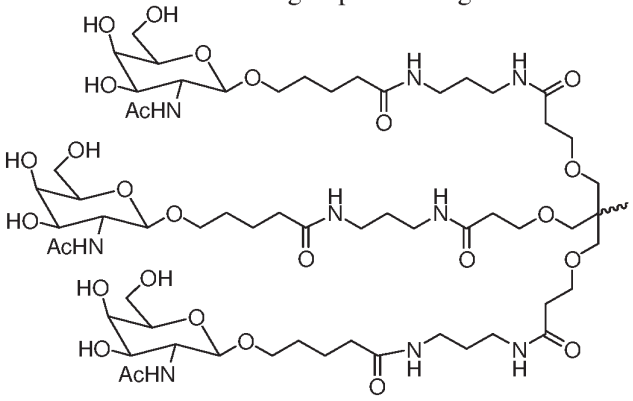
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate via a linker as shown in the following schematic, wherein X is O or S



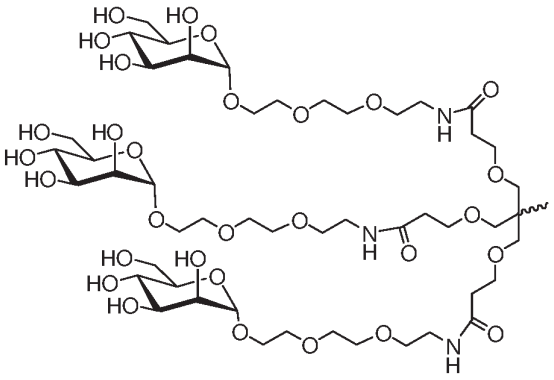
In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below:



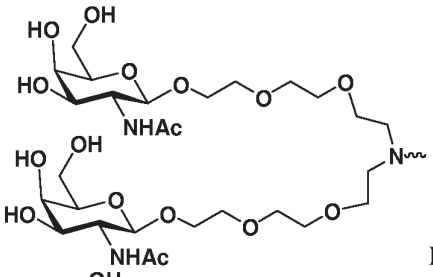
In certain embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



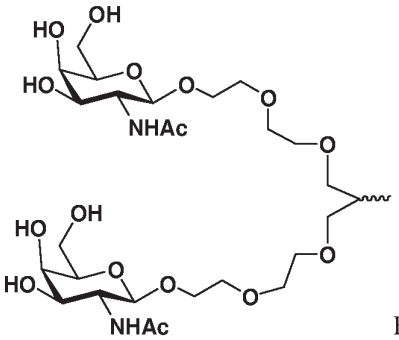
Formula II,



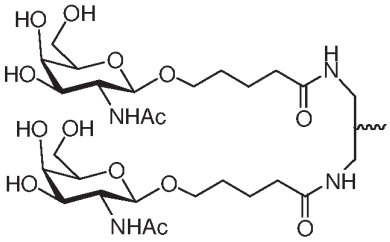
Formula III,



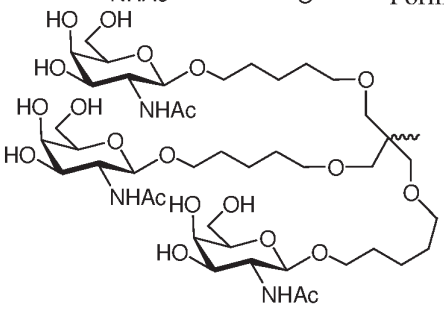
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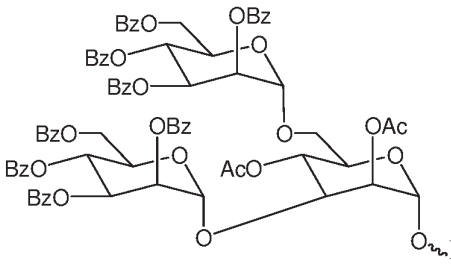
Formula V,



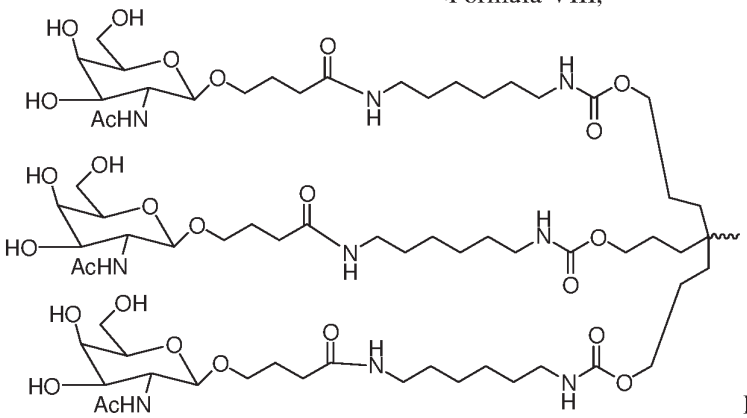
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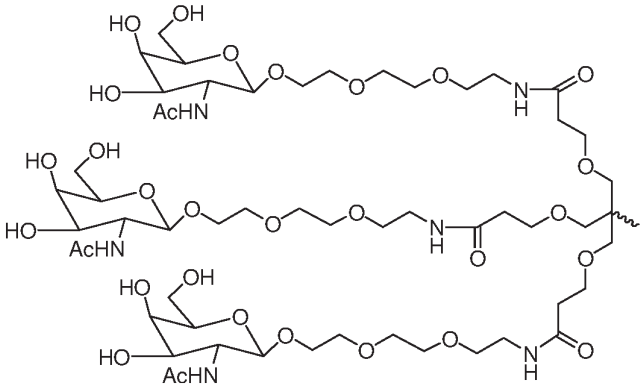
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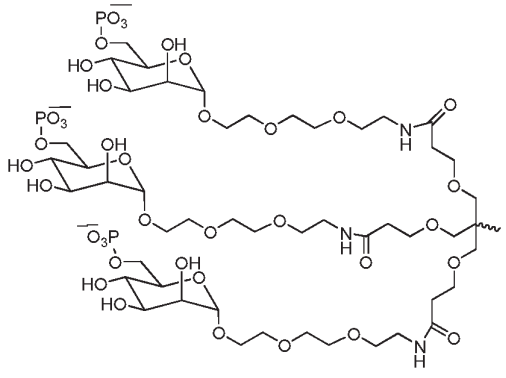
Formula VIII,



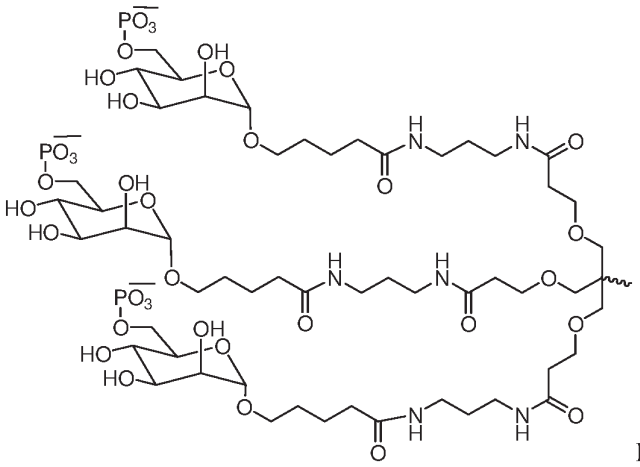
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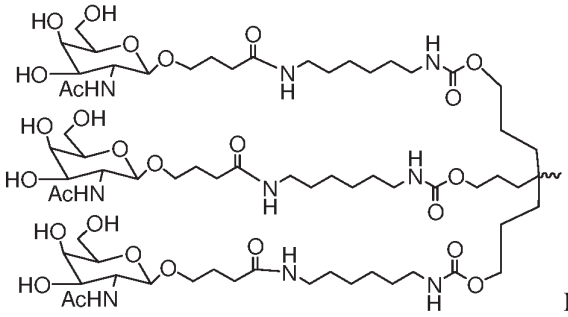
Formula X,



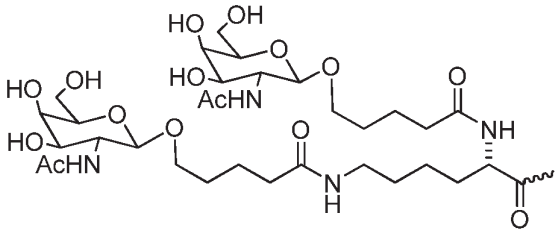
Formula XI,



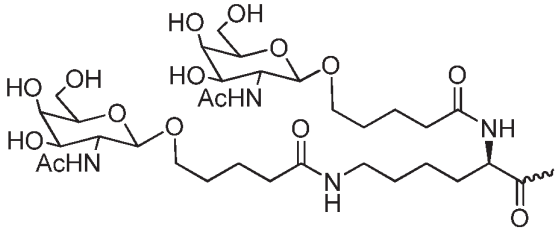
Formula XII,



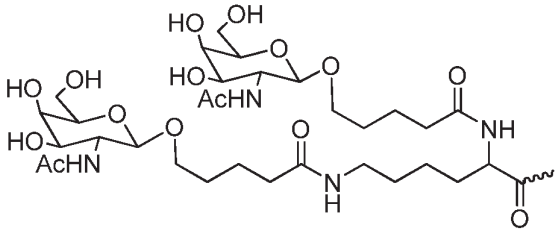
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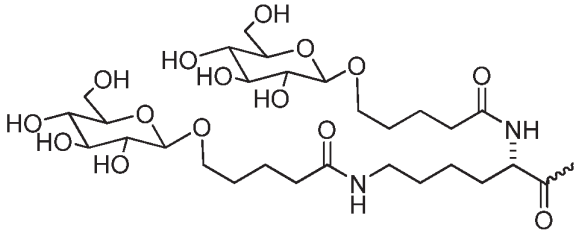
Formula XIV,



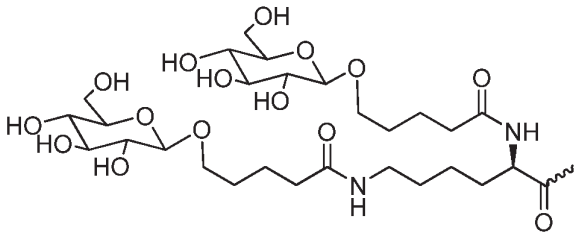
Formula XV,



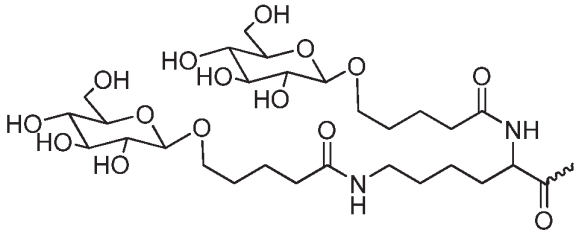
Formula XVI,



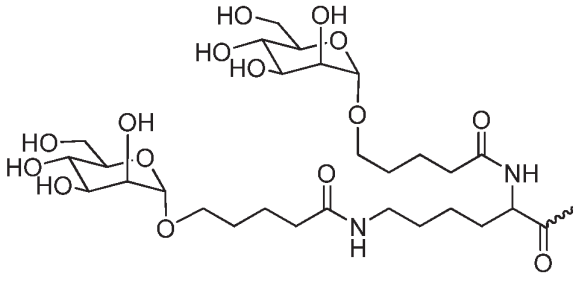
Formula XVII,



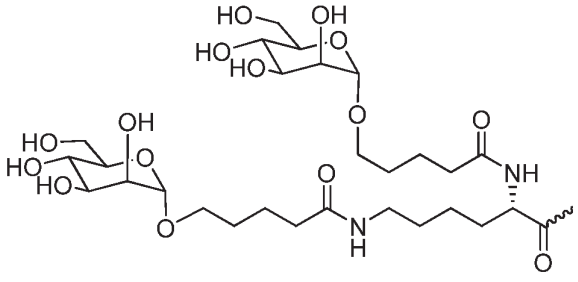
Formula XVIII,



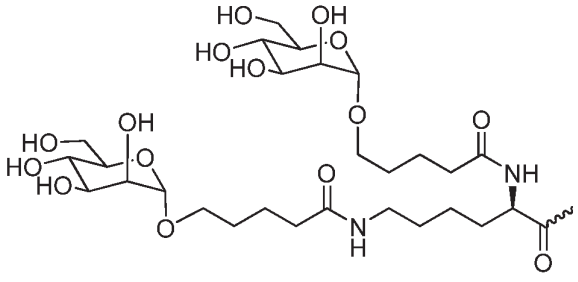
Formula XIX,



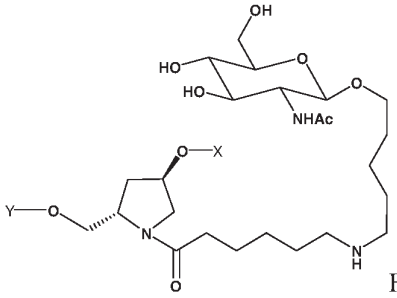
Formula XX,



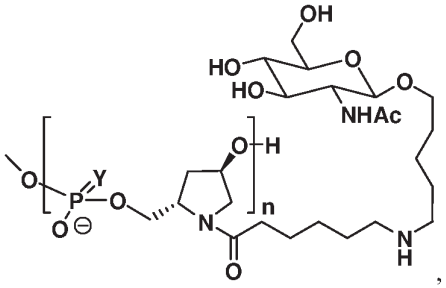
Formula XXI,



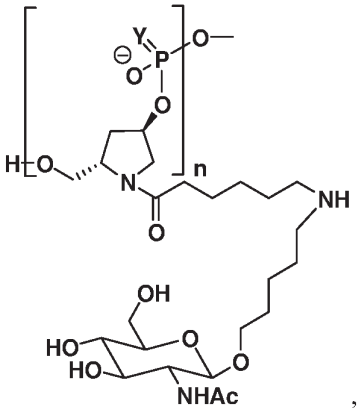
Formula XXII,



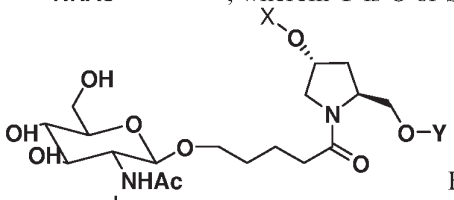
Formula XXIII;



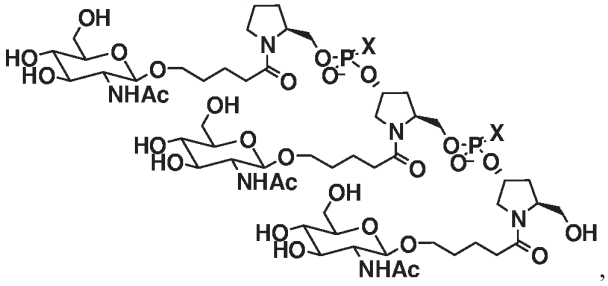
, wherein Y is O or S and n is 3 -6 (Formula XXIV);



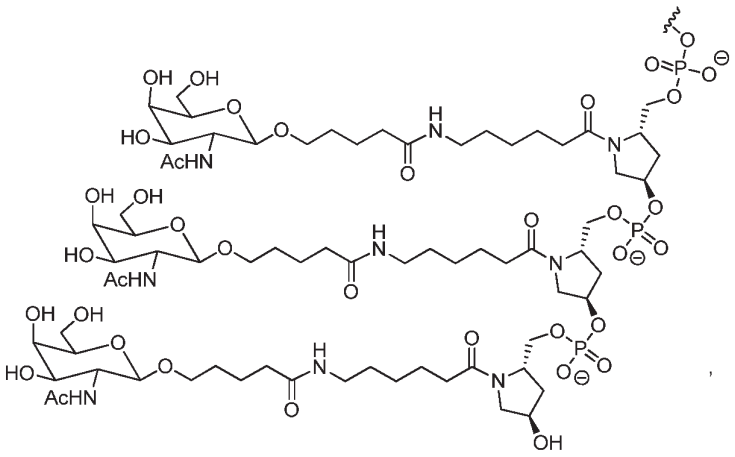
, wherein Y is O or S and n is 3-6 (Formula XXV);

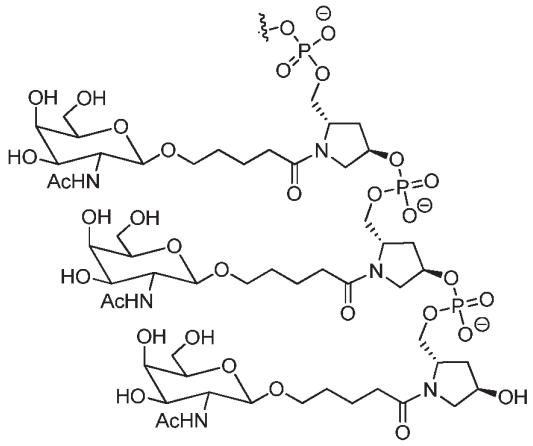


Formula XXVI;

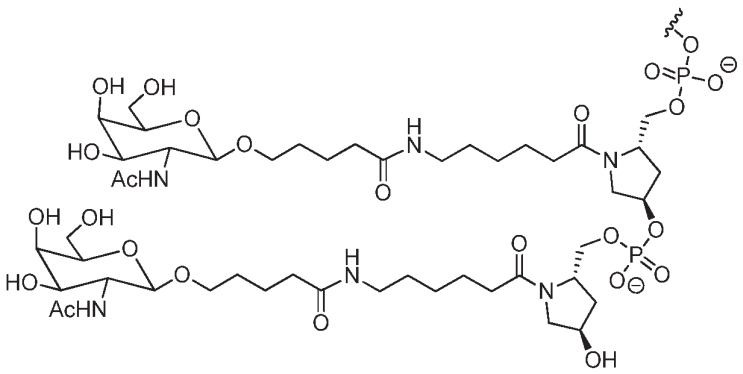


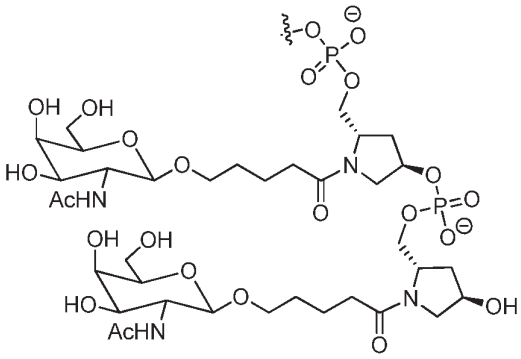
, wherein X is O or S (Formula XXVII);





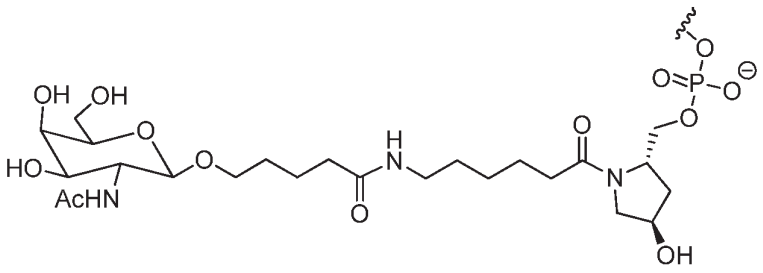
Formula XXVII; Formula XXIX;



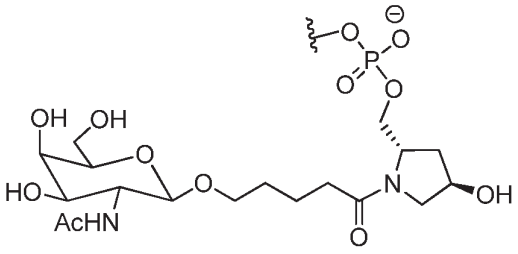


Formula XXX;

Formula XXXI;

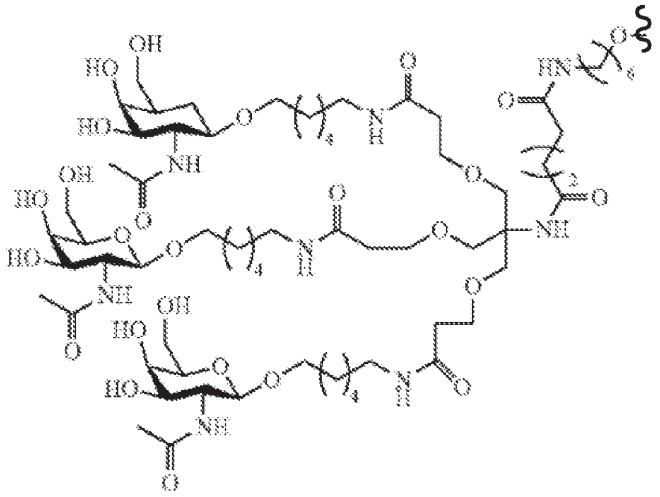


, and



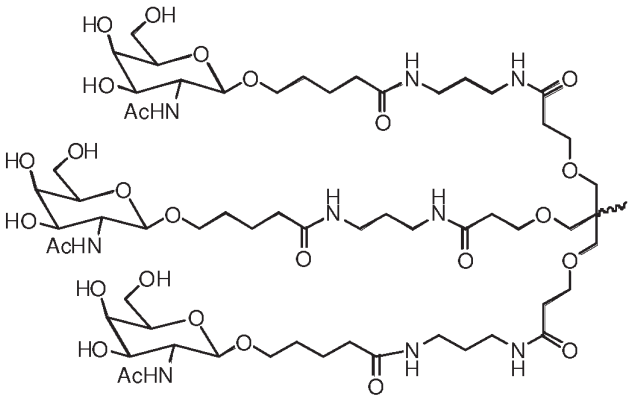
Formula XXXII;

Formula XXXIII.



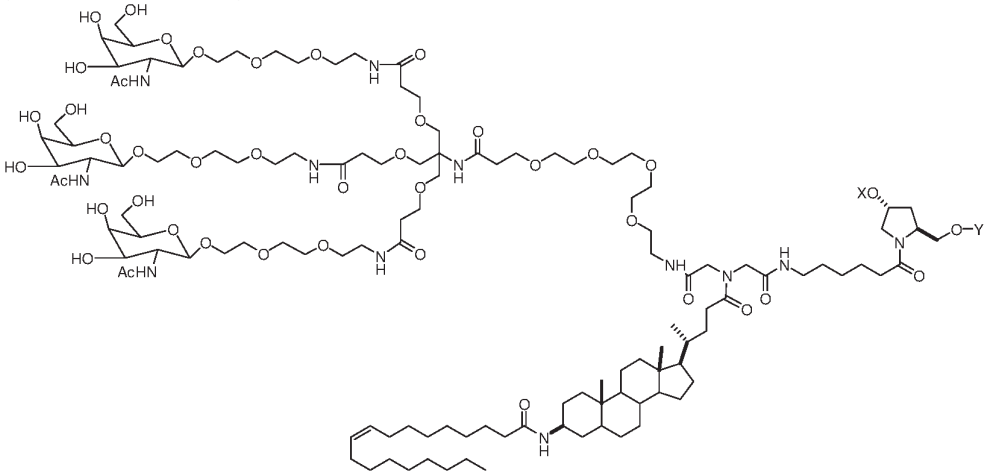
Formula XXXIV.

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



Formula II.

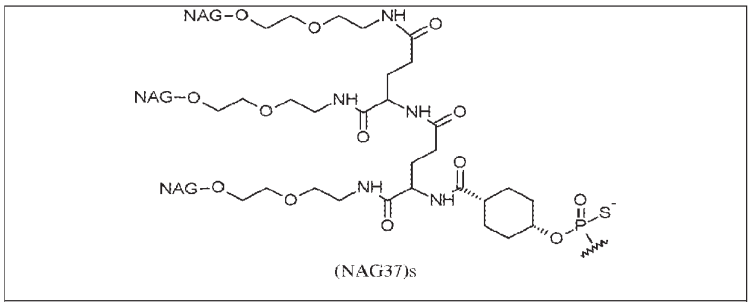
Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



(Formula XXXVI),

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

In some embodiments, a suitable ligand is a ligand disclosed in WO 2019/055633, the entire contents of which are incorporated herein by reference. In one embodiment the ligand comprises the structure below:



In certain embodiments, the RNAi agents of the disclosure may include GalNAc ligands, even if such GalNAc ligands are currently projected to be of limited value for the preferred intrathecal/ intracerebroventricular/ CNS delivery route(s) of the instant disclosure.

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

In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent, *e.g.,* the 5'end of the sense strand of a dsRNA agent, or the 5' end of one or both sense strands of a dual targeting RNAi agent as described herein. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.,* 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

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

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

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

D. Linkers

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

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl,

alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic or substituted aliphatic. In certain embodiments, the linker is between about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18 atoms, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

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

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

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

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

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

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

i. Redox cleavable linking groups

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

ii. Phosphate-based cleavable linking groups

In certain embodiments, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S -, wherein Rk at each occurrence can be, independently, C1-C20 alkyl, C1-C20 haloalkyl, C6-C10 aryl, or C7-C12 aralkyl. . Exemplary embodiments include -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-

P(S)(OH)-S-, -S-P(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O, -S-P(S)(H)-O-, -S-P(O)(H)-S-, and -O-P(S)(H)-S-. In certain embodiments a phosphate-based linking group is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

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

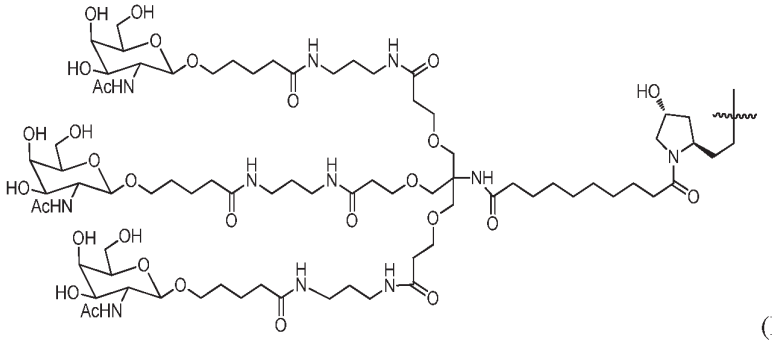
iv. Ester-based cleavable linking groups

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

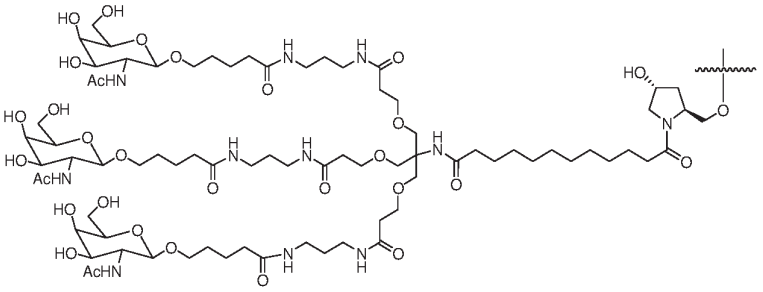
v. Peptide-based cleavable linking groups

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

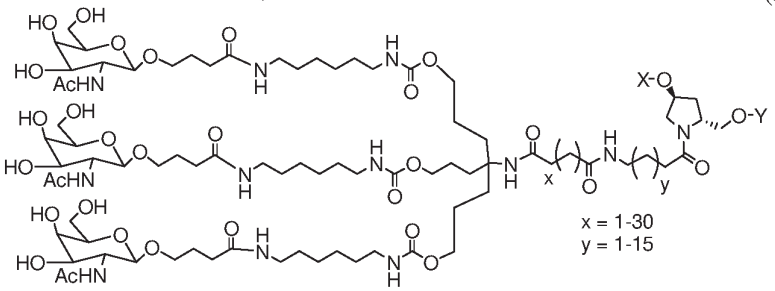
In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



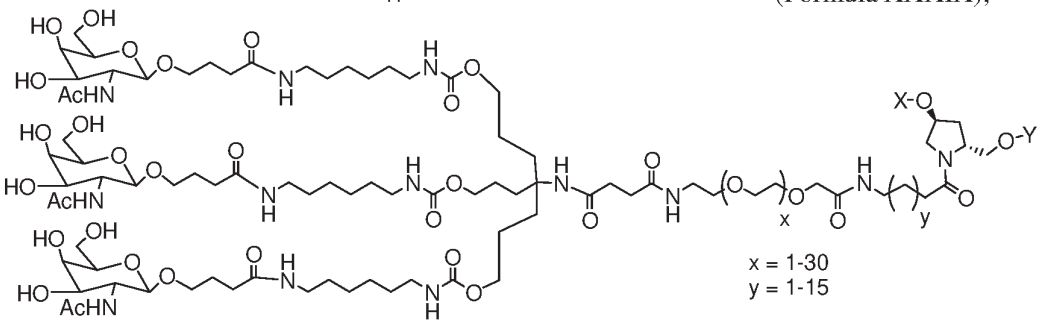
(Formula XXXVII),



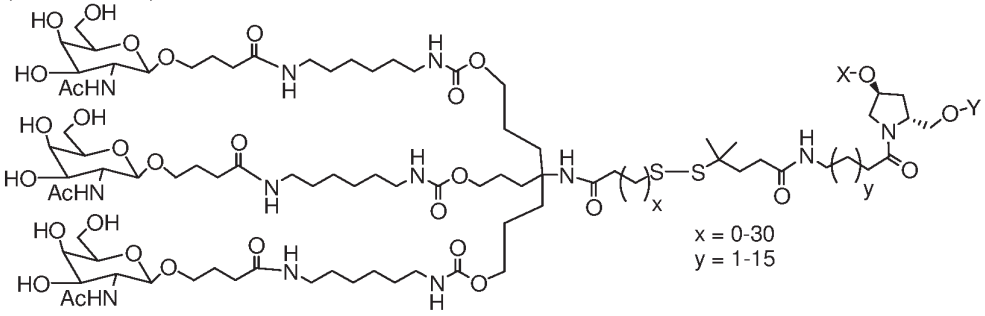
(Formula XXXVIII),



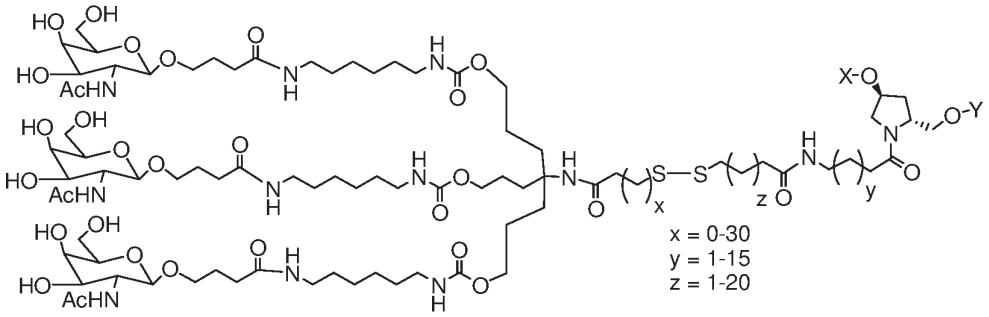
(Formula XXXIX),



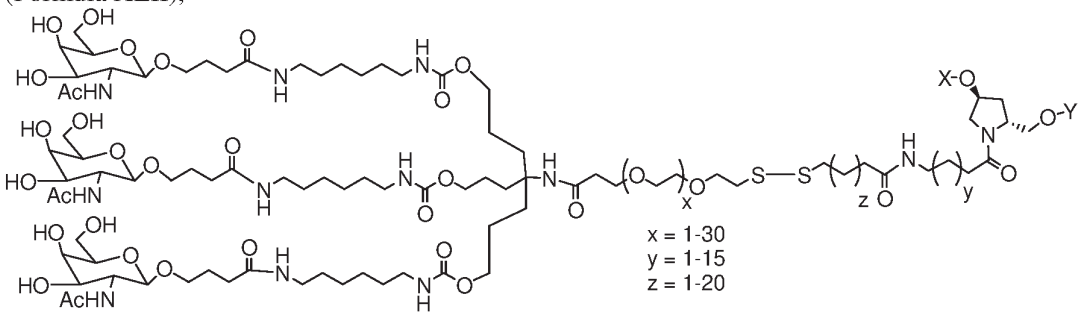
(Formula XL),



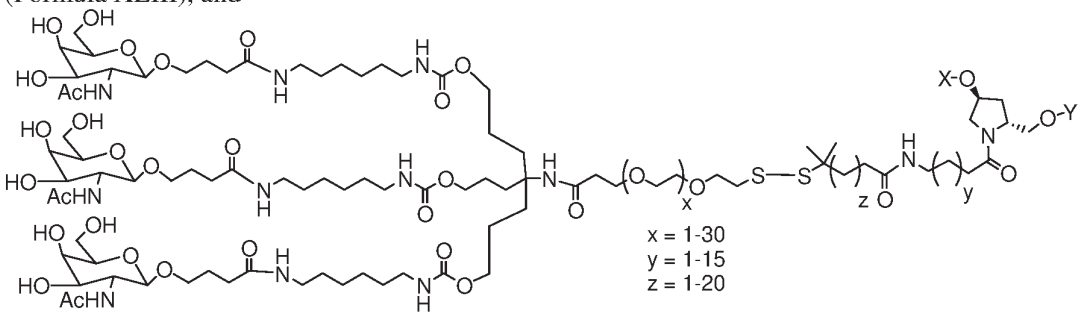
(Formula XLI),



(Formula XLII),



(Formula XLIII), and

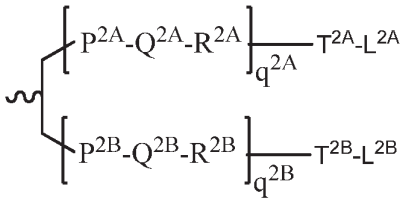


(Formula XLIV), when one of X or Y is an oligonucleotide, the other is a hydrogen.

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

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

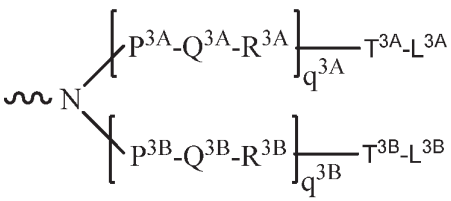
Formula XXXXV



(IV)

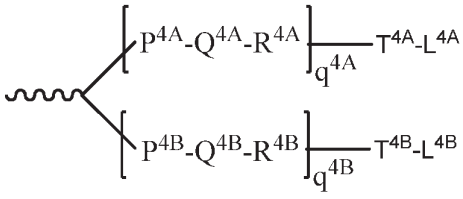
,

Formula XLVI

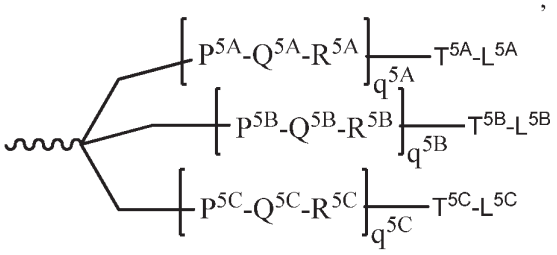


V)

,



Formula XLVII



Formula XLVIII

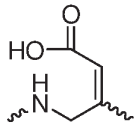
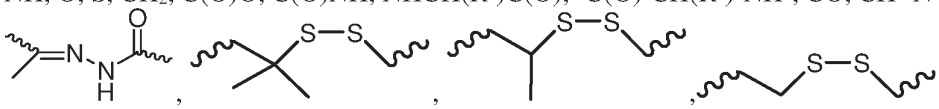
;

wherein:

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

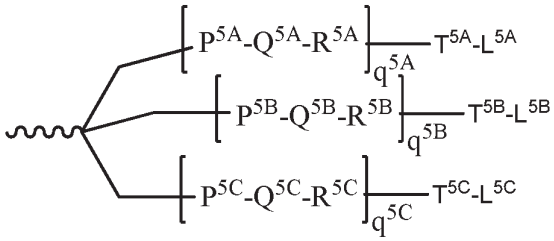
P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

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

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

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

Formula XLIX



Form ,

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

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

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

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

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

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

V. *In Vivo* Testing of SOD1 Knockdown

Mouse models of SOD1-associated neurodegenerative disease have been generated and can further be used to demonstrate the *in vivo* efficacy of the RNAi agents provided herein.

Such models may express, *e.g.,* overexpress, for example, human superoxide dismutase 1 (SOD1), in some instances comprising an ALS-related mutation (*e.g.,* a G93A, G37R, G86R, G85R, L84V, G127X, H46R, D90A, L126Z, A4V, or A4V/SOD1WT mutation) (see, *e.g.,* Mina M, *et al.* (2018) *J Transl Neurosci.* 3:9). Additionally, such models may contain constituitive or inducible expression, *e.g.,* overexpression, of, for example, human amyloid precursor protein (APP), in some instances comprising a pathogenic mutation (*e.g.,* a Swedish mutation (KM670/671NL)), constituitive or inducible expression, *e.g.,* overexpression, of, human presenilin 1 (PS1), in some instances comprising a pathogenic mutation (*e.g.,* dE9 mutation) (see, *e.g.,* Garcia-Alloza, M *et al* (2006) *Neurobiol Dis* 24(3): 516-24), and/or constituitive or inducible expression, *e.g.,* overexpression, of 1N4R human tau protein, in some instances comprising a pathogenic mutation (*e.g.,* a P301S mutation) (Wu, T *et al* (2019) *Cell Rep* 28(8): 2111-2123), superoxide dismutase 1 (SOD1) transgenic mice (see,

e.g., Aziza (2018) *In Vivo* 32(983), mouse toxin models of Parkinson's disease (*e.g.,* MPTP) and/or α-synuclein transgenic mice (Blesa and Przedborski (2014) *Front Neurosci* 8:155).

VI. Delivery of an RNAi Agent of the Disclosure

The delivery of a RNAi agent of the disclosure to a cell *e.g.,* a cell within a subject, such as a human subject (*e.g.,* a subject in need thereof, such as a subject having a SOD1-associated neurodegenerative disorder, *e.g.,*Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an RNAi agent of the disclosure either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an RNAi agent, *e.g.,* a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the RNAi agent. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with a RNAi agent of the disclosure (see *e.g.*, Akhtar S. and Julian RL., (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an RNAi agent include, for example, biological stability of the delivered agent, prevention of non-specific effects, and accumulation of the delivered agent in the target tissue. The non-specific effects of an RNAi agent can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the RNAi agent to be administered. Several studies have shown successful knockdown of gene products when an RNAi agent is administered locally. For example, intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ. *et al.*, (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ. *et al.* (2003) *Mol. Vis.* 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J. *et al.* (2005) *Mol. Ther.* 11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ. *et al.*, (2006) *Mol. Ther.* 14:343-350; Li, S. *et al.*, (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G. *et al.,* (2004) *Nucleic Acids* 32:e49; Tan, PH. *et al.* (2005) *Gene Ther.* 12:59-66; Makimura, H. *et a.l* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al.* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al.* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya,Y., *et al.* (2005) *J. Neurophysiol.* 93:594-602) and to the lungs by intranasal administration (Howard, KA. *et al.*, (2006) *Mol. Ther.* 14:476-484; Zhang, X. *et al.*, (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V. *et al.*, (2005) *Nat. Med.* 11:50-55). For administering a RNAi agent systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid

degradation of the dsRNA by endo- and exo-nucleases *in vivo*. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the RNAi agent to the target tissue and avoid undesirable off-target effects (*e.g.*, without wishing to be bound by theory, use of GNAs as described herein has been identified to destabilize the seed region of a dsRNA, resulting in enhanced preference of such dsRNAs for on-target effectiveness, relative to off-target effects, as such off-target effects are significantly weakened by such seed region destabilization). RNAi agents can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, a RNAi agent directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J. *et al.*, (2004) *Nature* 432:173-178). Conjugation of an RNAi agent to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO. *et al.*, (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the RNAi agent can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of molecule RNAi agent (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an RNAi agent by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an RNAi agent, or induced to form a vesicle or micelle (see *e.g.*, Kim SH. *et al.*, (2008) *Journal of Controlled Release* 129(2):107-116) that encases an RNAi agent. The formation of vesicles or micelles further prevents degradation of the RNAi agent when administered systemically. Methods for making and administering cationic- RNAi agent complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al.* (2003) *J. Mol. Biol* 327:761-766; Verma, UN. *et al.*, (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *et al.* (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of RNAi agents include DOTAP (Sorensen, DR., et al (2003), supra; Verma, UN. *et al.*, (2003), supra), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS. *et al.,* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY. *et al.*, (2005) *Cancer Gene Ther.* 12:321-328; Pal, A. *et al.*, (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME. *et al.*, (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA. *et al.*, (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H. *et al.*, (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, a RNAi agent forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of RNAi agents and cyclodextrins can be found in U.S. Patent No. 7, 427, 605, which is herein incorporated by reference in its entirety.

Certain aspects of the instant disclosure relate to a method of reducing the expression of a SOD1 target gene in a cell, comprising contacting said cell with the double-stranded RNAi agent of the disclosure. In one embodiment, the cell is a hepatic cell, optionally a hepatocyte. In one embodiment, the cell is an extrahepatic cell, optionally a CNS cell.

Another aspect of the disclosure relates to a method of reducing the expression of a SOD1 target gene in a subject, comprising administering to the subject the double-stranded RNAi agent of the disclosure.

Another aspect of the disclosure relates to a method of treating a subject having a SOD1-associated neurodegenerative disorder, comprising administering to the subject a therapeutically effective amount of the double-stranded RNAi agent of the disclosure, thereby treating the subject. Exemplary CNS disorders that can be treated by the method of the disclosure include Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

In one embodiment, the double-stranded RNAi agent is administered subcutaneously.

In one embodiment, the double-stranded RNAi agent is administered by intraventricular administration.

In one embodiment, the double-stranded RNAi agent is administered intrathecally. In one embodiment, the double-stranded RNAi agent is administered intracerebroventricularly. By intrathecal or intracerebroventricular administration of the double-stranded RNAi agent, the method can reduce the expression of a SOD1 target gene in a brain (*e.g.,* striatum) or spine tissue, for instance, cortex, cerebellum, cervical spine, lumbar spine, and thoracic spine.

For ease of exposition the formulations, compositions and methods in this section are discussed largely with regard to modified siRNA compounds. It may be understood, however, that these formulations, compositions and methods can be practiced with other siRNA compounds, *e.g.*, unmodified siRNA compounds, and such practice is within the disclosure. A composition that includes a RNAi agent can be delivered to a subject by a variety of routes. Exemplary routes include intrathecal, subcutaneous, intravenous, intraventricular (also known as intracerebroventricular), intraperitoneal, intravitreal, topical, rectal, anal, vaginal, and ocular.

The RNAi agents of the disclosure can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically include one or more species of RNAi agent and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The pharmaceutical compositions of the present disclosure may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, transdermal), oral, or parenteral. Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal, or intramuscular injection, or intrathecal or intraventricular administration.

The route and site of administration may be chosen to enhance targeting. For example, to target muscle cells, intramuscular injection into the muscles of interest would be a logical choice. Lung cells might be targeted by administering the RNAi agent in powder or aerosol form. The vascular endothelial cells could be targeted by coating a balloon catheter with the RNAi agent and mechanically introducing the RNA.

Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids, and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves, and the like may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water, syrups, elixirs or non-aqueous media, tablets, capsules, lozenges, or troches. In the case of tablets, carriers that can be used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc, are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use, the nucleic acid compositions can be combined with emulsifying and suspending agents. If desired, certain sweetening or flavoring agents can be added.

Compositions for intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents, and other suitable additives.

Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents, and other suitable additives. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir. For intravenous use, the total concentration of solutes may be controlled to render the preparation isotonic.

In one embodiment, the administration of the siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, composition is parenteral, *e.g.,* intravenous (*e.g.*, as a bolus or as a diffusible infusion), intradermal, intraperitoneal, intramuscular, intrathecal, intraventricular, intracranial, subcutaneous, transmucosal, buccal, sublingual, endoscopic, rectal, oral, vaginal, topical, urethral, or ocular. Administration can be provided by the subject or by another person, *e.g.*, a health care provider. The medication can be provided in measured doses or in a dispenser which delivers a metered dose. Selected modes of delivery are discussed in more detail below.

Intrathecal Administration.

In one embodiment, the double-stranded RNAi agent is delivered by intrathecal injection (*i.e.*, injection into the spinal fluid which bathes the brain and spinal cord tissue). Intrathecal injection of RNAi agents into the spinal fluid can be performed as a bolus injection or via minipumps which can be implanted beneath the skin, providing a regular and constant delivery of siRNA into the spinal fluid. The circulation of the spinal fluid from the choroid plexus, where it is produced, down around the spinal cord and dorsal root ganglia and subsequently up past the cerebellum and over the cortex to the

arachnoid granulations, where the fluid can exit the CNS, that, depending upon size, stability, and solubility of the compounds injected, molecules delivered intrathecally could hit targets throughout the entire CNS.

In some embodiments, the intrathecal administration is via a pump. The pump may be a surgically implanted osmotic pump. In one embodiment, the osmotic pump is implanted into the subarachnoid space of the spinal canal to facilitate intrathecal administration.

In some embodiments, the intrathecal administration is via an intrathecal delivery system for a pharmaceutical including a reservoir containing a volume of the pharmaceutical agent, and a pump configured to deliver a portion of the pharmaceutical agent contained in the reservoir. More details about this intrathecal delivery system may be found in WO 2015/116658, which is incorporated by reference in its entirety.

The amount of intrathecally injected RNAi agents may vary from one target gene to another target gene and the appropriate amount that has to be applied may have to be determined individually for each target gene. Typically, this amount ranges from 10 μg to 2 mg, preferably 50 μg to 1500 μg, more preferably 100 μg to 1000 μg.

Vector encoded RNAi agents of the Disclosure

RNAi agents targeting the SOD1 gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al*., *TIG.* (1996), 12:5-10; WO 00/22113, WO 00/22114, and US 6,054,299). Expression is preferablysustained (months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al*., (1995) *Proc. Natl. Acad. Sci. USA* 92:1292).

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

RNAi agent expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of a RNAi agent as described herein. Delivery of RNAi agent expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc*.; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of a RNAi agent will generally require regulatory elements, *e.g.*, promoters, enhancers, *etc*., to ensure the expression of the RNAi agent in target cells. Other aspects to consider for vectors and constructs are known in the art.

VII. Pharmaceutical Compositions of the Invention

The present disclosure also includes pharmaceutical compositions and formulations which include the RNAi agents of the disclosure. In one embodiment, provided herein are pharmaceutical compositions containing an RNAi agent, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the RNAi agent are useful for treating a disease or disorder associated with the expression or activity of SOD1, *e.g.*, a SOD1-associated neurodegenerative disease, such as Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery, *e.g.*, by intravenous (IV), intramuscular (IM), or for subcutaneous (subQ) delivery. Another example is compositions that are formulated for direct delivery into the CNS, *e.g.*, by intrathecal or intraventricular routes of injection, optionally by infusion into the brain (*e.g.,* striatum), such as by continuous pump infusion.

In some embodiments, the pharmaceutical compositions of the invention are pyrogen free or non-pyrogenic.

The pharmaceutical compositions of the disclosure may be administered in dosages sufficient to inhibit expression of a SOD1 gene. In general, a suitable dose of an RNAi agent of the disclosure will be a flat dose in the range of about 0.001 to about 200.0 mgabout once per month to about once per year, typically about once per quarter (i.e., about once every three months) to about once per year, generally a flat dose in the range of about 1 to 50 mg about once per month to about once per year, typically about once per quarter to about once per year.

After an initial treatment regimen (*e.g.*, loading dose), the treatments can be administered on a less frequent basis.

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments.

Advances in mouse genetics have generated a number of mouse models for the study of various SOD1-associated neurodegenerative diseases that would benefit from reduction in the expression of SOD1. Such models can be used for *in vivo* testing of RNAi agents, as well as for determining a therapeutically effective dose. Suitable mouse models are known in the art and include, for example, the mouse models described elsewhere herein.

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

The RNAi agents can be delivered in a manner to target a particular tissue, such as the liver, the CNS (*e.g.*, neuronal, glial or vascular tissue of the brain), or both the liver and CNS.

Pharmaceutical compositions and formulations for topical administration can include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be useful. Suitable topical formulations include those in which the RNAi agents featured in the disclosure are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include neutral (*e.g.,* dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (*e.g.,* dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.,* dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). RNAi agents featured in the disclosure can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, RNAi agents can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-20} alkyl ester (*e.g.,* isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in US 6,747,014, which is incorporated herein by reference.

A. RNAi Agent Formulations Comprising Membranous Molecular Assemblies

A RNAi agent for use in the compositions and methods of the disclosure can be formulated for delivery in a membranous molecular assembly, *e.g.*, a liposome or a micelle. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, *e.g.*, one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the RNAi agent composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the RNAi agent composition, although in some examples, it may. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the RNAi agent are delivered into the cell where the RNAi agent can specifically bind to a target RNA and can mediate RNAi. In some cases the liposomes are also specifically targeted, *e.g.*, to direct the RNAi agent to particular cell types.

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

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

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

small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew *et al.*, (1984) *Biochim. Biophys. Acta* 775:169. These methods are readily adapted to packaging RNAi agent preparations into liposomes.

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

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

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

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

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

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

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

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

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

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

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

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse

with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") (Boehringer Mannheim, Indianapolis, Indiana) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

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

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

Liposomal formulations are particularly suited for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer RNAi agent into the skin. In some implementations, liposomes are used for delivering RNAi agent to epidermal cells and also to enhance the penetration of RNAi agent into dermal tissues, *e.g.*, into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, *e.g.*, Weiner *et al.*, (1992) *Journal of Drug Targeting*, vol. 2,405-410 and du Plessis *et al.*, (1992) *Antiviral Research*, 18:259-265; Mannino, R. J. and Fould-Fogerite, S., (1998) *Biotechniques* 6:682-690; Itani, T. *et al.*, (1987) *Gene* 56:267-276; Nicolau, C. *et al.* (1987) *Meth. Enzymol.* 149:157-176; Straubinger, R. M. and Papahadjopoulos, D. (1983) *Meth. Enzymol.* 101:512-527; Wang, C. Y. and Huang, L., (1987) *Proc. Natl. Acad. Sci. USA* 84:7851-7855).

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

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

Other formulations amenable to the present disclosure are described in PCT publication No. WO 2008/042973.

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

Surfactants find wide application in formulations such as those described herein, particularlay in emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

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

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl

sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

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

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

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

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

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

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

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

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

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

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

Lipid particles

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

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

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

Certain specific LNP formulations for delivery of RNAi agents have been described in the art, including, *e.g.*, “LNP01” formulations as described in, *e.g.*, WO 2008/042973, which is hereby incorporated by reference.

Additional exemplary lipid-dsRNA formulations are identified in the table below.

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

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

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

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

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

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

SNALP (l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in WO 2009/127060, which is hereby incorporated by reference.

XTC comprising formulations are described in WO 2010/088537, the entire contents of which are hereby incorporated herein by reference.

MC3 comprising formulations are described, *e.g.*, in United States Patent Publication No. 2010/0324120, the entire contents of which are hereby incorporated by reference.

ALNY-100 comprising formulations are described in WO 2010/054406, the entire contents of which are hereby incorporated herein by reference.

C12-200 comprising formulations are described in WO 2010/129709, the entire contents of which are hereby incorporated herein by reference.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the disclosure are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids or esters or salts thereof, bile acids or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.,* sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the disclosure can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (*e.g.,* p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate,

poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Patent 6,887,906, U.S. 2003/0027780, and U.S. Patent No. 6,747,014, each of which is incorporated herein by reference.

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

Pharmaceutical compositions of the present disclosure include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly preferred are formulations that target the brain when treating SOD-1-associated diseases or disorders.

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

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

Additional Formulations

i. Emulsions

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

phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution in either aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise, a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

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

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

Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

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

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

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

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

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

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

ii. Microemulsions

In one embodiment of the present disclosure, the compositions of RNAi agents and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically, microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used, and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

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

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol

monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase can include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

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

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

iii. Microparticles

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

iv. Penetration Enhancers

In one embodiment, the present disclosure employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly RNAi agents, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

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

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

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

The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman *et al.* Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various

natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Suitable bile salts include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto *et al.*, J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita *et al.*, J. Pharm. Sci., 1990, 79, 579-583).

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

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

Agents that enhance uptake of RNAi agents at the cellular level can also be added to the pharmaceutical and other compositions of the present disclosure. For example, cationic lipids, such as lipofectin (Junichi *et al*, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic

molecules, such as polylysine (WO 97/30731), are also known to enhance the cellular uptake of dsRNAs.

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

vi. Excipients

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

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

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

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

vii. Other Components

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

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

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

In some embodiments, pharmaceutical compositions featured in the disclosure include (a) one or more RNAi agents and (b) one or more agents which function by a non-RNAi mechanism and which are useful in treating a SOD1-associated neurodegenerative disorder. Examples of such agents include, but are not lmited to SSRIs, venlafaxine, bupropion, and atypical antipsychotics.

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

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the disclosure lies generally within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the disclosure, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC_{50} (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the RNAi agents featured in the disclosure can be administered in combination with other known agents effective in treatment of pathological processes mediated by nucleotide repeat expression. In any event, the administering physician can adjust the amount and timing of RNAi agent administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VIII. Kits

In certain aspects, the instant disclosure provides kits that include a suitable container containing a pharmaceutical formulation of a siRNA compound, *e.g.*, a double-stranded siRNA compound, or siRNA compound, (*e.g.*, a precursor, *e.g.*, a larger siRNA compound which can be processed into a siRNA compound, or a DNA which encodes an siRNA compound, *e.g.*, a double-stranded siRNA compound, or siRNA compound, or precursor thereof). In certain embodiments the individual components of the pharmaceutical formulation may be provided in one container. Alternatively, it may be desirable to provide the components of the pharmaceutical formulation separately in two or more containers, *e.g.*, one container for a siRNA compound preparation, and at least another for a carrier compound. The kit may be packaged in a number of different configurations such as one or more containers in a single box. The different components can be combined, *e.g.*, according to instructions provided with the kit. The components can be combined according to a method described herein, *e.g.*, to prepare and administer a pharmaceutical composition. The kit can also include a delivery device.

IX. Methods for Inhibiting SOD1 Expression

The present disclosure also provides methods of inhibiting expression of a SOD1 gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNAi agent, in an amount effective to inhibit expression of SOD1 in the cell, thereby inhibiting expression of SOD1 in the cell. In certain embodiments of the disclosure, SOD1 is inhibited preferentially in CNS (*e.g.*, brain) cells. In other embodiments of the disclosure, SOD1 is inhibited preferentially in the liver (*e.g.*, hepatocytes). In certain embodiments of the disclosure, SOD1 is inhibited in CNS (*e.g.*, brain) cells and in liver (*e.g.*, hepatocytes) cells.

Contacting of a cell with a RNAi agent, *e.g.*, a double stranded RNAi agent, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the RNAi agent includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the RNAi agent. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible.

Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In some embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a GalNAc ligand, or any other ligand that directs the RNAi agent to a site of interest.

The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating," "suppressing" and other similar terms, and includes any level of inhibition. In certain embodiments, a level of inhibition, *e.g.*, for an RNAi agent of the instant disclosure, can be assessed in cell culture conditions, *e.g.*, wherein cells in cell culture are transfected *via* Lipofectamine™-mediated transfection at a concentration in the vicinity of a cell of 10 nM or less, 1 nM or less, *etc*. Knockdown of a given RNAi agent can be determined *via* comparison of pre-treated levels in cell culture versus post-treated levels in cell culture, optionally also comparing against cells treated in parallel with a scrambled

or other form of control RNAi agent. Knockdown in cell culture of, *e.g.*, preferably 50% or more, can thereby be identified as indicative of "inhibiting" or "reducing", "downregulating" or "suppressing", *etc*. having occurred. It is expressly contemplated that assessment of targeted mRNA or encoded protein levels (and therefore an extent of "inhibiting", *etc*. caused by a RNAi agent of the disclosure) can also be assessed in *in vivo* systems for the RNAi agents of the instant disclosure, under properly controlled conditions as described in the art.

The phrase "inhibiting expression of a SOD1 gene" or "inhibiting expression of SOD1," as used herein, includes inhibition of expression of any SOD1 gene (such as, *e.g.*, a mouse SOD1 gene, a rat SOD1 gene, a monkey SOD1 gene, or a human SOD1 gene) as well as variants or mutants of a SOD1 gene that encode a SOD1 protein. Thus, the SOD1 gene may be a wild-type SOD1 gene, a mutant SOD1 gene, or a transgenic SOD1 gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of a SOD1 gene" includes any level of inhibition of a SOD1 gene, *e.g.*, at least partial suppression of the expression of a SOD1 gene, such as an inhibition by at least 20%. In certain embodiments, inhibition is by at least 30%, at least 40%, preferably at least 50%, at least about 60%, at least 70%, at least about 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%; or to below the level of detection of the assay method. In a preferred method, inhibition is measured at a 10 nM concentration of the siRNA using the luciferase assay provided in Example 1.

The expression of a SOD1 gene may be assessed based on the level of any variable associated with SOD1 gene expression, *e.g.*, SOD1 mRNA level or SOD1 protein level, or, for example, the level of neuroinflammation, *e.g.,* microglial and astrocyte activation, and SOD1 deposition in areas of the brain associated with neuronal cell death.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more of these variables compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In some embodiments of the methods of the disclosure, expression of a SOD1 gene is inhibited by at least 20%, 30%, 40%, preferably at least 50%, 60%, 70%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In certain embodiments, the methods include a clinically relevant inhibition of expression of SOD1, *e.g.* as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of SOD1.

Inhibition of the expression of a SOD1 gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a SOD1 gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with a RNAi agent of the disclosure, or by administering a RNAi agent of the disclosure to a subject in which the cells are or were present) such that the expression of a SOD1

gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s) not treated with a RNAi agent or not treated with a RNAi agent targeted to the gene of interest). The degree of inhibition may be expressed in terms of:

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

In other embodiments, inhibition of the expression of a SOD1 gene may be assessed in terms of a reduction of a parameter that is functionally linked to a SOD1 gene expression, *e.g.*, SOD1 protein expression. SOD1 gene silencing may be determined in any cell expressing SOD1, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of a SOD1 protein may be manifested by a reduction in the level of the SOD1 protein that is expressed by a cell or group of cells (*e.g.*, the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibiton of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

A control cell or group of cells that may be used to assess the inhibition of the expression of a SOD1 gene includes a cell or group of cells that has not yet been contacted with an RNAi agent of the disclosure. For example, the control cell or group of cells may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent.

The level of SOD1 mRNA that is expressed by a cell or group of cells may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of SOD1 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.,* mRNA of the SOD1 gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy™ RNA preparation kits (Qiagen®) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, *in situ* hybridization, and microarray analysis. Circulating SOD1 mRNA may be detected using methods the described in WO2012/177906, the entire contents of which are hereby incorporated herein by reference.

In some embodiments, the level of expression of SOD1 is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific SOD1 nucleic acid or protein, or fragment thereof. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to SOD1 mRNA. In one embodiment, the mRNA is

immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix® gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of SOD1 mRNA.

An alternative method for determining the level of expression of SOD1 in a sample involves the process of nucleic acid amplification or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, US Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.*, US Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the disclosure, the level of expression of SOD1 is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System), by a Dual-Glo® Luciferase assay, or by other art-recognized method for measurement of SOD1 expression or mRNA level.

The expression level of SOD1 mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See US Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of SOD1 expression level may also comprise using nucleic acid probes in solution.

In some embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of this PCR method is described and exemplified in the Examples presented herein. Such methods can also be used for the detection of SOD1 nucleic acids.

The level of SOD1 protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like. Such assays can also be used for the detection of proteins indicative of the presence or replication of SOD1 proteins.

In some embodiments, the efficacy of the methods of the disclosure in the treatment of a SOD1-related disease is assessed by a decrease in SOD1 mRNA level (*e.g*, by assessment of a CSF sample for SOD1 level, by brain biopsy, or otherwise).

In some embodiments, the efficacy of the methods of the disclosure in the treatment of a SOD1-related disease is assessed by a decrease in SOD1 mRNA level (*e.g*, by assessment of a liver sample for SOD1 level, by biopsy, or otherwise).

In some embodiments of the methods of the disclosure, the RNAi agent is administered to a subject such that the RNAi agent is delivered to a specific site within the subject. The inhibition of expression of SOD1 may be assessed using measurements of the level or change in the level of SOD1 mRNA or SOD1 protein in a sample derived from a specific site within the subject, *e.g.*, CNS cells. In certain embodiments, the methods include a clinically relevant inhibition of expression of SOD1, *e.g.* as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of SOD1.

As used herein, the terms detecting or determining a level of an analyte are understood to mean performing the steps to determine if a material, *e.g.*, protein, RNA, is present. As used herein, methods of detecting or determining include detection or determination of an analyte level that is below the level of detection for the method used.

X. Methods of Treating or Preventing SOD1-Associated Neurodegenerative Diseases

The present disclosure also provides methods of using a RNAi agent of the disclosure or a composition containing a RNAi agent of the disclosure to reduce or inhibit SOD1 expression in a cell. The methods include contacting the cell with a dsRNA of the disclosure and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of a SOD1 gene, thereby inhibiting expression of the SOD1 gene in the cell. Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of SOD1 may be determined by determining the mRNA expression level of SOD1 using methods routine to one of ordinary skill in the art, *e.g.,* northern blotting, qRT-PCR; by determining the protein level of SOD1 using methods routine to one of ordinary skill in the art, such as western blotting, immunological techniques.

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

A cell suitable for treatment using the methods of the disclosure may be any cell that expresses a SOD1 gene. A cell suitable for use in the methods of the disclosure may be a mammalian cell, *e.g.,* a primate cell (such as a human cell or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), a non-primate cell (such as a rat cell, or a mouse cell. In one embodiment, the cell is a human cell, *e.g.,* a human CNS cell. In one embodiment, the cell is a human cell, *e.g.,* a human liver cell. In one embodiment, the cell is a human cell, *e.g.,* a human CNS cell and a human liver cell.

SOD1 expression is inhibited in the cell by at least about 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or about 100%, *i.e.*, to below the level of detection. In preferred embodiments, SOD1 expression is inhibited by at least 50 %.

The *in vivo* methods of the disclosure may include administering to a subject a composition containing a RNAi agent, where the RNAi agent includes a nucleotide sequence that is complementary

to at least a part of an RNA transcript of the SOD1 gene of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular (also referred to asintracerebroventricular), intraparenchymal, and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection. In certain embodiments, the compositions are administered by intrathecal injection. In certain embodiments, the compositions are administered by intracerebroventricular injection.

In some embodiments, the administration is *via* a depot injection. A depot injection may release the RNAi agent in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, *e.g.*, a desired inhibition of SOD1, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

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

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

In one aspect, the present disclosure also provides methods for inhibiting the expression of a SOD1 gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a SOD1 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the SOD1 gene, thereby inhibiting expression of the SOD1 gene in the cell. Reduction in gene expression can be assessed by any methods known it the art and by methods, *e.g.* qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known it the art and by methods, *e.g.* ELISA, described herein. In one embodiment, a CNS biopsy sample or a cerebrospinal fluid (CSF) sample serves as the tissue material for monitoring the reduction in SOD1 gene or protein expression (or of a proxy therefore).

The present disclosure further provides methods of treatment of a subject in need thereof. The treatment methods of the disclosure include administering an RNAi agent of the disclosure to a subject, *e.g.*, a subject that would benefit from inhibition of SOD1 expression, in a therapeutically effective

amount of a RNAi agent targeting a SOD1 gene or a pharmaceutical composition comprising a RNAi agent targeting a SOD1 gene.

In addition, the present disclosure provides methods of preventing, treating or inhibiting the progression of a SOD1-associated neurodegenerative disease or disorder, such as Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

The methods include administering to the subject a therapeutically effective amount of any of the RNAi agent, *e.g.,* dsRNA agents, or the pharmaceutical composition provided herein, thereby preventing, treating, or inhibiting the progression of the SOD1-associated neurodegenerative disease or disorder in the subject.

An RNAi agent of the disclosure may be administered as a "free RNAi agent." A free RNAi agent is administered in the absence of a pharmaceutical composition. The naked RNAi agent may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the RNAi agent can be adjusted such that it is suitable for administering to a subject.

Alternatively, an RNAi agent of the disclosure may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

Subjects that would benefit from a reduction or inhibition of SOD1 gene expression are those having a SOD1-associated neurodegenerative disease.

The disclosure further provides methods for the use of a RNAi agent or a pharmaceutical composition thereof, *e.g.*, for treating a subject that would benefit from reduction or inhibition of SOD1 expression, *e.g.,* a subject having a SOD1-associated neurodegenerative disorder, in combination with other pharmaceuticals or other therapeutic methods, *e.g.*, with known pharmaceuticals or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders. For example, in certain embodiments, an RNAi agent targeting SOD1 is administered in combination with, *e.g.*, an agent useful in treating a SOD1-associated neurodegenerative disorder as described elsewhere herein or as otherwise known in the art. For example, additional agents and treatments suitable for treating a subject that would benefit from reducton in SOD1 expression, *e.g.,* a subject having a SOD1-associated neurodegenerative disorder, may include agents currently used to treat symptoms of SOD1. The RNAi agent and additional therapeutic agents may be administered at the same time or in the same combination, *e.g.*, intrathecally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times or by another method known in the art or described herein. The RNAi agent and additional therapeutic agents may be administered at the same time or in the same combination or the additional therapeutic agent can be administered as part of a separate composition or at separate times or by another method known in the art or described herein.

Exemplary additional therapeutics and treatments include, for example, sedatives, antidepressants, clonazepam, sodium valproate, opiates, antiepileptic drugs, cholinesterase inhibitors, memantine, benzodiazepines, levodopa, COMT inhibitors (*e.g.,* tolcapone and entacapone), dopamine

agonists (*e.g.,* bromocriptine, pergolide, pramipexole, ropinirole, piribedil, cabergoline, apomorphine and lisuride), MAO-B inhibitors (*e.g.,* safinamide, selegiline and rasagiline), amantadine, an anticholinergic, modafinil, pimavanserin, doxepin, rasagline, an antipsychotic, an atypical antipsychotic (*e.g.,* amisulpride, olanzapine, risperidone, and clozapine), riluzole, edaravone , deep brain stimulation, non-invasive ventilation (NIV), invasive ventilation physical therapy, occupational therapy, speech therapy, dietary changes and swallowing technique a feeding tube, a PEG tube, probiotics, and psychological therapy.

In one embodiment, the method includes administering a composition featured herein such that expression of the target SOD1 gene is decreased, for at least one month. In preferred embodiments, expression is decreased for at least 2 months, or 6 months.

Preferably, the RNAi agents useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target SOD1 gene. Compositions and methods for inhibiting the expression of these genes using RNAi agents can be prepared and performed as described herein.

Administration of the dsRNA according to the methods of the disclosure may result in a reduction of the severity, signs, symptoms, or markers of such diseases or disorders in a patient with a SOD1-associated neurodegenerative disorder. By “reduction” in this context is meant a statistically significant or clinically significant decrease in such level. The reduction can be, for example, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of a SOD1-associated neurodegenerative disorder may be assessed, for example, by periodic monitoring of a subject’s cognition, learning, or memory. Comparisons of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of a RNAi agent targeting SOD1 or pharmaceutical composition thereof, "effective against" a SOD1-associated neurodegenerative disorder indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as an improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating SOD1-associated neurodegenerative disorders and the related causes.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50%, or more can be indicative of effective treatment. Efficacy for a given RNAi agent drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Alternatively, the efficacy can be measured by a reduction in the severity of disease as determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading scale. Any positive change resulting in *e.g.*, lessening of severity of disease measured using the appropriate scale, represents adequate treatment using a RNAi agent or RNAi agent formulation as described herein.

Subjects can be administered a therapeutic amount of dsRNA, such as about 0.01 mg/kg to about 200 mg/kg.

The RNAi agent can be administered intrathecally, intraventricularly, or by intravenous infusion over a period of time, on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. Administration of the RNAi agent can reduce SOD1 levels, *e.g.*, in a cell, tissue, blood, CSF sample or other compartment of the patient by at least 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70,% 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or at least about 99% or more. In a preferred embodiment, administration of the RNAi agent can reduce SOD1 levels, *e.g.*, in a cell, tissue, blood, CSF sample or other compartment of the patient by at least 50%.

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

Alternatively, the RNAi agent can be administered subcutaneously, *i.e.*, by subcutaneous injection.

One or more injections may be used to deliver the desired, *e.g.*, monthly dose of RNAi agent to a subject. The injections may be repeated over a period of time. The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimine may include administration of a therapeutic amount of RNAi agent on a regular basis, such as monthly or extending to once a quarter, twice per year, once per year. In certain embodiments, the RNAi agent is administered about once per month to about once per quarter (*i.e.*, about once every three months).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the RNAi agents and methods featured in the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

An informal Sequence Listing is filed herewith and forms part of the specification as filed.

EXAMPLES

Example 1. RNAi Agent Design, Synthesis, Selection, and *In Vitro* Evaluation

This Example describes methods for the design, synthesis, selection, and *in vitro* evaluation of SOD1 RNAi agents.

Source of reagents

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

Bioinformatics

siRNAs targeting the human superoxide dismutase 1 (SOD1) gene (human: NCBI refseqID NM_000454.4; NCBI GeneID: 6647), mouse SOD1 gene (refseqID NM_011434.1; NCBI GeneID: 20655), or *Macaca fascicularis* SOD1 gene (refseqID NM_001285406.1; NCBI GeneID: 102118687) were designed using custom R and Python scripts. The human NM_000454.4 REFSEQ mRNA has a length of 981 bases; the mouse NM_011434.1 REFSEQ mRNA has a length of 661 bases; and the *Macaca fascicularis* SOD1 NM_001285406.1 REFSEQ mRNA has a length of 465 bases.

Detailed lists of the unmodified SOD1 sense and antisense strand nucleotide sequences are shown in Tables 2, 4 and 6. Detailed lists of the modified SOD1 sense and antisense strand nucleotide sequences are shown in Tables 3, 5 and 7.

It is to be understood that, throughout the application, a duplex name without a decimal is equivalent to a duplex name with a decimal which merely references the batch number of the duplex. For example, AD-266859 is equivalent to AD-266859.1.

siRNA Synthesis

Briefly, siRNA sequences were synthesized on a 1 µmol scale using a Mermade 192 synthesizer (BioAutomation) with phosphoramidite chemistry on solid supports. The solid support was controlled pore glass (500-1000 Å) loaded with a custom GalNAc ligand (3'-GalNAc conjugates), universal solid

support (AM Chemicals), or the first nucleotide of interest. Ancillary synthesis reagents and standard 2-cyanoethyl phosphoramidite monomers (2'-deoxy-2'-fluoro, 2'-O-methyl, RNA, DNA) were obtained from Thermo-Fisher (Milwaukee, WI), Hongene (China), or Chemgenes (Wilmington, MA, USA). Additional phosphoramidite monomers were procured from commercial suppliers, prepared in-house, or procured using custom synthesis from various CMOs. Phosphoramidites were prepared at a concentration of 100 mM in either acetonitrile or 9:1 acetonitrile:DMF and were coupled using 5-Ethylthio-1H-tetrazole (ETT, 0.25 M in acetonitrile) with a reaction time of 400 s. Phosphorothioate linkages were generated using a 100 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (9:1 v/v). Oxidation time was 5 minutes. All sequences were synthesized with final removal of the DMT group ("DMT-Off").

Upon completion of the solid phase synthesis, solid-supported oligoribonucleotides were treated with 300 µL of Methylamine (40% aqueous) at room temperature in 96 well plates for approximately 2 hours to afford cleavage from the solid support and subsequent removal of all additional base-labile protecting groups. For sequences containing any natural ribonucleotide linkages (2'-OH) protected with a tert-butyl dimethyl silyl (TBDMS) group, a second deprotection step was performed using TEA.3HF (triethylamine trihydrofluoride). To each oligonucleotide solution in aqueous methylamine was added 200 µL of dimethyl sulfoxide (DMSO) and 300 µL TEA.3HF and the solution was incubated for approximately 30 mins at 60 °C. After incubation, the plate was allowed to come to room temperature and crude oligonucleotides were precipitated by the addition of 1 mL of 9:1 acetontrile:ethanol or 1:1 ethanol:isopropanol. The plates were then centrifuged at 4 °C for 45 mins and the supernatant carefully decanted with the aid of a multichannel pipette. The oligonucleotide pellet was resuspended in 20 mM NaOAc and subsequently desalted using a HiTrap size exclusion column (5 mL, GE Healthcare) on an Agilent LC system equipped with an autosampler, UV detector, conductivity meter, and fraction collector. Desalted samples were collected in 96 well plates and then analyzed by LC-MS and UV spectrometry to confirm identity and quantify the amount of material, respectively.

Duplexing of single strands was performed on a Tecan liquid handling robot. Sense and antisense single strands were combined in an equimolar ratio to a final concentration of 10 µM in 1x PBS in 96 well plates, the plate sealed, incubated at 100 °C for 10 minutes, and subsequently allowed to return slowly to room temperature over a period of 2-3 hours. The concentration and identity of each duplex was confirmed and then subsequently utilized for in vitro screening assays.

Cell culture and transfections

Cells were transfected by adding 4.9 µL of Opti-MEM plus 0.1 µL of RNAiMAX per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 µL of siRNA duplexes per well, with 4 replicates of each siRNA duplex, into a 384-well plate, and incubated at room temperature for 15 minutes. Forty µL of MEDIA containing ~5 x10^3 cells were then added to the siRNA mixture. Cells were incubated for 24

hours prior to RNA purification. Experiments were performed at 10nM and 0.1nM in primary mouse hepatocytes (PMH) or primary cynomolgus hepatocytes (PCH).

Total RNA isolation using DYNABEADS mRNA Isolation Kit

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

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

Ten µL of a master mix containing 1 µL 10X Buffer, 0.4 µL 25X dNTPs, 1 µL 10x Random primers, 0.5 µL Reverse Transcriptase, 0.5 µL RNase inhibitor and 6.6 µL of H_2O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 hour incubation at 37°C.

Real time PCR

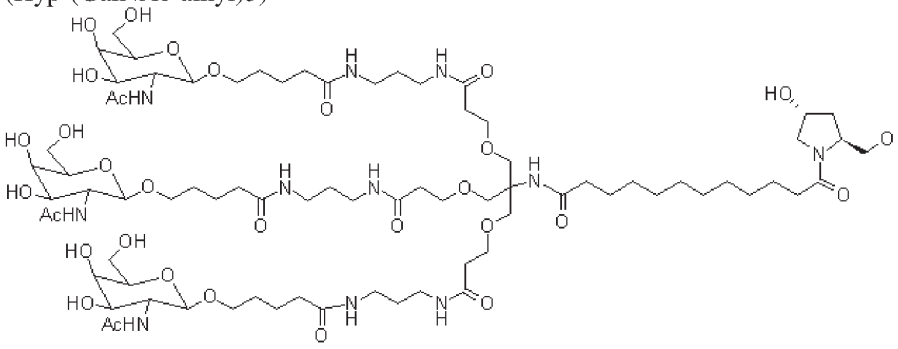
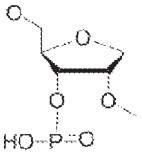
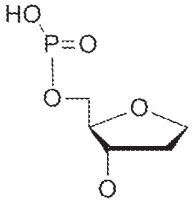
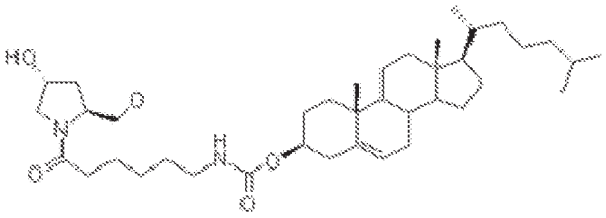
Two µL of cDNA were added to a master mix containing 0.5 µL of human or mouse GAPDH TaqMan Probe (ThermoFisher cat 4352934E or 4351309) and 0.5 µL of appropriate SOD1 probe (commercially available, *e.g.*, from Thermo Fisher) and 5 µL Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche). Each duplex was tested with N=4 and data were normalized to cells transfected with a non-targeting control siRNA. To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with a non-targeting control siRNA.

The results of single dose screens in primary Cynomolgus hepatocytes (PCH) of the duplexes in Tables 2 and 3 are provided in Table 8; the results of the single dose screens in primary mouse hepatocytes (PMH) of the duplexes in Tables 2 and 3 are provided in Table 9; the results of the single dose screens in primary Cynomolgus hepatocytes (PCH) of the duplexes in Tables 4 and 5 are provided in Table 10; and the results of the single dose screens in primary Cynomolgus hepatocytes (PCH) of the duplexes in Tables 6 and 7 are provided in Table 11.

Table 1. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds; and it is understood that when the nucleotide contains a 2'-fluoro modification,

then the fluoro replaces the hydroxy at that position in the parent nucleotide (i.e., it is a 2'-deoxy-2'-fluoronucleotide).

Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3`-phosphate
Abs	beta-L-adenosine-3`-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3`-phosphate
Cbs	beta-L-cytidine-3'-phosphorothioate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gb	beta-L-guanosine-3`-phosphate
Gbs	beta-L-guanosine-3`-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
Us	uridine -3'-phosphorothioate
N	any nucleotide, modified or unmodified
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'- phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'- phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'- phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage

Abbreviation	Nucleotide(s)
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol (Hyp-(GalNAc-alkyl)3) 
Y34	2-hydroxymethyl-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose) 
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofurane-5-phosphate) 
L10	N-(cholesterylcarboxamidocaproyl)-4-hydroxyprolinol (Hyp-C6-Chol) 
(Agn)	Adenosine-glycol nucleic acid (GNA) S-Isomer
(Cgn)	Cytidine-glycol nucleic acid (GNA) S-Isomer
(Ggn)	Guanosine-glycol nucleic acid (GNA) S-Isomer
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
P	Phosphate
VP	Vinyl-phosphonate
dA	2`-deoxyadenosine-3`-phosphate
dAs	2`-deoxyadenosine-3`-phosphorothioate
dC	2`-deoxycytidine-3`-phosphate
dCs	2`-deoxycytidine-3`-phosphorothioate
dG	2`-deoxyguanosine-3`-phosphate
dGs	2`-deoxyguanosine-3`-phosphorothioate
dT	2`-deoxythymidine-3`-phosphate
dTs	2`-deoxythymidine-3`-phosphorothioate
dU	2`-deoxyuridine
dUs	2`-deoxyuridine-3`-phosphorothioate
(C2p)	cytidine-2`-phosphate

Abbreviation	Nucleotide(s)
(G2p)	guanosine-2`-phosphate
(U2p)	uridine-2`-phosphate
(A2p)	adenosine-2`-phosphate
(Ahd)	2'-O-hexadecyl-adenosine-3'-phosphate
(Ahds)	2'-O-hexadecyl-adenosine-3'-phosphorothioate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Chds)	2'-O-hexadecyl-cytidine-3'-phosphorothioate
(Ghd)	2'-O-hexadecyl-guanosine-3'-phosphate
(Ghds)	2'-O-hexadecyl-guanosine-3'-phosphorothioate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate
(Uhds)	2'-O-hexadecyl-uridine-3'-phosphorothioate
s	phosphorothioate

Table 2. Unmodified Sense and Antisense Strand Sequences of Superoxide Dismutase 1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in Source, NM_001285406.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in Source, NM_001285406.1
AD-266859.1	AUCAGUUUGGAGAUAAUACAU	35	NM_001285406.1_146-166_C21U_s	146-166	AUGUAUUAUCUCCAAACUGAUGA	118	NM_001285406.1_144-166_G1A_as	144-166
AD-266997.1	UCUUUCGAAGAUUCUGUGAUU	36	NM_001285406.1_295-315_C21U_s	295-315	AAUCACAGAAUCUUCGAAAGACA	119	NM_001285406.1_293-315_G1A_as	293-315
AD-266992.1	AGGUGUCUUUCGAAGAUUCUU	37	NM_001285406.1_290-310_G21U_s	290-310	AAGAAUCUUCGAAAGACACCUUG	120	NM_001285406.1_288-310_C1A_as	288-310
AD-266903.1	CUUUAAUCCUCUAUCCAGACA	38	NM_001285406.1_192-212_s	192-212	UGUCUGGAUAGAGGAUUAAAGUG	121	NM_001285406.1_190-212_as	190-212
AD-266891.1	UGCAGGUCCUCACUUUAAUCU	39	NM_001285406.1_180-200_C21U_s	180-200	AGAUUAAAGUGAGGACCUGCACU	122	NM_001285406.1_178-200_G1A_as	178-200
AD-266996.1	GUCUUUCGAAGAUUCUGUGAU	40	NM_001285406.1_294-314_s	294-314	AUCACAGAAUCUUCGAAAGACAC	123	NM_001285406.1_292-314_as	292-314
AD-266893.1	CAGGUCCUCACUUUAAUCCUU	41	NM_001285406.1_182-202_C21U_s	182-202	AAGGAUUAAAGUGAGGACCUGCA	124	NM_001285406.1_180-202_G1A_as	180-202
AD-266898.1	CCUCACUUUAAUCCUCUAUCU	42	NM_001285406.1_187-207_C21U_s	187-207	AGAUAGAGGAUUAAAGUGAGGAC	125	NM_001285406.1_185-207_G1A_as	185-207
AD-266886.1	ACCAGUGCAGGUCCUCACUUU	43	NM_001285406.1_175-195_s	175-195	AAAGUGAGGACCUGCACUGGUAC	126	NM_001285406.1_173-195_as	173-195
AD-267072.1	AGCAGAUGACUUGGGCAAAGU	44	NM_001285406.1_369-389_G21U_s	369-389	ACUUUGCCCAAGUCAUCUGCUUU	127	NM_001285406.1_367-389_C1A_as	367-389
AD-267067.1	GAAAAAGCAGAUGACUUGGGU	45	NM_001285406.1_364-384_C21U_s	364-384	ACCCAAGUCAUCUGCUUUUUCAU	128	NM_001285406.1_362-384_G1A_as	362-384
AD-266791.1	UUCGAGCAGAAGGAAAGUAAU	46	NM_001285406.1_61-81_s	61-81	AUUACUUUCCUUCUGCUCGAAAU	129	NM_001285406.1_59-81_as	59-81
AD-266789.1	AUUUCGAGCAGAAGGAAAGUA	47	NM_001285406.1_59-79_s	59-79	UACUUUCCUUCUGCUCGAAAUUG	130	NM_001285406.1_57-79_as	57-79
AD-266861.1	CAGUUUGGAGAUAAUACACAA	48	NM_001285406.1_148-168_s	148-168	UUGUGUAUUAUCUCCAAACUGAU	131	NM_001285406.1_146-168_as	146-168
AD-266856.1	UUCAUCAGUUUGGAGAUAAUA	49	NM_001285406.1_143-163_s	143-163	UAUUAUCUCCAAACUGAUGAACA	132	NM_001285406.1_141-163_as	141-163
AD-266899.1	CUCACUUUAAUCCUCUAUCCA	50	NM_001285406.1_188-208_s	188-208	UGGAUAGAGGAUUAAAGUGAGGA	133	NM_001285406.1_186-208_as	186-208
AD-267000.1	UUCGAAGAUUCUGUGAUCUCU	51	NM_001285406.1_298-318_G21U_s	298-318	AGAGAUCACAGAAUCUUCGAAAG	134	NM_001285406.1_296-318_C1A_as	296-318
AD-267071.1	AAGCAGAUGACUUGGGCAAAU	52	NM_001285406.1_368-388_G21U_s	368-388	AUUUGCCCAAGUCAUCUGCUUUU	135	NM_001285406.1_366-388_C1A_as	366-388

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in Source, NM_001285406.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in Source, NM_001285406.1
AD-266895.1	GGUCCUCACUUUAAUCCUCUA	53	NM_001285406.1_184-204_s	184-204	UAGAGGAUUAAAGUGAGGACCUG	136	NM_001285406.1_182-204_as	182-204
AD-266888.1	CAGUGCAGGUCCUCACUUUAA	54	NM_001285406.1_177-197_s	177-197	UUAAAGUGAGGACCUGCACUGGU	137	NM_001285406.1_175-197_as	175-197
AD-266817.1	CAUUACAGGAUUGACUGAAGU	55	NM_001285406.1_105-125_G21U_s	105-125	ACUUCAGUCAAUCCUGUAAUGCU	138	NM_001285406.1_103-125_C1A_as	103-125
AD-267083.1	AAAGUAAAAAAGACAGGAAACU	56	NM_001285406.1_401-421_G21U_s	401-421	AGUUUCCUGUCUUUUUACUUUCU	139	NM_001285406.1_399-421_C1A_as	399-421
AD-266862.1	AGUUUGGAGAUAAAUACACAAU	57	NM_001285406.1_149-169_G21U_s	149-169	AUUGUGUAUUAUCUCCAAACUGA	140	NM_001285406.1_147-169_C1A_as	147-169
AD-267002.1	CGAAGAUUCUGUGAUCUCGCU	58	NM_001285406.1_300-320_s	300-320	AGCGAGAUCACAGAAUCUUCGAA	141	NM_001285406.1_298-320_as	298-320
AD-266816.1	GCAUUACAGGAUUGACUGAAU	59	NM_001285406.1_104-124_G21U_s	104-124	AUUCAGUCAAUCCUGUAAUGCUU	142	NM_001285406.1_102-124_C1A_as	102-124
AD-266857.1	UCAUCAGUUUGGAGAUAAUAU	60	NM_001285406.1_144-164_C21U_s	144-164	AUAUUAUCUCCAAACUGAUGAAC	143	NM_001285406.1_142-164_G1A_as	142-164
AD-266902.1	ACUUUAAUCCUCUAUCCAGAU	61	NM_001285406.1_191-211_C21U_s	191-211	AUCUGGAUAGAGGAUUAAAGUGA	144	NM_001285406.1_189-211_G1A_as	189-211
AD-267086.1	GUAAAAAGACAGGAAACGCUU	62	NM_001285406.1_404-424_G21U_s	404-424	AAGCGUUUCCUGUCUUUUUACUU	145	NM_001285406.1_402-424_C1A_as	402-424
AD-266785.1	AUCAAUUUCGAGCAGAAGGAA	63	NM_001285406.1_55-75_s	55-75	UUCCUUCUGCUCGAAAUUGAUGG	146	NM_001285406.1_53-75_as	53-75
AD-266897.1	UCCUCACUUUAAUCCUCUAUU	64	NM_001285406.1_186-206_C21U_s	186-206	AAUAGAGGAUUAAAGUGAGGACC	147	NM_001285406.1_184-206_G1A_as	184-206
AD-266896.1	GUCCUCACUUUAAUCCUCUAU	65	NM_001285406.1_185-205_s	185-205	AUAGAGGAUUAAAGUGAGGACCU	148	NM_001285406.1_183-205_as	183-205
AD-266858.1	CAUCAGUUUGGAGAUAAUACA	66	NM_001285406.1_145-165_s	145-165	UGUAUUAUCUCCAAACUGAUGAA	149	NM_001285406.1_143-165_as	143-165
AD-267084.1	AAGUAAAAAAGACAGGAAACGU	67	NM_001285406.1_402-422_C21U_s	402-422	ACGUUUCCUGUCUUUUUACUUUC	150	NM_001285406.1_400-422_G1A_as	400-422
AD-266815.1	AGCAUUACAGGAUUGACUGAA	68	NM_001285406.1_103-123_s	103-123	UUCAGUCAAUCCUGUAAUGCUUC	151	NM_001285406.1_101-123_as	101-123
AD-267007.1	AUUCUGUGAUCUCGCUCUCAU	69	NM_001285406.1_305-325_G21U_s	305-325	AUGAGAGCGAGAUCACAGAAUCU	152	NM_001285406.1_303-325_C1A_as	303-325
AD-266855.1	GUUCAUCAGUUUGGAGAUAAU	70	NM_001285406.1_142-162_s	142-162	AUUAUCUCCAAACUGAUGAACAU	153	NM_001285406.1_140-162_as	140-162
AD-266901.1	CACUUUAAUCCUCUAUCCAGA	71	NM_001285406.1_190-210_s	190-210	UCUGGAUAGAGGAUUAAAGUGAG	154	NM_001285406.1_188-210_as	188-210

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in Source, NM_001285406.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in Source, NM_001285406.1
AD-266994.1	GUGUCUUUCGAAGAUUCUGUU	72	NM_001285406.1_292-312_G21U_s	292-312	AACAGAAUCUUCGAAAGACACCU	155	NM_001285406.1_290-312_C1A_as	290-312
AD-266793.1	CGAGCAGAAGGAAAGUAAUGU	73	NM_001285406.1_63-83_G21U_s	63-83	ACAUUACUUUCCUUCUGCUCGAA	156	NM_001285406.1_61-83_C1A_as	61-83
AD-266850.1	UCCAUGUUCAUCAGUUUGGAU	74	NM_001285406.1_137-157_G21U_s	137-157	AUCCAAACUGAUGAACAUGGAAU	157	NM_001285406.1_135-157_C1A_as	135-157
AD-266887.1	CCAGUGCAGGUCCUCACUUUA	75	NM_001285406.1_176-196_s	176-196	UAAAGUGAGGACCUGCACUGGUA	158	NM_001285406.1_174-196_as	174-196
AD-266894.1	AGGUCCUCACUUUAAUCCUCU	76	NM_001285406.1_183-203_s	183-203	AGAGGAUUAAAGUGAGGACCUGC	159	NM_001285406.1_181-203_as	181-203
AD-266988.1	GCCAAGGUGUCUUUCGAAGAU	77	NM_001285406.1_286-306_s	286-306	AUCUUCGAAAGACACCUUGGCCA	160	NM_001285406.1_284-306_as	284-306
AD-267085.1	AGUAAAAAGACAGGAAACGCU	78	NM_001285406.1_403-423_s	403-423	AGCGUUUCCUGUCUUUUUACUUU	161	NM_001285406.1_401-423_as	401-423
AD-266873.1	AUACACAAGGCUGUACCAGUU	79	NM_001285406.1_161-181_G21U_s	161-181	AACUGGUACAGCCUUGUGUAUUA	162	NM_001285406.1_159-181_C1A_as	159-181
AD-266907.1	AAUCCUCUAUCCAGACAACAU	80	NM_001285406.1_196-216_C21U_s	196-216	AUGUUGUCUGGAUAGAGGAUUAA	163	NM_001285406.1_194-216_G1A_as	194-216
AD-266792.1	UCGAGCAGAAGGAAAGUAAUU	81	NM_001285406.1_62-82_G21U_s	62-82	AAUUACUUUCCUUCUGCUCGAAA	164	NM_001285406.1_60-82_C1A_as	60-82
AD-266900.1	UCACUUUAAUCCUCUAUCCAU	82	NM_001285406.1_189-209_G21U_s	189-209	AUGGAUAGAGGAUUAAAGUGAGG	165	NM_001285406.1_187-209_C1A_as	187-209
AD-266797.1	CAGAAGGAAAGUAAUGGACCA	83	NM_001285406.1_67-87_s	67-87	UGGUCCAUUACUUUCCUUCUGCU	166	NM_001285406.1_65-87_as	65-87
AD-266787.1	CAAUUUCGAGCAGAAGGAAAU	84	NM_001285406.1_57-77_G21U_s	57-77	AUUUCCUUCUGCUCGAAAUUGAU	167	NM_001285406.1_55-77_C1A_as	55-77
AD-266800.1	AAGGAAAGUAAUGGACCAGUU	85	NM_001285406.1_70-90_G21U_s	70-90	AACUGGUCCAUUACUUUCCUUCU	168	NM_001285406.1_68-90_C1A_as	68-90
AD-266889.1	AGUGCAGGUCCUCACUUUAAU	86	NM_001285406.1_178-198_s	178-198	AUUAAAGUGAGGACCUGCACUGG	169	NM_001285406.1_176-198_as	176-198
AD-266847.1	GAUUCCAUGUUCAUCAGUUUG	87	NM_001285406.1_134-154_s	134-154	CAAACUGAUGAACAUGGAAUCCA	170	NM_001285406.1_132-154_as	132-154
AD-266998.1	CUUUCGAAGAUUCUGUGAUCU	88	NM_001285406.1_296-316_s	296-316	AGAUCACAGAAUCUUCGAAAGAC	171	NM_001285406.1_294-316_as	294-316
AD-266790.1	UUUCGAGCAGAAGGAAAGUAA	89	NM_001285406.1_60-80_s	60-80	UUACUUUCCUUCUGCUCGAAAUU	172	NM_001285406.1_58-80_as	58-80
AD-266906.1	UAAUCCUCUAUCCAGACAACA	90	NM_001285406.1_195-215_s	195-215	UGUUGUCUGGAUAGAGGAUUAAA	173	NM_001285406.1_193-215_as	193-215

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in Source, NM_001285406.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in Source, NM_001285406.1
AD-266854.1	UGUUCAUCAGUUUGGAGAUAA	91	NM_001285406.1_141-161_s	141-161	UUAUCUCCAAACUGAUGAACAUG	174	NM_001285406.1_139-161_as	139-161
AD-266890.1	GUGCAGGUCCUCACUUUAAUU	92	NM_001285406.1_179-199_C21U_s	179-199	AAUUAAAGUGAGGACCUGCACUG	175	NM_001285406.1_177-199_G1A_as	177-199
AD-266808.1	UAAUGGACCAGUGAAGGUGUU	93	NM_001285406.1_78-98_G21U_s	78-98	AACACCUUCACUGGUCCAUUACU	176	NM_001285406.1_76-98_C1A_as	76-98
AD-266905.1	UUAAUCCUCUAUCCAGACAAU	94	NM_001285406.1_194-214_C21U_s	194-214	AUUGUCUGGAUAGAGGAUUAAAG	177	NM_001285406.1_192-214_G1A_as	192-214
AD-267024.1	UCAGGAGACCAUUCCAUCAUU	95	NM_001285406.1_322-342_s	322-342	AAUGAUGGAAUGGUCUCCUGAGA	178	NM_001285406.1_320-342_as	320-342
AD-266781.1	CACCAUCAAUUUCGAGCAGAA	96	NM_001285406.1_51-71_s	51-71	UUCUGCUCGAAAUUGAUGGUGCC	179	NM_001285406.1_49-71_as	49-71
AD-266892.1	GCAGGUCCUCACUUUAAUCCU	97	NM_001285406.1_181-201_s	181-201	AGGAUUAAAGUGAGGACCUGCAC	180	NM_001285406.1_179-201_as	179-201
AD-266999.1	UUUCGAAGAUUCUGUGAUCUU	98	NM_001285406.1_297-317_C21U_s	297-317	AAGAUCACAGAAUCUUCGAAAGA	181	NM_001285406.1_295-317_G1A_as	295-317
AD-266841.1	UGCAUGGAUUCCAUGUUCAUU	99	NM_001285406.1_128-148_C21U_s	128-148	AAUGAACAUGGAAUCCAUGCAGG	182	NM_001285406.1_126-148_G1A_as	126-148
AD-266908.1	AUCCUCUAUCCAGACAACACU	100	NM_001285406.1_197-217_G21U_s	197-217	AGUGUUGUCUGGAUAGAGGAUUA	183	NM_001285406.1_195-217_C1A_as	195-217
AD-267005.1	AGAUUCUGUGAUCUCGCUCUU	101	NM_001285406.1_303-323_C21U_s	303-323	AAGAGCGAGAUCACAGAAUCUUC	184	NM_001285406.1_301-323_G1A_as	301-323
AD-266942.1	GAAGAGAGGCAUGUUGGAGAU	102	NM_001285406.1_232-252_C21U_s	232-252	AUCUCCAACAUGCCUCUCUUCAU	185	NM_001285406.1_230-252_G1A_as	230-252
AD-135967.3	UUGGGCAAAGGUGGAAAUGAA	103	NM_011434.1_495-515_s	495-515	UUCAUUUCCACCUUUGCCCAAGU	186	NM_011434.1_493-515_as	493-515
AD-266786.1	UCAAUUUCGAGCAGAAGGAAA	104	NM_001285406.1_56-76_s	56-76	UUUCCUUCUGCUCGAAAUUGAUG	187	NM_001285406.1_54-76_as	54-76
AD-267064.1	CAUGAAAAAGCAGAUGACUUU	105	NM_001285406.1_361-381_G21U_s	361-381	AAAGUCAUCUGCUUUUUCAUGGA	188	NM_001285406.1_359-381_C1A_as	359-381
AD-266845.1	UGGAUUCCAUGUUCAUCAGUU	106	NM_001285406.1_132-152_s	132-152	AACUGAUGAACAUGGAAUCCAUG	189	NM_001285406.1_130-152_as	130-152
AD-266944.1	GAGAGGCAUGUUGGAGACCUU	107	NM_001285406.1_235-255_G21U_s	235-255	AAGGUCUCCAACAUGCCUCUCUU	190	NM_001285406.1_233-255_C1A_as	233-255
AD-267003.1	GAAGAUUCUGUGAUCUCGCUU	108	NM_001285406.1_301-321_C21U_s	301-321	AAGCGAGAUCACAGAAUCUUCGA	191	NM_001285406.1_299-321_G1A_as	299-321
AD-266860.1	UCAGUUUGGAGAUAAUACACA	109	NM_001285406.1_147-167_s	147-167	UGUGUAUUAUCUCCAAACUGAUG	192	NM_001285406.1_145-167_as	145-167

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in Source, NM_001285406.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in Source, NM_001285406.1
AD-266990.1	CAAGGUGUCUUUCGAAGAUUU	110	NM_001285406.1_288-308_C21U_s	288-308	AAAUCUUCGAAAGACACCUUGGC	193	NM_001285406.1_286-308_G1A_as	286-308
AD-266853.1	AUGUUCAUCAGUUUGGAGAUA	111	NM_001285406.1_140-160_s	140-160	UAUCUCCAAACUGAUGAACAUGG	194	NM_001285406.1_138-160_as	138-160
AD-266782.1	ACCAUCAAUUUCGAGCAGAAU	112	NM_001285406.1_52-72_G21U_s	52-72	AUUCUGCUCGAAAUUGAUGGUGC	195	NM_001285406.1_50-72_C1A_as	50-72
AD-266962.1	AUGUGACUGCUGGCAAAGAUU	113	NM_001285406.1_260-280_G21U_s	260-280	AAUCUUUGCCAGCAGUCACAUUG	196	NM_001285406.1_258-280_C1A_as	258-280
AD-267079.1	GGUGGAAAUGAAGAAAGUAAA	114	NM_001285406.1_388-408_s	388-408	UUUACUUUCUUCAUUUCCACCUU	197	NM_001285406.1_386-408_as	386-408
AD-266846.1	GGAUUCCAUGUUCAUCAGUUU	115	NM_001285406.1_133-153_s	133-153	AAACUGAUGAACAUGGAAUCCAU	198	NM_001285406.1_131-153_as	131-153
AD-266961.1	AAUGUGACUGCUGGCAAAGAU	116	NM_001285406.1_259-279_s	259-279	AUCUUUGCCAGCAGUCACAUUGC	199	NM_001285406.1_257-279_as	257-279
AD-267061.1	GUCCAUGAAAAAGCAGAUGAU	117	NM_001285406.1_358-378_C21U_s	358-378	AUCAUCUGCUUUUUCAUGGACCA	200	NM_001285406.1_356-378_G1A_as	356-378

Table 3. Modified Sense and Antisense Strand Sequences of Superoxide Dismutase 1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-266859.1	asuscaguUfuGfGfAfgauaauacauL96	201	asUfsguaUfuAfUfcuccAfaAfcugausgsa	284	UCAUCAGUUUGGAGAUAAUACAC	367
AD-266997.1	uscsuuucGfaAfGfAfuucugugauuL96	202	asAfsucaCfaGfAfaucuUfcGfaaagascsa	285	UGUCUUUCGAAGAUUCUGUGAUC	368
AD-266992.1	asgsguguCfuUfUfCfgaagauucuuL96	203	asAfsgaaUfcUfUfcgaaAfgAfcaccususg	286	CAAGGUGUCUUUCGAAGAUUCUG	369
AD-266903.1	csusuuaaUfcCfUfCfuauccagacaL96	204	usGfsucuGfgAfUfagagGfaUfuaaagsusg	287	CACUUUAAUCCUCUAUCCAGACA	370
AD-266891.1	usgscaggUfcCfUfCfacuuuaaucuL96	205	asGfsauuAfaAfGfugagGfaCfcugcascsu	288	AGUGCAGGUCCUCACUUUAAUCC	371
AD-266996.1	gsuscuuuCfgAfAfGfauucugugauL96	206	asUfscacAfgAfAfucuuCfgAfaagacsasc	289	GUGUCUUUCGAAGAUUCUGUGAU	372
AD-266893.1	csasggucCfuCfAfCfuuuaauccuuL96	207	asAfsggaUfuAfAfagugAfgGfaccugscsa	290	UGCAGGUCCUCACUUUAAUCCUC	373
AD-266898.1	cscsucacUfuUfAfAfuccucuaucuL96	208	asGfsauaGfaGfGfauuaAfaGfugaggsasc	291	GUCCUCACUUUAAUCCUCUAUCC	374
AD-266886.1	ascscaguGfcAfGfGfuccucacuuuL96	209	asAfsaguGfaGfGfaccuGfcAfcuggusasc	292	GUACCAGUGCAGGUCCUCACUUU	375
AD-267072.1	asgscagaUfgAfCfUfugggcaaaguL96	210	asCfsuuuGfcCfCfaaguCfaUfcugcususu	293	AAAGCAGAUGACUUGGGCAAAGG	376

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-267067.1	gsasaaaaGfcAfGfAfugacuuggguL96	211	asCfsccaAfgUfCfaucuGfcUfuuuucsasu	294	AUGAAAAAGCAGAUGACUUGGGC	377
AD-266791.1	ususcgagCfaGfAfAfggaaaguaauL96	212	asUfsuacUfuUfCfcuucUfgCfucgaasasu	295	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-266789.1	asusuucgAfgCfAfGfaaggaaaguaL96	213	usAfscuuUfcCfUfucugCfuCfgaaaususg	296	CAAUUUCGAGCAGAAGGAAAGUA	379
AD-266861.1	csasguuuGfgAfGfAfuaauacacaaL96	214	usUfsgugUfaUfUfaucuCfcAfaacugsasu	297	AUCAGUUUGGAGAUAAUACACAA	380
AD-266856.1	ususcaucAfgUfUfUfggagauaauaL96	215	usAfsuuaUfcUfCfcaaaCfuGfaugaascsa	298	UGUUCAUCAGUUUGGAGAUAAUA	381
AD-266899.1	csuscacuUfuAfAfUfccucuauccaL96	216	usGfsgauAfgAfGfgauuAfaAfgugagsgsa	299	UCCUCACUUUAAUCCUCUAUCCA	382
AD-267000.1	ususcgaaGfaUfUfCfugugaucucuL96	217	asGfsagaUfcAfCfagaaUfcUfucgaasasg	300	CUUUCGAAGAUUCUGUGAUCUCG	383
AD-267071.1	asasgcagAfuGfAfCfuugggcaaauL96	218	asUfsuugCfcCfCfAfagucAfuCfugcuususu	301	AAAAGCAGAUGACUUGGGCAAAG	384
AD-266895.1	gsgsuccuCfaCfUfUfuaauccuctaL96	219	usAfsgagGfaUfUfUfaaagUfgAfggaccsusg	302	CAGGUCCUCACUUUAAUCCUCUA	385
AD-266888.1	csasgugcAfgGfUfCfcucacuuuaaL96	220	usUfsaaaGfuGfAfggacCfuGfcacugsgsu	303	ACCAGUGCAGGUCCUCACUUUAA	386
AD-266817.1	csasuuacAfgGfAfUfugacugaaguL96	221	asCfsuucAfgUfCfaaucCfuGfuaaugscsu	304	AGCAUUACAGGAUUGACUGAAGG	387
AD-267083.1	asasaguaAfaAfAfGfacaggaaacuL96	222	asGfsuuuCfcUfGfucuuUfuUfacuuuscsu	305	AGAAAGUAAAAAAGACAGGAAACG	388
AD-266862.1	asgsuuugGfaGfAfUfaauacacaauL96	223	asUfsuguGfuAfUfuaucUfcCfaaacusgsa	306	UCAGUUUGGAGAUAAUACACAAG	389
AD-267002.1	csgsaagaUfuCfUfGfugaucucgcuL96	224	asGfscgaGfaUfCfacagAfaUfcuucgsasa	307	UUCGAAGAUUCUGUGAUCUCGCU	390
AD-266816.1	gscsauuaCfaGfGfAfuugacugaauL96	225	asUfsucaGfuCfAfauccUfgUfaaugcsusu	308	AAGCAUUACAGGAUUGACUGAAG	391
AD-266857.1	uscsaucaGfuUfUfGfgagauaauauL96	226	asUfsauuAfuCfUfccaaAfcUfgaugasasc	309	GUUCAUCAGUUUGGAGAUAAUAC	392
AD-266902.1	ascsuuuaAfuCfCfUfcuauccagauL96	227	asUfscugGfaUfAfgaggAfuUfaaagusgsa	310	UCACUUUAAUCCUCUAUCCAGAC	393
AD-267086.1	gsusaaaaAfgAfCfAfggaaacgcuuL96	228	asAfsgcgUfuUfCfcuguCfuUfuuuacsusu	311	AAGUAAAAAGACAGGAAACGCUG	394
AD-266785.1	asuscaauUfuCfGfAfgcagaaggaaL96	229	usUfscguUfcUfGfcucgAfaAfuuguasgsg	312	CCAUCAAUUUCGAGCAGAAGGAA	395
AD-266897.1	uscscucaCfuUfUfAfaucccuauuuL96	230	asAfsuagAfgGfAfuuaaAfgUfgaggascsc	313	GGUCCUCACUUUAAUCCUCUAUC	396
AD-266896.1	gsuscccucAfcUfUfUfaauccucuauL96	231	asUfsagaGfgAfUfuaaaGfuGfaggacscsu	314	AGGUCCUCACUUUAAUCCUCUAU	397
AD-266858.1	csasucagUfuUfGfGfagauaauacaL96	232	usGfsuauUfaUfCfuccaAfaCfugaugsasa	315	UUCAUCAGUUUGGAGAUAAUACA	398
AD-267084.1	asasguaaAfaAfGfAfcaggaaacguL96	233	asCfsguuUfcCfUfgucuUfuUfuacuususc	316	GAAAGUAAAAAGACAGGAAACGC	399
AD-266815.1	asgscauuAfcAfGfGfauugacugaaL96	234	usUfscagUfcAfAfuccuGfuAfaugcususc	317	GAAGCAUUACAGGAUUGACUGAA	400
AD-267007.1	asususcugUfgAfUfCfucgcucucauL96	235	asUfsgagAfgCfGfagauCfaCfagaauscsu	318	AGAUUCUGUGAUCUCGCUCUCAG	401
AD-266855.1	gsusucauCfaGfUfUfuggagauaauL96	236	asUfsuauCfuCfCfaaacUfgAfugaacsasu	319	AUGUUCAUCAGUUUGGAGAUAAU	402
AD-266901.1	csascuuuAfaUfCfCfucuauccagaL96	237	usCfsuggAfuAfGfaggaUfuAfaagugsasg	320	CUCACUUUAAUCCUCUAUCCAGA	403

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-266994.1	gsusgucuUfuCfGfAfagauucuguuL96	238	asAfscagAfaUfCfuucgAfaAfgacacscsu	321	AGGUGUCUUUCGAAGAUUCUGUG	404
AD-266793.1	csgsagcaGfaAfGfGfaaaguaauguL96	239	asCfsauuAfcUfUfuccuUfcUfgcucgsasa	322	UUCGAGCAGAAGGAAAGUAAUGG	405
AD-266850.1	uscscaugUfuCfAfUfcaguuuggauL96	240	asUfsccaAfaCfUfgaugAfaCfauggasasu	323	AUUCCAUGUUCAUCAGUUUGGAG	406
AD-266887.1	cscsagugCfaGfGfUfccucacuuuaL96	241	usAfsaagUfgAfGfgaccUfgCfacuggsusa	324	UACCAGUGCAGGUCCUCACUUUA	407
AD-266894.1	asgsguccUfcAfCfUfuuaauccucuL96	242	asGfsaggAfuUfAfaaguGfaGfgaccusgsc	325	GCAGGUCCUCACUUUAAUCCUCU	408
AD-266988.1	gscscaagGfuGfUfCfuuucgaagauL96	243	asUfscuuCfgAfAfagacAfcCfuuggcscsa	326	UGGCCAAGGUGUCUUUCGAAGAU	409
AD-267085.1	asgsuaaaAfaGfAfCfaggaaacgcuL96	244	asGfscguUfuCfCfugucUfuUfuuacususu	327	AAAGUAAAAAGACAGGAAACGCU	410
AD-266873.1	asusacacAfaGfGfCfuguaccaguuL96	245	asAfscugGfuAfCfagccUfuGfuguaususa	328	UAAUACACAAGGCUGUACCAGUG	411
AD-266907.1	asasuccuCfuAfUfCfcagacaacauL96	246	asUfsguuGfuCfUfggauAfgAfggauusasa	329	UUAAUCCUCUAUCCAGACAACAC	412
AD-266792.1	uscsgagcAfgAfAfGfgaaaguaauuL96	247	asAfsuuaCfuUfUfccuuCfuGfcucgasasa	330	UUUCGAGCAGAAGGAAAGUAAUG	413
AD-266900.1	uscsacuuUfaAfUfCfcucuauccauL96	248	asUfsggaUfaGfAfggauUfaAfagugasgsg	331	CCUCACUUUAAUCCUCUAUCCAG	414
AD-266797.1	csasgaagGfaAfAfGfuaauggaccaL96	249	usGfsgucCfaUfUfacuuUfcCfuucugscsu	332	AGCAGAAGGAAAGUAAUGGACCA	415
AD-266787.1	csasauuuCfgAfGfCfagaaggaaauL96	250	asUfsuucCfuUfCfugcuCfgAfaauugsasu	333	AUCAAUUUCGAGCAGAAGGAAAG	416
AD-266800.1	asasggaaAfgUfAfAfuggaccaguuL96	251	asAfscugGfuCfCfauuaCfuUfuccuuscsu	334	AGAAGGAAAGUAAUGGACCAGUG	417
AD-266889.1	asgsugcaGfgUfCfCfucacuuuaauL96	252	asUfsuaaAfgUfGfaggaCfcUfgcacusgsg	335	CCAGUGCAGGUCCUCACUUUAAU	418
AD-266847.1	gsasuuccAfuGfUfUfcaucaguuugL96	253	csAfsaacUfgAfUfgaacAfuGfgaaucscsa	336	UGGAUUCCAUGUUCAUCAGUUUG	419
AD-266998.1	csusuucgAfaGfAfUfucugugaucuL96	254	asGfsaucAfcAfGfaaucUfuCfgaaagsasc	337	GUCUUUCGAAGAUUCUGUGAUCU	420
AD-266790.1	ususucgaGfcAfGfAfaggaaaguaaL96	255	usUfsacuUfuCfCfuucuGfcUfcgaaasusu	338	AAUUUCGAGCAGAAGGAAAGUAA	421
AD-266906.1	usasauccUfcUfAfUfccagacaacaL96	256	usGfsuugUfcUfGfgauaGfaGfgauuasasa	339	UUUAAUCCUCUAUCCAGACAACA	422
AD-266854.1	usgsuucaUfcAfGfUfuuggagauaaL96	257	usUfsaucUfcCfAfaacuGfaUfgaacasusg	340	CAUGUUCAUCAGUUUGGAGAUAA	423
AD-266890.1	gsusgcagGfuCfCfUfcacuuuaauuL96	258	asAfsuuaAfaGfGfUfgaggAfcCfugcacsusg	341	CAGUGCAGGUCCUCACUUUAAUC	424
AD-266808.1	usasauggAfcCfAfGfugaaggugauL96	259	asAfscacCfuUfUfcfacugGfuCfcauuascsu	342	AGUAAUGGACCAGUGAAGGUGUG	425
AD-266905.1	ususaaucCfuCfUfAfuccagacaauL96	260	asUfsuguCfuGfGfauagAfgGfauuaasasg	343	CUUUAAUCCUCUAUCCAGACAAC	426
AD-267024.1	uscsaggaGfaCfCfAfuuccaucauuL96	261	asAfsugaUfgGfAfauggUfcUfccugasgsa	344	UCUCAGGAGACCAUUCCAUCAUU	427
AD-266781.1	csasccauCfaAfUfUfucgagcagaaL96	262	usUfscugCfuCfGfaaauUfgAfugugscsc	345	GGCACCAUCAAUUUCGAGCAGAA	428
AD-266892.1	gscsagguCfcUfCfAfcuuuaauccuL96	263	asGfsgauUfaAfAfgugaGfgAfccugcsasc	346	GUGCAGGUCCUCACUUUAAUCCU	429
AD-266999.1	ususucgaAfgAfUfUfcugugaucuuL96	264	asAfsgauCfaCfAfgaauCfuUfcgaaasgsa	347	UCUUUCGAAGAUUCUGUGAUCUC	430

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-266841.1	usgscaugGfaUfUfCfcauguucauuL96	265	asAfsugaAfcAfUfggaaUfcCfaugcasgsg	348	CCUGCAUGGAUUCCAUGUUCAUC	431
AD-266908.1	asusccucUfaUfCfCfagacaacacuL96	266	asGfsuguUfgUfCfuggaUfaGfaggaususa	349	UAAUCCUCUAUCCAGACAACACG	432
AD-267005.1	asgsauucUfgUfGfAfucucgcucuuL96	267	asAfsgagCfgAfGfaucaCfaGfaaucususc	350	GAAGAUUCUGUGAUCUCGCUCUC	433
AD-266942.1	gsasagagAfgGfCfAfuguuggagauL96	268	asUfscucCfaAfCfaugcCfuCfucuucsasu	351	AUGAAGAGAGGCAUGUUGGAGAC	434
AD-135967.3	ususgggcAfaAfGfGfuggaaaugaaL96	269	usUfscauUfuCfCfaccuUfuGfcccaasgsu	352	ACUUGGGCAAAGGUGGAAAUGAA	435
AD-266786.1	uscsaauuUfcGfAfGfcagaaggaaaL96	270	usUfsuccUfuCfUfgcucGfaAfauugasusg	353	CAUCAAUUUCGAGCAGAAGGAAA	436
AD-267064.1	csasugaaAfaAfGfCfagaugacuuuL96	271	asAfsaguCfaUfCfugcuUfuUfucaugsgsa	354	UCCAUGAAAAAGCAGAUGACUUG	437
AD-266845.1	usgsgauuCfcAfUfGfuucaucaguuL96	272	asAfscugAfuGfAfacauGfgAfauccasusg	355	CAUGGAUUCCAUGUUCAUCAGUU	438
AD-266944.1	gsasgaggCfaUfGfUfuggagaccuuL96	273	asAfsgguCfuCfCfaacaUfgCfcucucsusu	356	AAGAGAGGCAUGUUGGAGACCUG	439
AD-267003.1	gsasagauUfcUfGfUfgaucucgcuuL96	274	asAfsgcgAfgAfUfcacaGfaAfucuucsgsa	357	UCGAAGAUUCUGUGAUCUCGCUC	440
AD-266860.1	uscsaguuUfgGfAfGfauaauacacaL96	275	usGfsuguAfuUfAfucucCfaAfacugasusg	358	CAUCAGUUUGGAGAUAAUACACA	441
AD-266990.1	csasagguGfuCfUfUfucgaagauuuL96	276	asAfsaucUfuCfGfaaagAfcAfccuugsgsc	359	GCCAAGGUGUCUUUCGAAGAUUC	442
AD-266853.1	asusguucAfuCfAfGfuuuggagauaL96	277	usAfsucuCfcAfAfacugAfuGfaacausgsg	360	CCAUGUUCAUCAGUUUGGAGAUA	443
AD-266782.1	ascscaucAfaUfUfUfcgagcagaauL96	278	asUfsucuGfcUfCfgaaaUfuGfauggusgsc	361	GCACCAUCAAUUUCGAGCAGAAG	444
AD-266962.1	asusgugaCfuGfCfUfggcaaagauuL96	279	asAfsucuUfuGfCfcagcAfgUfcacaususg	362	CAAUGUGACUGCUGGCAAAGAUG	445
AD-267079.1	gsgsuggaAfaUfGfAfagaaaguaaaL96	280	usUfsuacUfuUfCfuucaUfuUfccaccsusu	363	AAGGUGGAAAUGAAGAAAGUAAA	446
AD-266846.1	gsgsauucCfaUfGfUfucaucaguuuL96	281	asAfsacuGfaUfGfaacaUfgGfaauccsasu	364	AUGGAUUCCAUGUUCAUCAGUUU	447
AD-266961.1	asasugugAfcUfGfCfuggcaaagauL96	282	asUfscuuUfgCfCfagcaGfuCfacauusgsc	365	GCAAUGUGACUGCUGGCAAAGAU	448
AD-267061.1	gsusccauGfaAfAfAfagcagaugauL96	283	asUfscauCfuGfCfuuuuUfcAfuggacscsa	366	UGGUCCAUGAAAAAGCAGAUGAC	449

Table 4. Unmodified Sense and Antisense Strand Sequences of Superoxide Dismutase 1 dsRNA Agents[

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
AD-	AUGACUUGGGCAAAGGUGGA	450	NM_011434.1_490-	490-510	UUCCACCUUUGCCCAAGUCAU	529	NM_011434.1_488-	488-510

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
135962.1	A		510_s		CU		510_as	
AD-135963.1	UGACUUGGGCAAAGGUGGAA A	451	NM_011434.1_491-511_s	491-511	UUUCCACCUUUGCCCAAGUCA UC	530	NM_011434.1_489-511_as	489-511
AD-135964.1	GACUUGGGCAAAGGUGGAAA U	452	NM_011434.1_492-512_s	492-512	AUUUCCACCUUUGCCCAAGUC AU	531	NM_011434.1_490-512_as	490-512
AD-135967.5	UUGGGCAAAGGUGGAAAUGA A	103	NM_011434.1_495-515_s	495-515	UUCAUUUCCACCUUUGCCCAA GU	186	NM_011434.1_493-515_as	493-515
AD-135974.3	AAGGUGGAAAUGAAGAAAGU A	453	NM_011434.1_502-522_s	502-522	UACUUUCUUCAUUUCCACCUU UG	532	NM_011434.1_500-522_as	500-522
AD-266788.1	AAUUUCGAGCAGAAGGAAAG U	454	NM_001285406.1_58-78_s	58-78	ACUUUCCUUCUGCUCGAAAUU GA	533	NM_001285406.1_56-78_as	56-78
AD-266789.2	AUUUCGAGCAGAAGGAAAGU A	47	NM_001285406.1_59-79_s	59-79	UACUUUCCUUCUGCUCGAAAU UG	130	NM_001285406.1_57-79_as	57-79
AD-266790.2	UUUCGAGCAGAAGGAAAGUA A	89	NM_001285406.1_60-80_s	60-80	UUACUUUCCUUCUGCUCGAAA UU	172	NM_001285406.1_58-80_as	58-80
AD-266791.3	UUCGAGCAGAAGGAAAGUAA U	46	NM_001285406.1_61-81_s	61-81	AUUACUUUCCUUCUGCUCGAA AU	129	NM_001285406.1_59-81_as	59-81
AD-266794.1	GAGCAGAAGGAAAGUAAUGG A	455	NM_001285406.1_64-84_s	64-84	UCCAUUACUUUCCUUCUGCUC GA	534	NM_001285406.1_62-84_as	62-84
AD-266798.1	AGAAGGAAAGUAAUGGACCA U	456	NM_001285406.1_68-88_G21U_s	68-88	AUGGUCCAUUACUUUCCUUCU GC	535	NM_001285406.1_66-88_C1A_as	66-88
AD-266799.1	GAAGGAAAGUAAUGGACCAG U	457	NM_001285406.1_69-89_s	69-89	ACUGGUCCAUUACUUUCCUUC UG	536	NM_001285406.1_67-89_as	67-89
AD-266801.1	AGGAAAGUAAUGGACCAGUG A	458	NM_001285406.1_71-91_s	71-91	UCACUGGUCCAUUACUUUCCU UC	537	NM_001285406.1_69-91_as	69-91
AD-266802.1	GGAAAGUAAUGGACCAGUGA A	459	NM_001285406.1_72-92_s	72-92	UUCACUGGUCCAUUACUUUCC UU	538	NM_001285406.1_70-92_as	70-92
AD-266803.1	GAAAGUAAUGGACCAGUGAA U	460	NM_001285406.1_73-93_G21U_s	73-93	AUUCACUGGUCCAUUACUUUC CU	539	NM_001285406.1_71-93_C1A_as	71-93
AD-266804.1	AAAGUAAUGGACCAGUGAAG U	461	NM_001285406.1_74-94_G21U_s	74-94	ACUUCACUGGUCCAUUACUUU CC	540	NM_001285406.1_72-94_C1A_as	72-94
AD-266805.1	AAGUAAUGGACCAGUGAAGG U	462	NM_001285406.1_75-95_s	75-95	ACCUUCACUGGUCCAUUACUU UC	541	NM_001285406.1_73-95_as	73-95
AD-266806.1	AGUAAUGGACCAGUGAAGGU U	463	NM_001285406.1_76-96_G21U_s	76-96	AACCUUCACUGGUCCAUUACU UU	542	NM_001285406.1_74-96_C1A_as	74-96
AD-266808.2	UAAUGGACCAGUGAAGGUGU U	93	NM_001285406.1_78-98_G21U_s	78-98	AACACCUUCACUGGUCCAUUA CU	176	NM_001285406.1_76-98_C1A_as	76-98

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
AD-266832.1	UGAAGGCCUGCAUGGAUUCCA	464	NM_001285406.1_1 20-140_s	120-140	UGGAAUCCAUGCAGGCCUUCAGU	543	NM_001285406.1_ 118-140_as	118-140
AD-266834.1	GAAGGCCUGCAUGGAUUCCAU	465	NM_001285406.1_1 21-141_s	121-141	AUGGAAUCCAUGCAGGCCUUCAG	544	NM_001285406.1_ 119-141_as	119-141
AD-266836.1	AGGCCUGCAUGGAUUCCAUGU	466	NM_001285406.1_1 23-143_s	123-143	ACAUGGAAUCCAUGCAGGCCUUC	545	NM_001285406.1_ 121-143_as	121-143
AD-266837.1	GGCCUGCAUGGAUUCCAUGUU	467	NM_001285406.1_1 24-144_s	124-144	AACAUGGAAUCCAUGCAGGCCUU	546	NM_001285406.1_ 122-144_as	122-144
AD-266838.1	GCCUGCAUGGAUUCCAUGUUU	468	NM_001285406.1_1 25-145_C21U_s	125-145	AAACAUGGAAUCCAUGCAGGCCU	547	NM_001285406.1_ 123-145_G1A_as	123-145
AD-266839.1	CCUGCAUGGAUUCCAUGUUCA	469	NM_001285406.1_1 26-146_s	126-146	UGAACAUGGAAUCCAUGCAGGCC	548	NM_001285406.1_ 124-146_as	124-146
AD-266840.1	CUGCAUGGAUUCCAUGUUCA	470	NM_001285406.1_1 27-147_s	127-147	AUGAACAUGGAAUCCAUGCAGGC	549	NM_001285406.1_ 125-147_as	125-147
AD-266841.3	UGCAUGGAUUCCAUGUUCAU	99	NM_001285406.1_1 28-148_C21U_s	128-148	AAUGAACAUGGAAUCCAUGCAGG	182	NM_001285406.1_ 126-148_G1A_as	126-148
AD-266886.2	ACCAGUGCAGGUCCUCACUU	43	NM_001285406.1_1 75-195_s	175-195	AAAGUGAGGACCUGCACUGGU AC	126	NM_001285406.1_ 173-195_as	173-195
AD-266887.3	CCAGUGCAGGUCCUCACUUU A	75	NM_001285406.1_1 76-196_s	176-196	UAAAGUGAGGACCUGCACUGG UA	158	NM_001285406.1_ 174-196_as	174-196
AD-266888.2	CAGUGCAGGUCCUCACUUUA A	54	NM_001285406.1_1 77-197_s	177-197	UUAAAGUGAGGACCUGCACUG GU	137	NM_001285406.1_ 175-197_as	175-197
AD-266890.3	GUGCAGGUCCUCACUUUAAU U	92	NM_001285406.1_1 79-199_C21U_s	179-199	AAUUAAAGUGAGGACCUGCAC UG	175	NM_001285406.1_ 177-199_G1A_as	177-199
AD-266891.2	UGCAGGUCCUCACUUUAAUC U	39	NM_001285406.1_1 80-200_C21U_s	180-200	AGAUUAAAGUGAGGACCUGCA CU	122	NM_001285406.1_ 178-200_G1A_as	178-200
AD-266892.2	GCAGGUCCUCACUUUAAUCC U	97	NM_001285406.1_1 81-201_s	181-201	AGGAUUAAAGUGAGGACCUGC AC	180	NM_001285406.1_ 179-201_as	179-201
AD-266899.2	CUCACUUUAAUCCUCUAUCC A	50	NM_001285406.1_1 88-208_s	188-208	UGGAUAGAGGAUUAAAGUGAG GA	133	NM_001285406.1_ 186-208_as	186-208
AD-266900.3	UCACUUUAAUCCUCUAUCCA U	82	NM_001285406.1_1 89-209_G21U_s	189-209	AUGGAUAGAGGAUUAAAGUGA GG	165	NM_001285406.1_ 187-209_C1A_as	187-209
AD-266901.2	CACUUUAAUCCUCUAUCCAG A	71	NM_001285406.1_1 90-210_s	190-210	UCUGGAUAGAGGAUUAAAGUG AG	154	NM_001285406.1_ 188-210_as	188-210
AD-266928.1	GGUGGGCCAAAGGAUGAAGA U	471	NM_001285406.1_2 17-237_G21U_s	217-237	AUCUUCAUCCUUUGGCCCACC GU	550	NM_001285406.1_ 215-237_C1A_as	215-237
AD-	GAUGAAGAGAGGCAUGUUGG	472	NM_011434.1_345-	345-365	UCCAACAUGCCUCUCUUCAUC	551	NM_011434.1	343-365

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
266934.1	A		365_s		CU			
AD-266936.1	CAAAGGAUGAAGAGAGGCAU U	473	NM_001285406.1_2 24-244_G21U_s	224-244	AAUGCCUCUCUUCAUCCUUUG GC	552	NM_001285406.1_ 222-244_C1A_as	222-244
AD-266938.1	AAGGAUGAAGAGAGGCAUGU U	474	NM_001285406.1_2 26-246_s	226-246	AACAUGCCUCUCUUCAUCCUU UG	553	NM_001285406.1_ 224-246_as	224-246
AD-266939.1	AGGAUGAAGAGAGGCAUGUU U	475	NM_001285406.1_2 27-247_G21U_s	227-247	AAACAUGCCUCUCUUCAUCCU UU	554	NM_001285406.1_ 225-247_C1A_as	225-247
AD-266940.1	GGAUGAAGAGAGGCAUGUUG U	476	NM_001285406.1_2 28-248_G21U_s	228-248	ACAACAUGCCUCUCUUCAUCC UU	555	NM_001285406.1_ 226-248_C1A_as	226-248
AD-266941.1	AUGAAGAGAGGCAUGUUGGA U	477	NM_001285406.1_2 30-250_G21U_s	230-250	AUCCAACAUGCCUCUCUUCAU CC	556	NM_001285406.1_ 228-250_C1A_as	228-250
AD-266943.1	AAGAGAGGCAUGUUGGAGAC U	478	NM_001285406.1_2 33-253_C21U_s	233-253	AGUCUCCAACAUGCCUCUCUU CA	557	NM_001285406.1_ 231-253_G1A_as	231-253
AD-267035.1	GAUGACUUGGGCAAAGGUGG A	479	NM_011434.1_489-509_s	489-509	UCCACCUUUGCCCAAGUCAUC UG	558	NM_011434.1	487-509
AD-267058.1	GUGGUCCAUGAAAAAGCAGA U	480	NM_001285406.1_3 55-375_s	355-375	AUCUGCUUUUUUCAUGGACCAC CA	559	NM_001285406.1_ 353-375_as	353-375
AD-267059.1	UGGUCCAUGAAAAAGCAGAU U	481	NM_001285406.1_3 56-376_G21U_s	356-376	AAUCUGCUUUUUUCAUGGACCA CC	560	NM_001285406.1_ 354-376_C1A_as	354-376
AD-267060.1	GGUCCAUGAAAAAGCAGAUG A	482	NM_001285406.1_3 57-377_s	357-377	UCAUCUGCUUUUUUCAUGGACC AC	561	NM_001285406.1_ 355-377_as	355-377
AD-267073.1	GCAGAUGACUUGGGCAAAGG U	483	NM_001285406.1_3 70-390_s	370-390	ACCUUUGCCCAAGUCAUCUGC UU	562	NM_001285406.1_ 368-390_as	368-390
AD-267075.1	AGAUGACUUGGGCAAAGGUG U	484	NM_001285406.1_3 72-392_G21U_s	372-392	ACACCUUUGCCCAAGUCAUCU GC	563	NM_001285406.1_ 370-392_C1A_as	370-392
AD-267076.1	ACUUGGGCAAAGGUGGAAAU U	485	NM_001285406.1_3 77-397_G21U_s	377-397	AAUUUCCACCUUUGCCCAAGU CA	564	NM_001285406.1_ 375-397_C1A_as	375-397
AD-267118.1	GCUUGUGGUGUAAUUGGGAU U	486	NM_001285406.1_4 36-456_C21U_s	436-456	AAUCCCAAUUACACCACAAGC CA	565	NM_001285406.1_ 434-456_G1A_as	434-456
AD-267119.1	CUUGUGGUGUAAUUGGGAUC U	487	NM_001285406.1_4 37-457_G21U_s	437-457	AGAUCCCAAUUACACCACAAG CC	566	NM_001285406.1_ 435-457_C1A_as	435-457
AD-267120.1	UUGUGGUGUAAUUGGGAUCG U	488	NM_001285406.1_4 38-458_C21U_s	438-458	ACGAUCCCAAUUACACCACAA GC	567	NM_001285406.1_ 436-458_G1A_as	436-458
AD-267121.1	UGUGGUGUAAUUGGGAUCGC U	489	NM_001285406.1_4 39-459_C21U_s	439-459	AGCGAUCCCAAUUACACCACA AG	568	NM_001285406.1_ 437-459_G1A_as	437-459
AD-267122.1	GUGGUGUAAUUGGGAUCGCC U	490	NM_001285406.1_4 40-460_C21U_s	440-460	AGGCGAUCCCAAUUACACCAC AA	569	NM_001285406.1_ 438-460_G1A_as	438-460

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
AD-295644.1	UGCAGGGCAUCAUCAAUUUCU	491	NM_000454.4_192-212_G21U_s	192-212	AGAAAUUGAUGAUGCCCUGCA	570	NM_000454.4_190-212_C1A_as	190-212
AD-295645.1	GCAGGGCAUCAUCAAUUUCG	492	NM_000454.4_193-213_s	193-213	UCGAAAUUGAUGAUGCCCUGC	571	NM_000454.4_191-213_as	191-213
AD-295646.1	CAGGGCAUCAUCAAUUUCGA	493	NM_000454.4_194-214_G21U_s	194-214	AUCGAAAUUGAUGAUGCCCUG	572	NM_000454.4_192-214_C1A_as	192-214
AD-295647.1	AGGGCAUCAUCAAUUUCGAG	494	NM_000454.4_195-215_C21U_s	195-215	ACUCGAAAUUGAUGAUGCCCU	573	NM_000454.4_193-215_G1A_as	193-215
AD-295648.1	GGGCAUCAUCAAUUUCGAGC	495	NM_000454.4_196-216_s	196-216	UGCUCGAAAUUGAUGAUGCCC	574	NM_000454.4_194-216_as	194-216
AD-295649.1	GGCAUCAUCAAUUUCGAGCA	496	NM_000454.4_197-217_G21U_s	197-217	AUGCUCGAAAUUGAUGAUGCC	575	NM_000454.4_195-217_C1A_as	195-217
AD-295651.1	CAUCAUCAAUUUCGAGCAGA	497	NM_000454.4_199-219_s	199-219	UUCUGCUCGAAAUUGAUGAUG	576	NM_000454.4_197-219_as	197-219
AD-295652.1	AUCAUCAAUUUCGAGCAGAA	498	NM_000454.4_200-220_G21U_s	200-220	AUUCUGCUCGAAAUUGAUGAU	577	NM_000454.4_198-220_C1A_as	198-220
AD-295653.1	UCAUCAAUUUCGAGCAGAAG	499	NM_000454.4_201-221_G21U_s	201-221	ACUUCUGCUCGAAAUUGAUGA	578	NM_000454.4_199-221_C1A_as	199-221
AD-295661.1	AUUAAAGGACUGACUGAAGG	500	NM_000454.4_254-274_C21U_s	254-274	ACCUUCAGUCAGUCCUUUAAU	579	NM_000454.4_252-274_G1A_as	252-274
AD-295685.1	CAUGUUCAUGAGUUUGGAGA	501	NM_000454.4_287-307_s	287-307	AUCUCCAAACUCAUGAACAUG	580	NM_000454.4_285-307_as	285-307
AD-295688.1	GUUCAUGAGUUUGGAGAUAA	502	NM_000454.4_290-310_s	290-310	AUUAUCUCCAAACUCAUGAAC	581	NM_000454.4_288-310_as	288-310
AD-295689.1	UUCAUGAGUUUGGAGAUAAU	503	NM_000454.4_291-311_s	291-311	UAUUAUCUCCAAACUCAUGAA	582	NM_000454.4_289-311_as	289-311
AD-295692.1	AUGAGUUUGGAGAUAAUACA	504	NM_000454.4_294-314_G21U_s	294-314	AUGUAUUAUCUCCAAACUCAU	583	NM_000454.4_292-314_C1A_as	292-314
AD-295795.1	UGUGUCUAUUGAAGAUUCUG	505	NM_000454.4_439-459_s	439-459	ACAGAAUCUUCAAUAGACACA	584	NM_000454.4_437-459_as	437-459
AD-295796.1	GUGUCUAUUGAAGAUUCUGU	506	NM_000454.4_440-460_G21U_s	440-460	AACAGAAUCUUCAAUAGACAC	585	NM_000454.4_438-460_C1A_as	438-460
AD-295827.1	UGGUGGUCCAUGAAAAAGCA	507	NM_001285406.1_353-373_G21U_s	353-373	AUGCUUUUUCAUGGACCACCA	586	NM_001285406.1	351-373
AD-295828.1	GGUGGUCCAUGAAAAAGCAG	508	NM_001285406.1_354-374_s	354-374	UCUGCUUUUUCAUGGACCACC	587	NM_001285406.1	352-374
AD-	GAGACCAUUGCAUCAUUGGC	509	NM_000454.4_474-	474-494	AGCCAAUGAUGCAAUGGUCUC	588	NM_000454.4_472-	472-494

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
295831.1	U		494_C21U_s		CU		494_G1A_as	
AD-295856.1	ACUGGUGGUCCAUGAAAAAG U	510	NM_000454.4_499-519_C21U_s	499-519	ACUUUUUCAUGGACCACCAGU GU	589	NM_000454.4_497-519_G1A_as	497-519
AD-295857.1	CUGGUGGUCCAUGAAAAAGC A	511	NM_000454.4_500-520_s	500-520	UGCUUUUUCAUGGACCACCAG UG	590	NM_000454.4_498-520_as	498-520
AD-295858.1	AGGUGGAAAUGAAGAAAGUA U	512	NM_000454.4_535-555_C21U_s	535-555	AUACUUUCUUCAUUUCCACCU UU	591	NM_000454.4_533-555_G1A_as	533-555
AD-295867.1	GAAAGUACAAAGACAGGAAA U	513	NM_000454.4_548-568_C21U_s	548-568	AUUUCCUGUCUUUGUACUUUC UU	592	NM_000454.4_546-568_G1A_as	546-568
AD-295868.1	AAAGUACAAAGACAGGAAAC U	514	NM_000454.4_549-569_G21U_s	549-569	AGUUUCCUGUCUUUGUACUUU CU	593	NM_000454.4_547-569_C1A_as	547-569
AD-295869.1	AAGUACAAAGACAGGAAACG U	515	NM_000454.4_550-570_C21U_s	550-570	ACGUUUCCUGUCUUUGUACUU UC	594	NM_000454.4_548-570_G1A_as	548-570
AD-295870.1	AGUACAAAGACAGGAAACGC U	516	NM_000454.4_551-571_s	551-571	AGCGUUUCCUGUCUUUGUACU UU	595	NM_000454.4_549-571_as	549-571
AD-295871.1	GUACAAAGACAGGAAACGCU U	517	NM_000454.4_552-572_G21U_s	552-572	AAGCGUUUCCUGUCUUUGUAC UU	596	NM_000454.4_550-572_C1A_as	550-572
AD-295872.1	UACAAAGACAGGAAACGCUG U	518	NM_000454.4_553-573_G21U_s	553-573	ACAGCGUUUCCUGUCUUUGUA CU	597	NM_000454.4_551-573_C1A_as	551-573
AD-295874.1	CAAAGACAGGAAACGCUGGA A	519	NM_000454.4_555-575_s	555-575	UUCCAGCGUUUCCUGUCUUUG UA	598	NM_000454.4_553-575_as	553-575
AD-295879.1	UGGCUUGUGGUGUAAUUGGG A	520	NM_001285406.1_434-454_s	434-454	UCCCAAUUACACCACAAGCCA AA	599	NM_001285406.1	432-454
AD-295880.1	GGCUUGUGGUGUAAUUGGGA U	521	NM_001285406.1_435-455_s	435-455	AUCCCAAUUACACCACAAGCC AA	600	NM_001285406.1	433-455
AD-295897.1	GUCGUUUUGGCUUGUGGUGUA A	522	NM_000454.4_576-596_s	576-596	UUACACCACAAGCCAAACGAC UU	601	NM_000454.4_574-596_as	574-596
AD-295898.1	UCGUUUUGGCUUGUGGUGUAA U	523	NM_000454.4_577-597_s	577-597	AUUACACCACAAGCCAAACGA CU	602	NM_000454.4_575-597_as	575-597
AD-295899.1	CGUUUUGGCUUGUGGUGUAAU U	524	NM_000454.4_578-598_s	578-598	AAUUACACCACAAGCCAAACG AC	603	NM_000454.4_576-598_as	576-598
AD-295900.1	GUUUUGGCUUGUGGUGUAAUU U	525	NM_000454.4_579-599_G21U_s	579-599	AAAUUACACCACAAGCCAAAC GA	604	NM_000454.4_577-599_C1A_as	577-599
AD-295901.1	UUUGGCUUGUGGUGUAAUUG U	526	NM_000454.4_580-600_G21U_s	580-600	ACAAUUACACCACAAGCCAAA CG	605	NM_000454.4_578-600_C1A_as	578-600
AD-295902.1	UUGGCUUGUGGUGUAAUUGG U	527	NM_000454.4_581-601_G21U_s	581-601	ACCAAUUACACCACAAGCCAA AC	606	NM_000454.4_579-601_C1A_as	579-601

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
AD-295904.1	GUAAUUGGGAUCGCCCAAUAA	528	NM_000454.4_593-613_s	593-613	UUAUUGGGCGAUCCCAAUUACAC	607	NM_000454.4_591-613_as	591-613

Table 5. Modified Sense and Antisense Strand Sequences of Superoxide Dismutase 1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antiense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-135962.1	asusgacuUfgGfGfCfaaagguggaaL96	608	usUfsccaCfcUfUfugccCfaAfgucauscsu	687	AGAUGACUUGGGCAAAGGUGGAA	766
AD-135963.1	usgsacuuGfgGfCfAfaagguggaaaL96	609	usUfsuccAfcCfUfuugcCfcAfagucasusc	688	GAUGACUUGGGCAAAGGUGGAAA	767
AD-135964.1	gsascuugGfgCfAfAfagguggaaauL96	610	asUfsuucCfaCfCfuuugCfcCfaagucsasu	689	AUGACUUGGGCAAAGGUGGAAAU	768
AD-135967.5	ususgggcAfaAfGfGfuggaaaugaaL96	269	usUfscauUfuCfCfaccuUfuGfcccaasgsu	352	ACUUGGGCAAAGGUGGAAAUGAA	435
AD-135974.3	asasggugGfaAfAfUfgaagaaaguaL96	611	usAfscuuUfcUfUfcauuUfcCfaccuususg	690	CAAAGGUGGAAAUGAAGAAAGUA	769
AD-266788.1	asasuuucGfaGfCfAfgaaggaaaguL96	612	asCfsuuuCfcUfUfcugcUfcGfaaauusgsa	691	UCAAUUUCGAGCAGAAGGAAAGU	770
AD-266789.2	asusuucgAfgCfAfGfaaggaaaguaL96	213	usAfscuuUfcCfUfucugCfuCfgaaaususg	296	CAAUUUCGAGCAGAAGGAAAGUA	379
AD-266790.2	ususucgaGfcAfGfAfaggaaaguaaL96	255	usUfsacuUfuCfCfuucuGfcUfcgaaasusu	338	AAUUUCGAGCAGAAGGAAAGUAA	421
AD-266791.3	ususcgagCfaGfAfAfggaaaguaauL96	212	asUfsuacUfuUfCfcuucUfgCfucgaasasu	295	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-266794.1	gsasgcagAfaGfGfAfaaguaauggaL96	613	usCfscauUfaCfUfuuccUfuCfugcucsgsa	692	UCGAGCAGAAGGAAAGUAAUGGA	771
AD-266798.1	asgsaaggAfaAfGfUfaauggaccauL96	614	asUfsgguCfcAfUfuacuUfuCfcuucusgsc	693	GCAGAAGGAAAGUAAUGGACCAG	772
AD-266799.1	gsasaggaAfaGfUfAfauggaccaguL96	615	asCfsuggUfcCfAfuuacUfuUfccuucsusg	694	CAGAAGGAAAGUAAUGGACCAGU	773
AD-266801.1	asgsgaaaGfuAfAfUfggaccagugaL96	616	usCfsacuGfgUfCfcauuAfcUfuuccususc	695	GAAGGAAAGUAAUGGACCAGUGA	774
AD-266802.1	gsgsaaagUfaAfUfGfgaccagugaaL96	617	usUfscacUfgGfUfccauUfaCfuuuccsusu	696	AAGGAAAGUAAUGGACCAGUGAA	775
AD-266803.1	gsasaaguAfaUfGfGfaccagugaauL96	618	asUfsucaCfuGfGfuccaUfuAfcuuucscsu	697	AGGAAAGUAAUGGACCAGUGAAG	776
AD-266804.1	asasaguaAfuGfGfAfccagugaaguL96	619	asCfsuucAfcUfGfguccAfuUfacuuuscsc	698	GGAAAGUAAUGGACCAGUGAAGG	777
AD-266805.1	asasguaaUfgGfAfCfcagugaagguL96	620	asCfscuuCfaCfUfggucCfaUfuacuususc	699	GAAAGUAAUGGACCAGUGAAGGU	778
AD-266806.1	asgsuaauGfgAfCfCfagugaagguuL96	621	asAfsccuUfcAfCfugguCfcAfuuacususu	700	AAAGUAAUGGACCAGUGAAGGUG	779
AD-266808.2	usasauggAfcCfAfGfugaagguguuL96	259	asAfscacCfuUfCfacugGfuCfcauuascsu	342	AGUAAUGGACCAGUGAAGGUGUG	425

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antiense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-266832.1	usgsaaggCfcUfGfCfauggauuccaL96	622	usGfsgaaUfcCfAfugcaGfgCfcuucasgsu	701	ACUGAAGGCCUGCAUGGAUUCCA	780
AD-266834.1	gsasaggcCfuGfCfAfuggauuccauL96	623	asUfsggaAfuCfCfaugcAfgGfccuucsasg	702	CUGAAGGCCUGCAUGGAUUCCAU	781
AD-266836.1	asgsgccuGfcAfUfGfgauuccauguL96	624	asCfsaugGfaAfUfccauGfcAfggccususc	703	GAAGGCCUGCAUGGAUUCCAUGU	782
AD-266837.1	gsgsccugCfaUfGfGfauuccauguuL96	625	asAfscauGfgAfAfuccaUfgCfaggccsusu	704	AAGGCCUGCAUGGAUUCCAUGUU	783
AD-266838.1	gscscugcAfuGfGfAfauuccauguuuL96	626	asAfsacaUfgGfAfauccAfuGfcaggcscsu	705	AGGCCUGCAUGGAUUCCAUGUUC	784
AD-266839.1	cscsugcaUfgGfAfUfuccauguucaL96	627	usGfsaacAfuGfGfaauccCfaUfgcaggscsc	706	GGCCUGCAUGGAUUCCAUGUUCA	785
AD-266840.1	csusgcauGfgAfUfUfccauguucauL96	628	asUfsgaaCfaUfGfgaauCfcAfugcagsgsc	707	GCCUGCAUGGAUUCCAUGUUCAU	786
AD-266841.3	usgscaugGfaUfUfCfcauguucauuL96	265	asAfsugaAfcAfUfggaaUfcCfaugcasgsg	348	CCUGCAUGGAUUCCAUGUUCAUC	431
AD-266886.2	ascscaguGfcAfGfGfuccucacuuuL96	209	asAfsaguGfaGfGfaccuGfcAfcuggusasc	292	GUACCAGUGCAGGUCCUCACUUU	375
AD-266887.3	cscsagugCfaGfGfUfccucacuuuaL96	241	usAfsaagUfgAfGfgaccUfgCfacuggsusa	324	UACCAGUGCAGGUCCUCACUUUA	407
AD-266888.2	csasgugcAfgGfUfCfcucacuuuaaL96	220	usUfsaaaGfuGfAfggacCfuGfcacugsgsu	303	ACCAGUGCAGGUCCUCACUUUAA	386
AD-266890.3	gsusgcagGfuCfCfUfcacuuuaaauL96	258	asAfsuuaAfaGfUfgaggAfcCfugcacsusg	341	CAGUGCAGGUCCUCACUUUAAUC	424
AD-266891.2	usgscaggUfcCfUfCfacuuuaaucuL96	205	asGfsauuAfaAfGfugagGfaCfcugcascsu	288	AGUGCAGGUCCUCACUUUAAUCC	371
AD-266892.2	gscsagguCfcUfCfAfcuuuaaucccuL96	263	asGfsgauUfaAfAfgugaGfgAfccugcsasc	346	GUGCAGGUCCUCACUUUAAUCCU	429
AD-266899.2	csuscacuUfuAfAfUfccucuauccaL96	216	usGfsgauAfgAfGfgauuAfaAfgugagsgsa	299	UCCUCACUUUAAUCCUCUAUCCA	382
AD-266900.3	uscsacuuUfaAfUfCfcucuauccauL96	248	asUfsggaUfaGfAfggauUfaAfagugasgsg	331	CCUCACUUUAAUCCUCUAUCCAG	414
AD-266901.2	csascuuuAfaUfCfCfucuauccagaL96	237	usCfsuggAfuAfGfaggaUfuAfaagugsasg	320	CUCACUUUAAUCCUCUAUCCAGA	403
AD-266928.1	gsgsugggCfcAfAfAfggaugaagauL96	629	asUfscuuCfaUfCfcuuuGfgCfccaccsgsu	708	ACGGUGGGCCAAAGGAUGAAGAG	787
AD-266934.1	gsasugaaGfaGfAfGfgcauguuggaL96	630	usCfscaaCfaUfGfccucUfcUfucauccscsu	709	AGGAUGAAGAGAGGCAUGUUGGA	788
AD-266936.1	csasaaggAfuGfAfAfgagaggcauuL96	631	asAfsugcCfuCfUfcuucAfuCfcuuugsgsc	710	GCCAAAGGAUGAAGAGAGGCAUG	789
AD-266938.1	asasggauGfaAfGfAfgaggcauguuL96	632	asAfscauGfcCfUfcucuUfcAfuccuususg	711	CAAAGGAUGAAGAGAGGCAUGUU	790
AD-266939.1	asgsgaugAfaGfAfGfaggcauguuuL96	633	asAfsacaUfgCfCfucucUfuCfauccususu	712	AAAGGAUGAAGAGAGGCAUGUUG	791
AD-266940.1	gsgsaugaAfgAfGfAfggcauguuguL96	634	asCfsaacAfuGfCfcucuCfuUfcauccsusu	713	AAGGAUGAAGAGAGGCAUGUUGG	792
AD-266941.1	asusgaagAfgAfGfGfcauguuggauL96	635	asUfsccaAfcAfUfgccuCfuCfuucauscsc	714	GGAUGAAGAGAGGCAUGUUGGAG	793
AD-266943.1	asasgagaGfgCfAfUfguuggagacuL96	636	asGfsucuCfcAfAfcaugCfcUfcucuuscsa	715	UGAAGAGAGGCAUGUUGGAGACC	794
AD-267035.1	gsasugacUfuGfGfGfcaaagguggaL96	637	usCfscacCfuUfUfgcccAfaGfucaucsusg	716	CAGAUGACUUGGGCAAAGGUGGA	795
AD-267058.1	gsusggucCfaUfGfAfaaaagcagauL96	638	asUfscugCfuUfUfuucaUfgGfaccacscsa	717	UGGUGGUCCAUGAAAAAGCAGAU	796

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antiense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-267059.1	usgsguccAfuGfAfAfaaagcagauuL96	639	asAfsucuGfcUfUfuuucAfuGfgaccascsc	718	GGUGGUCCAUGAAAAAGCAGAUG	797
AD-267060.1	gsgsuccaUfgAfAfAfaagcagaugaL96	640	usCfsaucUfgCfUfuuuuCfaUfggaccsasc	719	GUGGUCCAUGAAAAAGCAGAUGA	798
AD-267073.1	gscsagauGfaCfUfUfgggcaaagguL96	641	asCfscuuUfgCfCfcaagUfcAfucugcsusu	720	AAGCAGAUGACUUGGGCAAAGGU	799
AD-267075.1	asgsaugaCfuUfGfGfgcaaagguguL96	642	asCfsaccUfuUfGfcccaAfgUfcaucusgsc	721	GCAGAUGACUUGGGCAAAGGUGG	800
AD-267076.1	ascsuuggGfcAfAfAfgguggaaauuL96	643	asAfsuuuCfcAfCfcuuuGfcCfcaaguscsa	722	UGACUUGGGCAAAGGUGGAAAUG	801
AD-267118.1	gscsuuguGfgUfGfUfaauugggauuL96	644	asAfsuccCfaAfUfuacaCfcAfcaagcscsa	723	UGGCUUGUGGGUGUAAUUGGGAUC	802
AD-267119.1	csusugugGfuGfUfAfauugggaucuL96	645	asGfsaucCfcAfAfuuacAfcCfacaagcscsc	724	GGCUUGUGGGUGUAAUUGGGAUCG	803
AD-267120.1	ususgugguUfgUfAfAfuugggaucguL96	646	asCfsgauCfcCfAfauuaCfaCfcacaagsgsc	725	GCUUGUGGGUGUAAUUGGGAUCGC	804
AD-267121.1	usgsugguGfuAfAfUfugggaucgcuL96	647	asGfscgaUfcCfCfCfaauuAfcAfccacasasg	726	CUUGUGGGUGUAAUUGGGAUCGCC	805
AD-267122.1	gsusgguGfuAfAfUfUfgggaucgccuL96	648	asGfsgcgAfuCfCfcaauUfaCfaccacsasa	727	UUGUGGGUGUAAUUGGGAUCGCCC	806
AD-295644.1	usgscaggGfcAfUfCfaucaauuucuL96	649	asGfsaaaUfuGfAfugauGfcCfcugcascsu	728	AGUGCAGGGCAUCAUCAAUUUCG	807
AD-295645.1	gscsagggCfaUfCfAfucaauuucgaL96	650	usCfsgaaAfuUfGfaugaUfgCfccugcsasc	729	GUGCAGGGCAUCAUCAAUUUCGA	808
AD-295646.1	csasgggcAfuCfAfUfcaauuucgauL96	651	asUfscgaAfaUfUfgaugAfuGfcccugscsa	730	UGCAGGGCAUCAUCAAUUUCGAG	809
AD-295647.1	asgsggcaUfcAfUfCfaauuucgaguL96	652	asCfsucgAfaAfUfugauGfaUfgcccusgsc	731	GCAGGGCAUCAUCAAUUUCGAGC	810
AD-295648.1	gsgsgcauCfaUfCfAfauuucgagcaL96	653	usGfscucGfaAfAfuugaUfgAfugcccsusg	732	CAGGGCAUCAUCAAUUUCGAGCA	811
AD-295649.1	ggscaucAfuCfAfAfuuucgagcauL96	654	asUfsgcuCfgAfAfauugAfuGfaugccscsu	733	AGGGCAUCAUCAAUUUCGAGCAG	812
AD-295651.1	csasucauCfaAfUfUfucgagcagaaL96	655	usUfscugCfuCfGfaaauUfgAfugaugcscsc	734	GGCAUCAUCAAUUUCGAGCAGAA	813
AD-295652.1	asuscaucAfaUfUfUfcgagcagaauL96	656	asUfsucuGfcUfCfgaaaUfuGfaugausgsc	735	GCAUCAUCAAUUUCGAGCAGAAG	814
AD-295653.1	uscsaucaAfuUfUfCfgagcagaaguL96	657	asCfsuucUfgCfUfcgaaAfuUfgaugasusg	736	CAUCAUCAAUUUCGAGCAGAAGG	815
AD-295661.1	asusuaaaGfgAfCfUfgacugaagguL96	658	asCfscuuCfaGfUfcaguCfcUfuuaausgsc	737	GCAUUAAAGGACUGACUGAAGGC	816
AD-295685.1	csasuguuCfaUfGfAfguuuggagauL96	659	asUfscucCfaAfAfcucaUfgAfacaugsgsa	738	UCCAUGUUCAUGAGUUUGGAGAU	817
AD-295688.1	gsusucauGfaGfUfUfuggagauaauL96	660	asUfsuauCfuCfCfaaacUfcAfugaacsasu	739	AUGUUCAUGAGUUUGGAGAUAAU	818
AD-295689.1	ususcaugAfgUfUfUfggagauaauaL96	661	usAfsuuaUfcUfCfcaaaCfuCfaugaascsa	740	UGUUCAUGAGUUUGGAGAUAAUA	819
AD-295692.1	asusgaguUfuGfGfAfgauaauacauL96	662	asUfsguaUfuAfUfcuccAfaAfcucausgsa	741	UCAUGAGUUUGGAGAUAAUACAG	820
AD-295795.1	usgsgucUfaUfUfGfaagauucuguL96	663	asCfsagaAfuCfUfucaaUfaGfacacasusc	742	GAUGUGUCUAUUGAAGAUUCUGU	821
AD-295796.1	gsusgucuAfuUfGfAfagauucuguuL96	664	asAfscagAfaUfCfUfucaAfuAfgacacsasu	743	AUGUGUCUAUUGAAGAUUCUGUG	822
AD-295827.1	usgsgugUfcCfAfUfgaaaagcauuL96	665	asUfsgcuUfuUfUfcaugGfaCfcaccasgsu	744	ACUGGUGGUCCAUGAAAAAGCAU	823

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antiense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-295828.1	gsgsuggu CfcAfUfGfaaaaagcagaL96	666	usCfsugcUfuUfUfucauGfgAfccaccsasg	745	CUGGUGGUCCAUGAAAAAGCAGA	824
AD-295831.1	gsasgaccAfuUfGfCfaucauuggcuL96	667	asGfsccaAfuGfAfugcaAfuGfgucucscsu	746	AGGAGACCAUUGCAUCAUUGGCC	825
AD-295856.1	ascsuggu GfgUfCfCfaugaaaaaguL96	668	asCfsuuuUfuCfAfuggaCfcAfccagusgsu	747	ACACUGGUGGUCCAUGAAAAAGC	826
AD-295857.1	csusggugGfuCfCfAfugaaaaagcaL96	669	usGfscuuUfuUfCfauggAfcCfaccagsusg	748	CACUGGUGGUCCAUGAAAAAGCA	827
AD-295858.1	asgsuggAfaAfUfGfaagaaaguauL96	670	asUfsacuUfuCfUfucauUfuCfcaccususu	749	AAAGGUGGAAAUGAAGAAAGUAC	828
AD-295867.1	gsasaaguAfcAfAfAfgacaggaaauL96	671	asUfsuucCfuGfUfcuuuGfuAfcuuucsusu	750	AAGAAAGUACAAAGACAGGAAAC	829
AD-295868.1	asasaguaCfaAfAfGfacaggaaacuL96	672	asGfsuuuCfcUfGfucuuUfgUfacuuuscsu	751	AGAAAGUACAAAGACAGGAAACG	830
AD-295869.1	asasguacAfaAfGfAfcaggaaacguL96	673	asCfsguuUfcCfUfgucuUfuGfuacuususc	752	GAAAGUACAAAGACAGGAAACGC	831
AD-295870.1	asgsuacaAfaGfAfCfaggaaacgcuL96	674	asGfscguUfuCfCfugucUfuUfguacususu	753	AAAGUACAAAGACAGGAAACGCU	832
AD-295871.1	gsusacaaAfgAfCfAfggaaacgcuuL96	675	asAfsgcgUfuUfCfcuguCfuUfguacsusu	754	AAGUACAAAGACAGGAAACGCUG	833
AD-295872.1	usascaaaGfaCfAfGfgaaacgcuguL96	676	asCfsagcGfuUfUfccugUfcUfuuguascsu	755	AGUACAAAGACAGGAAACGCUGG	834
AD-295874.1	csasaagaCfaGfGfAfaacgcuggaaL96	677	usUfsccaGfcGfUfuuceUfgUfcuuugsusa	756	UACAAAGACAGGAAACGCUGGAA	835
AD-295879.1	usgsgcuuGfuGfGfUfguaauugggaL96	678	usCfsccaAfuUfAfcaccAfcAfagccasasa	757	UUUGGCUUGUGGUGUAAUUGGGA	836
AD-295880.1	gsgscuugUfgGfUfGfuaauugggauL96	679	asUfsccc AfaUfUfacacCfaCfaagccsasa	758	UUGGCUUGUGGUGUAAUUGGGAU	837
AD-295897.1	gsuscguuUfgGfCfUfugugguguaaL96	680	usUfsacaCfcAfCfaagcCfaAfacgacsusu	759	AAGUCGUUUGGCUUGUGGUGUAA	838
AD-295898.1	uscsguuuGfgCfUfUfgugguguaauL96	681	asUfsuacAfcCfAfcaagCfcAfaacgascsu	760	AGUCGUUUGGCUUGUGGUGUAAU	839
AD-295899.1	csgsuuugGfcUfUfGfugguguaauuL96	682	asAfsuuaCfaCfCfacaaGfcCfaaacgsasc	761	GUCGUUUGGCUUGUGGUGUAAUU	840
AD-295900.1	gsusuuggCfuUfGfUfgguguaauuuL96	683	asAfsauuAfcAfCfcacaAfgCfcaaacsgsa	762	UCGUUUGGCUUGUGGUGUAAUUG	841
AD-295901.1	ususuggcUfuGfUfGfguguaauuguL96	684	asCfsaauUfaCfAfccacAfaGfccaaascsg	763	CGUUUGGCUUGUGGUGUAAUUGG	842
AD-295902.1	ususggcuUfgUfGfGfuguaauuggcuL96	685	asCfscaaUfuAfCfaccaCfaAfgccaasasc	764	GUUUGGCUUGUGGUGUAAUUGGG	843
AD-295904.1	gsusaauuGfgGfAfUfcgcccaauaaL96	686	usUfsauuGfgGfCfgaucCfcAfauuacsasc	765	GUGUAAUUGGGAUCGCCCAAUAA	844

Table 6. Unmodified Sense and Antisense Strand Sequences of Superoxide Dismutase 1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
AD-301535.1	UGCAGGGCAUCAUCAAUUUCU	491	NM_000454.4_192-212_G21U_s	192-212	AGAAAUTGAUGAUGCCCUGCACU	846	NM_000454.4_190-212_C1A_as	190-212
AD-301536.1	GCAGGGCAUCAUCAAUUUCGA	492	NM_000454.4_193-213_s	193-213	UCGAAATUGAUGAUGCCCUGCAC	847	NM_000454.4_191-213_as	191-213
AD-301537.1	CAGGGCAUCAUCAAUUUCGAU	493	NM_000454.4_194-214_G21U_s	194-214	AUCGAAAUUGAUGAUGCCCUGCA	572	NM_000454.4_192-214_C1A_as	192-214
AD-301538.1	AGGGCAUCAUCAAUUUCGAGU	494	NM_000454.4_195-215_C21U_s	195-215	ACUCGAAAUUGAUGAUGCCCUGC	573	NM_000454.4_193-215_G1A_as	193-215
AD-301539.1	GGGCAUCAUCAAUUUCGAGCA	495	NM_000454.4_196-216_s	196-216	UGCUCGAAAUUGAUGAUGCCCUG	574	NM_000454.4_194-216_as	194-216
AD-301540.1	GGCAUCAUCAAUUUCGAGCAU	496	NM_000454.4_197-217_G21U_s	197-217	AUGCUCGAAAUUGAUGAUGCCCU	575	NM_000454.4_195-217_C1A_as	195-217
AD-301542.1	CAUCAUCAAUUUCGAGCAGAA	497	NM_000454.4_199-219_s	199-219	UUCUGCTCGAAAUUGAUGAUGCC	848	NM_000454.4_197-219_as	197-219
AD-301543.1	AUCAUCAAUUUCGAGCAGAAU	498	NM_000454.4_200-220_G21U_s	200-220	AUUCUGCUCGAAAUUGAUGAUGC	577	NM_000454.4_198-220_C1A_as	198-220
AD-301544.1	UCAUCAAUUUCGAGCAGAAGU	499	NM_000454.4_201-221_G21U_s	201-221	ACUUCUGCUCGAAAUUGAUGAUG	578	NM_000454.4_199-221_C1A_as	199-221
AD-301549.1	AAUUUCGAGCAGAAGGAAAGU	454	NM_001285406.1_58-78_s	58-78	ACUUUCCUUCUGCUCGAAAUUGA	533	NM_001285406.1	56-78
AD-301550.1	AUUUCGAGCAGAAGGAAAGUA	47	NM_001285406.1_59-79_s	59-79	UACUUUCCUUCUGCUCGAAAUUG	130	NM_001285406.1	57-79
AD-301551.1	UUUCGAGCAGAAGGAAAGUAA	89	NM_001285406.1_60-80_s	60-80	UUACUUTCCUUCUGCUCGAAAUU	849	NM_001285406.1	58-80
AD-301552.1	UUCGAGCAGAAGGAAAGUAAU	46	NM_001285406.1_61-81_s	61-81	AUUACUTUCCUUCUGCUCGAAAU	850	NM_001285406.1	59-81
AD-301555.1	GAGCAGAAGGAAAGUAAUGGA	455	NM_001285406.1_64-84_s	64-84	UCCAUUACUUUCCUUCUGCUCGA	534	NM_001285406.1	62-84
AD-301559.1	AGAAGGAAAGUAAUGGACCAU	456	NM_001285406.1_68-88_G21U_s	68-88	AUGGUCCAUUACUUUCCUUCUGC	535	NM_001285406.1	66-88
AD-301560.1	GAAGGAAAGUAAUGGACCAGU	457	NM_001285406.1_69-89_s	69-89	ACUGGUCCAUUACUUUCCUUCUG	536	NM_001285406.1	67-89
AD-301562.1	AGGAAAGUAAUGGACCAGUGA	458	NM_001285406.1_71-91_s	71-91	UCACUGGUCCAUUACUUUCCUUC	537	NM_001285406.1	69-91
AD-301563.1	GGAAAGUAAUGGACCAGUGAA	459	NM_001285406.1_72-92_s	72-92	UUCACUGGUCCAUUACUUUCCUU	538	NM_001285406.1	70-92
AD-301564.1	GAAAGUAAUGGACCAGUGAAU	460	NM_001285406.1_73-93_G21U_s	73-93	AUUCACTGGUCCAUUACUUUCCU	851	NM_001285406.1	71-93
AD-301565.1	AAAGUAAUGGACCAGUGAAGU	461	NM_001285406.1_74-94_G21U_s	74-94	ACUUCACUGGUCCAUUACUUUCC	540	NM_001285406.1	72-94

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
AD-301566.1	AAGUAAUGGACCAGUGAAGGU	462	NM_001285406.1_75-95_s	75-95	ACCUUCACUGGUCCAUUACUUUC	541	NM_001285406.1	73-95
AD-301567.1	AGUAAUGGACCAGUGAAGGUU	463	NM_001285406.1_76-96_G21U_s	76-96	AACCUUCACUGGUCCAUUACUUU	542	NM_001285406.1	74-96
AD-301569.1	UAAUGGACCAGUGAAGGUGUU	93	NM_001285406.1_78-98_G21U_s	78-98	AACACCTUCACUGGUCCAUUACU	852	NM_001285406.1	76-98
AD-301579.1	AUUAAAGGACUGACUGAAGGU	500	NM_000454.4_254-274_C21U_s	254-274	ACCUUCAGUCAGUCCUUUAAUGC	579	NM_000454.4_252-274_G1A_as	252-274
AD-301593.1	UGAAGGCCUGCAUGGAUUCCA	464	NM_001285406.1_120-140_s	120-140	UGGAAUCCAUGCAGGCCUUCAGU	543	NM_001285406.1	118-140
AD-301594.1	GAAGGCCUGCAUGGAUUCCAU	465	NM_001285406.1_121-141_s	121-141	AUGGAATCCAUGCAGGCCUUCAG	853	NM_001285406.1	119-141
AD-301596.1	AGGCCUGCAUGGAUUCCAUGU	466	NM_001285406.1_123-143_s	123-143	ACAUGGAAUCCAUGCAGGCCUUC	545	NM_001285406.1	121-143
AD-301597.1	GGCCUGCAUGGAUUCCAUGUU	467	NM_001285406.1_124-144_s	124-144	AACAUGGAAUCCAUGCAGGCCUU	546	NM_001285406.1	122-144
AD-301598.1	GCCUGCAUGGAUUCCAUGUUU	468	NM_001285406.1_125-145_C21U_s	125-145	AAACAUGGAAUCCAUGCAGGCCU	547	NM_001285406.1	123-145
AD-301599.1	CCUGCAUGGAUUCCAUGUUCA	469	NM_001285406.1_126-146_s	126-146	UGAACATGGAAUCCAUGCAGGCC	854	NM_001285406.1	124-146
AD-301600.1	CUGCAUGGAUUCCAUGUUCAU	470	NM_001285406.1_127-147_s	127-147	AUGAACAUGGAAUCCAUGCAGGC	549	NM_001285406.1	125-147
AD-301601.1	UGCAUGGAUUCCAUGUUCAUU	99	NM_001285406.1_128-148_C21U_s	128-148	AAUGAACAUGGAAUCCAUGCAGG	182	NM_001285406.1	126-148
AD-301612.1	CAUGUUCAUGAGUUUGGAGAU	501	NM_000454.4_287-307_s	287-307	AUCUCCAAACUCAUGAACAUGGA	580	NM_000454.4_285-307_as	285-307
AD-301615.1	GUUCAUGAGUUUGGAGAUAAU	502	NM_000454.4_290-310_s	290-310	AUUAUCTCCAAACUCAUGAACAU	855	NM_000454.4_288-310_as	288-310
AD-301616.1	UUCAUGAGUUUGGAGAUAAUA	503	NM_000454.4_291-311_s	291-311	UAUUAUCUCCAAACUCAUGAACA	582	NM_000454.4_289-311_as	289-311
AD-301619.1	AUGAGUUUGGAGAUAAUACAU	504	NM_000454.4_294-314_G21U_s	294-314	AUGUAUTAUCUCCAAACUCAUGA	856	NM_000454.4_292-314_C1A_as	292-314
AD-301648.1	ACCAGUGCAGGUCCUCACUUU	43	NM_001285406.1_175-195_s	175-195	AAAGUGAGGACCUGCACUGGUAC	126	NM_001285406.1	173-195
AD-301649.1	CCAGUGCAGGUCCUCACUUUA	75	NM_001285406.1_176-196_s	176-196	UAAAGUGAGGACCUGCACUGGUA	158	NM_001285406.1	174-196
AD-301650.1	CAGUGCAGGUCCUCACUUUAA	54	NM_001285406.1_177-197_s	177-197	UUAAAGTGAGGACCUGCACUGGU	857	NM_001285406.1	175-197
AD-301652.1	GUGCAGGUCCUCACUUUAAUU	92	NM_001285406.1_179-199_C21U_s	179-199	AAUUAAAGUGAGGACCUGCACUG	175	NM_001285406.1	177-199
AD-301653.1	UGCAGGUCCUCACUUUAAUCU	39	NM_001285406.1_180-200_C21U_s	180-200	AGAUUAAAGUGAGGACCUGCACU	122	NM_001285406.1	178-200

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
AD-301654.1	GCAGGUCCUCACUUUAAUCCU	97	NM_001285406.1_181-201_s	181-201	AGGAUUAAAGUGAGGACCUGCAC	180	NM_001285406.1	179-201
AD-301661.1	CUCACUUUAAUCCUCUAUCCA	50	NM_001285406.1_188-208_s	188-208	UGGAUAGAGGAUUAAAGUGAGGA	133	NM_001285406.1	186-208
AD-301662.1	UCACUUUAAUCCUCUAUCCAU	82	NM_001285406.1_189-209_G21U_s	189-209	AUGGAUAGAGGAUUAAAGUGAGG	165	NM_001285406.1	187-209
AD-301663.1	CACUUUAAUCCUCUAUCCAGA	71	NM_001285406.1_190-210_s	190-210	UCUGGATAGAGGAUUAAAGUGAG	858	NM_001285406.1	188-210
AD-301688.1	ACGGUGGGCCAAAGGAUGAAU	845	NM_001285406.1_215-235_G21U_s	215-235	AUUCAUCCUUUGGCCCACCGUGU	859	NM_001285406.1	213-235
AD-301690.1	GGUGGGCCAAAGGAUGAAGAU	471	NM_001285406.1_217-237_G21U_s	217-237	AUCUUCAUCCUUUGGCCCACCGU	550	NM_001285406.1	215-237
AD-301697.1	CAAAGGAUGAAGAGAGGCAUU	473	NM_001285406.1_224-244_G21U_s	224-244	AAUGGCCTCUCUUCAUCCUUUGGC	860	NM_001285406.1	222-244
AD-301699.1	AAGGAUGAAGAGAGGCAUGUU	474	NM_001285406.1_226-246_s	226-246	AACAUGCCUCUCUUCAUCCUUUG	553	NM_001285406.1	224-246
AD-301700.1	AGGAUGAAGAGAGGCAUGUUU	475	NM_001285406.1_227-247_G21U_s	227-247	AAACAUGCCUCUCUUCAUCCUUU	554	NM_001285406.1	225-247
AD-301701.1	GGAUGAAGAGAGGCAUGUUGU	476	NM_001285406.1_228-248_G21U_s	228-248	ACAACATGCCUCUCUUCAUCCUU	861	NM_001285406.1	226-248
AD-301702.1	GAUGAAGAGAGGCAUGUUGGA	472	NM_011434.1_345-365_s	345-365	UCCAACAUGCCUCUCUUCAUCCU	551	NM_000454.4	304-326
AD-301703.1	AUGAAGAGAGGCAUGUUGGAU	477	NM_001285406.1_230-250_G21U_s	230-250	AUCCAACAUGCCUCUCUUCAUCC	556	NM_001285406.1	228-250
AD-301706.1	AAGAGAGGCAUGUUGGAGACU	478	NM_001285406.1_233-253_C21U_s	233-253	AGUCUCCAACAUGCCUCUCUUCA	557	NM_001285406.1	231-253
AD-301764.1	UGUGUCUAUUGAAGAUUCUGU	505	NM_000454.4_439-459_s	439-459	ACAGAATCUUCAAUAGACACAUC	862	NM_000454.4_437-459_as	437-459
AD-301765.1	GUGUCUAUUGAAGAUUCUGUU	506	NM_000454.4_440-460_G21U_s	440-460	AACAGAAUCUUCAAUAGACACAU	585	NM_000454.4_438-460_C1A_as	438-460
AD-301799.1	GAGACCAUUGCAUCAUUGGCU	509	NM_000454.4_474-494_C21U_s	474-494	AGCCAATGAUGCAAUGGUCUCCU	863	NM_000454.4_472-494_G1A_as	472-494
AD-301824.1	ACUGGUGGUCCAUGAAAAAGU	510	NM_000454.4_499-519_C21U_s	499-519	ACUUUUTCAUGGACCACCAGUGU	864	NM_000454.4_497-519_G1A_as	497-519
AD-301825.1	CUGGUGGUCCAUGAAAAAGCA	511	NM_000454.4_500-520_s	500-520	UGCUUUTUCAUGGACCACCAGUG	865	NM_000454.4_498-520_as	498-520
AD-301826.1	UGGUGGUCCAUGAAAAAGCAU	507	NM_001285406.1_353-373_G21U_s	353-373	AUGCUUTUUCAUGGACCACCAGU	866	NM_000454.4_499-521_C1A_as	351-373
AD-301827.1	GGUGGUCCAUGAAAAAGCAGA	508	NM_001285406.1_354-374_s	354-374	UCUGCUTUUUCAUGGACCACCAG	867	NM_000454.4_500-522_as	352-374
AD-301828.1	GUGGUCCAUGAAAAAGCAGAU	480	NM_001285406.1_355-375_s	355-375	AUCUGCTUUUUCAUGGACCACCA	868	NM_001285406.1	353-375

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
AD-301829.1	UGGUCCAUGAAAAAGCAGAUU	481	NM_001285406.1_356-376_G21U_s	356-376	AAUCUGCUUUUUCAUGGACCACC	560	NM_001285406.1	354-376
AD-301830.1	GGUCCAUGAAAAAGCAGAUGA	482	NM_001285406.1_357-377_s	357-377	UCAUCUGCUUUUUCAUGGACCAC	561	NM_001285406.1	355-377
AD-301843.1	GCAGAUGACUUGGGCAAAGGU	483	NM_001285406.1_370-390_s	370-390	ACCUUUGCCCAAGUCAUCUGCUU	562	NM_001285406.1	368-390
AD-301845.1	AGAUGACUUGGGCAAAGGUGU	484	NM_001285406.1_372-392_G21U_s	372-392	ACACCUUGCCCAAGUCAUCUGC	869	NM_001285406.1	370-392
AD-301846.1	GAUGACUUGGGCAAAGGUGGA	479	NM_011434.1_489-509_s	489-509	UCCACCUUGCCCAAGUCAUCUG	870	NM_011434.1	486-509
AD-301847.1	AUGACUUGGGCAAAGGUGGAA	450	NM_011434.1_490-510_s	490-510	UUCCACCUUGGCCCAAGUCAUCU	529	NM_011434.1	488-510
AD-301848.1	UGACUUGGGCAAAGGUGGAAA	451	NM_011434.1_491-511_s	491-511	UUUCCACCUUUGCCCAAGUCAUC	530	NM_011434.1	489-511
AD-301849.1	GACUUGGGCAAAGGUGGAAAU	452	NM_011434.1_492-512_s	492-512	AUUUCCACCUUUGCCCAAGUCAU	531	NM_011434.1	490-512
AD-301850.1	ACUUGGGCAAAGGUGGAAAUU	485	NM_001285406.1_377-397_G21U_s	377-397	AAUUUCCACCUUUGCCCAAGUCA	564	NM_001285406.1	375-397
AD-301852.1	UUGGGCAAAGGUGGAAAUGAA	103	NM_011434.1_495-515_s	495-515	UUCAUUTCCACCUUUGCCCAAGU	871	NM_011434.1	493-515
AD-301859.1	AAGGUGGAAAUGAAGAAAGUA	453	NM_011434.1_502-522_s	502-522	UACUUUCUUCAUUUCCACCUUUG	532	NM_011434.1	500-522
AD-301860.1	AGGUGGAAAUGAAGAAAGUAU	512	NM_000454.4_535-555_C21U_s	535-555	AUACUUTCUUCAUUUCCACCUUU	872	NM_000454.4_533-555_G1A_as	533-555
AD-301873.1	GAAAGUACAAAGACAGGAAAU	513	NM_000454.4_548-568_C21U_s	548-568	AUUUCCTGUCUUUGUACUUUCUU	873	NM_000454.4_546-568_G1A_as	546-568
AD-301874.1	AAAGUACAAAGACAGGAAACU	514	NM_000454.4_549-569_G21U_s	549-569	AGUUUCCUGUCUUUGUACUUUCU	593	NM_000454.4_547-569_C1A_as	547-569
AD-301875.1	AAGUACAAAGACAGGAAACGU	515	NM_000454.4_550-570_C21U_s	550-570	ACGUUUCCUGUCUUUGUACUUUC	594	NM_000454.4_548-570_G1A_as	548-570
AD-301876.1	AGUACAAAGACAGGAAACGCU	516	NM_000454.4_551-571_s	551-571	AGCGUUTCCUGUCUUUGUACUUU	874	NM_000454.4_549-571_as	549-571
AD-301877.1	GUACAAAGACAGGAAACGCUU	517	NM_000454.4_552-572_G21U_s	552-572	AAGCGUTTCCUGUCUUUGUACUU	875	NM_000454.4_550-572_C1A_as	550-572
AD-301878.1	UACAAAGACAGGAAACGCUGU	518	NM_000454.4_553-573_G21U_s	553-573	ACAGCGTUUCCUGUCUUUGUACU	876	NM_000454.4_551-573_C1A_as	551-573
AD-301880.1	CAAAGACAGGAAACGCUGGAA	519	NM_000454.4_555-575_s	555-575	UUCCAGCGUUUCCUGUCUUUGUA	598	NM_000454.4_553-575_as	553-575
AD-301901.1	GUCGUUUGGCUUGUGGUGUAA	522	NM_000454.4_576-596_s	576-596	UUACACCACAAGCCAAACGACUU	601	NM_000454.4_574-596_as	574-596
AD-301902.1	UCGUUUGGCUUGUGGUGUAAU	523	NM_000454.4_577-597_s	577-597	AUUACACCACAAGCCAAACGACU	602	NM_000454.4_575-597_as	575-597

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name	Antisense Sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Acession No. in Source Name
AD-301903.1	CGUUUGGCUUGUGGUGUAAUU	524	NM_000454.4_578-598_s	578-598	AAUUACACCACAAGCCAAACGAC	603	NM_000454.4_576-598_as	576-598
AD-301904.1	GUUUGGCUUGUGGUGUAAUUU	525	NM_000454.4_579-599_G21U_s	579-599	AAAUUACACCACAAGCCAAACGA	604	NM_000454.4_577-599_C1A_as	577-599
AD-301905.1	UUUGGCUUGUGGUGUAAUUGU	526	NM_000454.4_580-600_G21U_s	580-600	ACAAUUACACCACAAGCCAAACG	605	NM_000454.4_578-600_C1A_as	578-600
AD-301906.1	UUGGCUUGUGGUGUAAUUGGU	527	NM_000454.4_581-601_G21U_s	581-601	ACCAAUTACACCACAAGCCAAAC	877	NM_000454.4_579-601_C1A_as	579-601
AD-301907.1	UGGCUUGUGGUGUAAUUGGGA	520	NM_001285406.1_434-454_s	434-454	UCCCAATUACACCACAAGCCAAA	878	NM_001285406.1	432-454
AD-301908.1	GGCUUGUGGUGUAAUUGGGAU	521	NM_001285406.1_435-455_s	435-455	AUCCCAAUUACACCACAAGCCAA	600	NM_001285406.1	433-455
AD-301909.1	GCUUGUGGUGUAAUUGGGAUU	486	NM_001285406.1_436-456_C21U_s	436-456	AAUCCCAAUUACACCACAAGCCA	565	NM_001285406.1	434-456
AD-301910.1	CUUGUGGUGUAAUUGGGAUCU	487	NM_001285406.1_437-457_G21U_s	437-457	AGAUCCCAAUUACACCACAAGCC	566	NM_001285406.1	435-457
AD-301911.1	UUGUGGUGUAAUUGGGAUCGU	488	NM_001285406.1_438-458_C21U_s	438-458	ACGAUCCCAAUUACACCACAAGC	567	NM_001285406.1	436-458
AD-301912.1	UGUGGUGUAAUUGGGAUCGCU	489	NM_001285406.1_439-459_C21U_s	439-459	AGCGAUCCCAAUUACACCACAAG	568	NM_001285406.1	437-459
AD-301918.1	GUAAUUGGGAUCGCCCAAUAA	528	NM_000454.4_593-613_s	593-613	UUAUUGGGCGAUCCCAAUUACAC	607	NM_000454.4_591-613_as	591-613

Table 7. Modified Sense and Antisense Strand Sequences of Superoxide Dismutase 1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-301535.1	usgscaggGfcAfUfCfaucaauuucuL96	649	asGfsaaau(Tgn)gaugauGfcCfcugcascsu	880	AGUGCAGGGCAUCAUCAAUUUCG	807
AD-301536.1	gscsagggCfaUfCfAfucaauuucgaL96	650	usCfsgaaa(Tgn)ugaugaUfgCfccugcsasc	881	GUGCAGGGCAUCAUCAAUUUCGA	808
AD-301537.1	csasgggcAfuCfAfUfcaauuucgauL96	651	asUfscgaa(Agn)uugaugAfuGfcccugscsa	882	UGCAGGGCAUCAUCAAUUUCGAG	809
AD-301538.1	asgsggcaUfcAfUfCfaauuucgaguL96	652	asCfsucga(Agn)auugauGfaUfgcccusgsc	883	GCAGGGCAUCAUCAAUUUCGAGC	810
AD-301539.1	gsgsgcauCfaUfCfAfauuucgagcaL96	653	usGfscucg(Agn)aauugaUfgAfugcccsusg	884	CAGGGCAUCAUCAAUUUCGAGCA	811
AD-301540.1	gsgscaucAfuCfAfAfuuucgagcauL96	654	asUfsgcuc(Ggn)aaauugAfuGfaugccscsu	885	AGGGCAUCAUCAAUUUCGAGCAG	812

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-301542.1	csasucauCfaAfUfUfucgagcagaaL96	655	usUfscugc(Tgn)cgaaauUfgAfugaugscsc	886	GGCAUCAUCAAUUUCGAGCAGAA	813
AD-301543.1	asuscaucAfaUfUfUfcgagcagaauL96	656	asUfsucug(Cgn)ucgaaaUfuGfaugausgsc	887	GCAUCAUCAAUUUCGAGCAGAAG	814
AD-301544.1	uscsaucaAfuUfUfCfgagcagaaguL96	657	asCfsuucu(Ggn)cucgaaAfuUfgaugasusg	888	CAUCAUCAAUUUCGAGCAGAAGG	815
AD-301549.1	asasuuucGfaGfCfAfgaaggaaaguL96	612	asCfsuuuc(Cgn)uucugcUfcGfaaauusgsa	889	UCAAUUUCGAGCAGAAGGAAAGU	770
AD-301550.1	asusuucgAfgCfAfGfaaggaaaguaL96	213	usAfscuuu(Cgn)cuucugCfuCfgaaaususg	890	CAAUUUCGAGCAGAAGGAAAGUA	379
AD-301551.1	ususucgaGfcAfGfAfaggaaaguaaL96	255	usUfsacuu(Tgn)ccuucuGfcUfcgaaasusu	891	AAUUUCGAGCAGAAGGAAAGUAA	421
AD-301552.1	ususcgagCfaGfAfAfggaaaguaauL96	212	asUfsuacu(Tgn)uccuucUfgCfucgaasasu	892	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-301555.1	gsasgcagAfaGfGfAfaaguaauggaL96	613	usCfscauu(Agn)cuuuccUfuCfugcucsgsa	893	UCGAGCAGAAGGAAAGUAAUGGA	771
AD-301559.1	asgsaaggAfaAfGfUfaauggaccauL96	614	asUfsgguc(Cgn)auuacuUfuCfcuucusgsc	894	GCAGAAGGAAAGUAAUGGACCAG	772
AD-301560.1	gsasaggaAfaGfUfAfauggaccaguL96	615	asCfsuggu(Cgn)cauuacUfuUfccuucsusg	895	CAGAAGGAAAGUAAUGGACCAGU	773
AD-301562.1	asggsaaaGfuAfAfUfggaccagugaL96	616	usCfsacug(Ggn)uccauuuAfcUfuccususc	896	GAAGGAAAGUAAUGGACCAGUGA	774
AD-301563.1	gsgsaaagUfaAfUfGfgaccagugaaL96	617	usUfscacu(Ggn)guccauUfaCfuuuccsusu	897	AAGGAAAGUAAUGGACCAGUGAA	775
AD-301564.1	gsasaaguAfaUfGfGfaccagugaauL96	618	asUfsucac(Tgn)gguccaUfuAfcuuuccscsu	898	AGGAAAGUAAUGGACCAGUGAAG	776
AD-301565.1	asasaguaAfuGfGfAfccagugaaguL96	619	asCfsuuca(Cgn)uggucc AfuUfacuuuscsc	899	GGAAAGUAAUGGACCAGUGAAGG	777
AD-301566.1	asasguaaUfgGfAfCfcagugaaggUL96	620	asCfscuuc(Agn)cuggucCfaUfuacuususc	900	GAAAGUAAUGGACCAGUGAAGGU	778
AD-301567.1	asgsuaauGfgAfCfCfagugaaggUuL96	621	asAfsccuu(Cgn)acuggu CfcAfuuacususu	901	AAAGUAAUGGACCAGUGAAGGUG	779
AD-301569.1	usasauggAfcCfAfGfugaaggugUuL96	259	asAfscacc(Tgn)ucacugGfuCfcauuascsu	902	AGUAAUGGACCAGUGAAGGUGUG	425
AD-301579.1	asusuaaaGfgAfCfUfgacugaaggUL96	658	asCfscuuc(Agn)gucaguCfcUfuuaausgsc	903	GCAUUAAAGGACUGACUGAAGGC	816
AD-301593.1	usgsaaggCfcUfGfCfaugaauuccaL96	622	usGfsgaau(Cgn)caugcaGfgCfcuucasgsu	904	ACUGAAGGCCUGCAUGGAUUCCA	780
AD-301594.1	gsasaggcCfuGfCfAfuggauuccauL96	623	asUfsggaa(Tgn)ccaugcAfgGfccuucsasg	905	CUGAAGGCCUGCAUGGAUUCCAU	781
AD-301596.1	asgsgccuGfcAfUfGfgauuccauguL96	624	asCfsaugg(Agn)auccauGfcAfggccususc	906	GAAGGCCUGCAUGGAUUCCAUGU	782
AD-301597.1	gsgsccugCfaUfGfGfauuccauguuL96	625	asAfscaug(Ggn)aauccaUfgCfaggccsusu	907	AAGGCCUGCAUGGAUUCCAUGUU	783
AD-301598.1	gscscugcAfuGfGfAfuuccauguuuL96	626	asAfsacau(Ggn)gaauccAfuGfcaggcscsu	908	AGGCCUGCAUGGAUUCCAUGUUC	784
AD-301599.1	cscsugcaUfgGfAfUfuccauguucaL96	627	usGfsaaca(Tgn)ggaauccCfaUfgcaggscsc	909	GGCCUGCAUGGAUUCCAUGUUCA	785
AD-301600.1	csusgcauGfgAfUfUfccauguucauL96	628	asUfsgaac(Agn)uggaauCfcAfugcagsgsc	910	GCCUGCAUGGAUUCCAUGUUCAU	786
AD-301601.1	usgscaugGfaUfUfCfcauguucauuL96	265	asAfsugaa(Cgn)auggaaUfcCfaugcasgsg	911	CCUGCAUGGAUUCCAUGUUCAUC	431
AD-301612.1	csasuguuCfaUfGfAfguuuggagauL96	659	asUfscucc(Agn)aacucaUfgAfacaugsgsa	912	UCCAUGUUCAUGAGUUUGGAGAU	817

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-301615.1	gsusucauGfaGfUfUfuggagauaauL96	660	asUfsuauc(Tgn)ccaaacUfcAfugaacsasu	913	AUGUUCAUGAGUUUGGAGAUAAU	818
AD-301616.1	ususcaugAfgUfUfUfggagauaauaL96	661	usAfsuuau(Cgn)uccaaaCfuCfaugaascsa	914	UGUUCAUGAGUUUGGAGAUAAUA	819
AD-301619.1	asusgaguUfuGfGfAfgauaauacauL96	662	asUfsguau(Tgn)aucuccAfaAfcucausgsa	915	UCAUGAGUUUGGAGAUAAUACAG	820
AD-301648.1	ascscaguGfcAfGfGfuccucacuuuL96	209	asAfsagug(Agn)ggaccuGfcAfcuggusasc	916	GUACCAGUGCAGGUCCUCACUUU	375
AD-301649.1	cscsagugCfaGfGfUfccucacuuuaL96	241	usAfsaagu(Ggn)aggaccUfgCfacuggsusa	917	UACCAGUGCAGGUCCUCACUUUA	407
AD-301650.1	csasgugcAfgGfUfCfcucacuuuaaL96	220	usUfsaaag(Tgn)gaggacCfuGfcacugsgsu	918	ACCAGUGCAGGUCCUCACUUUAA	386
AD-301652.1	gsusgcagGfuCfCfUfcacuuuaauuL96	258	asAfsuuaa(Agn)gugaggAfcCfugcacsusg	919	CAGUGCAGGUCCUCACUUUAAUC	424
AD-301653.1	usgscaggUfcCfUfCfacuuuaaucuL96	205	asGfsauua(Agn)agugagGfaCfcugcascsu	920	AGUGCAGGUCCUCACUUUAAUCC	371
AD-301654.1	gscsagguCfcUfCfAfcuuuaauccuL96	263	asGfsgauu(Agn)aagugaGfgAfccugcsasc	921	GUGCAGGUCCUCACUUUAAUCCU	429
AD-301661.1	csuscacuUfuAfAfUfCfcucuauccaL96	216	usGfsgaua(Ggn)aggauuAfaAfgugagsgsa	922	UCCUCACUUUAAUCCUCUAUCCA	382
AD-301662.1	uscsacuuUfaAfUfCfCfucuauccauL96	248	asUfsggau(Agn)gaggauUfaAfagugasgsg	923	CCUCACUUUAAUCCUCUAUCCAG	414
AD-301663.1	csascuuuAfaUfCfCfUfcuauccagaL96	237	usCfsugga(Tgn)agaggaUfuAfaagugsasg	924	CUCACUUUAAUCCUCUAUCCAGA	403
AD-301688.1	ascsggugGfgCfCfAfaaggaugaauL96	879	asUfsucau(Cgn)cuuuggCfcCfaccgusgsu	925	ACACGGUGGGCCAAAGGAUGAAG	974
AD-301690.1	gsgsugggCfcAfAfAfggaugaagauL96	629	asUfscuuc(Agn)uccuuuGfgCfccaccsgsu	926	ACGGUGGGCCAAAGGAUGAAGAG	787
AD-301697.1	csasaaggAfuGfAfAfgagaggcauuL96	631	asAfsugcc(Tgn)cucuucAfuCfcuuugsgsc	927	GCCAAAGGAUGAAGAGAGGCAUG	789
AD-301699.1	asasggauGfaAfGfAfgaggcauguuL96	632	asAfscaug(Cgn)cucucuUfcAfuccuususg	928	CAAAGGAUGAAGAGAGGCAUGUU	790
AD-301700.1	asgsgaugAfaGfAfGfaggcauguuuL96	633	asAfsacau(Ggn)ccucucUfuCfauccususu	929	AAAGGAUGAAGAGAGGCAUGUUG	791
AD-301701.1	gsgsaugaAfgAfGfAfggcauguugL96	634	asCfsaaca(Tgn)gccucuCfuUfcauccsusu	930	AAGGAUGAAGAGAGGCAUGUUGG	792
AD-301702.1	gsasugaaGfaGfAfGfgcauguuggaL96	630	usCfscaac(Agn)ugccucUfcUfucaucscsu	931	AGGAUGAAGAGAGGCAUGUUGGA	788
AD-301703.1	asusgaagAfgAfGfGfcauguuggauL96	635	asUfsccaa(Cgn)augccuCfuCfuucauscsc	932	GGAUGAAGAGAGGCAUGUUGGAG	793
AD-301706.1	asasgagaGfgCfAfUfguuggagacuL96	636	asGfsucuc(Cgn)aacaugCfcUfcucuuscsa	933	UGAAGAGAGGCAUGUUGGAGACC	794
AD-301764.1	usgsugucUfaUfUfGfaagauucuguL96	663	asCfsagaa(Tgn)cuucaaUfaGfacacasusc	934	GAUGUGUCUAUUGAAGAUUCUGU	821
AD-301765.1	gsusgucuAfuUfGfAfagauucuguuL96	664	asAfscag(Agn)ucuucaAfuAfgacacsasu	935	AUGUGUCUAUUGAAGAUUCUGUG	822
AD-301799.1	gsasgaccAfuUfGfCfaucauuggcuL96	667	asGfsccaa(Tgn)gaugcaAfuGfgucucscsu	936	AGGAGACCAUUGCAUCAUUGGCC	825
AD-301824.1	ascsugguGfgUfCfCfaugaaaaaguL96	668	asCfsuuuu(Tgn)cauggaCfcAfccagusgsu	937	ACACUGGUGGUCCAUGAAAAAGC	826
AD-301825.1	csusggugGfuCfCfAfugaaaaagcaL96	669	usGfscuuu(Tgn)ucauggAfcCfaccagsusg	938	CACUGGUGGUCCAUGAAAAAGCA	827
AD-301826.1	usgsguggUfcCfAfUfgaaaaagcauL96	665	asUfsgcuu(Tgn)uucaugGfaCfcaccasgsu	939	ACUGGUGGUCCAUGAAAAAGCAU	823

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-301827.1	gsgsuggucCfcAfUfGfaaaaagcagaL96	666	usCfsugcu(Tgn)uuucauGfgAfccaccsasg	940	CUGGUGGUCCAUGAAAAAGCAGA	824
AD-301828.1	gsusggucCfaUfGfAfaaaagcagauL96	638	asUfscugc(Tgn)uuuucaUfgGfaccacscsa	941	UGGUGGUCCAUGAAAAAGCAGAU	796
AD-301829.1	usgsguccAfuGfAfAfaaagcagauuL96	639	asAfsucug(Cgn)uuuuuucAfuGfgaccascsc	942	GGUGGUCCAUGAAAAAGCAGAUG	797
AD-301830.1	gsgsuccaUfgAfAfAfaagcagaugaL96	640	usCfsaucu(Ggn)cuuuuuCfaUfggaccsasc	943	GUGGUCCAUGAAAAAGCAGAUGA	798
AD-301843.1	gscsagauGfaCfUfUfgggcaaagguL96	641	asCfscuuu(Ggn)cccaagUfcAfucugcsusu	944	AAGCAGAUGACUUGGGCAAAGGU	799
AD-301845.1	asgsaugaCfuUfGfGfgcaaaggugL96	642	asCfsaccu(Tgn)ugcccaAfgUfcaucusgsc	945	GCAGAUGACUUGGGCAAAGGUGG	800
AD-301846.1	gsasugacUfuGfGfGfcaaagguggaL96	637	usCfscacc(Tgn)uugcccAfaGfucaucsusg	946	CAGAUGACUUGGGCAAAGGUGGA	795
AD-301847.1	asusgacuUfgGfGfCfaaagguggaaL96	608	usUfsccac(Cgn)uuugcccCfaAfgucauscsu	947	AGAUGACUUGGGCAAAGGUGGAA	766
AD-301848.1	usgsacuuGfgGfCfAfaagguggaaaL96	609	usUfsucca(Cgn)cuuugcCfcAfagucasusc	948	GAUGACUUGGGCAAAGGUGGAAA	767
AD-301849.1	gsascuugGfgCfAfAfagguggaaauL96	610	asUfsuucc(Agn)ccuuugCfcCfaagucsasu	949	AUGACUUGGGCAAAGGUGGAAAU	768
AD-301850.1	ascsuuggGfcAfAfAfgguggaaauuL96	643	asAfsuuuc(Cgn)accuuuGfcCfcaaguscsa	950	UGACUUGGGCAAAGGUGGAAAUG	801
AD-301852.1	ususgggcAfaAfGfGfuggaaaugaaL96	269	usUfscauu(Tgn)ccaccuUfuGfcccaasgsu	951	ACUUGGGCAAAGGUGGAAAUGAA	435
AD-301859.1	asasggugGfaAfAfUfgaagaaaguaL96	611	usAfscuuu(Cgn)uucauuUfcCfaccuususg	952	CAAAGGUGGAAAUGAAGAAAGUA	769
AD-301860.1	asgsguggAfaAfUfGfaagaaaguauL96	670	asUfsacuu(Tgn)cuucauUfuCfcaccususu	953	AAAGGUGGAAAUGAAGAAAGUAC	828
AD-301873.1	gsasaaguAfcAfAfAfAfgacaggaaauL96	671	asUfsuucc(Tgn)gucuuuGfuAfcuuucsusu	954	AAGAAAGUACAAAGACAGGAAAC	829
AD-301874.1	asasaguaCfaAfAfGfacaggaaacuL96	672	asGfsuuuc(Cgn)ugucuuUfgUfacuuuscsu	955	AGAAAGUACAAAGACAGGAAACG	830
AD-301875.1	asasguacAfaAfGfAfcaggaaacguL96	673	asCfsguuu(Cgn)cugucuUfuGfuacuususc	956	GAAAGUACAAAGACAGGAAACGC	831
AD-301876.1	asgsuacaAfaGfAfCfaggaaacgcuL96	674	asGfscguu(Tgn)ccugucUfuUfuguacususu	957	AAAGUACAAAGACAGGAAACGCU	832
AD-301877.1	gsusacaaAfgAfCfAfggaaacgcuuL96	675	asAfsgcgu(Tgn)uccugucUfuUfuguacsusu	958	AAGUACAAAGACAGGAAACGCUG	833
AD-301878.1	usascaaaGfaCfAfGfgaaacgcuguL96	676	asCfsagcg(Tgn)uuccugUfcUfuuguascsu	959	AGUACAAAGACAGGAAACGCUGG	834
AD-301880.1	csasaagaCfaGfGfAfaacgcuggaaL96	677	usUfsccag(Cgn)guuuccUfgUfcuuugsusa	960	UACAAAGACAGGAAACGCUGGAA	835
AD-301901.1	gsuscguuUfgGfCfUfugugguguaaL96	680	usUfsacac(Cgn)acaagcCfaAfacgacsusu	961	AAGUCGUUUGGCUUGUGGUGUAA	838
AD-301902.1	uscsguuuGfgCfUfUfgugguguaauL96	681	asUfsuaca(Cgn)cacaagCfcAfaacgascsu	962	AGUCGUUUGGCUUGUGGUGUAAU	839
AD-301903.1	csgsuuugGfcUfUfGfugguguaauuL96	682	asAfsuuac(Agn)ccacaaGfcCfaaacgsasc	963	GUCGUUUGGCUUGUGGUGUAAUU	840
AD-301904.1	gsusuuggCfuUfGfUfgguguaauuuL96	683	asAfsauua(Cgn)accacaAfgCfcaaacgsga	964	UCGUUUGGCUUGUGGUGUAAUUG	841
AD-301905.1	ususuggcUfuGfUfGfguguaauugL96	684	asCfsaauu(Agn)caccacAfaGfccaaascsg	965	CGUUUGGCUUGUGGUGUAAUUGG	842
AD-301906.1	ususggcuUfgUfGfGfuguaauuggL96	685	asCfscaau(Tgn)acaccaCfaAfgccaasasc	966	GUUUGGCUUGUGGUGUAAUUGGG	843

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-301907.1	usgsgcuuGfuGfGfUfguaauugggaL96	678	usCfsccaa(Tgn)uacaccAfcAfagccasasa	967	UUUGGCUUGUGGUGUAAUUGGGA	836
AD-301908.1	gsgscuugUfgGfUfGfuaauugggauL96	679	asUfsccca(Agn)uuacacCfaCfaagccsasa	968	UUGGCUUGUGGUGUAAUUGGGAU	837
AD-301909.1	gscsuuguGfgUfGfUfaauugggauuL96	644	asAfsuccc(Agn)auuacaCfcAfcaagcscsa	969	UGGCUUGUGGUGUAAUUGGGAUC	802
AD-301910.1	csusugugGfuGfUfAfauugggaucuL96	645	asGfsaucc(Cgn)aauuacAfcCfacaagscsc	970	GGCUUGUGGUGUAAUUGGGAUCG	803
AD-301911.1	ususguggUfgUfAfAfuugggaucguL96	646	asCfsgauc(Cgn)caauuaCfaCfcacaasgsc	971	GCUUGUGGUGUAAUUGGGAUCGC	804
AD-301912.1	usgsugguGfuAfAfUfugggaucgcuL96	647	asGfscgau(Cgn)ccaauuAfcAfccacasasg	972	CUUGUGGUGUAAUUGGGAUCGCC	805
AD-301918.1	gsusaauuGfgGfAfUfcgcccaauaaL96	686	usUfsauug(Ggn)gcgaucCfcAfauuacsasc	973	GUGUAAUUGGGAUCGCCCAAUAA	844

Table 8. Superoxide Dismutase 1 *In Vitro* Single Dose Screens in Primary Cynomolgus Hepatocytes (PCH) cells

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-266859.1	1.6	0.3	23.5	4.1
AD-266997.1	1.2	0.4	15.3	4.3
AD-266992.1	0.8	0.1	11.4	1.5
AD-266903.1	6.5	0.8	81.9	8.0
AD-266891.1	7.4	1.7	81.4	9.4
AD-266996.1	10.8	7.1	78.4	6.0
AD-266893.1	1.7	1.0	29.0	5.0
AD-266898.1	3.5	2.1	60.5	7.6
AD-266886.1	4.2	0.9	77.1	23.0
AD-267072.1	7.9	2.0	104.4	22.5
AD-267067.1	25.1	2.0	110.8	14.4
AD-266791.1	1.3	0.3	11.8	2.0
AD-266789.1	11.4	1.7	85.0	5.2
AD-266861.1	1.9	0.3	33.6	1.9
AD-266856.1	2.3	0.4	27.0	1.4
AD-266899.1	7.8	0.9	65.6	4.9
AD-267000.1	1.3	0.2	18.9	2.3
AD-267071.1	1.9	0.5	26.1	4.9
AD-266895.1	6.6	0.4	58.3	4.2
AD-266888.1	3.8	0.7	51.4	16.5
AD-266817.1	7.7	1.8	102.7	26.6
AD-267083.1	5.1	2.0	77.8	17.5
AD-266862.1	1.9	0.3	34.4	2.7
AD-267002.1	2.1	0.3	33.3	2.9
AD-266816.1	1.6	0.4	19.6	3.1
AD-266857.1	1.6	0.2	21.8	1.6
AD-266902.1	3.9	0.6	51.7	5.0
AD-267086.1	2.8	0.1	39.0	4.4
AD-266785.1	4.6	0.4	56.5	3.5
AD-266897.1	2.7	0.4	34.1	4.4
AD-266896.1	17.2	2.1	73.2	9.5
AD-266858.1	2.8	0.6	49.9	12.5
AD-267084.1	5.8	1.7	80.7	26.6
AD-266815.1	9.6	1.2	76.0	4.2
AD-267007.1	1.6	0.4	24.1	3.0
AD-266855.1	1.2	0.3	14.0	1.5
AD-266901.1	3.3	1.1	38.8	3.2

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-266994.1	1.9	0.4	25.0	1.9
AD-266793.1	3.6	0.5	50.0	4.7
AD-266850.1	2.9	0.2	42.0	4.9
AD-266887.1	1.8	0.0	22.8	3.2
AD-266894.1	4.3	0.4	50.6	5.4
AD-266988.1	2.2	0.4	37.4	8.8
AD-267085.1	6.6	1.4	78.5	20.6
AD-266873.1	2.4	0.4	41.7	6.0
AD-266907.1	8.3	0.9	60.5	5.7
AD-266792.1	4.8	0.6	44.5	3.8
AD-266900.1	3.0	0.6	33.8	1.4
AD-266797.1	4.9	0.4	54.4	6.1
AD-266787.1	2.5	0.6	32.0	1.8
AD-266800.1	2.1	0.2	31.9	3.6
AD-266889.1	1.9	0.2	18.1	2.2
AD-266847.1	7.2	0.8	71.6	31.4
AD-266998.1	3.8	0.5	65.0	26.6
AD-266790.1	2.7	0.2	52.9	4.8
AD-266906.1	57.9	5.2	81.0	4.8
AD-266854.1	3.1	0.7	42.7	3.1
AD-266890.1	2.4	0.2	25.6	2.1
AD-266808.1	6.0	1.5	63.3	4.7
AD-266905.1	1.7	0.4	19.7	1.6
AD-267024.1	1.1	0.2	12.4	1.4
AD-266781.1	2.3	0.3	26.2	4.1
AD-266892.1	11.0	2.8	68.7	7.7
AD-266999.1	7.5	2.9	88.9	7.0
AD-266841.1	1.5	0.3	16.1	1.8
AD-266908.1	2.7	0.1	42.2	6.2
AD-267005.1	1.9	0.3	20.8	3.4
AD-266942.1	9.4	1.9	70.3	5.6
AD-135967.3	2.4	0.3	31.6	1.4
AD-266786.1	11.2	0.9	74.2	26.2
AD-267064.1	1.7	0.4	25.3	8.1
AD-266845.1	2.7	0.4	48.8	12.7
AD-266944.1	3.3	1.1	40.5	14.5
AD-267003.1	5.9	1.3	100.7	5.4

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-266860.1	5.9	0.7	75.1	7.3
AD-266990.1	1.4	0.0	13.7	1.5
AD-266853.1	2.2	0.3	31.7	3.7
AD-266782.1	1.8	0.3	44.6	7.5
AD-266962.1	2.1	0.6	33.3	4.2

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-267079.1	1.7	0.1	29.1	6.6
AD-266846.1	1.9	0.6	41.7	13.0
AD-266961.1	5.1	2.8	72.8	19.3
AD-267061.1	4.8	1.7	65.6	11.1

Table 9. Superoxide Dismutase 1 *In Vitro* Single Dose Screens in Primary Mouse Hepatocytes (PMH)

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-266859.1	76.4	36.1	51.0	22.7
AD-266997.1	60.0	20.9	51.8	4.2
AD-266992.1	29.1	34.9	24.7	27.9
AD-266903.1	48.1	13.8	47.6	16.2
AD-266891.1	36.1	7.9	47.3	7.4
AD-266996.1	67.0	28.3	67.5	25.6
AD-266893.1	0.5	0.5	21.0	23.7
AD-266898.1	58.6	17.9	89.5	56.8
AD-266886.1	65.0	7.4	77.3	33.0
AD-267072.1	4.8	9.2	104.7	66.4
AD-267067.1	64.5	9.7	98.0	55.1
AD-266791.1	2.2	2.8	52.1	7.0
AD-266789.1	43.9	6.8	52.7	7.8
AD-266861.1	60.5	23.4	50.9	7.5
AD-266856.1	52.6	18.3	51.7	6.7
AD-266899.1	72.0	12.2	58.1	14.2
AD-267000.1	67.3	14.4	65.8	16.5
AD-267071.1	0.3	0.1	27.9	7.2
AD-266895.1	63.4	11.5	79.5	13.7
AD-266888.1	2.1	0.6	75.6	27.2
AD-266817.1	82.2	47.3	113.0	37.6
AD-267083.1	89.1	42.8	122.5	36.2
AD-266862.1	63.3	29.2	53.6	12.6
AD-267002.1	46.8	9.0	64.5	9.9
AD-266816.1	0.9	0.4	49.2	0.5
AD-266857.1	58.6	14.7	64.5	6.3

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-266902.1	74.0	21.2	70.1	19.7
AD-267086.1	67.0	17.7	84.8	17.6
AD-266785.1	21.9	10.5	71.9	10.0
AD-266897.1	62.2	15.8	80.2	22.7
AD-266896.1	72.7	18.4	66.8	16.7
AD-266858.1	67.0	15.8	80.7	14.9
AD-267084.1	79.2	28.4	110.7	45.7
AD-266815.1	56.9	21.6	54.6	7.2
AD-267007.1	0.4	0.1	31.6	7.3
AD-266855.1	62.3	12.9	59.6	1.8
AD-266901.1	64.5	13.2	61.6	13.7
AD-266994.1	75.0	16.8	75.0	12.9
AD-266793.1	68.3	9.5	84.9	15.4
AD-266850.1	87.1	23.7	88.8	12.4
AD-266887.1	5.6	3.0	96.5	10.9
AD-266894.1	7.8	5.7	97.4	7.4
AD-266988.1	71.6	14.6	86.0	11.7
AD-267085.1	17.8	11.0	116.2	52.3
AD-266873.1	0.1	0.0	21.6	5.2
AD-266907.1	48.3	13.4	49.6	2.9
AD-266792.1	55.4	16.5	65.4	16.5
AD-266900.1	63.9	20.6	69.0	5.7
AD-266797.1	64.6	20.7	72.8	11.8
AD-266787.1	41.6	8.9	78.8	7.8
AD-266800.1	83.6	21.8	86.3	11.2
AD-266889.1	0.8	0.3	80.4	11.7

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-266847.1	78.3	16.4	104.9	34.1
AD-266998.1	79.2	23.4	114.2	52.0
AD-266790.1	38.1	7.4	54.4	15.0
AD-266906.1	44.1	8.5	49.5	4.1
AD-266854.1	57.1	20.8	53.8	5.8
AD-266890.1	4.8	3.7	48.7	3.0
AD-266808.1	72.0	18.8	74.9	17.7
AD-266905.1	72.0	12.3	71.2	3.1
AD-267024.1	0.6	0.3	30.8	4.4
AD-266781.1	73.5	13.8	88.8	14.6
AD-266892.1	76.1	15.8	90.7	29.4
AD-266999.1	96.0	59.3	114.2	44.5
AD-266841.1	7.7	4.4	42.4	13.5
AD-266908.1	44.5	4.9	50.5	16.1
AD-267005.1	2.6	1.4	48.4	10.5
AD-266942.1	1.2	0.6	47.2	11.7

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-135967.3	0.2	0.1	38.8	27.3
AD-266786.1	62.0	14.7	93.1	40.3
AD-267064.1	71.8	15.9	70.7	11.3
AD-266845.1	75.7	27.7	99.4	61.4
AD-266944.1	0.6	0.2	72.2	38.7
AD-267003.1	118.9	75.9	156.4	51.6
AD-266860.1	39.3	8.1	24.6	16.1
AD-266990.1	38.7	9.2	21.2	14.6
AD-266853.1	37.8	37.7	15.2	17.2
AD-266782.1	38.6	32.7	41.6	34.4
AD-266962.1	0.4	0.2	28.8	19.9
AD-267079.1	0.2	0.1	20.1	23.0
AD-266846.1	62.8	8.1	85.3	66.2
AD-266961.1	40.3	20.5	59.0	11.0
AD-267061.1	87.4	31.4	110.3	49.9

Table 10. Superoxide Dismutase 1 *In Vitro* Single Dose Screens in Primary Cynomolgus Hepatocytes (PCH) cells

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-135962.1	83.19	8.92	101.60	7.55
AD-135963.1	45.58	1.94	98.64	9.66
AD-135964.1	5.28	0.63	49.17	5.26
AD-135967.5	4.25	0.39	37.19	3.98
AD-135974.3	5.42	1.40	45.31	3.47
AD-266788.1	3.93	0.78	37.67	4.27
AD-266789.2	26.33	3.94	96.69	5.67
AD-266790.2	6.26	0.53	44.79	2.51
AD-266791.3	2.20	0.29	24.12	3.18
AD-266794.1	12.48	1.11	74.79	3.70
AD-266798.1	11.78	1.66	66.55	9.45

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-266799.1	8.19	1.19	70.59	4.84
AD-266801.1	13.29	1.01	87.14	4.40
AD-266802.1	19.56	4.86	74.12	6.09
AD-266803.1	4.12	0.71	54.10	12.04
AD-266804.1	7.57	1.06	75.49	7.11
AD-266805.1	79.23	3.60	91.30	7.81
AD-266806.1	18.66	0.31	82.02	4.98
AD-266808.2	9.95	1.04	81.05	4.63
AD-266832.1	43.37	3.77	99.19	3.54
AD-266834.1	4.39	1.02	36.56	8.26
AD-266836.1	74.92	2.31	91.29	5.81

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-266837.1	28.04	3.97	101.92	7.61
AD-266838.1	3.40	0.72	32.93	1.02
AD-266839.1	7.64	1.23	61.20	5.51
AD-266840.1	4.65	0.80	51.48	7.19
AD-266841.3	2.35	0.51	20.69	3.24
AD-266886.2	5.26	1.22	60.65	4.88
AD-266887.3	2.21	0.16	36.90	7.14
AD-266888.2	4.78	0.28	49.47	1.84
AD-266890.3	6.38	1.33	34.83	4.96
AD-266891.2	13.25	0.53	79.79	5.06
AD-266892.2	20.45	3.03	82.90	5.65
AD-266899.2	13.48	2.79	75.57	6.33
AD-266900.3	5.86	1.58	40.45	2.63
AD-266901.2	4.08	1.07	46.32	3.22
AD-266928.1	87.89	3.19	104.83	10.57
AD-266934.1	10.50	2.75	89.20	1.11
AD-266936.1	5.20	1.59	57.85	12.38
AD-266938.1	5.76	0.67	55.29	3.26
AD-266939.1	3.47	0.62	37.20	7.60
AD-266940.1	8.36	1.54	83.30	13.87
AD-266941.1	9.85	1.24	81.42	3.29
AD-266943.1	17.56	2.41	88.20	4.40
AD-267035.1	21.32	1.33	87.91	4.72
AD-267058.1	4.60	0.36	45.53	3.39
AD-267059.1	5.06	0.87	45.02	6.93
AD-267060.1	23.86	2.69	108.98	16.46
AD-267073.1	11.04	1.93	83.91	9.60
AD-267075.1	15.92	2.72	187.79	125.27
AD-267076.1	63.80	5.42	87.64	8.46
AD-267118.1	24.21	1.80	88.65	9.90
AD-267119.1	54.00	4.01	97.77	3.05
AD-267120.1	14.75	0.67	89.26	7.34
AD-267121.1	8.76	1.55	77.47	8.50
AD-267122.1	16.43	1.70	89.44	4.75
AD-295644.1	50.36	7.62	96.21	5.50
AD-295645.1	6.17	1.20	64.53	6.70
AD-295646.1	11.96	1.00	73.74	2.20
AD-295647.1	13.07	3.08	97.30	3.11
AD-295648.1	15.63	0.98	88.39	8.08
AD-295649.1	2.44	0.75	41.73	17.58
AD-295651.1	2.73	1.12	34.65	3.73
AD-295652.1	4.15	0.60	36.36	2.77
AD-295653.1	19.69	2.24	76.48	3.43
AD-295661.1	82.29	10.31	109.30	4.05
AD-295685.1	96.98	6.95	107.38	2.09
AD-295688.1	29.20	1.19	90.67	7.33
AD-295689.1	83.92	12.63	96.81	3.43
AD-295692.1	4.09	1.47	39.47	3.40
AD-295795.1	90.87	13.20	91.02	8.08
AD-295796.1	93.36	6.57	101.37	9.46
AD-295827.1	36.07	4.64	84.17	4.85
AD-295828.1	59.84	2.83	105.17	14.92
AD-295831.1	89.14	4.03	109.23	12.98

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-295856.1	12.43	2.28	74.44	6.83
AD-295857.1	14.73	1.83	73.35	5.77
AD-295858.1	4.41	0.68	43.87	2.79
AD-295867.1	72.40	3.25	105.71	1.62
AD-295868.1	18.79	1.83	92.79	5.86
AD-295869.1	16.91	2.13	86.65	12.21
AD-295870.1	13.84	1.24	80.22	4.93
AD-295871.1	8.36	1.51	84.76	7.34
AD-295872.1	12.59	2.47	67.76	2.99
AD-295874.1	9.68	1.33	86.08	14.84

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-295879.1	26.38	1.68	102.12	3.87
AD-295880.1	56.67	5.40	92.87	7.90
AD-295897.1	7.20	2.16	72.12	5.62
AD-295898.1	7.50	1.96	66.17	3.69
AD-295899.1	18.36	1.08	75.37	6.79
AD-295900.1	34.17	4.15	82.75	4.97
AD-295901.1	78.50	9.63	99.36	8.09
AD-295902.1	39.46	0.94	85.36	8.06
AD-295904.1	81.60	3.70	122.37	38.87

Table 11. Superoxide Dismutase 1 *In Vitro* Single Dose Screens in Primary Cynomolgus Hepatocytes (PCH) cells

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-301535.1	98.03	4.84	103.16	4.23
AD-301536.1	13.08	3.95	91.41	4.56
AD-301537.1	90.15	2.62	98.10	3.83
AD-301538.1	78.20	1.84	99.10	2.94
AD-301539.1	17.73	1.91	93.38	5.78
AD-301540.1	4.00	1.11	18.66	3.72
AD-301542.1	4.10	0.15	16.46	1.70
AD-301543.1	19.93	2.34	42.34	2.20
AD-301544.1	46.92	1.85	91.07	7.70
AD-301549.1	7.47	2.11	36.82	2.61

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-301550.1	81.19	4.70	93.06	2.17
AD-301551.1	12.82	2.60	59.20	4.04
AD-301552.1	4.02	0.81	11.54	3.27
AD-301555.1	16.41	2.27	79.37	2.67
AD-301559.1	63.74	4.62	86.68	6.17
AD-301560.1	26.30	2.05	81.69	5.23
AD-301562.1	11.59	0.41	62.13	4.15
AD-301563.1	13.26	3.63	82.33	8.84
AD-301564.1	8.00	2.00	44.21	2.37
AD-301565.1	93.58	18.36	91.71	5.14

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-301566.1	91.41	6.74	94.53	6.85
AD-301567.1	81.95	2.20	92.89	1.58
AD-301569.1	13.38	2.26	85.15	7.45
AD-301579.1	98.66	3.16	93.09	6.57
AD-301593.1	92.38	3.29	98.75	2.28
AD-301594.1	7.28	0.98	64.65	3.75
AD-301596.1	100.90	2.95	101.04	4.27
AD-301597.1	9.98	1.83	67.09	2.22
AD-301598.1	4.24	0.87	27.83	2.99
AD-301599.1	9.11	0.46	66.87	2.62
AD-301600.1	8.37	0.52	65.13	1.96
AD-301601.1	27.44	1.54	74.81	1.51
AD-301612.1	105.54	2.23	101.38	3.70
AD-301615.1	86.32	2.18	93.33	4.73
AD-301616.1	96.28	2.34	95.39	2.41
AD-301619.1	56.80	2.91	76.46	3.60
AD-301648.1	7.79	0.88	77.88	5.67
AD-301649.1	12.76	1.77	70.13	3.81
AD-301650.1	53.37	7.81	81.48	2.45
AD-301652.1	22.62	0.96	63.95	3.43
AD-301653.1	52.02	3.26	88.05	6.54
AD-301654.1	47.36	2.57	95.09	4.89
AD-301661.1	43.53	4.13	85.28	4.94
AD-301662.1	47.23	5.69	88.54	3.58
AD-301663.1	5.86	0.94	48.62	2.08
AD-301688.1	91.87	1.85	98.28	4.04
AD-301690.1	88.47	3.32	98.93	2.85

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-301697.1	4.24	0.46	34.14	5.74
AD-301699.1	4.18	0.12	16.49	3.39
AD-301700.1	10.31	1.39	53.80	6.12
AD-301701.1	14.10	2.33	87.21	3.78
AD-301702.1	37.88	3.57	99.22	6.26
AD-301703.1	87.54	6.72	96.03	1.28
AD-301706.1	75.02	2.08	94.98	5.30
AD-301764.1	106.66	3.34	104.25	4.60
AD-301765.1	92.11	4.56	97.95	4.52
AD-301799.1	85.29	2.57	97.06	2.86
AD-301824.1	51.16	1.47	92.79	4.17
AD-301825.1	41.28	3.29	83.80	4.54
AD-301826.1	78.25	14.04	88.09	3.75
AD-301827.1	83.51	5.58	98.20	7.25
AD-301828.1	5.26	0.41	39.91	3.31
AD-301829.1	7.69	0.33	39.97	1.79
AD-301830.1	21.72	1.98	91.45	6.31
AD-301843.1	26.53	2.76	89.45	3.20
AD-301845.1	19.80	4.82	93.62	7.53
AD-301846.1	22.54	3.19	94.07	2.15
AD-301847.1	89.51	1.30	99.77	2.02
AD-301848.1	99.23	4.92	100.84	7.65
AD-301849.1	5.32	0.70	30.99	3.27
AD-301850.1	88.76	2.72	94.06	3.16
AD-301852.1	4.61	0.15	20.03	3.42
AD-301859.1	7.06	0.40	44.19	1.61

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-301860.1	5.80	1.55	33.00	5.38
AD-301873.1	93.07	4.80	95.72	2.66
AD-301874.1	94.25	5.82	99.41	6.79
AD-301875.1	97.60	6.57	98.62	4.21
AD-301876.1	38.17	1.34	90.73	3.16
AD-301877.1	48.77	2.53	93.26	2.44
AD-301878.1	51.91	2.81	89.98	4.17
AD-301880.1	36.41	2.32	78.14	2.74
AD-301901.1	98.04	5.09	98.26	3.62
AD-301902.1	89.05	1.95	100.83	2.70
AD-301903.1	95.54	5.42	102.78	3.31

	Dose - Unit		Dose - Unit	
	10 - nM		0.1 - nM	
Duplex	**Avg**	**SD**	**Avg**	**SD**
AD-301904.1	96.36	5.22	97.39	2.37
AD-301905.1	90.02	3.46	97.35	2.27
AD-301906.1	77.52	1.57	99.69	6.26
AD-301907.1	82.84	3.43	91.36	4.32
AD-301908.1	81.82	2.51	95.34	6.79
AD-301909.1	61.89	1.32	89.93	3.70
AD-301910.1	70.37	2.00	95.73	4.15
AD-301911.1	12.59	1.24	84.00	5.08
AD-301912.1	53.89	4.10	97.43	4.16
AD-301918.1	69.96	8.26	91.14	5.80

Example 2. Design, Synthesis and *In Vitro* Screening of Additional dsRNA Duplexes

Based on the results provided in Example 1, additional siRNAs targeting identified hot spots in SOD1 mRNA were designed, synthesized and prepared using methods known in the art and described above in Example 1.

Detailed lists of the additional unmodified SOD1 sense and antisense strand nucleotide sequences are shown in Table 12. Detailed lists of the modified SOD1 sense and antisense strand nucleotide sequences are shown in Table 13.

Single dose screens of the additional agents were performed by transfection. Experiments were performed at 50 mM, 10nM, 1 nM and 0.1nM in primary cynomolgus hepatocytes (PCH) or neuroblastoma Be(2)C cells.

Total RNA isolation was performed using DYNABEADS. Briefly, cells were lysed in 10µl of Lysis/Binding Buffer containing 3 µL of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 3µL) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 12µL RT mixture was added to each well, as described below.

For cDNA synthesis, a master mix of 1.5µl 10X Buffer, 0.6µl 10X dNTPs, 1.5µl Random primers, 0.75µl Reverse Transcriptase, 0.75µl RNase inhibitor and 9.9µl of H2O per reaction were

added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37°C for 2 hours. Following this, the plates were agitated at 80°C for 8 minutes.

RT-qPCR was performed as described above and relative fold change was calculated as described above.

The results of the transfection assays in PCH cells are shown in Table 14 and the results of the transfection assays in BE(2)C cells are shown in Table 15, and illustrated as stacked bar graphs in Figures 12A-12H (as mapped to NM_00454.6).

Table 12. Unmodified Sense and Antisense Strand Sequences of Superoxide Dismutase 1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Acession No. NM_		Antisense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Acession No. NM_	
			001285406.1	000454.4			001285406.1	000454.5
AD-1321202.1	UCAUCAAUUUCGAGCAGAAGU	499	53-73	201-221	ACUUCUGCUCGAAAUUGAUGAUG	578	51-73	199-221
AD-1321203.1	CAUCAAUUUCGAGCAGAAGGA	975	54-74	202-222	ACCUUCUGCUCGAAAUUGAUGAU	1009	52-74	200-222
AD-1321204.1	AUCAAUUUCGAGCAGAAGGAA	63	55-75	203-223	UUCCTUCUGCUCGAAAUUGAUGA	1010	53-75	201-223
AD-1321205.1	UCAAUUUCGAGCAGAAGGAAA	104	56-76	204-224	UUUCCUTCUGCUCGAAAUUGAUG	1011	54-76	202-224
AD-1321206.1	CAAUUUCGAGCAGAAGGAAAU	84	57-77	205-225	AUUUCCTUCUGCUCGAAAUUGAU	1012	55-77	203-225
AD-1321207.1	AAUUUCGAGCAGAAGGAAAGU	454	58-78	206-226	ACUUTCCUUCUGCUCGAAAUUGA	1013	56-78	204-226
AD-1321208.1	AUUUCGAGCAGAAGGAAAGUA	47	59-79	207-227	UACUTUCCUUCUGCUCGAAAUUG	1014	57-79	205-227
AD-1321209.1	UUUCGAGCAGAAGGAAAGUAA	89	60-80	208-228	UUACTUTCCUUCUGCUCGAAAUU	1015	58-80	206-228
AD-1321210.1	UUCGAGCAGAAGGAAAGUAAU	46	61-81	209-229	AUUACUTUCCUUCUGCUCGAAAU	850	59-81	207-229
AD-1321211.1	UCGAGCAGAAGGAAAGUAAUU	81	62-82	210-230	AAUUACTUUCCUUCUGCUCGAAA	1016	60-82	208-230
AD-1321212.1	CGAGCAGAAGGAAAGUAAUGU	73	63-83	211-231	ACAUUACUUUCCUUCUGCUCGAA	156	61-83	209-231
AD-1321213.1	GAGCAGAAGGAAAGUAAUGGA	455	64-84	212-232	ACCAUUACUUUCCUUCUGCUCGA	1017	62-84	210-232
AD-1321214.1	AGCAGAAGGAAAGUAAUGGAU	976	65-85	213-233	AUCCAUUACUUUCCUUCUGCUCG	1018	63-85	211-233
AD-1321215.1	GCAGAAGGAAAGUAAUGGACU	977	66-86	214-234	AGUCCATUACUUUCCUUCUGCUC	1019	64-86	212-234
AD-1321216.1	CAGAAGGAAAGUAAUGGACCA	83	67-87	215-235	UGGUCCAUUACUUUCCUUCUGCU	166	65-87	213-235
AD-1321217.1	AGAAGGAAAGUAAUGGACCAU	456	68-88	216-236	AUGGTCCAUUACUUUCCUUCUGC	1020	66-88	214-236
AD-1321218.1	GAAGGAAAGUAAUGGACCAGU	457	69-89	217-237	ACUGGUCCAUUACUUUCCUUCUG	536	67-89	215-237
AD-1321219.1	AAGGAAAGUAAUGGACCAGUU	85	70-90	218-238	AACUGGTCCAUUACUUUCCUUCU	1021	68-90	216-238
AD-1321220.1	AGGAAAGUAAUGGACCAGUGA	458	71-91	219-239	UCACTGGUCCAUUACUUUCCUUC	1022	69-91	217-239
AD-1321221.1	GGAAAGUAAUGGACCAGUGAA	459	72-92	220-240	UUCACUGGUCCAUUACUUUCCUU	538	70-92	218-240
AD-1321222.1	GAAAGUAAUGGACCAGUGAAU	460	73-93	221-241	AUUCACTGGUCCAUUACUUUCCU	851	71-93	219-241
AD-1321223.1	AAAGUAAUGGACCAGUGAAGU	461	74-94	222-242	ACUUCACUGGUCCAUUACUUUCC	540	72-94	220-242
AD-1321224.1	AAGUAAUGGACCAGUGAAGGU	462	75-95	223-243	ACCUUCACUGGUCCAUUACUUUC	541	73-95	221-243
AD-1321225.1	AGUAAUGGACCAGUGAAGGUU	463	76-96	224-244	AACCTUCACUGGUCCAUUACUUU	1023	74-96	222-244
AD-1321226.1	GUAAUGGACCAGUGAAGGUGU	978	77-97	225-245	ACACCUUCACUGGUCCAUUACUU	1024	75-97	223-245

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Acession No. NM_		Antisense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Acession No. NM_	
			001285406.1	000454.4			001285406.1	000454.5
AD-1321227.1	UAAUGGACCAGUGAAGGUGUU	93	78-98	226-246	AACACCUUCACUGGUCCAUUACU	176	76-98	224-246
AD-1321228.1	AAUGGACCAGUGAAGGUGUGU	979	79-99	227-247	ACACACCUUCACUGGUCCAUUAC	1025	77-99	225-247
AD-1321229.1	GUACCAGUGCAGGUCCUCACU	980	173-193	321-341	AGUGAGGACCUGCACUGGUACAG	1026	171-193	319-341
AD-1321230.1	UACCAGUGCAGGUCCUCACUU	981	174-194	322-342	AAGUGAGGACCUGCACUGGUACA	1027	172-194	320-342
AD-1321231.1	ACCAGUGCAGGUCCUCACUUU	43	175-195	323-343	AAAGTGAGGACCUGCACUGGUAC	1028	173-195	321-343
AD-1321232.1	CCAGUGCAGGUCCUCACUUUA	75	176-196	324-344	UAAAGUGAGGACCUGCACUGGUA	158	174-196	322-344
AD-1321233.1	CAGUGCAGGUCCUCACUUUAA	54	177-197	325-345	UUAAAGTGAGGACCUGCACUGGU	857	175-197	323-345
AD-1321234.1	AGUGCAGGUCCUCACUUUAAU	86	178-198	326-346	AUUAAAGUGAGGACCUGCACUGG	169	176-198	324-346
AD-1321235.1	GUGCAGGUCCUCACUUUAAUU	92	179-199	327-347	AAUUAAAGUGAGGACCUGCACUG	175	177-199	325-347
AD-1321236.1	UGCAGGUCCUCACUUUAAUCU	39	180-200	328-348	AGAUUAAAGUGAGGACCUGCACU	122	178-200	326-348
AD-1321237.1	GCAGGUCCUCACUUUAAUCCU	97	181-201	329-349	AGGAUUAAAGUGAGGACCUGCAC	180	179-201	327-349
AD-1321238.1	CAGGUCCUCACUUUAAUCCUU	41	182-202	330-350	AAGGAUUAAAGUGAGGACCUGCA	124	180-202	328-350
AD-1321239.1	AGGUCCUCACUUUAAUCCUCU	76	183-203	331-351	AGAGGAUUAAAGUGAGGACCUGC	159	181-203	329-351
AD-1321240.1	GGUCCUCACUUUAAUCCUCUA	53	184-204	332-352	UAGAGGAUUAAAGUGAGGACCUG	136	182-204	330-352
AD-1321241.1	GUCCUCACUUUAAUCCUCUAU	65	185-205	333-353	AUAGAGGAUUAAAGUGAGGACCU	148	183-205	331-353
AD-1321242.1	UCCUCACUUUAAUCCUCUAUU	64	186-206	334-354	AAUAGAGGAUUAAAGUGAGGACC	147	184-206	332-354
AD-1321243.1	CCUCACUUUAAUCCUCUAUCU	42	187-207	335-355	AGAUAGAGGAUUAAAGUGAGGAC	125	185-207	333-355
AD-1321244.1	CUCACUUUAAUCCUCUAUCCA	50	188-208	336-356	AGGAUAGAGGAUUAAAGUGAGGA	1029	186-208	334-356
AD-1321245.1	UCACUUUAAUCCUCUAUCCAU	82	189-209	337-357	AUGGAUAGAGGAUUAAAGUGAGG	165	187-209	335-357
AD-1321246.1	CACUUUAAUCCUCUAUCCAGA	71	190-210	338-358	ACUGGAUAGAGGAUUAAAGUGAG	1030	188-210	336-358
AD-1321247.1	ACUUUAAUCCUCUAUCCAGAA	982	191-211	339-359	UUCUGGAUAGAGGAUUAAAGUGA	1031	189-211	337-359
AD-1321248.1	GUGGGCCAAAGGAUGAAGAGA	983	218-238	366-386	UCUCTUCAUCCUUUGGCCCACCG	1032	216-238	364-386
AD-1321249.1	UGGGCCAAAGGAUGAAGAGAU	984	219-239	367-387	AUCUCUTCAUCCUUUGGCCCACC	1033	217-239	365-387
AD-1321250.1	GGGCCAAAGGAUGAAGAGAGU	985	220-240	368-388	ACUCTCTUCAUCCUUUGGCCCAC	1034	218-240	366-388
AD-1321251.1	GGCCAAAGGAUGAAGAGAGGU	986	221-241	369-389	ACCUCUCUUCAUCCUUUGGCCCA	1035	219-241	367-389
AD-1321252.1	GCCAAAGGAUGAAGAGAGGCA	987	222-242	370-390	UGCCTCTCUUCAUCCUUUGGCCC	1036	220-242	368-390

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Acession No. NM_		Antisense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Acession No. NM_	
			001285406.1	000454.4			001285406.1	000454.5
AD-1321253.1	CCAAAGGAUGAAGAGAGGCAU	988	223-243	371-391	AUGCCUCUCUUCAUCCUUUGGCC	1037	221-243	369-391
AD-1321254.1	CAAAGGAUGAAGAGAGGCAUU	473	224-244	372-392	AAUGCCTCUCUUCAUCCUUUGGC	860	222-244	370-392
AD-1321255.1	AAAGGAUGAAGAGAGGCAUGU	989	225-245	373-393	ACAUGCCUCUCUUCAUCCUUUGG	1038	223-245	371-393
AD-1321256.1	AAGGAUGAAGAGAGGCAUGUU	474	226-246	374-394	AACATGCCUCUCUUCAUCCUUUG	1039	224-246	372-394
AD-1321257.1	AGGAUGAAGAGAGGCAUGUUU	475	227-247	375-395	AAACAUGCCUCUCUUCAUCCUUU	554	225-247	373-395
AD-1321258.1	GGAUGAAGAGAGGCAUGUUGU	476	228-248	376-396	ACAACAUGCCUCUCUUCAUCCUU	555	226-248	374-396
AD-1321259.1	GAUGAAGAGAGGCAUGUUGGA	472	229-249	377-397	ACCAACAUGCCUCUCUUCAUCCU	1040	227-249	375-397
AD-1321260.1	AUGAAGAGAGGCAUGUUGGAU	477	230-250	378-398	AUCCAACAUGCCUCUCUUCAUCC	556	228-250	376-398
AD-1321261.1	UGAAGAGAGGCAUGUUGGAGA	990	231-251	379-399	ACUCCAACAUGCCUCUCUUCAUC	1041	229-251	377-399
AD-1321262.1	GAAGAGAGGCAUGUUGGAGAU	102	232-252	380-400	AUCUCCAACAUGCCUCUCUUCAU	185	230-252	378-400
AD-1321263.1	AAGAGAGGCAUGUUGGAGACU	478	233-253	381-401	AGUCTCCAACAUGCCUCUCUUCA	1042	231-253	379-401
AD-1321264.1	AGAGAGGCAUGUUGGAGACUU	991	234-254	382-402	AAGUCUCCAACAUGCCUCUCUUC	1043	232-254	380-402
AD-1321265.1	GAGAGGCAUGUUGGAGACUUU	992	235-255	383-403	AAAGTCTCCAACAUGCCUCUCUU	1044	233-255	381-403
AD-1321266.1	AGAGGCAUGUUGGAGACUUGU	993	236-256	384-404	ACAAGUCUCCAACAUGCCUCUCU	1045	234-256	382-404
AD-1321267.1	GCAGAUGACUUGGGCAAAGGU	483	370-390	518-538	ACCUTUGCCCAAGUCAUCUGCUU	1046	368-390	516-538
AD-1321268.1	CAGAUGACUUGGGCAAAGGUU	994	371-391	519-539	AACCUUUGCCCAAGUCAUCUGCU	1047	369-391	517-539
AD-1321269.1	AGAUGACUUGGGCAAAGGUGU	484	372-392	520-540	ACACCUUUGCCCAAGUCAUCUGC	563	370-392	518-540
AD-1321270.1	GAUGACUUGGGCAAAGGUGGA	479	373-393	521-541	ACCACCUUUGCCCAAGUCAUCUG	1048	371-393	519-541
AD-1321271.1	AUGACUUGGGCAAAGGUGGAA	450	374-394	522-542	UUCCACCUUUGCCCAAGUCAUCU	529	372-394	520-542
AD-1321272.1	UGACUUGGGCAAAGGUGGAAA	451	375-395	523-543	UUUCCACCUUUGCCCAAGUCAUC	530	373-395	521-543
AD-1321273.1	GACUUGGGCAAAGGUGGAAAU	452	376-396	524-544	AUUUCCACCUUUGCCCAAGUCAU	531	374-396	522-544
AD-1321274.1	ACUUGGGCAAAGGUGGAAAUU	485	377-397	525-545	AAUUTCCACCUUUGCCCAAGUCA	1049	375-397	523-545
AD-1321275.1	CUUGGGCAAAGGUGGAAAUGA	995	378-398	526-546	ACAUUUCCACCUUUGCCCAAGUC	1050	376-398	524-546
AD-1321276.1	UUGGGCAAAGGUGGAAAUGAA	103	379-399	527-547	UUCATUTCCACCUUUGCCCAAGU	1051	377-399	525-547
AD-1321277.1	UGGGCAAAGGUGGAAAUGAAU	996	380-400	528-548	AUUCAUTUCCACCUUUGCCCAAG	1052	378-400	526-548
AD-1321278.1	GGGCAAAGGUGGAAAUGAAGA	997	381-401	529-549	ACUUCAUUUCCACCUUUGCCCAA	1053	379-401	527-549

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Acession No. NM_		Antisense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Acession No. NM_	
			001285406.1	000454.4			001285406.1	000454.5
AD-1321279.1	GGCAAAGGUGGAAAUGAAGAA	998	382-402	530-550	UUCUTCAUUUCCACCUUUGCCCA	1054	380-402	528-550
AD-1321280.1	GCAAAGGUGGAAAUGAAGAAA	999	383-403	531-551	UUUCTUCAUUUCCACCUUUGCCC	1055	381-403	529-551
AD-1321281.1	CAAAGGUGGAAAUGAAGAAAU	1000	384-404	532-552	AUUUCUTCAUUUCCACCUUUGCC	1056	382-404	530-552
AD-1321282.1	AAAGGUGGAAAUGAAGAAAGU	1001	385-405	533-553	ACUUTCTUCAUUUCCACCUUUGC	1057	383-405	531-553
AD-1321283.1	AAGGUGGAAAUGAAGAAAGUA	453	386-406	534-554	UACUTUCUUCAUUUCCACCUUUG	1058	384-406	532-554
AD-1321284.1	AGGUGGAAAUGAAGAAAGUAU	512	387-407	535-555	AUACTUTCUUCAUUUCCACCUUU	1059	385-407	533-555
AD-1321285.1	GGUGGAAAUGAAGAAAGUACA	1002	388-408	536-556	AGUACUUUCUUCAUUUCCACCUU	1060	386-408	534-556
AD-1321286.1	GUGGAAAUGAAGAAAGUACAA	1003	389-409	537-557	UUGUACTUUCUUCAUUUCCACCU	1061	387-409	535-557
AD-1321287.1	UGGAAAUGAAGAAAGUACAAA	1004	390-410	538-558	UUUGTACUUUCUUCAUUUCCACC	1062	388-410	536-558
AD-1321288.1	GGAAAUGAAGAAAGUACAAAU	1005	391-411	539-559	AUUUGUACUUUCUUCAUUUCCAC	1063	389-411	537-559
AD-1321289.1	GAAAUGAAGAAAGUACAAAGA	1006	392-412	540-560	UCUUTGTACUUUCUUCAUUUCCA	1064	390-412	538-560
AD-1321290.1	AAAUGAAGAAAGUACAAAGAU	1007	393-413	541-561	AUCUTUGUACUUUCUUCAUUUCC	1065	391-413	539-561
AD-1321291.1	AAUGAAGAAAGUACAAAGACA	1008	394-414	542-562	UGUCTUTGUACUUUCUUCAUUUC	1066	392-414	540-562

Table 13. Modified Sense and Antisense Strand Sequences of Superoxide Dismutase 1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1321202.1	uscsauc(Ahd)AfuUfUfCfgagcagaaguL96	1067	asCfsuudCu(G2p)cucgaaAfuUfgaugasusg	1157	CACCAUCAAUUUCGAGCAGAAGG	1247
AD-1321203.1	csasuca(Ahd)UfuUfCfGfagcagaaggaL96	1068	asCfscuuCfugcucgaAfaUfugaugsasu	1158	ACCAUCAAUUUCGAGCAGAAGGA	1248
AD-1321204.1	asuscaa(Uhd)UfuCfGfAfgcagaaggaaL96	1069	usUfsccdTu(C2p)ugcucgAfaAfuugausgsa	1159	CCAUCAAUUUCGAGCAGAAGGAA	395

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1321205.1	uscsaau(Uhd)UfcGfAfGfcagaaggaaaL96	1070	usUfsucdCu(Tgn)cugcucGfaAfauugasusg	1160	CAUCAAUUUCGAGCAGAAGGAAA	436
AD-1321206.1	csasauu(Uhd)CfgAfGfCfagaaggaaauL96	1071	asUfsuudCc(Tgn)ucugcuCfgAfaauugsasu	1161	AUCAAUUUCGAGCAGAAGGAAAG	416
AD-1321207.1	asasuuu(Chd)GfaGfCfAfgaaggaaagL96	1072	asCfsuudTc(C2p)uucugcUfcGfaaauusgsa	1162	UCAAUUUCGAGCAGAAGGAAAGU	770
AD-1321208.1	asusuuc(Ghd)AfgCfAfGfaaggaaaguaL96	1073	usAfscudTu(C2p)cuucugCfuCfgaaaususg	1163	CAAUUUCGAGCAGAAGGAAAGUA	379
AD-1321209.1	ususucg(Ahd)GfcAfGfAfaggaaaguaaL96	1074	usUfsacdTu(Tgn)ccuucuGfcUfcgaaasusu	1164	AAUUUCGAGCAGAAGGAAAGUAA	421
AD-1321210.1	ususcga(Ghd)CfaGfAfAfggaaaguaaauL96	1075	asUfsuadCu(Tgn)uccuucUfgCfucgaasasu	1165	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-1321211.1	uscsgag(Chd)AfgAfAfGfgaaaguaaauuL96	1076	asAfsuudAc(Tgn)uuccuuCfuGfcucgasasa	1166	UUUCGAGCAGAAGGAAAGUAAUG	413
AD-1321212.1	csgsagc(Ahd)GfaAfGfGfaaaguaauguL96	1077	asCfsauuAfcuuuccuUfcUfgcucgsasa	1167	UUCGAGCAGAAGGAAAGUAAUGG	405
AD-1321213.1	gsasgca(Ghd)AfaGfGfAfaaguaauggaL96	1078	asCfscauUfacuuuccUfuCfugcucsgsa	1168	UCGAGCAGAAGGAAAGUAAUGGA	771
AD-1321214.1	asgscag(Ahd)AfgGfAfAfaguaauggauL96	1079	asUfsccaUfuacuuucCfuUfcugcuscsg	1169	CGAGCAGAAGGAAAGUAAUGGAC	1249
AD-1321215.1	gscsaga(Ahd)GfgAfAfAfguaauggacuL96	1080	asGfsucdCa(Tgn)uacuuuCfcUfucugcsusc	1170	GAGCAGAAGGAAAGUAAUGGACC	1250
AD-1321216.1	csasgaa(Ghd)GfaAfAfGfuaauggaccaL96	1081	usGfsgudCc(Agn)uuacuuUfcCfuucugscsu	1171	AGCAGAAGGAAAGUAAUGGACCA	415
AD-1321217.1	asgsaag(Ghd)AfaAfGfUfaauggaccauL96	1082	asUfsggdTc(C2p)auuacuUfuCfcuucusgsc	1172	GCAGAAGGAAAGUAAUGGACCAG	772
AD-1321218.1	gsasagg(Ahd)AfaGfUfAfauggaccaguL96	1083	asCfsugdGu(C2p)cauuacUfuUfccuucsusg	1173	CAGAAGGAAAGUAAUGGACCAGU	773
AD-1321219.1	asasgga(Ahd)AfgUfAfAfuggaccaguuL96	1084	asAfscudGg(Tgn)ccauuaCfuUfuccuuscsu	1174	AGAAGGAAAGUAAUGGACCAGUG	417
AD-1321220.1	asgsgaa(Ahd)GfuAfAfUfggaccagugaL96	1085	usCfsacdTg(G2p)uccauuAfcUfuuccususc	1175	GAAGGAAAGUAAUGGACCAGUGA	774
AD-1321221.1	gsgsaaa(Ghd)UfaAfUfGfgaccagugaaL96	1086	usUfscadCu(G2p)guccauUfaCfuuuccsusu	1176	AAGGAAAGUAAUGGACCAGUGAA	775
AD-1321222.1	gsasaag(Uhd)AfaUfGfGfaccagugaauL96	1087	asUfsucdAc(Tgn)gguccaUfuAfcuuuccscsu	1177	AGGAAAGUAAUGGACCAGUGAAG	776

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1321223.1	asasagu(Ahd)AfuGfGfAfccagugaaguL96	1088	asCfsuucAfcugguccAfuUfacuuuscsc	1178	GGAAAGUAAUGGACCAGUGAAGG	777
AD-1321224.1	asasgua(Ahd)UfgGfAfCfcagugaaggUL96	1089	asCfscuuCfacuggucCfaUfuacuususc	1179	GAAAGUAAUGGACCAGUGAAGGU	778
AD-1321225.1	asgsuaa(Uhd)GfgAfCfCfagugaaggUUL96	1090	asAfsccdTu(C2p)acugguCfcAfuuacususu	1180	AAAGUAAUGGACCAGUGAAGGUG	779
AD-1321226.1	gsusaau(Ghd)GfaCfCfAfgugaaggUgUL96	1091	asCfsaccUfucacuggUfcCfauuacsusu	1181	AAGUAAUGGACCAGUGAAGGUGU	1251
AD-1321227.1	usasaug(Ghd)AfcCfAfGfugaaggUguUL96	1092	asAfscacCfuucacugGfuCfcauuascsu	1182	AGUAAUGGACCAGUGAAGGUGUG	425
AD-1321228.1	asasugg(Ahd)CfcAfGfUfgaaggUguUL96	1093	asCfsacaCfcuucacuGfgUfccauusasc	1183	GUAAUGGACCAGUGAAGGUGUGG	1252
AD-1321229.1	gsusacc(Ahd)GfuGfCfAfgguccucacuL96	1094	asGfsugdAg(G2p)accugcAfcUfgguacsasg	1184	CUGUACCAGUGCAGGUCCUCACU	1253
AD-1321230.1	usascca(Ghd)UfgCfAfGfguccucacuuL96	1095	asAfsgudGa(G2p)gaccugCfaCfugguascsa	1185	UGUACCAGUGCAGGUCCUCACUU	1254
AD-1321231.1	ascscag(Uhd)GfcAfGfGfuccucacuuuL96	1096	asAfsagdTg(Agn)ggaccuGfcAfcuggusasc	1186	GUACCAGUGCAGGUCCUCACUUU	375
AD-1321232.1	cscsagu(Ghd)CfaGfGfUfccucacuuuaL96	1097	usAfsaadGu(G2p)aggaccUfgCfacuggsusa	1187	UACCAGUGCAGGUCCUCACUUUA	407
AD-1321233.1	csasgug(Chd)AfgGfUfCfcucacuuuaaL96	1098	usUfsaadAg(Tgn)gaggacCfuGfcacugsgsu	1188	ACCAGUGCAGGUCCUCACUUUAA	386
AD-1321234.1	asgsugc(Ahd)GfgUfCfCfucacuuuaauL96	1099	asUfsuadAa(G2p)ugaggaCfcUfgcacusgsg	1189	CCAGUGCAGGUCCUCACUUUAAU	418
AD-1321235.1	gsusgca(Ghd)GfuCfCfUfcacuuuaauuL96	1100	asAfsuuaAfagugaggAfcCfugcacsusg	1190	CAGUGCAGGUCCUCACUUUAAUC	424
AD-1321236.1	usgscag(Ghd)UfcCfUfCfacuuuaaucuL96	1101	asGfsauuAfaagugagGfaCfcugcascsu	1191	AGUGCAGGUCCUCACUUUAAUCC	371
AD-1321237.1	gscsagg(Uhd)CfcUfCfAfcuuuaauccuL96	1102	asGfsgauUfaaagugaGfgAfccugcsasc	1192	GUGCAGGUCCUCACUUUAAUCCU	429
AD-1321238.1	csasggu(Chd)CfuCfAfCfuuuaauccuuL96	1103	asAfsggaUfuaaagugAfgGfaccugscsa	1193	UGCAGGUCCUCACUUUAAUCCUC	373
AD-1321239.1	asgsguc(Chd)UfcAfCfUfuuaaucccucuL96	1104	asGfsaggAfuuaaaguGfaGfgaccusgsc	1194	GCAGGUCCUCACUUUAAUCCUCU	408
AD-1321240.1	gsgsucc(Uhd)CfaCfUfUfuaaucccucuaL96	1105	usAfsgadGg(Agn)uuaaagUfgAfggaccsusg	1195	CAGGUCCUCACUUUAAUCCUCUA	385

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1321241.1	gsusccu(Chd)AfcUfUfUfaauccucuauL96	1106	asUfsagdAg(G2p)auuaaaGfuGfaggacscsu	1196	AGGUCCUCACUUUAAUCCUCUAU	397
AD-1321242.1	uscscuc(Ahd)CfuUfUfAfauccucuauuL96	1107	asAfsuadGa(G2p)gauuaaAfgUfgaggascsc	1197	GGUCCUCACUUUAAUCCUCUAUC	396
AD-1321243.1	cscsuca(Chd)UfuUfAfAfuccucuaucuL96	1108	asGfsaudAg(Agn)ggauuaAfaGfugaggsasc	1198	GUCCUCACUUUAAUCCUCUAUCC	374
AD-1321244.1	csuscac(Uhd)UfuAfAfUfccucuauccaL96	1109	asGfsgauAfgaggauuAfaAfgugagsgsa	1199	UCCUCACUUUAAUCCUCUAUCCA	382
AD-1321245.1	uscsacu(Uhd)UfaAfUfCfcucuauccauL96	1110	asUfsggaUfagaggauUfaAfagugasgsg	1200	CCUCACUUUAAUCCUCUAUCCAG	414
AD-1321246.1	csascuu(Uhd)AfaUfCfCfucuauccagaL96	1111	asCfsuggAfuagaggaUfuAfaagugsasg	1201	CUCACUUUAAUCCUCUAUCCAGA	403
AD-1321247.1	ascsuuu(Ahd)AfuCfCfUfcuauccagaaL96	1112	usUfscudGg(Agn)uagaggAfuUfaaagusgsa	1202	UCACUUUAAUCCUCUAUCCAGAC	393
AD-1321248.1	gsusggg(Chd)CfaAfAfGfgaugaagagaL96	1113	usCfsucdTu(C2p)auccuuUfgGfcccacscsg	1203	CGGUGGGCCAAAGGAUGAAGAGA	1255
AD-1321249.1	usgsggc(Chd)AfaAfGfGfaugaagagauL96	1114	asUfscudCu(Tgn)cauccuUfuGfgcccascsc	1204	GGUGGGCCAAAGGAUGAAGAGAG	1256
AD-1321250.1	gsgsgcc(Ahd)AfaGfGfAfugaagagaguL96	1115	asCfsucdTc(Tgn)ucauccUfuUfggcccsasc	1205	GUGGGCCAAAGGAUGAAGAGAGG	1257
AD-1321251.1	gsgscca(Ahd)AfgGfAfUfgaagagagguL96	1116	asCfscudCu(C2p)uucaucCfuUfuggccscsa	1206	UGGGCCAAAGGAUGAAGAGAGGC	1258
AD-1321252.1	gscscaa(Ahd)GfgAfUfGfaagagaggcaL96	1117	usGfsccdTc(Tgn)cuucauCfcUfuuggcscsc	1207	GGGCCAAAGGAUGAAGAGAGGCA	1259
AD-1321253.1	cscsaaa(Ghd)GfaUfGfAfagagaggcauL96	1118	asUfsgcdCu(C2p)ucuucaUfcCfuuuggscsc	1208	GGCCAAAGGAUGAAGAGAGGCAU	1260
AD-1321254.1	csasaag(Ghd)AfuGfAfAfgagaggcauuL96	1119	asAfsugdCc(Tgn)cucuucAfuCfcuuugsgsc	1209	GCCAAAGGAUGAAGAGAGGCAUG	789
AD-1321255.1	asasagg(Ahd)UfgAfAfGfagaggcauguL96	1120	asCfsaudGc(C2p)ucucuuCfaUfccuuusgsg	1210	CCAAAGGAUGAAGAGAGGCAUGU	1261
AD-1321256.1	asasgga(Uhd)GfaAfGfAfgaggcauguuL96	1121	asAfscadTg(C2p)cucucuUfcAfuccuususg	1211	CAAAGGAUGAAGAGAGGCAUGUU	790
AD-1321257.1	asgsgau(Ghd)AfaGfAfGfaggcauguuuL96	1122	asAfsacdAu(G2p)ccucucUfuCfauccususu	1212	AAAGGAUGAAGAGAGGCAUGUUG	791
AD-1321258.1	gsgsaug(Ahd)AfgAfGfAfggcauguuguL96	1123	asCfsaacAfugccucuCfuUfcauccsusu	1213	AAGGAUGAAGAGAGGCAUGUUGG	792

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1321259.1	gsasuga(Ahd)GfaGfAfGfgcauguuggaL96	1124	asCfscaaCfaugccucUfcUfucaucscsu	1214	AGGAUGAAGAGAGGCAUGUUGGA	788
AD-1321260.1	asusgaa(Ghd)AfgAfGfGfcauguuggauL96	1125	asUfsccaAfcaugccuCfuCfuucauscsc	1215	GGAUGAAGAGAGGCAUGUUGGAG	793
AD-1321261.1	usgsaag(Ahd)GfaGfGfCfauguuggagaL96	1126	asCfsuccAfacaugccUfcUfcuucasusc	1216	GAUGAAGAGAGGCAUGUUGGAGA	1262
AD-1321262.1	gsasaga(Ghd)AfgGfCfAfuguuggagauL96	1127	asUfscucCfaacaugcCfuCfucuucsasu	1217	AUGAAGAGAGGCAUGUUGGAGAC	434
AD-1321263.1	asasgag(Ahd)GfgCfAfUfguuggagacuL96	1128	asGfsucdTc(C2p)aacaugCfcUfcucuuscsa	1218	UGAAGAGAGGCAUGUUGGAGACC	794
AD-1321264.1	asgsaga(Ghd)GfcAfUfGfuuggagacuuL96	1129	asAfsgudCu(C2p)caacauGfcCfucucususc	1219	GAAGAGAGGCAUGUUGGAGACCU	1263
AD-1321265.1	gsasgag(Ghd)CfaUfGfUfuggagacuuuL96	1130	asAfsagdTc(Tgn)ccaacaUfgCfcucucsusu	1220	AAGAGAGGCAUGUUGGAGACCUG	439
AD-1321266.1	asgsagg(Chd)AfuGfUfUfggagacuugL96	1131	asCfsaadGu(C2p)uccaacAfuGfccucuscsu	1221	AGAGAGGCAUGUUGGAGACCUGG	1264
AD-1321267.1	gscsaga(Uhd)GfaCfUfUfgggcaaaggL96	1132	asCfscudTu(G2p)cccaagUfcAfucugcsusu	1222	AAGCAGAUGACUUGGGCAAAGGU	799
AD-1321268.1	csasgau(Ghd)AfcUfUfGfggcaaaggUL96	1133	asAfsccuUfugcccaaGfuCfaucugscsu	1223	AGCAGAUGACUUGGGCAAAGGUG	1265
AD-1321269.1	asgsaug(Ahd)CfuUfGfGfgcaaaggugL96	1134	asCfsaccUfuugcccaAfgUfcaucusgsc	1224	GCAGAUGACUUGGGCAAAGGUGG	800
AD-1321270.1	gsasuga(Chd)UfuGfGfGfcaaaggUggaL96	1135	asCfscacCfuuugcccAfaGfucaucsusg	1225	CAGAUGACUUGGGCAAAGGUGGA	795
AD-1321271.1	asusgac(Uhd)UfgGfGfCfaaaggUggaaL96	1136	usUfsccdAc(C2p)uuugccCfaAfgucauscsu	1226	AGAUGACUUGGGCAAAGGUGGAA	766
AD-1321272.1	usgsacu(Uhd)GfgGfCfAfaaggUggaaaL96	1137	usUfsucdCa(C2p)cuuugcCfcAfagucasusc	1227	GAUGACUUGGGCAAAGGUGGAAA	767
AD-1321273.1	gsascuu(Ghd)GfgCfAfAfaggUggaaauL96	1138	asUfsuudCc(Agn)ccuuugCfcCfaagucsasu	1228	AUGACUUGGGCAAAGGUGGAAAU	768
AD-1321274.1	ascsuug(Ghd)GfcAfAfAfgguggaaauuL96	1139	asAfsuudTc(C2p)accuuuGfcCfcaaguscsa	1229	UGACUUGGGCAAAGGUGGAAAUG	801
AD-1321275.1	csusugg(Ghd)CfaAfAfGfguggaaaugaL96	1140	asCfsauuUfccaccuuUfgCfccaagsusc	1230	GACUUGGGCAAAGGUGGAAAUGA	1266
AD-1321276.1	ususggg(Chd)AfaAfGfGfguggaaaugaaL96	1141	usUfscadTu(Tgn)ccaccuUfuGfcccaasgsu	1231	ACUUGGGCAAAGGUGGAAAUGAA	435

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1321277.1	usgsggc(Ahd)AfaGfGfUfggaaaugaauL96	1142	asUfsucdAu(Tgn)uccaccUfuUfgcccasasg	1232	CUUGGGCAAAGGUGGAAAUGAAG	1267
AD-1321278.1	ggsggca(Ahd)AfgGfUfGfgaaaugaagaL96	1143	asCfsuucAfuuuccacCfuUfugcccsasa	1233	UUGGGCAAAGGUGGAAAUGAAGA	1268
AD-1321279.1	ggscaa(Ahd)GfgUfGfGfaaaugaagaaL96	1144	usUfscudTc(Agn)uuuccaCfcUfuugccscsa	1234	UGGGCAAAGGUGGAAAUGAAGAA	1269
AD-1321280.1	gscsaaa(Ghd)GfuGfGfAfaaugaagaaaL96	1145	usUfsucdTu(C2p)auuuccAfcCfuuugcscsc	1235	GGGCAAAGGUGGAAAUGAAGAAA	1270
AD-1321281.1	csasaag(Ghd)UfgGfAfAfaugaagaaauL96	1146	asUfsuudCu(Tgn)cauuucCfaCfcuuugscsc	1236	GGCAAAGGUGGAAAUGAAGAAAG	1271
AD-1321282.1	asasagg(Uhd)GfgAfAfAfugaagaaaguL96	1147	asCfsuudTc(Tgn)ucauuuCfcAfccuuusgsc	1237	GCAAAGGUGGAAAUGAAGAAAGU	1272
AD-1321283.1	asasggu(Ghd)GfaAfAfUfgaagaaaguaL96	1148	usAfscudTu(C2p)uucauuUfcCfaccuususg	1238	CAAAGGUGGAAAUGAAGAAAGUA	769
AD-1321284.1	asgsgug(Ghd)AfaAfUfGfaagaaaguauL96	1149	asUfsacdTu(Tgn)cuucauUfuCfcaccususu	1239	AAAGGUGGAAAUGAAGAAAGUAA	1273
AD-1321285.1	gsgsugg(Ahd)AfaUfGfAfagaaaguacaL96	1150	asGfsuacUfuucuucaUfuUfccaccsusu	1240	AAGGUGGAAAUGAAGAAAGUAAA	446
AD-1321286.1	gsusgga(Ahd)AfuGfAfAfgaaaguacaaL96	1151	usUfsgudAc(Tgn)uucuucAfuUfuccacscsu	1241	AGGUGGAAAUGAAGAAAGUAAAA	1274
AD-1321287.1	usgsgaa(Ahd)UfgAfAfGfaaaguacaaaL96	1152	usUfsugdTa(C2p)uuucuuCfaUfuuccascsc	1242	GGUGGAAAUGAAGAAAGUAAAAA	1275
AD-1321288.1	ggsgsaaa(Uhd)GfaAfGfAfaaguacaaauL96	1153	asUfsuudGu(Agn)cuuucuUfcAfuuuccsasc	1243	GUGGAAAUGAAGAAAGUAAAAAG	1276
AD-1321289.1	gsasaauu(Ghd)AfaGfAfAfaguacaaagaL96	1154	usCfsuudTg(Tgn)acuuucUfuCfauuucscsa	1244	UGGAAAUGAAGAAAGUAAAAAGA	1277
AD-1321290.1	asasaug(Ahd)AfgAfAfAfguacaaagauL96	1155	asUfscudTu(G2p)uacuuuCfuUfcauuuscsc	1245	GGAAAUGAAGAAAGUAAAAAGAC	1278
AD-1321291.1	asasuga(Ahd)GfaAfAfGfuacaaagacaL96	1156	usGfsucdTu(Tgn)guacuuUfcUfucauususc	1246	GAAAUGAAGAAAGUAAAAAGACA	1279

Table 14. Superoxide Dismutase 1 *In Vitro* Single Dose Screens in Primary Cynomolgus Hepatocytes (PCH) cells

	50 mM		10 nM		1 nM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1321202.1	40.5	4.8	38.0	4.1	63.0	6.8	91.8	3.6
AD-1321203.1	24.5	1.5	29.0	2.0	72.8	5.4	89.5	2.7
AD-1321204.1	27.2	1.1	20.2	1.5	31.5	2.3	70.9	2.6
AD-1321205.1	96.4	5.1	86.7	2.0	95.3	4.9	97.3	2.3
AD-1321206.1	21.5	1.2	20.7	1.7	37.6	4.2	69.5	2.8
AD-1321207.1	9.2	1.1	9.9	1.3	22.9	3.7	53.7	4.1
AD-1321208.1	41.0	4.8	27.7	1.0	49.9	2.4	81.8	2.0
AD-1321209.1	66.0	1.8	55.7	4.0	67.5	3.4	90.3	2.0
AD-1321210.1	6.9	1.1	8.0	1.3	13.9	2.8	34.8	3.6
AD-1321211.1	101.9	4.3	99.1	1.6	88.3	3.4	100.2	3.5
AD-1321212.1	21.7	2.3	25.4	2.1	57.8	5.9	88.7	4.3
AD-1321213.1	31.1	3.1	29.0	2.4	60.6	6.6	93.2	3.2
AD-1321214.1	76.6	8.9	75.7	9.1	93.7	8.1	98.0	3.3
AD-1321215.1	30.3	2.9	35.0	4.4	83.2	10.4	94.5	4.6
AD-1321216.1	17.7	4.0	13.5	3.1	31.1	5.0	64.7	3.3
AD-1321217.1	47.9	5.0	35.5	4.3	54.9	5.9	80.8	6.2
AD-1321218.1	28.6	4.0	37.5	7.0	58.3	5.2	81.4	6.1
AD-1321219.1	7.5	0.6	10.4	1.2	26.2	4.8	60.8	4.2
AD-1321220.1	9.0	1.6	10.3	0.5	29.8	3.1	68.7	2.5
AD-1321221.1	26.6	1.2	28.8	3.9	53.6	5.1	90.8	2.4
AD-1321222.1	18.2	2.2	17.4	1.3	39.9	1.5	78.6	5.8
AD-1321223.1	92.2	10.4	72.0	9.8	99.8	4.6	108.7	11.5
AD-1321224.1	91.7	5.5	85.1	6.0	110.4	12.0	102.3	4.3
AD-1321225.1	30.7	3.9	41.7	4.1	72.7	10.8	91.4	2.2
AD-1321226.1	49.0	6.3	65.4	6.1	86.6	6.6	100.4	7.4
AD-1321227.1	64.6	6.7	63.0	6.7	83.2	6.5	99.1	5.1
AD-1321228.1	89.9	6.7	88.4	2.5	100.9	8.3	102.7	7.6
AD-1321229.1	62.5	8.6	61.1	3.3	78.3	4.9	83.2	32.2
AD-1321230.1	77.5	7.3	73.6	2.1	88.6	9.5	102.8	7.0
AD-1321231.1	17.0	2.6	21.8	5.1	48.1	3.5	80.0	4.1
AD-1321232.1	67.1	1.9	52.0	2.4	46.8	8.7	72.2	5.8
AD-1321233.1	99.8	8.2	104.4	10.3	99.7	6.0	102.4	4.9
AD-1321234.1	74.6	6.3	69.0	8.1	87.3	7.7	96.9	5.3
AD-1321235.1	65.4	2.3	52.6	5.3	68.4	9.4	87.1	2.7
AD-1321236.1	63.7	3.4	60.8	1.3	87.6	2.1	98.6	5.9
AD-1321237.1	42.5	1.3	54.2	1.5	96.8	4.1	104.7	5.7
AD-1321238.1	22.3	2.3	23.3	3.0	45.5	6.4	73.5	2.8
AD-1321239.1	66.4	5.6	50.9	3.4	76.6	9.0	98.3	6.7

	50 mM		10 nM		1 nM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1321240.1	98.9	1.4	100.6	9.7	104.9	10.4	102.5	2.4
AD-1321241.1	95.2	8.9	89.6	10.7	101.7	13.1	101.7	5.1
AD-1321242.1	17.0	1.9	19.2	1.9	43.0	4.9	78.7	1.6
AD-1321243.1	8.7	1.7	10.0	1.2	32.6	3.3	66.3	2.9
AD-1321244.1	43.9	2.8	34.4	2.3	73.8	9.5	93.0	4.1
AD-1321245.1	25.1	0.6	26.0	1.8	61.0	5.8	90.3	6.7
AD-1321246.1	6.4	1.7	6.8	1.0	20.9	2.8	51.3	6.9
AD-1321247.1	86.5	5.8	80.7	6.6	85.3	4.2	92.1	4.9
AD-1321248.1	98.5	6.9	99.9	11.4	102.8	7.5	105.8	6.3
AD-1321249.1	63.4	3.0	69.2	6.0	92.7	3.5	104.5	3.9
AD-1321250.1	40.2	1.8	56.5	1.8	96.8	5.2	100.7	0.6
AD-1321251.1	98.2	3.8	97.2	12.0	107.0	13.0	103.6	5.1
AD-1321252.1	71.0	2.3	63.8	7.5	94.0	5.3	100.8	5.6
AD-1321253.1	7.8	0.6	11.3	0.8	36.6	10.7	75.2	9.7
AD-1321254.1	36.8	3.1	27.4	2.0	47.4	7.6	78.6	5.1
AD-1321255.1	73.6	5.5	61.4	6.5	76.2	11.1	92.4	4.9
AD-1321256.1	8.3	0.6	8.5	0.7	20.3	4.4	49.8	5.2
AD-1321257.1	7.4	0.4	9.8	2.3	21.9	2.5	51.1	0.8
AD-1321258.1	16.8	1.2	29.4	2.8	81.9	8.3	107.0	2.1
AD-1321259.1	19.2	1.7	21.9	1.9	65.6	6.8	94.0	4.1
AD-1321260.1	49.6	2.6	53.9	3.5	86.8	7.6	101.9	1.1
AD-1321261.1	19.6	1.6	28.2	2.7	75.7	3.0	102.2	9.2
AD-1321262.1	52.5	1.7	56.4	2.3	91.1	10.7	105.8	8.3
AD-1321263.1	86.3	3.5	95.2	11.5	101.5	9.8	95.9	10.7
AD-1321264.1	92.0	7.4	91.8	8.7	93.9	7.1	103.0	8.1
AD-1321265.1	58.9	4.1	62.3	5.0	78.3	5.6	92.2	3.5
AD-1321266.1	89.0	3.7	94.2	8.4	103.9	9.2	100.4	10.1
AD-1321267.1	24.6	3.2	36.0	7.6	84.3	5.2	95.7	5.9
AD-1321268.1	98.4	3.4	91.4	9.5	107.5	11.1	101.4	9.1
AD-1321269.1	61.0	2.1	63.7	1.4	97.1	7.2	99.4	4.5
AD-1321270.1	65.6	2.1	68.9	11.4	92.6	12.7	99.2	8.1
AD-1321271.1	94.0	7.5	96.9	10.1	108.1	15.0	110.0	13.5
AD-1321272.1	92.4	6.7	94.4	11.3	98.8	10.7	106.2	12.6
AD-1321273.1	19.8	2.0	23.0	3.3	49.5	9.4	77.4	2.4
AD-1321274.1	100.5	9.1	83.5	9.3	104.1	5.4	99.3	13.4
AD-1321275.1	76.2	6.5	61.2	3.0	91.6	5.8	94.6	8.6
AD-1321276.1	11.8	0.8	10.6	1.8	23.5	2.9	50.9	3.1
AD-1321277.1	62.2	2.8	61.1	6.1	81.0	7.6	91.3	4.4
AD-1321278.1	89.1	6.7	88.1	5.7	101.9	2.0	101.9	6.0
AD-1321279.1	79.5	3.1	66.2	5.0	82.8	3.9	95.9	8.6
AD-1321280.1	33.9	5.1	18.0	1.8	26.5	2.6	54.9	11.6

	50 mM		10 nM		1 nM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1321281.1	67.6	5.5	52.4	6.2	85.3	6.8	98.1	9.4
AD-1321282.1	10.4	0.6	12.4	2.3	58.9	16.7	83.3	6.3
AD-1321283.1	11.7	1.6	9.6	0.9	28.2	3.2	57.7	7.8
AD-1321284.1	32.8	2.1	19.9	2.7	29.1	2.8	64.2	4.8
AD-1321285.1	13.4	0.8	14.1	1.1	36.8	2.6	76.3	5.5
AD-1321286.1	81.8	3.8	70.8	5.8	82.1	4.0	95.5	4.6
AD-1321287.1	83.5	5.0	68.8	3.4	80.6	4.4	94.8	0.6
AD-1321288.1	33.5	2.7	30.3	2.3	54.3	2.4	89.9	3.1
AD-1321289.1	84.7	7.1	70.2	4.8	90.5	4.9	98.6	2.0
AD-1321290.1	25.7	1.3	22.2	1.2	45.2	3.4	77.9	2.1
AD-1321291.1	92.9	3.9	80.9	1.8	93.8	3.7	101.7	5.2

Table 15. Superoxide Dismutase 1 *In Vitro* Single Dose Screens in BE(2)C cells

	50 mM		10 nM		1 mM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1321202.1	21.9	2.6	25.0	1.5	69.4	11.3	77.1	6.1
AD-1321203.1	20.4	4.2	39.9	6.8	79.6	11.8	79.8	7.0
AD-1321204.1	18.4	3.7	20.2	2.0	51.0	6.7	75.6	3.3
AD-1321205.1	71.6	2.6	80.8	8.7	80.1	17.4	81.9	6.6
AD-1321206.1	17.2	1.7	25.3	1.7	53.8	8.0	83.1	7.3
AD-1321207.1	11.8	2.5	18.4	1.3	34.3	3.4	55.7	4.8
AD-1321208.1	24.7	2.8	32.9	3.0	61.9	8.7	75.8	7.5
AD-1321209.1	27.8	1.3	33.0	3.9	48.9	1.7	72.6	1.5
AD-1321210.1	12.4	1.8	15.9	1.3	30.9	8.2	50.6	4.2
AD-1321211.1	44.7	6.1	42.6	5.1	78.3	12.9	100.4	9.7
AD-1321212.1	21.8	1.3	27.4	4.9	68.4	13.4	108.3	18.5
AD-1321213.1	32.5	15.9	24.4	2.1	63.1	15.3	120.6	39.0
AD-1321214.1	29.4	8.3	44.4	8.6	107.8	28.8	107.3	17.2
AD-1321215.1	31.5	8.2	37.4	2.1	74.7	12.0	92.0	15.1
AD-1321216.1	19.9	4.7	27.1	3.4	54.0	14.3	76.2	7.4
AD-1321217.1	19.0	2.0	30.7	7.6	45.8	5.3	78.3	2.4
AD-1321218.1	23.9	5.6	33.7	5.7	69.4	4.6	91.1	9.0
AD-1321219.1	23.2	5.1	24.5	3.3	84.9	30.5	103.3	10.1
AD-1321220.1	15.5	2.7	18.6	1.3	53.1	11.9	78.9	21.9
AD-1321221.1	31.0	6.9	38.9	4.0	76.0	6.8	96.2	25.2
AD-1321222.1	17.2	1.2	26.4	0.4	51.7	10.1	109.5	29.4
AD-1321223.1	50.6	8.4	54.1	16.0	105.3	25.4	103.4	20.0
AD-1321224.1	66.2	13.1	90.9	24.5	83.7	16.7	88.3	10.1
AD-1321225.1	37.9	11.8	44.7	9.3	89.5	9.9	92.3	17.3
AD-1321226.1	51.0	15.0	50.4	11.2	77.7	4.9	114.1	15.0

	50 mM		10 nM		1 mM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1321227.1	33.5	1.8	41.9	12.3	88.9	11.7	99.4	12.6
AD-1321228.1	99.3	67.0	71.7	6.3	91.8	8.1	91.0	4.0
AD-1321229.1	26.5	2.3	33.1	4.0	76.5	10.9	121.7	34.2
AD-1321230.1	37.8	10.5	45.0	11.9	81.7	25.0	97.6	20.9
AD-1321231.1	16.7	5.3	21.9	5.5	40.3	16.0	64.6	15.2
AD-1321232.1	25.0	4.5	24.0	7.4	57.7	16.2	76.8	9.7
AD-1321233.1	72.8	15.1	68.0	23.8	114.4	27.2	102.7	14.3
AD-1321234.1	25.6	4.6	33.5	6.3	63.2	7.9	99.1	7.4
AD-1321235.1	17.4	3.7	20.1	2.1	45.2	6.5	85.2	4.0
AD-1321236.1	35.7	15.1	36.0	2.8	73.4	8.5	106.5	33.8
AD-1321237.1	34.0	1.8	42.9	2.7	79.4	3.5	211.3	62.3
AD-1321238.1	22.4	11.5	22.9	5.8	50.2	18.5	119.1	31.9
AD-1321239.1	25.9	6.2	31.8	6.6	71.6	25.7	98.4	20.3
AD-1321240.1	81.8	18.9	92.0	17.7	115.4	22.1	112.4	20.5
AD-1321241.1	56.8	14.7	68.4	10.8	114.5	12.4	112.0	12.8
AD-1321242.1	20.9	0.8	27.2	6.8	60.5	5.3	105.5	36.8
AD-1321243.1	16.3	3.8	20.9	3.8	40.0	5.4	87.3	41.9
AD-1321244.1	25.9	2.6	33.4	2.3	72.2	23.6	142.4	55.0
AD-1321245.1	19.6	3.2	26.5	3.3	53.4	3.3	87.5	25.1
AD-1321246.1	13.2	1.1	18.8	2.8	51.6	16.9	72.2	17.2
AD-1321247.1	51.2	6.7	58.7	18.8	106.1	24.6	110.8	29.0
AD-1321248.1	72.4	26.1	68.2	16.5	127.1	27.3	104.4	4.6
AD-1321249.1	50.0	2.6	63.0	7.4	104.3	6.6	120.2	26.5
AD-1321250.1	42.9	7.2	85.7	22.3	99.3	14.1	147.4	32.8
AD-1321251.1	73.8	17.4	95.7	12.4	102.3	4.7	122.5	17.5
AD-1321252.1	67.0	12.9	74.3	11.8	107.9	18.3	98.8	10.7
AD-1321253.1	19.7	3.5	29.9	13.6	92.2	20.1	119.7	30.0
AD-1321254.1	17.5	3.7	31.6	12.1	68.1	28.4	82.7	5.0
AD-1321255.1	28.9	8.6	34.5	7.8	99.4	31.3	101.2	10.5
AD-1321256.1	22.5	4.9	21.2	3.1	63.8	18.1	80.1	8.2
AD-1321257.1	16.5	1.8	23.7	3.8	41.4	15.2	78.4	9.8
AD-1321258.1	24.0	3.1	37.7	7.4	84.8	14.6	106.0	15.1
AD-1321259.1	23.1	1.8	30.6	3.1	73.6	5.5	132.6	38.2
AD-1321260.1	34.0	7.0	44.6	19.7	79.6	10.4	102.5	23.9
AD-1321261.1	20.1	3.0	34.9	12.3	78.3	18.5	109.5	28.6
AD-1321262.1	40.8	4.8	63.8	14.1	101.9	37.9	97.4	15.7
AD-1321263.1	46.9	6.2	54.8	7.4	88.2	17.4	112.6	32.6
AD-1321264.1	34.3	2.4	43.1	11.3	101.2	31.5	125.3	29.6
AD-1321265.1	19.1	2.5	21.7	4.5	53.6	1.0	78.5	5.8
AD-1321266.1	37.1	3.4	51.1	15.7	85.1	11.7	106.4	13.8
AD-1321267.1	30.9	7.9	45.6	10.0	80.9	7.5	88.7	9.2
AD-1321268.1	91.7	22.7	110.6	34.2	93.5	24.1	92.9	12.1

	50 mM		10 nM		1 mM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1321269.1	40.8	2.0	63.9	16.8	87.6	25.6	108.8	40.7
AD-1321270.1	29.1	1.6	38.0	5.0	87.4	19.0	114.4	43.7
AD-1321271.1	73.6	4.5	95.7	35.7	118.4	16.7	106.0	18.9
AD-1321272.1	65.8	3.9	70.6	20.3	134.7	50.9	159.4	62.2
AD-1321273.1	18.8	2.7	32.7	8.3	58.3	4.5	143.3	40.1
AD-1321274.1	43.4	1.9	72.3	13.9	104.3	31.6	154.1	37.6
AD-1321275.1	35.0	4.0	69.5	6.7	104.2	28.2	142.6	53.5
AD-1321276.1	13.4	1.4	24.2	10.8	38.0	11.4	65.8	8.5
AD-1321277.1	23.7	4.5	31.7	2.5	67.8	9.1	78.3	3.6
AD-1321278.1	44.1	3.9	64.6	12.3	90.8	7.4	112.7	16.7
AD-1321279.1	33.5	3.8	50.9	12.8	100.6	45.8	104.9	22.6
AD-1321280.1	15.7	2.2	29.2	9.7	47.9	10.5	106.6	17.4
AD-1321281.1	31.0	3.0	67.9	14.8	101.8	20.2	143.5	22.6
AD-1321282.1	20.1	3.6	40.9	12.3	74.5	18.4	163.2	37.1
AD-1321283.1	16.7	1.1	36.6	5.5	46.5	8.9	85.1	3.7
AD-1321284.1	19.0	1.4	26.0	9.1	37.1	4.3	67.3	12.0
AD-1321285.1	12.6	1.2	16.6	3.4	47.6	2.0	75.9	8.5
AD-1321286.1	13.5	1.3	15.8	4.1	33.8	6.5	70.6	13.0
AD-1321287.1	12.9	0.9	16.9	1.1	29.8	4.5	62.9	10.2
AD-1321288.1	13.7	2.1	17.1	2.7	31.6	3.5	65.2	4.8
AD-1321289.1	21.7	3.6	29.3	11.3	74.4	19.5	81.0	10.9
AD-1321290.1	15.8	2.2	24.1	8.2	50.3	12.3	111.6	32.3
AD-1321291.1	17.1	3.1	29.3	9.6	57.8	17.7	95.2	20.9

Example 3. *In Vivo* Evaluation in Mice

Duplexes of interest, identified from the above studies, were further evaluated in vivo. In particular, at pre-dose day -14 wild-type mice (C57BL/6) were transduced by retro-orbital administration of 2 x 10^{10} viral particles of an adeno-associated virus 8 (AAV8) vector encoding human SOD1.

At day 0, groups of three mice were subcutaneously administered a single 3 mg/kg dose of the agents of interest or PBS control. Table 16 provides the treatment groups and Table 18 provides the modifided and unmodified nucleotide sequences of the sense and antisense strands of the duplexes of interest. At day 7 post-dose animals were sacrificed, retro-orbital bleeding were performed. Liver samples were collected at day 7 post-dose, and snap-frozen in liquid nitrogen. Tissue mRNA was extracted and analyzed by the RT-QPCR method.

Human SOD1 mRNA levels were compared to housekeeping gene GAPDH. The values were then normalized to the average of PBS vehicle control group. The data were expressed as percent of baseline value, and presented as mean plus standard deviation. The results, listed in Table 17 and

shown in Figure 1, demonstrate that the exemplary duplex agents tested effectively reduce the level of the human SOD1 messenger RNA *in vivo*.

Table 16. Treatment Groups

Group #	Animal #	Treatment	Dose (mpk)	TD
1	1	PBS	n/a	d7
	2			
	3			
2	4	AAV + Ctrl (AD-64228.39)	3	
	5			
	6			
3	7	AD-1321210.2	3	
	8			
	9			
4	10	AD-1321207.2	3	
	11			
	12			
5	13	AD-1321276.2	3	
	14			
	15			
6	16	AD-1321257.2	3	
	17			
	18			
7	19	AD-1321246.2	3	
	20			
	21			
8	22	AD-1321280.2	3	
	23			
	24			
9	25	AD-1321232.2	3	
	26			
	27			
10	28	AD-1321238.2	3	
	29			
	30			
11	31	AD-1321220.2	3	
	32			
	33			
12	34	AD-1321219.2	3	
	35			
	36			

Group #	Animal #	Treatment	Dose (mpk)	TD
13	37	AD-1321204.2	3	
	38			
	39			
14	40	AD-1321222.2	3	
	41			
	42			
15	43	AD-1321243.2	3	
	44			
	45			
16	46	AD-1321256.2	3	
	47			
	48			
17	49	AD-1321284.2	3	
	50			
	51			
18	52	Naïve (AAV only)	n/a	
	53			
	54			
19	55			
	56			
	57			
19	55			
	56			
	57			

Table 17.

Duplex	% Message Remaining	SD	Sample
PBS	101.2654922	19.63002982	Liver
AAV + Ctrl (AD-64228.39)	35.31638173	0.262604802	Liver
AD-1321210.2	30.42438251	19.91605213	Liver
AD-1321207.2	30.25915539	6.758286564	Liver
AD-1321276.2	60.10435363	9.664373248	Liver
AD-1321257.2	42.2278116	12.20020318	Liver
AD-1321246.2	31.12908021	6.362946317	Liver
AD-1321280.2	60.34803578	3.949191278	Liver
AD-1321232.2	56.60772288	6.333661103	Liver
AD-1321238.2	14.6775853	5.863189424	Liver
AD-1321220.2	74.41995976	22.16007547	Liver
AD-1321219.2	55.78880525	1.781783363	Liver
AD-1321204.2	51.1507952	16.4638919	Liver

Duplex	% Message Remaining	SD	Sample
AD-1321222.2	53.99310372	9.219313647	Liver
AD-1321243.2	34.54117695	1.128855605	Liver
AD-1321256.2	27.38377094	11.20122452	Liver
AD-1321284.2	68.29130171	23.82285508	Liver
Naïve (AAV only)	75.69780392	23.65689142	Liver

Table 18. Unmodified and Modified Nucleotide Sequences of the Duplexes of Interest

Duplex Name	Strand	Modified Sequence 5' to 3'	SEQ ID NO:	Unmodified Sequence 5' to 3'	SEQ ID NO:
AD-1321204.2	sense	asuscaa(Uhd)UfuCfGfAfgcagaaggaaL96	1069	AUCAAUUUCGAGCAGAAGGAA	63
	antis	usUfsccdTu(C2p)ugcucgAfaAfuugausgsa	1159	UUCCTUCUGCUCGAAAUUGAUGA	1010
AD-1321207.2	sense	asasuuu(Chd)GfaGfCfAfgaaggaaaguL96	1072	AAUUUCGAGCAGAAGGAAAGU	454
	antis	asCfsuudTc(C2p)uucugcUfcGfaaauusgsa	1162	ACUUTCCUUCUGCUCGAAAUUGA	1013
AD-1321210.2	sense	ususcga(Ghd)CfaGfAfAfggaaaguaauL96	1075	UUCGAGCAGAAGGAAAGUAAU	46
	antis	asUfsuadCu(Tgn)uccuucUfgCfucgaasasu	1165	AUUACUTUCCUUCUGCUCGAAAU	850
AD-1321219.2	sense	asasgga(Ahd)AfgUfAfAfuggaccaguuL96	1084	AAGGAAAGUAAUGGACCAGUU	85
	antis	asAfscudGg(Tgn)ccauuaCfuUfuccuuscsu	1174	AACUGGTCCAUUACUUUCCUUCU	1021
AD-1321220.2	sense	asgsgaa(Ahd)GfuAfAfUfggaccagugaL96	1085	AGGAAAGUAAUGGACCAGUGA	458
	antis	usCfsacdTg(G2p)uccauuAfcUfuuccususc	1175	UCACTGGUCCAUUACUUUCCUUC	1022
AD-1321222.2	sense	gsasaag(Uhd)AfaUfGfGfaccagugaauL96	1087	GAAAGUAAUGGACCAGUGAAU	460
	antis	asUfsucdAc(Tgn)gguccaUfuAfcuuucscsu	1177	AUUCACTGGUCCAUUACUUUCCU	851
AD-1321232.2	sense	cscsagu(Ghd)CfaGfGfUfccucacuuuaL96	1097	CCAGUGCAGGUCCUCACUUUA	75
	antis	usAfsaadGu(G2p)aggaccUfgCfacuggsusa	1187	UAAAGUGAGGACCUGCACUGGUA	158
AD-1321238.2	sense	csasggu(Chd)CfuCfAfCfuuuaauccuuL96	1103	CAGGUCCUCACUUUAAUCCUU	41
	antis	asAfsggaUfuaaagugAfgGfaccugscsa	1193	AAGGAUUAAAGUGAGGACCUGCA	124
AD-1321243.2	sense	cscsuca(Chd)UfuUfAfAfuccucuaucuL96	1108	CCUCACUUUAAUCCUCUAUCU	42
	antis	asGfsaudAg(Agn)ggauuaAfaGfugaggsasc	1198	AGAUAGAGGAUUAAAGUGAGGAC	125
AD-1321246.2	sense	csascuu(Uhd)AfaUfCfCfucuauccagaL96	1111	CACUUUAAUCCUCUAUCCAGA	71
	antis	asCfsuggAfuagaggaUfuAfaagugsasg	1201	ACUGGAUAGAGGAUUAAAGUGAG	1030
AD-1321256.2	sense	asasgga(Uhd)GfaAfGfAfgaggcauguuL96	1121	AAGGAUGAAGAGAGGCAUGUU	474
	antis	asAfscadTg(C2p)cucucuUfcAfuccuususg	1211	AACATGCCUCUCUUCAUCCUUUG	1039
AD-1321257.2	sense	asgsgau(Ghd)AfaGfAfGfaggcauguuuL96	1122	AGGAUGAAGAGAGGCAUGUUU	475
	antis	asAfsacdAu(G2p)ccucucUfuCfauccususu	1212	AAACAUGCCUCUCUUCAUCCUUU	554
AD-1321276.2	sense	ususggg(Chd)AfaAfGfGfuggaaaugaaL96	1141	UUGGGCAAAGGUGGAAAUGAA	103
	antis	usUfscadTu(Tgn)ccaccuUfuGfcccaasgsu	1231	UUCATUTCCACCUUUGCCCAAGU	1051

Duplex Name	Strand	Modified Sequence 5' to 3'	SEQ ID NO:	Unmodified Sequence 5' to 3'	SEQ ID NO:
AD-1321280.2	sense	gscsaaa(Ghd)GfuGfGfAfaaugaagaaaL96	1145	GCAAAGGUGGAAAUGAAGAAA	999
	antis	usUfsucdTu(C2p)auuuccAfcCfuuugcscsc	1235	UUUCTUCAUUUCCACCUUUGCCC	1055
AD-1321284.2	sense	asgsgug(Ghd)AfaAfUfGfaagaaaguauL96	1149	AGGUGGAAAUGAAGAAAGUAU	512
	antis	asUfsacdTu(Tgn)cuucauUfuCfcaccususu	1239	AUACTUTCUUCAUUUCCACCUUU	1059

Example 4. *In vivo* Assessment of RNAi Agents Targeting SOD1 in G93A-SOD1 Transgenic Mice

In order to demonsrate that a single intracerebroventricular injection (ICV) of a dsRNA agent similarly inhibits the expression of SOD1 in therapeutically relevant regions of the brain and spinal cord, male G93A-SOD1 transgenic mice received a single 25 µg, 50 µg, 100 µg, 150 µg , 200 µg, or 300 µg dose in a volume of 5 µl of AD-401824, or 5 µl of artificial CSF (aCSF) control (n=3 per group) by ICV injection using using a Hamilton syringe and an angled 30G needle at Day 0. G93A-SOD1 mice express human SOD1 with the G93A mutation under control of the cistronic human SOD1 promotor. Mutations in this gene have been linked to familial Amyotrophic Lateral Sclerosis (ALS or Lou Gehrig's disease). The SOD1-G93A mice show a phenotype similar to Amyotrophic Lateral Sclerosis in humans. They develop paralysis in one or more limbs within a few weeks of age (see, *e.g.,* Henriques, *et al.* (2010) *PLoS One* 5(11): e15445).

At Day 14 post-dose, animals were sacrificed and brain samples (right hemisphere, left hemisphere, cerebellum and brainstem) and spinal cord samples were collected and flash frozen in liquid nitrogen. mRNA was extracted from the tissue and analyzed by the RT-QPCR method.

The results, depicted in Figure 2, demonstrate that a single ICV injection of between 50-300 ug of dsRNA agent provides consistent and robust knockdown of SOD1 in the CNS in a dose dependent manner. The results also demonstrate that a single dose of dsRNA agent between 150-300ug knocks down SOD1 mRNA to its lowest levels across all tissue types.

Table 19. Unmodified Sense and Antisense Strand Sequences of Superoxide Dismutase 1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_		Antisense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_	
			001285406.1	000454.4			001285406.1	000454.4
AD-1321204.3	AUCAAUUUCGAGCAGAAGGAA	63	55-75	203-223	UUCCTUCUGCUCGAAAUUGAUGA	1010	53-75	201-223
AD-1395718.1	AUCAAUUUCGAGCAGAAGGAA	63	55-75	203-223	UUCCTUCUGCUCGAAAUUGAUGG	1289	53-75	201-223
AD-1395719.1	AUCAAUUUCGAGCAGAAGGAA	63	55-75	203-223	UUCCTUCUGCUCGAAAUUGAUGG	1289	53-75	201-223
AD-1395720.1	AUCAAUUUCGAGCAGAAGGAA	63	55-75	203-223	UUCCTUCUGCUCGAAAUUGAUGG	1289	53-75	201-223
AD-1395721.1	AUCAAUUUCGAGCAGAAGGAA	63	55-75	203-223	UTCCTUCUGCUCGAAAUUGAUGG	1290	53-75	201-223
AD-1395722.1	AUCAAUUUCGAGCAGAAGGAA	63	55-75	203-223	UTCCTUCUGCUCGAAAUUGAUGG	1290	53-75	201-223
AD-1395723.1	AUCAAUUUCGAGCAGAAGGAA	63	55-75	203-223	UTCCTUCUGCUCGAAAUUGAUGG	1290	53-75	201-223
AD-1321207.3	AAUUUCGAGCAGAAGGAAAGU	454	58-78	206-226	ACUUTCCUUCUGCUCGAAAUUGA	1013	56-78	204-226
AD-1395724.1	AAUUUCGAGCAGAAGGAAAGA	1280	58-78	206-226	UCUUTCCUUCUGCUCGAAAUUGG	1291	56-78	204-226
AD-1395725.1	AAUUUCGAGCAGAAGGAAAGA	1280	58-78	206-226	UCUUTCCUUCUGCUCGAAAUUGG	1291	56-78	204-226
AD-1395726.1	AAUUUCGAGCAGAAGGAAAGA	1280	58-78	206-226	UCUUTCCUUCUGCUCGAAAUUGG	1291	56-78	204-226
AD-1395727.1	AAUUUCGAGCAGAAGGAAAGA	1280	58-78	206-226	UCUUTCCUUCUGCUCGAAAUUGG	1291	56-78	204-226
AD-1395728.1	AAUUUCGAGCAGAAGGAAAGA	1280	58-78	206-226	UCUUTCCUUCUGCUCGAAAUUGG	1291	56-78	204-226
AD-1395729.1	AAUUUCGAGCAGAAGGAAAGA	1280	58-78	206-226	UCUUTCCUUCUGCUCGAAAUUGG	1291	56-78	204-226
AD-1321210.3	UUCGAGCAGAAGGAAAGUAAU	46	61-81	209-229	AUUACUTUCCUUCUGCUCGAAAU	850	59-81	207-229
AD-1395730.1	UUCGAGCAGAAGGAAAGUAAU	46	61-81	209-229	AUUACUTUCCUUCUGCUCGAAAU	850	59-81	207-229

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_		Antisense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_	
			001285406.1	000454.4			001285406.1	000454.4
AD-1395731.1	UUCGAGCAGAAGGAAAGUAAA	1281	61-81	209-229	UUUACUTUCCUUCUGCUCGAAAU	1292	59-81	207-229
AD-1395732.1	UUCGAGCAGAAGGAAAGUAAA	1281	61-81	209-229	UUUACUTUCCUUCUGCUCGAAAU	1292	59-81	207-229
AD-1395733.1	UUCGAGCAGAAGGAAAGUAAA	1281	61-81	209-229	UUUACUTUCCUUCUGCUCGAAAU	1292	59-81	207-229
AD-1395734.1	UUCGAGCAGAAGGAAAGUAAA	1281	61-81	209-229	UTUACUTUCCUTCUGCUCGAAAU	1293	59-81	207-229
AD-1395735.1	UUCGAGCAGAAGGAAAGUAAA	1281	61-81	209-229	UTUACUTUCCUTCUGCUCGAAAU	1293	59-81	207-229
AD-1395736.1	UUCGAGCAGAAGGAAAGUAAA	1281	61-81	209-229	UTUACUTUCCUTCUGCUCGAAAU	1293	59-81	207-229
AD-1321219.3	AAGGAAAGUAAUGGACCAGUU	85	70-90	218-238	AACUGGTCCAUUACUUUCCUUCU	1021	68-90	216-238
AD-1395737.1	AAGGAAAGUAAUGGACCAGUA	1282	70-90	218-238	UACUGGTCCAUUACUUUCCUUCU	1294	68-90	216-238
AD-1395738.1	AAGGAAAGUAAUGGACCAGUA	1282	70-90	218-238	UACUGGTCCAUTACUUUCCUUCU	1295	68-90	216-238
AD-1395739.1	AAGGAAAGUAAUGGACCAGUA	1282	70-90	218-238	UACUGGTCCAUTACUTUCCUUCU	1296	68-90	216-238
AD-1321220.3	AGGAAAGUAAUGGACCAGUGA	458	71-91	219-239	UCACTGGUCCAUUACUUUCCUUC	1022	69-91	217-239
AD-1395740.1	AGGAAAGUAAUGGACCAGUGA	458	71-91	219-239	UCACTGGUCCAUUACUUUCCUUC	1022	69-91	217-239
AD-1395741.1	AGGAAAGUAAUGGACCAGUGA	458	71-91	219-239	UCACTGGUCCATUACUUUCCUUC	1297	69-91	217-239
AD-1395742.1	AGGAAAGUAAUGGACCAGUGA	458	71-91	219-239	UCACTGGUCCATUACTUUCCUUC	1298	69-91	217-239
AD-1321222.3	GAAAGUAAUGGACCAGUGAAU	460	73-93	221-241	AUUCACTGGUCCAUUACUUUCCU	851	71-93	219-241
AD-1395743.1	GAAAGUAAUGGACCAGUGAAA	1283	73-93	221-241	UUUCACTGGUCCAUUACUUUCCU	1299	71-93	219-241
AD-1395744.1	GAAAGUAAUGGACCAGUGAAA	1283	73-93	221-241	UUUCACTGGUCCAUUACUUUCCU	1299	71-93	219-241

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_		Antisense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_	
			001285406.1	000454.4			001285406.1	000454.4
AD-1395745.1	GAAAGUAAUGGACCAGUGAAA	1283	73-93	221-241	UUUCACTGGUCCAUUACUUUCCU	1299	71-93	219-241
AD-1395746.1	GAAAGUAAUGGACCAGUGAAA	1283	73-93	221-241	UTUCACTGGUCCAUUACUUUCCU	1300	71-93	219-241
AD-1395747.1	GAAAGUAAUGGACCAGUGAAA	1283	73-93	221-241	UTUCACTGGUCCAUUACUUUCCU	1300	71-93	219-241
AD-1395748.1	GAAAGUAAUGGACCAGUGAAA	1283	73-93	221-241	UTUCACTGGUCCAUUACUUUCCU	1300	71-93	219-241
AD-1321232.3	CCAGUGCAGGUCCUCACUUUA	75	176-196	324-344	UAAAGUGAGGACCUGCACUGGUA	158	174-196	322-344
AD-1395749.1	CCAGUGCAGGUCCUCACUUUA	75	176-196	324-344	UAAAGUGAGGACCUGCACUGGUA	158	174-196	322-344
AD-1395750.1	CCAGUGCAGGUCCUCACUUUA	75	176-196	324-344	UAAAGUGAGGACCUGCACUGGUG	1301	174-196	322-344
AD-1395751.1	CCAGUGCAGGUCCUCACUUUA	75	176-196	324-344	UAAAGUGAGGACCUGCACUGGUG	1301	174-196	322-344
AD-1395752.1	CCAGUGCAGGUCCUCACUUUA	75	176-196	324-344	UAAAGUGAGGACCUGCACUGGUG	1301	174-196	322-344
AD-1395753.1	CCAGUGCAGGUCCUCACUUUA	75	176-196	324-344	UAAAGUGAGGACCUGCACUGGUG	1301	174-196	322-344
AD-1395754.1	CCAGUGCAGGUCCUCACUUUA	75	176-196	324-344	UAAAGUGAGGACCUGCACUGGUG	1301	174-196	322-344
AD-1395755.1	CCAGUGCAGGUCCUCACUUUA	75	176-196	324-344	UAAAGUGAGGACCUGCACUGGUG	1301	174-196	322-344
AD-1321238.3	CAGGUCCUCACUUUAAUCCUU	41	182-202	330-350	AAGGAUUAAAGUGAGGACCUGCA	124	180-202	328-350
AD-1395756.1	CAGGUCCUCACUUUAAUCCUA	1284	182-202	330-350	UAGGAUTAAAGTGAGGACCUGCG	1302	180-202	328-350
AD-1395757.1	CAGGUCCUCACUUUAAUCCUA	1284	182-202	330-350	UAGGAUTAAAGTGAGGACCUGCG	1302	180-202	328-350
AD-1395758.1	CAGGUCCUCACUUUAAUCCUA	1284	182-202	330-350	UAGGAUTAAAGTGAGGACCUGCG	1302	180-202	328-350
AD-1321243.3	CCUCACUUUAAUCCUCUAUCU	42	187-207	335-355	AGAUAGAGGAUUAAAGUGAGGAC	125	185-207	333-355

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_		Antisense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_	
			001285406.1	000454.4			001285406.1	000454.4
AD-1395759.1	CCUCACUUUAAUCCUCUA UCA	1285	187-207	335-355	UGAUAGAGGAUUAAAGUGA GGAC	1303	185-207	333-355
AD-1395760.1	CCUCACUUUAAUCCUCUA UCA	1285	187-207	335-355	UGAUAGAGGAUTAAAGUGAG GAC	1304	185-207	333-355
AD-1395761.1	CCUCACUUUAAUCCUCUA UCA	1285	187-207	335-355	UGAUAGAGGAUTAAAGUGAG GAC	1304	185-207	333-355
AD-1321246.3	CACUUUAAUCCUCUAUCC AGA	71	190-210	338-358	ACUGGAUAGAGGAUUAAAG UGAG	1030	188-210	336-358
AD-1395762.1	CACUUUAAUCCUCUAUCC AGA	71	190-210	338-358	UCUGGATAGAGGAUUAAAGU GAG	858	188-210	336-358
AD-1395763.1	CACUUUAAUCCUCUAUCC AGA	71	190-210	338-358	UCUGGATAGAGGATUAAAGU GAG	1305	188-210	336-358
AD-1321256.3	AAGGAUGAAGAGAGGCAU GUU	474	226-246	374-394	AACATGCCUCUCUUCAUCCU UUG	1039	224-246	372-394
AD-1395764.1	AAGGAUGAAGAGAGGCAU GUA	1286	226-246	374-394	UACATGCCUCUCUUCAUCCU UUG	1306	224-246	372-394
AD-1395765.1	AAGGAUGAAGAGAGGCAU GUA	1286	226-246	374-394	UACATGCCUCUCUUCAUCCU UUG	1306	224-246	372-394
AD-1395766.1	AAGGAUGAAGAGAGGCAU GUA	1286	226-246	374-394	UACATGCCUCUCUUCAUCCU UUG	1306	224-246	372-394
AD-1395767.1	AAGGAUGAAGAGAGGCAU GUA	1286	226-246	374-394	UACATGCCUCUCUUCAUCCU UUG	1306	224-246	372-394
AD-1395768.1	AAGGAUGAAGAGAGGCAU GUA	1286	226-246	374-394	UACATGCCUCUCUUCAUCCU UUG	1306	224-246	372-394
AD-1395769.1	AAGGAUGAAGAGAGGCAU GUA	1286	226-246	374-394	UACATGCCUCUCUUCAUCCU UUG	1306	224-246	372-394
AD-1321257.3	AGGAUGAAGAGAGGCAUG UUU	475	227-247	375-395	AAACAUGCCUCUCUUCAUCC UUU	554	225-247	373-395
AD-1395770.1	AGGAUGAAGAGAGGCAUG UUU	475	227-247	375-395	AAACAUGCCUCUCUUCAUCC UUU	554	225-247	373-395
AD-1395771.1	AGGAUGAAGAGAGGCAUG UUA	1287	227-247	375-395	UAACAUGCCUCUCUUCAUCC UUU	1307	225-247	373-395
AD-1395772.1	AGGAUGAAGAGAGGCAUG UUA	1287	227-247	375-395	UAACAUGCCUCUCUUCAUCC UUU	1307	225-247	373-395

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_		Antisense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_	
			001285406.1	000454.4			001285406.1	000454.4
AD-1395773.1	AGGAUGAAGAGAGGGCAUG UUA	1287	227-247	375-395	UAACAUGCCUCUCUUCAUCC UUU	1307	225-247	373-395
AD-1395774.1	AGGAUGAAGAGAGGGCAUG UUA	1287	227-247	375-395	UAACAUGCCUCTCUUCAUCC UUU	1308	225-247	373-395
AD-1395775.1	AGGAUGAAGAGAGGGCAUG UUA	1287	227-247	375-395	UAACAUGCCUCTCUUCAUCC UUU	1308	225-247	373-395
AD-1321276.3	UUGGGCAAAGGUGGGAAAU GAA	103	379-399	527-547	UUCATUTCCACCUUUGCCCA AGU	1051	377-399	525-547
AD-1395776.1	UUGGGCAAAGGUGGGAAAU GAA	103	379-399	527-547	UUCATUTCCACCUUUGCCCA AGU	1051	377-399	525-547
AD-1395777.1	UUGGGCAAAGGUGGGAAAU GAA	103	379-399	527-547	UUCATUTCCACCUUUGCCCA AGU	1051	377-399	525-547
AD-1395778.1	UUGGGCAAAGGUGGGAAAU GAA	103	379-399	527-547	UUCATUTCCACCUUUGCCCA AGU	1051	377-399	525-547
AD-1395779.1	UUGGGCAAAGGUGGGAAAU GAA	103	379-399	527-547	UTCATUTCCACCUUUGCCCA AGU	1309	377-399	525-547
AD-1395780.1	UUGGGCAAAGGUGGGAAAU GAA	103	379-399	527-547	UTCATUTCCACCUUUGCCCA AGU	1309	377-399	525-547
AD-1321280.3	GCAAAGGUGGAAAUGAAG AAA	999	383-403	531-551	UUUCTUCAUUUCCACCUUUG CCC	1055	381-403	529-551
AD-1395781.1	GCAAAGGUGGAAAUGAAG AAA	999	383-403	531-551	UUUCTUCAUUUCCACCUUUG CCC	1055	381-403	529-551
AD-1395782.1	GCAAAGGUGGAAAUGAAG AAA	999	383-403	531-551	UUUCTUCAUUUCCACCUUUG CCC	1055	381-403	529-551
AD-1395783.1	GCAAAGGUGGAAAUGAAG AAA	999	383-403	531-551	UUUCTUCAUUUCCACCUUUG CCC	1055	381-403	529-551
AD-1395784.1	GCAAAGGUGGAAAUGAAG AAA	999	383-403	531-551	UTUCTUCAUUUCCACCUUUG CCC	1310	381-403	529-551
AD-1395785.1	GCAAAGGUGGAAAUGAAG AAA	999	383-403	531-551	UTUCTUCAUUUCCACCUUUG CCC	1310	381-403	529-551
AD-1321284.3	AGGUGGAAAUGAAGAAAG UAU	512	387-407	535-555	AUACTUTCUUCAUUUCCACC UUU	1059	385-407	533-555
AD-1395786.1	AGGUGGAAAUGAAGAAAG UAU	512	387-407	535-555	AUACTUTCUUCAUUUCCACC UUU	1059	385-407	533-555

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_		Antisense Sequence 5' to 3'	SEQ ID NO:	Range in GenBank Accession No. NM_	
			001285406.1	000454.4			001285406.1	000454.4
AD-1395787.1	AGGUGGAAAUGAAGAAAG UAA	1288	387-407	535-555	UUACTUTCUUCAUUUCCACC UUU	1311	385-407	533-555
AD-1395788.1	AGGUGGAAAUGAAGAAAG UAA	1288	387-407	535-555	UUACTUTCUUCAUUUCCACC UUU	1311	385-407	533-555
AD-1395789.1	AGGUGGAAAUGAAGAAAG UAA	1288	387-407	535-555	UUACTUTCUUCAUUUCCACC UUU	1311	385-407	533-555
AD-1395790.1	AGGUGGAAAUGAAGAAAG UAA	1288	387-407	535-555	UTACTUTCUUCAUUUCCACC UUU	1312	385-407	533-555
AD-1395791.1	AGGUGGAAAUGAAGAAAG UAA	1288	387-407	535-555	UTACTUTCUUCAUUUCCACC UUU	1312	385-407	533-555
AD-1395792.1	AGGUGGAAAUGAAGAAAG UAA	1288	387-407	535-555	UTACTUTCUUCAUUUCCACC UUU	1312	385-407	533-555

Table 20. Modified Sense and Antisense Strand Sequences of Superoxide Dismutase 1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1321204.3	asuscaa(Uhd)UfuCfGfAfgcagaaggaa L96	1069	usUfsccdTu(C2p)ugcucgAfaAfuugausgsa	1159	CCAUCAAUUUCGAGCAGAAGGAA	395
AD-1395718.1	asuscaa(Uhd)uuCfGfAfgcagaaggsasa	23	VPusUfsccdTu(C2p)ugcucgAfaAfuugausg sg	24	CCAUCAAUUUCGAGCAGAAGGAA	395
AD-1395719.1	asuscaa(Uhd)uuCfgAfgcagaaggsasa	1313	VPusUfsccdTu(C2p)ugcucgAfaAfuugausg sg	24	CCAUCAAUUUCGAGCAGAAGGAA	395
AD-1395720.1	asuscaa(Uhd)uuCfgAfdGcagaaggsas a	1314	VPusUfsccdTu(C2p)ugcucgAfaAfuugausg sg	24	CCAUCAAUUUCGAGCAGAAGGAA	395
AD-1395721.1	asuscaa(Uhd)uuCfGfAfgcagaaggsasa	23	VPusdTsccdTu(C2p)ugcudCgAfaauugaus gsg	1343	CCAUCAAUUUCGAGCAGAAGGAA	395
AD-1395722.1	asuscaa(Uhd)uuCfgAfgcagaaggsasa	1313	VPusdTsccdTu(C2p)ugcudCgAfaauugaus gsg	1343	CCAUCAAUUUCGAGCAGAAGGAA	395

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1395723.1	asuscaa(Uhd)uuCfgAfdGcagaaggsasa	1314	VPusdTsccdTu(C2p)ugcudCgAfaauugausgsg	1343	CCAUCAAUUUCGAGCAGAAGGAA	395
AD-1321207.3	asasuuu(Chd)GfaGfCfAfgaaggaaaguL96	1072	asCfsuudTc(C2p)uucugcUfcGfaaauusgsa	1162	UCAAUUUCGAGCAGAAGGAAAGU	770
AD-1395724.1	asasuuu(Chd)gaGfCfAfgaaggaaasgsa	29	VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg	30	UCAAUUUCGAGCAGAAGGAAAGU	770
AD-1395725.1	asasuuu(Chd)gagCfAfgaaggaaasgsa	1315	VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg	30	UCAAUUUCGAGCAGAAGGAAAGU	770
AD-1395726.1	asasuuu(Chd)gadGcAfdGaaggaaasgsa	1316	VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg	30	UCAAUUUCGAGCAGAAGGAAAGU	770
AD-1395727.1	asasuuu(Chd)gaGfCfAfgaaggaaasgsa	29	VPusdCsuudTc(C2p)uucudGcUfcgaaauusgsg	1344	UCAAUUUCGAGCAGAAGGAAAGU	770
AD-1395728.1	asasuuu(Chd)gagCfAfgaaggaaasgsa	1315	VPusdCsuudTc(C2p)uucudGcUfcgaaauusgsg	1344	UCAAUUUCGAGCAGAAGGAAAGU	770
AD-1395729.1	asasuuu(Chd)gadGcAfdGaaggaaasgsa	1316	VPusdCsuudTc(C2p)uucudGcUfcgaaauusgsg	1344	UCAAUUUCGAGCAGAAGGAAAGU	770
AD-1321210.3	ususcga(Ghd)CfaGfAfAfggaaaguaauL96	1075	asUfsuadCu(Tgn)uccuucUfgCfucgaasasu	1165	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-1395730.1	ususcgag(Chd)aGfAfAfggaaaguaauL96	1317	asUfsuadCu(Tgn)uccuucUfgCfucgaasasu	1165	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-1395731.1	ususcgag(Chd)aGfAfAfggaaaguasasa	15	VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu	16	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-1395732.1	ususcgag(Chd)agAfAfggaaaguasasa	1318	VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu	16	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-1395733.1	ususcgag(Chd)adGaAfdGgaaaguasasa	1319	VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu	16	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-1395734.1	ususcgag(Chd)aGfAfAfggaaaguasasa	15	VPusdTsuadCu(Tgn)uccudTcUfgcucgaasasu	1345	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-1395735.1	ususcgag(Chd)agAfAfggaaaguasasa	1318	VPusdTsuadCu(Tgn)uccudTcUfgcucgaasasu	1345	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-1395736.1	ususcgag(Chd)adGaAfdGgaaaguasasa	1319	VPusdTsuadCu(Tgn)uccudTcUfgcucgaasasu	1345	AUUUCGAGCAGAAGGAAAGUAAU	378
AD-1321219.3	asasgga(Ahd)AfgUfAfAfuggaccaguuL96	1084	asAfscudGg(Tgn)ccauuaCfuUfuccuuscsu	1174	AGAAGGAAAGUAAUGGACCAGUG	417
AD-1395737.1	asasgga(Ahd)agUfAfAfuggaccagsusa	21	VPusAfscudGg(Tgn)ccauuaCfuUfuccuuscsu	1346	AGAAGGAAAGUAAUGGACCAGUG	417

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1395738.1	asasgga(Ahd)agUfAfAfuggaccagsusa	21	VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu	22	AGAAGGAAAGUAAUGGACCAGUG	417
AD-1395739.1	asasgga(Ahd)agUfAfAfuggaccagsusa	21	VPusdAscudGg(Tgn)ccaudTadCudTuccuuscsu	1347	AGAAGGAAAGUAAUGGACCAGUG	417
AD-1321220.3	asgsgaa(Ahd)GfuAfAfUfggaccaguga L96	1085	usCfsacdTg(G2p)uccauuAfcUfuuccususc	1175	GAAGGAAAGUAAUGGACCAGUGA	774
AD-1395740.1	asgsgaa(Ahd)guAfAfUfggaccagusgsa	1320	VPusCfsacdTg(G2p)uccauuAfcUfuuccususc	1348	GAAGGAAAGUAAUGGACCAGUGA	774
AD-1395741.1	asgsgaa(Ahd)guAfAfUfggaccagusgsa	1320	VPusdCsacdTg(G2p)uccadTuAfcuuuccususc	1349	GAAGGAAAGUAAUGGACCAGUGA	774
AD-1395742.1	asgsgaa(Ahd)guAfAfUfggaccagusgsa	1320	VPusdCsacdTg(G2p)uccadTudAcdTuuccususc	1350	GAAGGAAAGUAAUGGACCAGUGA	774
AD-1321222.3	gsasaag(Uhd)AfaUfGfGfaccagugaau L96	1087	asUfsucdAc(Tgn)gguccaUfuAfcuuucscsu	1177	AGGAAAGUAAUGGACCAGUGAAG	776
AD-1395743.1	gsasaag(Uhd)aaUfGfGfaccagugasasa	17	VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu	18	AGGAAAGUAAUGGACCAGUGAAG	776
AD-1395744.1	gsasaag(Uhd)aaUfgdGaccagugasasa	1321	VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu	18	AGGAAAGUAAUGGACCAGUGAAG	776
AD-1395745.1	gsasaag(Uhd)aaUfgdGAfccagugasasa	1322	VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu	18	AGGAAAGUAAUGGACCAGUGAAG	776
AD-1395746.1	gsasaag(Uhd)aaUfGfGfaccagugasasa	17	VPusdTsucdAc(Tgn)ggucdCaUfuacuuucscsu	1351	AGGAAAGUAAUGGACCAGUGAAG	776
AD-1395747.1	gsasaag(Uhd)aaUfgdGaccagugasasa	1321	VPusdTsucdAc(Tgn)ggucdCaUfuacuuucscsu	1351	AGGAAAGUAAUGGACCAGUGAAG	776
AD-1395748.1	gsasaag(Uhd)aaUfgdGAfccagugasasa	1322	VPusdTsucdAc(Tgn)ggucdCaUfuacuuucscsu	1351	AGGAAAGUAAUGGACCAGUGAAG	776
AD-1321232.3	cscsagu(Ghd)CfaGfGfUfccucacuuua L96	1097	usAfsaadGu(G2p)aggaccUfgCfacuggsusa	1187	UACCAGUGCAGGUCCUCACUUUA	407
AD-1395749.1	cscsagug(Chd)aGfGfUfccucacuuuaL96	1323	usAfsaadGu(G2p)aggaccUfgCfacuggsusa	1187	UACCAGUGCAGGUCCUCACUUUA	407
AD-1395750.1	cscsagug(Chd)aGfGfUfccucacuususa	1324	VPusAfsaadGu(G2p)aggaccUfgCfacuggsusg	1352	UACCAGUGCAGGUCCUCACUUUA	407
AD-1395751.1	cscsagug(Chd)adGgUfccucacuususa	1325	VPusAfsaadGu(G2p)aggaccUfgCfacuggsusg	1352	UACCAGUGCAGGUCCUCACUUUA	407
AD-1395752.1	cscsagug(Chd)adGgUfCfcucacuususa	1326	VPusAfsaadGu(G2p)aggaccUfgCfacuggsusg	1352	UACCAGUGCAGGUCCUCACUUUA	407

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1395753.1	cscsagug(Chd)aGfGfUfccucacuususa	1324	VPusdAsaadGu(G2p)aggadCcUfgcacuggsusg	1353	UACCAGUGCAGGUCCUCACUUUA	407
AD-1395754.1	cscsagug(Chd)adGgUfccucacuususa	1325	VPusdAsaadGu(G2p)aggadCcUfgcacuggsusg	1353	UACCAGUGCAGGUCCUCACUUUA	407
AD-1395755.1	cscsagug(Chd)adGgUfCfcucacuususa	1326	VPusdAsaadGu(G2p)aggadCcUfgcacuggsusg	1353	UACCAGUGCAGGUCCUCACUUUA	407
AD-1321238.3	csasggu(Chd)CfuCfAfCfuuuaauccuuL96	1103	asAfsggaUfuaaagugAfgGfaccugscsa	1193	UGCAGGUCCUCACUUUAAUCCUC	373
AD-1395756.1	csasggu(Chd)cuCfAfCfuuuaauccsusa	13	VPusdAsggdAudTaaagdTgAfggaccugscsg	14	UGCAGGUCCUCACUUUAAUCCUC	373
AD-1395757.1	csasggu(Chd)cuCfAfCfuuuaauccsusa	13	VPusdAsggdAudTaaagdTgdAgdGaccugscsg	1354	UGCAGGUCCUCACUUUAAUCCUC	373
AD-1395758.1	csasggu(Chd)cuCfaCfUfuuaauccsusa	1327	VPusdAsggdAudTaaagdTgdAgdGaccugscsg	1354	UGCAGGUCCUCACUUUAAUCCUC	373
AD-1321243.3	cscsuca(Chd)UfuUfAfAfuccucuaucuL96	1108	asGfsaudAg(Agn)ggauuaAfaGfugaggsasc	1198	GUCCUCACUUUAAUCCUCUAUCC	374
AD-1395759.1	cscsuca(Chd)uuUfAfAfuccucuauscsa	25	VPusGfsaudAg(Agn)ggauuaAfaGfugaggsasc	1355	GUCCUCACUUUAAUCCUCUAUCC	374
AD-1395760.1	cscsuca(Chd)uuUfAfAfuccucuauscsa	25	VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc	26	GUCCUCACUUUAAUCCUCUAUCC	374
AD-1395761.1	cscsuca(Chd)uuUfAfAfuccucuauscsa	25	VPusdGsaudAg(Agn)ggaudTadAadGugaggsasc	1356	GUCCUCACUUUAAUCCUCUAUCC	374
AD-1321246.3	csascuu(Uhd)AfaUfCfCfucuauccagaL96	1111	asCfsuggAfuagaggaUfuAfaagugsasg	1201	CUCACUUUAAUCCUCUAUCCAGA	403
AD-1395762.1	csascuu(Uhd)aaUfCfCfucuauccasgsa	11	VPusdCsugdGadTagagdGaUfuaaagugsasg	12	CUCACUUUAAUCCUCUAUCCAGA	403
AD-1395763.1	csascuu(Uhd)aaUfCfCfucuauccasgsa	11	VPusdCsugdGadTagagdGadTudAaagugsasg	1357	CUCACUUUAAUCCUCUAUCCAGA	403
AD-1321256.3	asasgga(Uhd)GfaAfGfAfgaggcauguuL96	1121	asAfscadTg(C2p)cucucuUfcAfuccuususg	1211	CAAAGGAUGAAGAGAGGCAUGUU	790
AD-1395764.1	asasgga(Uhd)gaAfGfAfgaggcaugsusa	27	VPusAfscadTg(C2p)cucucuUfcAfuccuususg	28	CAAAGGAUGAAGAGAGGCAUGUU	790
AD-1395765.1	asasgga(Uhd)gaAfgAfdGaggcaugsusa	1328	VPusAfscadTg(C2p)cucucuUfcAfuccuususg	28	CAAAGGAUGAAGAGAGGCAUGUU	790
AD-1395766.1	asasgga(Uhd)gadAgdAgaggcaugsusa	1329	VPusAfscadTg(C2p)cucucuUfcAfuccuususg	28	CAAAGGAUGAAGAGAGGCAUGUU	790

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1395767.1	asasgga(Uhd)gaAfGfAfgaggcaugsusa	27	VPusdAscadTg(C2p)cucudCuUfcauccuususg	1358	CAAAGGAUGAAGAGAGGCAUGUU	790
AD-1395768.1	asasgga(Uhd)gaAfgAfdGaggcaugsusa	1328	VPusdAscadTg(C2p)cucudCuUfcauccuususg	1358	CAAAGGAUGAAGAGAGGCAUGUU	790
AD-1395769.1	asasgga(Uhd)gadAgdAgaggcaugsusa	1329	VPusdAscadTg(C2p)cucudCuUfcauccuususg	1358	CAAAGGAUGAAGAGAGGCAUGUU	790
AD-1321257.3	asgsgau(Ghd)AfaGfAfGfaggcauguuuL96	1122	asAfsacdAu(G2p)ccucucUfuCfauccususu	1212	AAAGGAUGAAGAGAGGCAUGUUG	791
AD-1395770.1	asgsga(Uhd)gAfaGfAfGfaggcauguuuL96	1330	asAfsacdAu(G2p)ccucucUfuCfauccususu	1212	AAAGGAUGAAGAGAGGCAUGUUG	791
AD-1395771.1	asgsga(Uhd)gaaGfAfGfaggcaugsusa	19	VPusAfsacdAu(G2p)ccucucUfuCfauccususu	20	AAAGGAUGAAGAGAGGCAUGUUG	791
AD-1395772.1	asgsga(Uhd)gaadGadGaggcaugususa	1331	VPusAfsacdAu(G2p)ccucucUfuCfauccususu	20	AAAGGAUGAAGAGAGGCAUGUUG	791
AD-1395773.1	asgsga(Uhd)gaadGadGAfggcaugsusa	1332	VPusAfsacdAu(G2p)ccucucUfuCfauccususu	20	AAAGGAUGAAGAGAGGCAUGUUG	791
AD-1395774.1	asgsga(Uhd)gaadGadGaggcaugususa	1331	VPusdAsacdAu(G2p)ccucdTcUfucauccususu	1359	AAAGGAUGAAGAGAGGCAUGUUG	791
AD-1395775.1	asgsga(Uhd)gaadGadGAfggcaugsusa	1332	VPusdAsacdAu(G2p)ccucdTcUfucauccususu	1359	AAAGGAUGAAGAGAGGCAUGUUG	791
AD-1321276.3	ususggg(Chd)AfaAfGfGfuggaaaugaaL96	1141	usUfscadTu(Tgn)ccaccuUfuGfcccaasgsu	1231	ACUUGGGCAAAGGUGGAAAUGAA	435
AD-1395776.1	ususggg(Chd)aaAfGfGfuggaaaugsasa	1333	VPusUfscadTu(Tgn)ccaccuUfuGfcccaasgsu	1360	ACUUGGGCAAAGGUGGAAAUGAA	435
AD-1395777.1	ususggg(Chd)aaAfgdGuggaaaugsasa	1334	VPusUfscadTu(Tgn)ccaccuUfuGfcccaasgsu	1360	ACUUGGGCAAAGGUGGAAAUGAA	435
AD-1395778.1	ususggg(Chd)aaAfgdGUfggaaaugsasa	1335	VPusUfscadTu(Tgn)ccaccuUfuGfcccaasgsu	1360	ACUUGGGCAAAGGUGGAAAUGAA	435
AD-1395779.1	ususggg(Chd)aaAfgdGuggaaaugsasa	1334	VPusdTscadTu(Tgn)ccacdCuUfugcccaasgsu	1361	ACUUGGGCAAAGGUGGAAAUGAA	435
AD-1395780.1	ususggg(Chd)aaAfgdGUfggaaaugsasa	1335	VPusdTscadTu(Tgn)ccacdCuUfugcccaasgsu	1361	ACUUGGGCAAAGGUGGAAAUGAA	435
AD-1321280.3	gscsaaa(Ghd)GfuGfGfAfaaugaagaaaL96	1145	usUfsucdTu(C2p)auuuccAfcCfuuugcscsc	1235	GGGCAAAGGUGGAAAUGAAGAAA	1270
AD-1395781.1	gscsaaagGfuGfGfAfaaug(Ahd)agaaaL96	1336	usUfsucdTu(C2p)auuuccAfcCfuuugcscsc	1235	GGGCAAAGGUGGAAAUGAAGAAA	1270

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1395782.1	gscsaaagguGfGfAfaaug(Ahd)agasasa	1337	VPusUfsucdTu(C2p)auuuccAfcCfuuugcsc sc	1362	GGGCAAAGGUGGAAAUGAAGAAA	1270
AD-1395783.1	gscsaaaggudGgAfaaug(Ahd)agasasa	1338	VPusUfsucdTu(C2p)auuuccAfcCfuuugcsc sc	1362	GGGCAAAGGUGGAAAUGAAGAAA	1270
AD-1395784.1	gscsaaagguGfGfAfaaug(Ahd)agasasa	1337	VPusdTsucdTu(C2p)auuudCcAfccuuugcs csc	1363	GGGCAAAGGUGGAAAUGAAGAAA	1270
AD-1395785.1	gscsaaaggudGgAfaaug(Ahd)agasasa	1338	VPusdTsucdTu(C2p)auuudCcAfccuuugcs csc	1363	GGGCAAAGGUGGAAAUGAAGAAA	1270
AD-1321284.3	asgsgug(Ghd)AfaAfUfGfaagaaaguau L96	1149	asUfsacdTu(Tgn)cuucauUfuCfcaccususu	1239	AAAGGUGGAAAUGAAGAAAGUAA	1273
AD-1395786.1	asgsguggAfaAfUfUfGfaaga(Ahd)aguau L96	1339	asUfsacdTu(Tgn)cuucauUfuCfcaccususu	1239	AAAGGUGGAAAUGAAGAAAGUAA	1273
AD-1395787.1	asgsguggaaAfUfGfaaga(Ahd)agusas a	1340	VPusUfsacdTu(Tgn)cuucauUfuCfcaccusu su	1364	AAAGGUGGAAAUGAAGAAAGUAA	1273
AD-1395788.1	asgsguggaaAfudGaaga(Ahd)agusasa	1341	VPusUfsacdTu(Tgn)cuucauUfuCfcaccusu su	1364	AAAGGUGGAAAUGAAGAAAGUAA	1273
AD-1395789.1	asgsguggaaAfudGAfaga(Ahd)agusas a	1342	VPusUfsacdTu(Tgn)cuucauUfuCfcaccusu su	1364	AAAGGUGGAAAUGAAGAAAGUAA	1273
AD-1395790.1	asgsguggaaAfUfGfaaga(Ahd)agusas a	1340	VPusdTsacdTu(Tgn)cuucdAuUfuccaccusu su	1365	AAAGGUGGAAAUGAAGAAAGUAA	1273
AD-1395791.1	asgsguggaaAfudGaaga(Ahd)agusasa	1341	VPusdTsacdTu(Tgn)cuucdAuUfuccaccusu su	1365	AAAGGUGGAAAUGAAGAAAGUAA	1273
AD-1395792.1	asgsguggaaAfudGAfaga(Ahd)agusas a	1342	VPusdTsacdTu(Tgn)cuucdAuUfuccaccusu su	1365	AAAGGUGGAAAUGAAGAAAGUAA	1273

Table 21. Superoxide Dismutase 1 *In Vitro* Single Dose Screens in Primary Cynomolgus Hepatocytes (PCH) cells

	50 nM		10 nM		1 nM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1321204.3	4.2	0.5	9.4	7.6	32.0	7.5	44.4	15.6
AD-1395718.1	10.2	1.2	7.0	1.5	16.1	6.8	16.1	2.0
AD-1395719.1	129.7	21.2	70.3	9.2	77.6	5.3	62.8	5.6
AD-1395720.1	142.6	13.6	112.8	39.7	97.6	8.4	86.1	11.1
AD-1395721.1	26.0	4.4	18.1	2.7	37.3	2.0	42.9	5.7
AD-1395722.1	83.9	33.3	71.4	15.4	106.4	12.8	109.9	25.9
AD-1395723.1	127.6	19.8	118.0	41.9	110.2	2.2	114.6	25.6
AD-1321207.3	3.1	1.0	3.3	1.0	17.2	5.7	27.7	7.6
AD-1395724.1	2.3	0.2	3.3	0.3	13.3	7.5	12.1	4.2
AD-1395725.1	29.8	9.8	13.0	0.7	23.2	3.1	26.2	1.9
AD-1395726.1	114.1	21.8	70.9	13.1	74.3	14.6	56.2	6.8
AD-1395727.1	7.3	1.2	5.5	0.4	12.7	2.8	19.2	3.7
AD-1395728.1	66.0	6.7	19.4	2.2	37.5	3.9	52.2	2.2
AD-1395729.1	57.4	8.0	48.7	14.0	62.5	8.9	63.9	15.7
AD-1321210.3	2.5	0.6	2.0	0.5	9.5	2.4	13.9	2.9
AD-1395730.1	1.7	0.6	2.5	0.8	15.4	7.0	24.1	6.9
AD-1395731.1	1.3	0.2	2.7	0.4	9.8	0.6	11.8	0.8
AD-1395732.1	59.2	9.9	21.8	4.2	34.3	2.2	46.3	6.0
AD-1395733.1	63.5	14.3	13.0	4.1	15.6	2.6	23.8	2.8
AD-1395734.1	2.0	0.7	2.4	0.4	8.1	1.6	13.9	1.3
AD-1395735.1	4.8	1.0	5.9	0.9	17.6	2.0	31.0	6.2
AD-1395736.1	22.7	4.3	6.3	1.0	14.9	4.4	23.8	5.5
AD-1321219.3	2.7	0.4	3.1	0.7	22.3	3.6	46.1	14.1
AD-1395737.1	2.7	0.7	3.9	0.1	14.6	1.0	17.3	5.9
AD-1395738.1	3.0	1.7	3.6	0.5	11.3	1.7	21.4	6.5
AD-1395739.1	6.7	2.3	7.0	1.1	18.0	3.5	35.0	10.0
AD-1321220.3	2.3	0.5	2.3	0.4	15.5	4.8	37.3	8.1
AD-1395740.1	1.5	0.2	3.0	0.6	9.0	2.9	13.0	4.0
AD-1395741.1	2.0	0.1	2.7	0.5	13.1	3.6	22.8	9.2
AD-1395742.1	2.8	0.7	3.2	0.5	12.2	5.3	24.5	5.8
AD-1321222.3	2.7	0.3	4.0	0.9	23.1	2.5	46.7	12.7
AD-1395743.1	7.4	3.0	5.3	2.0	17.1	4.7	25.2	6.5
AD-1395744.1	80.7	9.7	48.0	10.7	56.2	10.5	78.6	8.3
AD-1395745.1	106.6	11.9	56.1	20.2	53.4	2.9	81.7	8.8
AD-1395746.1	11.3	2.3	8.9	0.6	29.8	5.9	61.3	10.4
AD-1395747.1	34.9	6.1	43.3	2.1	66.6	4.6	89.5	8.3
AD-1395748.1	84.6	10.5	61.1	9.7	76.1	9.1	103.6	16.4
AD-1321232.3	23.9	4.4	7.0	1.0	21.0	2.6	36.8	11.9

	50 nM		10 nM		1 nM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1395749.1	9.6	3.3	4.8	0.5	38.1	11.0	60.9	10.6
AD-1395750.1	5.3	2.0	6.6	1.0	16.4	2.6	38.4	14.7
AD-1395751.1	92.3	7.9	58.5	15.2	63.6	6.2	87.6	11.6
AD-1395752.1	119.3	16.0	65.1	24.8	68.6	9.2	85.8	11.1
AD-1395753.1	10.9	3.5	6.7	0.8	16.3	2.8	40.5	9.3
AD-1395754.1	49.0	3.6	45.2	6.1	59.8	7.5	83.6	7.3
AD-1395755.1	65.5	7.2	26.3	10.9	48.8	5.2	75.5	10.9
AD-1321238.3	3.4	0.6	4.7	0.8	22.5	2.1	46.3	9.5
AD-1395756.1	12.2	3.1	8.1	0.7	18.1	4.0	32.8	14.7
AD-1395757.1	3.2	0.8	4.2	0.6	13.1	3.0	34.7	15.2
AD-1395758.1	80.0	13.6	39.0	12.2	41.6	1.8	53.8	5.3
AD-1321243.3	2.2	1.3	2.4	0.3	13.4	2.1	37.7	17.9
AD-1395759.1	1.5	0.9	2.9	0.9	8.6	1.4	16.8	8.9
AD-1395760.1	2.0	0.6	2.9	0.6	10.4	2.1	26.1	9.7
AD-1395761.1	4.6	1.2	4.6	1.1	18.7	3.8	38.7	8.0
AD-1321246.3	1.6	0.6	2.0	0.4	15.7	1.9	40.9	20.4
AD-1395762.1	8.3	2.5	6.4	1.2	13.7	2.2	32.7	9.7
AD-1395763.1	11.4	2.6	9.7	0.6	21.0	2.7	50.3	14.5
AD-1321256.3	1.5	0.2	1.7	0.2	7.5	2.0	22.7	5.3
AD-1395764.1	5.7	2.5	2.5	0.4	6.0	0.5	15.4	5.7
AD-1395765.1	76.1	12.4	37.6	6.6	30.4	1.9	41.6	9.6
AD-1395766.1	90.9	14.9	35.9	10.9	29.8	4.2	36.7	5.9
AD-1395767.1	4.7	0.9	3.0	0.6	9.2	2.0	17.8	4.8
AD-1395768.1	88.4	21.7	58.2	18.0	39.7	2.6	45.7	6.6
AD-1395769.1	93.1	23.8	79.7	2.5	67.7	29.9	69.8	5.0
AD-1321257.3	1.6	0.4	2.8	0.7	11.1	3.5	27.6	4.7
AD-1395770.1	3.0	0.6	2.5	0.4	10.6	4.8	23.3	2.7
AD-1395771.1	3.2	1.0	3.1	0.5	7.5	0.3	13.7	1.9
AD-1395772.1	42.6	18.2	16.7	2.5	22.9	7.7	31.4	3.5
AD-1395773.1	22.7	7.2	9.2	0.7	22.8	10.2	30.5	5.2
AD-1395774.1	30.2	9.0	12.0	1.4	28.3	7.4	36.6	5.3
AD-1395775.1	24.7	13.4	15.4	4.8	23.6	7.2	33.6	6.5
AD-1321276.3	2.1	0.3	3.0	0.7	10.8	5.0	20.3	2.7
AD-1395776.1	3.8	0.3	4.0	1.1	9.7	1.8	17.3	2.8
AD-1395777.1	63.4	15.6	38.5	6.0	43.7	11.2	56.0	14.3
AD-1395778.1	91.7	14.4	66.4	10.5	48.4	16.8	53.7	11.6
AD-1395779.1	99.2	17.5	87.2	16.3	120.4	52.7	99.6	29.0
AD-1395780.1	81.5	12.3	82.8	9.1	95.5	18.8	103.6	17.9
AD-1321280.3	6.0	0.8	5.3	1.5	18.5	9.6	35.4	7.2
AD-1395781.1	3.9	0.4	3.8	1.0	13.8	5.4	25.6	3.3
AD-1395782.1	5.2	4.1	4.0	0.4	10.5	4.9	16.2	0.9

	50 nM		10 nM		1 nM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1395783.1	66.8	17.7	46.6	8.4	83.7	36.1	84.0	15.5
AD-1395784.1	4.6	1.5	3.9	0.9	15.5	5.5	30.0	7.7
AD-1395785.1	80.5	16.4	56.2	13.0	66.2	9.1	106.8	29.1
AD-1321284.3	7.4	1.9	5.2	2.3	18.3	10.9	37.0	22.0
AD-1395786.1	12.5	1.0	9.4	6.1	46.3	28.2	80.0	25.2
AD-1395787.1	21.4	4.7	12.3	2.1	18.4	7.3	33.0	12.9
AD-1395788.1	131.2	18.7	132.1	23.4	77.1	23.8	93.8	28.1
AD-1395789.1	147.3	32.6	105.3	19.7	68.1	25.2	90.4	51.6
AD-1395790.1	11.0	1.9	7.9	2.4	20.6	9.0	40.8	15.5
AD-1395791.1	101.8	24.9	97.5	30.7	114.8	51.9	129.7	38.0
AD-1395792.1	122.6	25.4	167.1	42.2	155.0	40.6	129.3	50.0

Table 22. Superoxide Dismutase 1 *In Vitro* Single Dose Screens in BE(2)C cells

	50 nM		10 nM		1 nM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1321204.3	2.6	1.9	5.5	1.1	20.1	4.8	37.7	6.1
AD-1395718.1	4.7	1.5	6.1	5.5	5.2	1.2	10.9	2.8
AD-1395719.1	100.1	45.1	25.1	4.5	17.7	7.0	20.8	4.5
AD-1395720.1	99.7	33.1	36.4	4.8	27.4	3.5	37.8	5.2
AD-1395721.1	8.6	1.4	5.2	0.9	7.5	1.1	18.4	12.6
AD-1395722.1	72.9	10.4	61.6	26.4	30.6	2.5	38.8	6.5
AD-1395723.1	104.8	45.1	38.2	5.2	33.6	7.9	37.8	4.9
AD-1321207.3	2.1	0.5	5.1	1.9	12.9	1.6	25.7	5.9
AD-1395724.1	2.4	0.6	4.1	1.5	4.3	1.0	8.3	0.9
AD-1395725.1	56.9	10.0	11.4	2.5	7.6	3.1	11.1	2.7
AD-1395726.1	108.0	36.1	43.8	9.2	25.9	6.6	22.0	4.4
AD-1395727.1	6.7	1.2	4.5	2.1	7.2	2.0	15.7	8.2
AD-1395728.1	63.6	12.4	16.5	4.2	15.9	3.1	23.7	6.3
AD-1395729.1	80.8	17.0	37.5	8.9	24.5	8.0	33.3	4.9
AD-1321210.3	4.1	1.9	4.3	1.6	10.8	4.3	16.8	9.8
AD-1395730.1	2.5	0.7	4.4	1.4	12.1	3.6	28.4	21.1
AD-1395731.1	2.2	1.1	3.5	2.7	4.9	2.4	6.2	2.4
AD-1395732.1	46.5	11.0	11.1	4.5	8.8	2.2	15.6	1.9
AD-1395733.1	52.4	13.8	14.5	5.0	7.0	1.2	14.8	3.5
AD-1395734.1	3.3	1.9	3.5	1.2	7.8	4.5	12.7	2.8
AD-1395735.1	7.9	2.0	4.1	1.2	10.2	2.9	15.5	6.3
AD-1395736.1	20.0	7.8	7.0	2.6	7.9	3.6	9.8	2.4
AD-1321219.3	4.0	1.1	7.2	2.5	25.2	10.8	47.9	9.1
AD-1395737.1	4.2	2.5	5.3	3.8	10.9	6.1	13.8	10.8

	50 nM		10 nM		1 nM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1395738.1	2.1	0.6	3.4	1.1	6.0	2.4	8.7	3.4
AD-1395739.1	7.0	2.1	6.4	2.8	14.3	6.5	34.4	14.0
AD-1321220.3	3.1	1.3	6.9	3.7	22.8	4.7	50.6	24.7
AD-1395740.1	2.5	0.9	3.8	1.7	6.3	2.6	11.4	3.9
AD-1395741.1	3.0	1.0	4.1	2.0	8.1	2.1	10.4	4.6
AD-1395742.1	2.9	1.0	3.3	0.6	6.5	1.9	11.7	5.2
AD-1321222.3	5.1	2.9	12.0	7.1	35.8	19.3	55.3	27.1
AD-1395743.1	4.2	0.5	4.6	2.1	9.0	5.3	11.4	3.0
AD-1395744.1	74.6	18.0	39.0	6.8	34.4	5.6	58.5	9.8
AD-1395745.1	82.3	17.6	47.8	15.1	53.1	17.7	70.1	28.8
AD-1395746.1	7.4	0.9	8.5	4.1	21.9	6.7	41.2	14.8
AD-1395747.1	80.1	12.9	59.5	8.5	65.4	18.0	105.6	33.0
AD-1395748.1	124.8	1.3	80.2	16.6	60.1	25.5	116.2	22.8
AD-1321232.3	14.9	2.6	14.7	7.4	33.3	13.5	76.1	19.7
AD-1395749.1	6.0	1.4	7.8	5.8	30.3	20.9	62.5	24.8
AD-1395750.1	4.3	1.8	3.7	1.2	5.8	2.3	11.4	4.0
AD-1395751.1	76.0	9.7	44.2	9.0	48.1	16.3	60.7	6.8
AD-1395752.1	78.1	8.0	62.8	8.3	54.8	18.2	73.6	13.5
AD-1395753.1	6.8	2.3	8.1	1.9	16.4	4.0	34.4	8.8
AD-1395754.1	68.1	21.5	27.7	7.1	65.5	14.9	79.4	27.9
AD-1395755.1	98.0	24.7	37.0	16.2	67.5	18.0	83.0	20.7
AD-1321238.3	3.5	1.5	6.8	3.6	16.5	13.3	22.6	4.2
AD-1395756.1	5.6	1.1	3.3	1.2	4.7	1.9	5.6	2.0
AD-1395757.1	2.9	1.2	2.7	1.8	8.0	3.0	35.2	15.8
AD-1395758.1	36.1	7.6	15.0	3.6	16.8	3.3	29.6	11.3
AD-1321243.3	3.5	2.0	7.0	2.5	17.1	2.9	24.0	2.4
AD-1395759.1	3.8	1.8	3.2	1.5	5.8	1.8	9.0	4.6
AD-1395760.1	3.2	2.3	3.5	1.3	6.4	4.9	9.1	2.8
AD-1395761.1	5.3	1.1	5.9	1.5	10.7	1.9	19.4	8.8
AD-1321246.3	3.4	3.7	3.3	0.9	10.3	3.3	23.1	13.1
AD-1395762.1	1.6	1.3	2.1	0.6	2.9	0.8	5.6	2.6
AD-1395763.1	3.6	0.7	3.4	0.6	6.6	1.8	14.1	5.7
AD-1321256.3	3.1	2.2	6.3	5.1	10.9	3.3	20.3	1.1
AD-1395764.1	2.3	1.0	2.5	0.3	6.1	1.4	11.3	3.0
AD-1395765.1	86.7	33.4	31.8	8.0	19.0	7.6	23.7	4.1
AD-1395766.1	67.2	29.3	19.8	10.2	13.0	4.3	16.7	3.3
AD-1395767.1	5.0	1.1	7.0	3.0	10.5	4.4	16.4	5.2
AD-1395768.1	100.0	28.9	56.1	31.8	7.8	1.0	11.6	2.0
AD-1395769.1	49.7	34.7	28.2	6.5	11.2	2.4	16.9	3.9
AD-1321257.3	3.0	0.7	4.0	2.5	11.9	3.9	24.8	4.9
AD-1395770.1	3.5	2.1	4.5	1.3	12.7	1.8	24.3	8.1

	50 nM		10 nM		1 nM		0.1 nM	
Duplex	Avg	SD	Avg	SD	Avg	SD	Avg	SD
AD-1395771.1	2.7	1.1	1.9	0.8	5.6	2.0	8.1	2.2
AD-1395772.1	55.4	13.2	17.8	9.3	12.5	4.2	11.3	8.5
AD-1395773.1	43.6	15.7	8.6	2.1	13.6	5.4	16.4	1.4
AD-1395774.1	26.4	6.7	6.8	2.3	24.8	32.6	13.0	3.5
AD-1395775.1	13.4	6.7	4.9	1.0	10.3	10.2	9.3	1.6
AD-1321276.3	2.5	0.9	3.9	2.0	29.3	28.0	19.6	3.2
AD-1395776.1	2.4	0.8	12.8	14.4	26.7	26.0	12.7	4.4
AD-1395777.1	86.1	34.8	16.8	4.3	15.9	4.6	17.5	3.2
AD-1395778.1	80.3	37.1	33.8	11.2	20.9	14.3	17.6	3.6
AD-1395779.1	57.8	19.2	25.0	6.9	19.6	4.5	30.8	5.3
AD-1395780.1	62.9	18.7	21.0	6.3	15.0	7.1	26.4	5.5
AD-1321280.3	4.1	1.8	4.5	0.9	20.5	5.0	34.4	10.0
AD-1395781.1	5.2	2.0	5.2	1.3	16.3	9.8	34.4	24.1
AD-1395782.1	3.8	1.3	2.5	1.0	5.1	0.6	10.2	2.6
AD-1395783.1	50.6	15.8	15.3	4.1	18.6	3.7	42.6	10.3
AD-1395784.1	5.1	1.2	3.0	0.7	7.7	2.8	13.7	2.1
AD-1395785.1	63.8	28.5	20.2	10.0	22.9	6.5	46.9	13.7
AD-1321284.3	9.9	2.5	7.5	2.6	26.3	3.9	37.9	10.9
AD-1395786.1	24.7	21.5	13.7	3.1	91.2	59.0	94.0	39.7
AD-1395787.1	17.3	6.1	9.8	4.4	7.8	1.0	25.0	7.8
AD-1395788.1	69.3	16.3	70.6	28.2	56.4	40.4	36.8	12.4
AD-1395789.1	70.7	24.9	31.2	5.2	30.6	19.8	43.0	24.7
AD-1395790.1	9.4	3.2	8.4	6.3	9.6	3.7	21.0	6.2
AD-1395791.1	75.9	22.1	34.4	12.2	27.8	6.5	46.6	12.1
AD-1395792.1	74.7	29.5	27.8	4.7	19.2	1.6	51.3	19.8

Example 5. *In vivo* Assessment of RNAi Agents Targeting SOD1 in G93A-SOD1 Transgenic Rats

Based on the *in vitro* and *in vivo* studies above, three duplexes, AD-1395762, AD-1395756, and AD-1395731 were selected for further analysis (see, *e.g.,* Table 21). Figure 3A summarizes the effects of these three duplexes observed *in vitro*, demonstrating that all three duplexes reduce human SOD1 (hSOD1) mRNA expression by greater than 90% in BE(2)c cells on Day 14 post-dose across all doses tested (50 nM, 10 nM, 1 nM, and 0.1 nM).

These three duplexes were further assessed for *in vivo* activity in G93A rats. This transgenic rat model, which over-expresses the mutant hSOD1G93A gene, reproduces the pathology and symptoms observed in ALS patients, *e.g.,* paralysis in one or more limbs within a few weeks of age (see, *e.g.,* Matsumoto A, *et al.* (2006) *J Neurosc Res* 83: 119–133).

Briefly, male G93A-SOD1 rats received a single 0.9 mg dose in a volume of 5 µl of AD-1395762, AD-1395756, or AD-1395731, or 5 µl of artificial CSF (aCSF) control (n=3 per group) by

intrathecal injection using using a Hamilton syringe and an angled 30G needle at Day 0. At Day 14 post-dose, animals were sacrificed and tissue samples, including limbar, thoracic and cervical spinal cord, were collected and flash frozen. mRNA was extracted from the tissue and analyzed by the RT-QPCR method.

The results, depicted in Figure 3B, demonstrate that all three duplexes reduce target SOD1 (hSOD1) mRNA expression by greater than 90% in G93A rats on Day 14 post-dose in all three regions of the spinal cord (lumbar, thoracic, and cervical) following a single 0.9 mg intrathecally administered dose of the duplex.

Example 6. Preclinical Assessment of RNAi Agents Targeting SOD1

Metabolite Identification (MetID) was used to determine which metabolites and the amount of metabolites that were formed following administration of each of duplexes AD-1395762, AD-1395756, and AD-1395731, as well as the percent of parent duplex exposure (area under the curve (AUC)) in the brain and spine of treated wild-type rats.

Metabolite identification was performed on pooled rat cerebral cortex collected 4-1344 hr post-dose, and pooled lumbar spinal cord collected 4-1344 hr post-dose *via* LC-HRMS (see, *e.g.*, methods in Liu *et al., Bioanalysis* (2019) 11(21), 1967–1981).

The results of these studies are presented in Figures 4-5.

Figures 4A and 4B demonstrate that all three duplexes AD-1395762, AD-1395756, and AD-1395731, have similar exposure in rat spine (cervical, thoracic and lumbar; Figure 4A) and brain (cerebral cortex and brainstem; Figure 4B).

Figure 4C demonstrates that all three duplexes AD-1395762, AD-1395756, and AD-1395731, have similar retention in rat spine (cervical, thoracic and lumbar; Figure 4A) and brain (cerebral cortex and brainstem; Figure 4B).

In addition, it was determined that the half-life ($t_{1/2}$) of each of these three duplexes is long (>20 days) and that the $t_{1/2}$ are comparable even when considering potential inter-animal dosing variability.

Figure 5 demonstrates that all three duplexes AD-1395762, AD-1395756, and AD-1395731, have similar metabolite profiles in both brain and spine and that the antisense strand lacking the 3'-terminal nucleotide (3'N-1 AS) is the major active metabolite.

Figure 6 is a Table summarizing the tissue exposure and metabolite profiling of duplexes AD-1395762, AD-1395756, and AD-1395731. Table 23 shows the nucleotide sequences for exemplary observed or predicted 3'N-1 AS metabolites for certain duplexes described herein.

Table 23. 3'N-1 AS Metabolites.

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Antisense 3'N-1 Metabolite	SEQ ID NO:
AD-1395762	csascuu(Uhd)aaUfCfCfucuauccasgsa	11	VPusdCsugdGadTagagdGaUfuaaagugsasg	12	VPusdCsugdGadTagagdGaUfuaaagugsa	1369
AD-1395756	csasggu(Chd)cuCfAfCfuuuaauccsusa	13	VPusdAsggdAudTaaagdTgAfggaccugscsg	14	VPusdAsggdAudTaaagdTgAfggaccugsc	1370
AD-1395731	ususcgag(Chd)aGfAfAfggaaaguasasa	15	VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu	16	VPusUfsuadCu(Tgn)uccuucUfgCfucgaasa	1371
AD-1395743	gsasaag(Uhd)aaUfGfGfaccagugasasa	17	VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu	18	VPusUfsucdAc(Tgn)gguccaUfuAfcuuucsc	1372
AD-1395771	asgsga(Uhd)gaaGfAfGfaggcaugususa	19	VPusAfsacdAu(G2p)ccucucUfuCfaccususu	20	VPusAfsacdAu(G2p)ccucucUfuCfaccusu	1373
AD-1395738	asasgga(Ahd)agUfAfAfuggaccagsusa	21	VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu	22	VPusdAscudGg(Tgn)ccaudTaCfuuuccuusc	1374
AD-1395718	asuscaa(Uhd)uuCfGfAfgcagaaggsasa	23	VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg	24	VPusUfsccdTu(C2p)ugcucgAfaAfuugausg	1375
AD-1395760	cscsuca(Chd)uuUfAfAfuccucuauscsa	25	VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc	26	VPusdGsaudAg(Agn)ggaudTaAfagugaggsa	1376
AD-1395764	asasgga(Uhd)gaAfGfAfgaggcaugsusa	27	VPusAfscadTg(C2p)cucucuUfcAfuccususg	28	VPusAfscadTg(C2p)cucucuUfcAfuccusu	1377
AD-1395724	asasuuu(Chd)gaGfCfAfgaaggaaasgsa	29	VPusCfsuudTc(C2p)uucugcUfcGfaaausgsg	30	VPusCfsuudTc(C2p)uucugcUfcGfaaausg	1378

Example 7. *In vivo* Assessment of RNAi Agents in Non-Human Primates (NHP)

The effects of duplexes AD-1395762, AD-1395756, and AD-1395731 were also assessed *in vivo* in non-human primates (NHP).

As depicted in Figure 7, on Day 0 non-human primates were intrathecally administered a single 70 mg dose of AD-1395762, AD-1395756, or AD-1395731 in a volume of 2 mL, or a single 120 mg dose of AD-1395731 in a volume of 2 mL, or 2 mL of artificial cerebrospinal fluid (aCSF). Animals were sacrificed at Day 31, Day 85, or Day 169 post-dose, tissue samples were collected and the level of SOD1 mRNA was quantified as described above.

Due to differences in dosing of the animals (Figure 8A), the relative potencies of each duplex was unclear. However, by removing samples having drug exposure levels at 24 hours in the CSF that were lower than 1500 ng/ml, which are considered suboptimal doses, the data demonstrate that a single intrathecally administered 70 mg dose of AD-1395762, AD-1395756, or AD-1395731 resulted in the reduction of SOD1 mRNA in various CNS tissues and that the reduction in SOD1 mRNA in various CNS tissues was durable and maintained out to Day 85 post-dose (Figure 8B). Remarkably, as depicted in Figure 8C, a single intrathecally administered 70 mg dose of AD-1395762, AD-1395756, or AD-1395731 reduced SOD1 mRNA by 75% in the cervical spinal cord at Day 85 post-dose and by 60% in the cortex by Day 85 post-dose (Figure 8C).

In addition, as depicted in Figures 9A-9C, a single intrathecally administered 70 mg dose of AD-1395762, AD-1395756, or AD-1395731 resulted in the reduction of SOD1 protein in CSF samples by 60% out to Day 85 post-dose and, in three animals that were extended in the study, a single intrathecally administered 70 mg dose of AD-1395762, AD-1395756, or AD-1395731 resulted in the reduction of SOD1 protein in CSF samples by 60% out to Day 141 post-dose.

As depicted in Figure 10, the observed reductions in mRNA levels and protein levels in pre-frontal cortex samples and thoracic spinal cord samples are highly and significantly correlated in NHP. Figure 13 shows that there was no substantial knockdown of SOD1 in kidney and liver at days 31 and 85 post-intrathecal administration of the three tested duplexes. Additionally, no significant differences were seen in the minimal effects at the kidney and liver among the duplexes.

Figure 11A depicts a set of graphs showing the mRNA remaining versus siRNA exposure in tissues that were examined following intrathecal administration of a single 70 mg dose of AD-1395762, AD-1395756, or AD-1395731. Analysis of the data demonstrated that there is a strong correlation between both mRNA and protein reduction to the amount of siRNA exposure in both prefrontal cortex and thoracic spinal cord samples following a single intrathecally administered 70 mg dose of AD-1395762, AD-1395756, or AD-1395731 (Figure 11B).

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent, or a pharmaceutically acceptable salt thereof, comprising a sense strand and an antisense strand forming a double stranded region, wherein

a) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusdCsugdGadTagagdGaUfuaaagugsasg-3' (SEQ ID NO: 12);

b) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14);

c) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16);

d) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18);

e) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20);

f) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22);

g) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24);

h) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26);

i) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28); or

j) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30), wherein

VP is a 5'-vinyl phosphonate;
(Ahd) is 2'-O-hexadecyl-adenosine-3'-phosphate;
(Chd) is 2'-O-hexadecyl-cytidine-3'-phosphate;
(Uhd) is 2'-O-hexadecyl-uridine-3'-phosphate;
(Agn) is adenosine-glycol nucleic acid (GNA), S-isomer;
(Tgn) is thymidine-glycol nucleic acid (GNA), S-Isomer;
(C2p) is cytidine-2'-phosphate;
(G2p) is guanosine-2'-phosphate;
s is a phosphorothioate linkage;
a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U;
dA, dC, dG, and dT are 2′-deoxy A, C, G, and T; and
Af, Cf, Gf, and Uf are 2′-deoxy-2'-fluoro (2'-F) A, C, G, and U.

2. The dsRNA agent, or a pharmaceutically acceptable salt thereof, of claim 1, wherein

a) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusdCsugdGadTagagdGaUfuaaagugsasg-3' (SEQ ID NO: 12);

b) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14);

c) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16);

d) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the

nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18);

e) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20);

f) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22);

g) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3 (SEQ ID NO: 24);

h) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26);

i) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28); or

j) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30).

3. The dsRNA agent, or a pharmaceutically acceptable salt thereof, of claim 1, wherein

a) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusdCsugdGadTagagdGaUfuaaagugsasg-3' (SEQ ID NO: 12);

b) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14);

c) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16);

d) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18);

e) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20);

f) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22);

g) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24);

h) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26);

i) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28); or

j) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30).

4. The dsRNA agent, or a pharmaceutically acceptable salt thereof, of claim 1, wherein

a) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the

nucleotide sequence 5'-VPusdCsugdGadTagagdGaUfuaaagugsasg-3' (SEQ ID NO: 12);

b) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14);

c) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16);

d) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18);

e) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20);

f) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22);

g) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24);

h) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26);

i) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28); or

j) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30).

5. The dsRNA agent, or a pharmaceutically acceptable salt thereof, of claim 1, wherein

a) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusdCsugdGadTagagdGaUfuaaagugsasg-3'(SEQ ID NO: 12);

b) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14);

c) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16);

d) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18);

e) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20);

f) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22);

g) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand comprises from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24);

h) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26);

i) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28); or

j) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30).

6. The dsRNA agent, or a pharmaceutically acceptable salt thereof, of claim 1, wherein

a) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-csascuu(Uhd)aaUfCfCfucuauccasgsa-3' (SEQ ID NO: 11) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusdCsugdGadTagagdGaUfuaaagugsasg-3' (SEQ ID NO: 12);

b) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-csasggu(Chd)cuCfAfCfuuuaauccsusa-3' (SEQ ID NO: 13) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusdAsggdAudTaaagdTgAfggaccugscsg-3' (SEQ ID NO: 14);

c) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-ususcgag(Chd)aGfAfAfggaaaguasasa-3' (SEQ ID NO: 15) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusUfsuadCu(Tgn)uccuucUfgCfucgaasasu-3' (SEQ ID NO: 16);

d) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-gsasaag(Uhd)aaUfGfGfaccagugasasa-3' (SEQ ID NO: 17) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusUfsucdAc(Tgn)gguccaUfuAfcuuucscsu-3' (SEQ ID NO: 18);

e) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-asgsga(Uhd)gaaGfAfGfaggcaugususa-3' (SEQ ID NO: 19) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusAfsacdAu(G2p)ccucucUfuCfauccususu-3' (SEQ ID NO: 20);

f) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-asasgga(Ahd)agUfAfAfuggaccagsusa-3' (SEQ ID NO: 21) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusdAscudGg(Tgn)ccaudTaCfuuuccuuscsu-3' (SEQ ID NO: 22);

g) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-asuscaa(Uhd)uuCfGfAfgcagaaggsasa-3' (SEQ ID NO: 23) and the nucleotide sequence of the antisense strand consists of from the nucleotide sequence 5'-VPusUfsccdTu(C2p)ugcucgAfaAfuugausgsg-3' (SEQ ID NO: 24);

h) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-cscsuca(Chd)uuUfAfAfuccucuauscsa-3' (SEQ ID NO: 25) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusdGsaudAg(Agn)ggaudTaAfagugaggsasc-3' (SEQ ID NO: 26);

i) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-asasgga(Uhd)gaAfGfAfgaggcaugsusa-3' (SEQ ID NO: 27) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusAfscadTg(C2p)cucucuUfcAfuccuususg-3' (SEQ ID NO: 28); or

j) the nucleotide sequence of the sense strand consists of the nucleotide sequence 5'-asasuuu(Chd)gaGfCfAfgaaggaaasgsa-3' (SEQ ID NO: 29) and the nucleotide sequence of the antisense strand consists of the nucleotide sequence 5'-VPusCfsuudTc(C2p)uucugcUfcGfaaauusgsg-3' (SEQ ID NO: 30).

7. The dsRNA agent of any one of claims 1-6 that is a sodium salt.

8. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of superoxide dismutase 1 (SOD1), wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides from any one of the nucleotide sequences of nucleotides 201-223, 204-226, 207-229, 216-238, 219-241, 328-350, 333-355, 336-358, 372-394, or 373-395 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2, wherein

(i) the dsRNA agent comprises at least one modified nucleotide,

(ii) the double stranded region is 15-30 nucleotide pairs in length, and

(iii) the sense strand or the antisense strand is conjugated to one or more lipophilic moieties.

9. The dsRNA agent of claim 8, wherein the sense strand comprises at least 15 contiguous nucleotides from any one of the nucleotide sequences of nucleotides 207-229, 219-241, 328-350, or 336-358 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

10. The dsRNA agent of claim 8, wherein the sense strand comprises at least 15 contiguous nucleotides from any one of the nucleotide sequences of nucleotides 207-229, 328-350, or 336-358 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

11. The dsRNA agent of claim 8, wherein the sense strand comprises at least 15 contiguous nucleotides from nucleotides 336-358 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides from the corresponding nucleotide sequence of SEQ ID NO: 2.

12. The dsRNA agent of claim 8, wherein the antisense strand comprises at least 15 contiguous nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected

from the group consisting of AD-1395762, AD-1395756, AD-1395731, AD-1395743, AD-1395771, AD-1395738, AD-1395718, AD-1395760, AD-1395764, or AD-1395724.

13. The dsRNA agent of claim 8, wherein the antisense strand comprises at least 15 contiguous nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1395762, AD-1395756, AD-1395731, and AD-1395743.

14. The dsRNA agent of claim 8, wherein the antisense strand comprises at least 15 contiguous nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1395762, AD-1395756, and AD-1395731.

15. The dsRNA agent of claim 8, wherein the antisense strand comprises at least 15 contiguous nucleotides from the antisense strand nucleotide sequence of duplex AD-1395762.

16. The dsRNA agent of any one of claims 8-15, wherein all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

17. The dsRNA agent of any one of claims 8-16, wherein at least one of the modified nucleotides is selected from the group a deoxy-nucleotide, a 3’-terminal deoxythimidine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a 2’-5’-linked ribonucleotide (3’-RNA), a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2’-amino-modified nucleotide, a 2’-O-allyl-modified nucleotide, 2’-C-alkyl-modified nucleotide, a 2’-methoxyethyl modified nucleotide, a 2’-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide comprising a 5’ phosphate or 5’ phosphate mimic, a nucleotide comprising vinyl phosphonate, a glycol nucleic acid (GNA), a glycol nucleic acid S-Isomer (S-GNA), a nucleotide comprising 2-hydroxymethyl-tetrahydrofuran-5-phosphate, a nucleotide comprising 2’-deoxythymidine-3’phosphate, a nucleotide comprising 2’-deoxyguanosine-3’-phosphate, and a terminal nucleotide linked to a cholesteryl derivative and a dodecanoic acid bisdecylamide group; and combinations thereof.

18. The dsRNA agent of claim 17, wherein the modified nucleotide is selected from the group consisting of a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, 3’-terminal deoxythimidine nucleotides (dT), a locked nucleotide, an abasic nucleotide, a 2’-

amino-modified nucleotide, a 2'-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

19. The dsRNA agent of claim 17, wherein the modifications on the nucleotides are independently selected from the group consisting of 2'-deoxy, 2'-O-methyl, 3'-RNA, GNA, S-GNA, and 2'-deoxy-2'-fluoro modifications.

20. The dsRNA agent of any one of claims 8-19, further comprising at least one phosphorothioate internucleotide linkage.

21. The dsRNA agent of claim 20, wherein the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages.

22. The dsRNA agent of any one of claims 8-21, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

23. The dsRNA agent of claim 22, wherein at least one strand comprises a 3' overhang of 2 nucleotides.

24. The dsRNA agent of any one of claims 8-23, wherein the double stranded region is 17-23 nucleotide pairs in length.

25. The dsRNA agent of claim 24, wherein the double stranded region is 19-21 nucleotide pairs in length.

26. The dsRNA agent of claim 24, wherein the double stranded region is 21-23 nucleotide pairs in length.

27. The dsRNA agent of any one of claims 8-26, wherein each strand has 19-30 nucleotides.

28. The dsRNA agent of any one of claims 8-27, wherein the one or more lipophilic moieties are conjugated to one or more internal positions on at least one strand.

29. The dsRNA agent of claim 28, wherein one lipophilic moiety is conjugated an internal position selected from the group consisting of positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.

30. The dsRNA agent of claim 29, wherein the internal position is selected from the group consisting of positions 5, 6, 7, 15, and 17 on the sense strand, counting from the 5'-end of the strand.

31. The dsRNA agent of claim 29, wherein the internal position is selected from the group consisting of positions 15 and 17 on the antisense strand, counting from the 5'-end of the strand.

32. The dsRNA agent of claim 29,wherein the internal position is selected from the group consisting of positions 6 and 7 of the sense strand.

33. The dsRNA agent of any one of claims 28-32, wherein the lipophilic moiety is an aliphatic, alicyclic, or polyalicyclic compound.

34. The dsRNA agent of claim 33, wherein the lipophilic moiety contains a saturated or unsaturated C4-C30 hydrocarbon chain, and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

35. The dsRNA agent of claim 33, wherein the lipophilic moiety contains a saturated or unsaturated C6-C18 hydrocarbon chain.

36. The dsRNA agent of claim 33, wherein the lipophilic moiety contains a saturated or unsaturated C16 hydrocarbon chain.

37. The dsRNA agent of claim 33, wherein the saturated or unsaturated C16 hydrocarbon chain is conjugated to position 6 or 7 of the sense strand, counting from the 5'-end of the sense strand.

38. The dsRNA agent of any one of claims 8-37, wherein the agent further comprises a phosphate or phosphate mimic at the 5'-end of the antisense strand.

39. The dsRNA agent of claim 38, wherein the phosphate mimic is a 5'-vinyl phosphonate (VP).

40. The dsRNA agent of claim 38, wherein the phosphate mimic is a 5'-*E*-vinyl phosphonate (VP).

41. The dsRNA agent of any one of claims 8-40, wherein the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

42. The dsRNA agent of any one of claims 8-41, wherein the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

43. A cell containing the dsRNA agent of any one of claims 1-42.

44. A pharmaceutical composition comprising the dsRNA agent of any one of claims 1-42 and a pharmaceutically acceptable diluent.

45. A method of inhibiting expression of a SOD1 gene in a cell, the method comprising:
(a) contacting the cell with the dsRNA agent of any one of claims 1-42, or the pharmaceutical composition of claim 44; and
(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the SOD1 gene, thereby inhibiting expression of the SOD1 gene in the cell.

46. The method of claim 45, wherein the cell is within a human subject.

47. The method of claim 46, wherein the subject meets at least one diagnostic criterion for a SOD1-associated neurodegenerative disease or has been diagnosed with a SOD1-associated neurodegenerative disease.

48. The method of claim 47, wherein the SOD1-associated neurodegenerative disease is selected from the group consisting of Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

49. A method of treating a subject diagnosed with an SOD1-associated neurodegenerative disease, the method comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-42 or the pharmaceutical composition of claim 44, thereby treating the subject.

50. The method of claim 49, wherein treating comprises amelioration of at least one sign or symptom of the disease.

51. The method of claim 49, where treating comprises prevention of progression of the disease.

52. The method of claim 49, wherein the SOD1-associated neurodegenerative disease is is selected from the group consisting of Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

53. A method of preventing development of an SOD1-associated neurodegenerative disease in a subject meeting at least one diagnostic criterion for an SOD1-associated neurodegenerative

disease, the method comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-42 or the pharmaceutical composition of claim 44, thereby preventing the development of a SOD1-associated neurodegenerative disease in the subject meeting at least one diagnostic criterion for a SOD1-associated neurodegenerative disease.

54. The method of any one of claims 49-53, wherein the subject is human.

55. The method of claim 54, wherein the subject has been diagnosed with an SOD1-associated neurodegenerative disease.

56. The method of claim 55, wherein the SOD1-associated neurodegenerative disease is selected from the group consisting of Amyotrophic Lateral Sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Down's syndrome (DS).

57. The method of any one of claims 49-56, wherein the dsRNA agent is administered to the subject intrathecally or intracerebroventricularly.