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(54) **Title:** SHORT DUPLEX DNA AS A NOVEL GENE SILENCING TECHNOLOGY AND USE THEREOF

(57) **Abstract:** The present invention discloses a novel type of gene silencing technology for modulating target nucleic acid and/or protein in cells, tissues, organisms and animals. The new technology provides compositions for use in gene silencing applications, including prevention and treatment of human diseases. The composition comprises a short, duplex DNA molecule where the sense strand is at least equal to the antisense strand in length. The duplex DNA molecule further includes at least one interspersed ribonucleotide monomer. The present invention further provides methods of using the compositions for modulating expression or function of a target gene, or for treatment or prevention of diseases as well as for other medical or biological applications.

Short Duplex DNA as a Novel Gene Silencing Technology and Use Thereof

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of co-pending U.S. provisional patent application Serial No. 63/195,010, filed May 29, 2021, which application is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to short duplex DNA to be used as gene silencing technology and related compositions and methods that can be used in biological or medical research, in the treatment and prevention of diseases and for gene silencing applications in other biological fields.

BACKGROUND OF THE INVENTION

[0003] Modern medical therapeutics is dependent on two fundamental technologies, namely small molecule chemistry and protein/antibody technology. However, only about 10% of the targets identified by genomic and biomedical research can be addressed by the two aforementioned cornerstone technologies. Oligonucleotides hold promise for addressing numerous targets, including non-druggable ones by small molecule chemistry and antibody/protein technologies. More than four decades of research have created Antisense oligonucleotide (ASO) and small interfering RNA (siRNA) technologies (*Cy A. Stein et al., 2017*). However, despite of over 40 years of research, other than a small number of clinical orphan indications, significant druggability issues have blocked the development of ASO and siRNA technologies from becoming a mainstay therapeutic platform. Such druggability issues include, among others, low silencing efficiency, off-target effects, stimulation of non-intended immune response, tissue penetration challenges, and in vivo delivery, etc. There is hence a significant unmet need to create novel technologies to target genes of interest in various biological and medical applications.

[0004] ASO is a gene silencing technology based upon a concept originally proposed in 1978 (*Zamecnik P.C. et al., 1978*). Generally, the principle behind the ASO technology is that an antisense oligonucleotide hybridizes to a target nucleic acid and modulates gene expression activities or function, such as transcription/post-transcription or translation. The mechanisms can be broadly categorized as: (1) occupancy only without promoting RNA degradation, in which the binding of the ASO leads to translational arrest, inhibition of splicing, or induction of alternatively spliced variants, or (2) occupancy-induced destabilization, in which the binding of

the ASO promotes degradation of the RNA through endogenous enzymes, such as ribonuclease H1 (RNase H1); and (3) translation modulation: ASO can block upstream open reading frames (uORFs) or other inhibitory or regulatory elements in the 5'UTR, increasing or modulating translation efficiency (*Stanley T. Crooke et al., 2008; C. Frank Bennett, 2010; Richard G. Lee, 2013; Stanley T. Crooke, 2017*). The ASO structure is a single-stranded deoxyribonucleotide sequence that can bind to target RNA through base-pairing. After 40 years of research, ASO technology has been improved through various chemical modifications of the single stranded oligonucleotide, such as phosphorothioate substitution or other modified nucleotides *(See Iwamoto N et al 2017, Crooke ST, 2017; Crooke ST et al., 2018*; U.S. Pat. Nos. 7919472 and 9045754).

[0005] RNAi is a mechanism by which short double-stranded RNA triggers the loss of RNA of homologous sequence, and was first observed in plants and demonstrated in nematodes (*Caenorhabditis elegans*) (*A. Fire et al, 1998*). The mechanism involved degradation of a long dsRNA into short interfering duplex RNAs (siRNA), and interaction of the siRNA with a multi-protein RNA-Induced Silencing Complex (RISC); within the RISC, the siRNA is unwound, the sense strand is discarded, and the antisense or guide strand binds to the RISC endonuclease AGO2, which then cleaves the target RNA (*de Fougerolles et al., 2007; Ryszard Kole, 2016*). In mammalian cells, synthetic siRNA or asymmetric short interfering RNAs (aiRNA or asymmetric siRNA) can be used to induce gene silencing through RISC-dependent mechanism (*See Elbashir SM et al., 2001; Sun X et al., 2008*; U.S. Pat. Nos. 7056704 and 9328345).

[0006] Oligonucleotides have been studied for decades and are considered to hold significant promise for becoming a whole new class of therapeutics. However, limited silencing efficiency, delivery challenges and dose-dependent adverse effects, including hybridization-dependent toxicities and hybridization-independent toxicities, of oligonucleotides continue to limit the development of these novel classes of therapeutics (*C. Frank Bennett, 2010; C. Frank Bennett, 2019; Roberts TC et al., 2020; Crooke ST et al., 2018; and Setten RL et al., 2020*). In general, ASO compounds are less potent than siRNA-based compounds in inducing gene silencing, yet ASO compounds have some pharmaceutical advantages than siRNA compounds. Currently ASO and siRNAs remain the two equally important platform technologies for designing gene-silencing therapeutics *(Crooke ST et al 2018; Roberts TC et al 2020)*. The Hybridization-dependent toxicities of oligonucleotides are mainly attributed to hybridization to non-target genes ("off-target effects") (*Jackson et al., 2003; Lin X et al., 2005*). Hybridization-independent toxicities happened through the interactions of the oligonucleotide with proteins: the effects

include increased coagulation time, pro-inflammatory effects and activation of the complement pathway. These effects tend to occur at higher doses of oligonucleotides and are dose-dependent. For example, at higher concentrations, ASOs lead to renal tubule changes and thrombocytopenia (*Geary, RS. et al., 2007; Kwoh J T, 2008*). Clinically, the primary tolerability and safety issues for first-generation PS antisense oligodeoxynucleotides and second-generation 2'-MOE-modified antisense oligonucleotides have proven to be hybridization-independent effects such as prolongation of activated partial thromboplastin time, injection site reaction, and constitutional symptoms such as fever, chills, and headache (C. *Frank Bennett, 2010; Henry S P, 2008; Kwoh J T, 2008*). Even the most optimized ASOs generally are still far less potent than siRNA and have proven to have dosage-dependent stereotypic toxicity (*Kendall S. Frazier, 2015*). In order to alleviate oligonucleotides dose-dependent toxicities, efforts have been made in the past 40 years to overcome limited efficacy issues and associated safety problems through various chemical modifications (*Iwamoto N. et al 2017*; *Crooke ST et al., 2018; and Roberts TC et al., 2020*).

[0007] Compared to ASOs, off-target effects of siRNA duplex are considered to be mediated by sense strand-mediated silencing, competition with endogenous miRNA pathways and interaction with TLR or other proteins *(Setten RL et al 2019)*. In addition, the typical siRNA duplex of 21nt/19bp is not efficient in cell and tissue penetration, also requires extensive chemical modifications to enhance stability and other pharmaceutical properties.

[0008] In summary, after more than 40 years of innovation in ASO technology and more than 20 years of research in RNAi-based technologies, successful development of gene-targeted therapies against nearly 90% of targets implicated in human diseases remains challenging. Moreover, the current approved oligonucleotide drugs cost more than half a million USD per patient/per year, making it impossible to address diseases affecting the general populations. As such, novel technologies to overcome these challenges are urgently needed.

[0009] The references cited herein are not admitted to be prior art to the claimed invention.

SUMMARY OF THE INVENTION

[00010] The present invention is based on a surprising discovery of potent gene silencing triggered by short duplex deoxyribonucleotides (sdDNA). This novel type of gene silencing technology enabled by sdDNA with one or more interspersed segment of ribonucleotide monomer(s) ("ISR") employs a short, duplex molecule made up by linked nucleotide monomers that are each selected from the group of naturally occurring nucleotide, analogs thereof, and modified nucleotide (hereinafter, collectively referred to as "nucleotide monomers"). In other

words, nucleotide monomers used in an embodiment of the present invention include "deoxyribonucleotide monomers" selected from the group of naturally occurring deoxyribonucleotides, analogs thereof, and modified deoxyribonucleotides. Furthermore, the gene silencing function of sdDNA can be dramatically enabled or enhanced by incorporating one or a few interspersed ribonucleotide monomers. The "ribonucleotide monomers" can be selected from the group of naturally occurring ribonucleotides, analogs thereof, and modified ribonucleotides.

[00011] In the present invention, a short duplex DNA (sdDNA) molecule is further interspersed with one or a few ribonucleotide monomers, which form at least one interspersed segment of ribonucleotide monomer(s) ("ISR").

[00012] The great gene silencing effect of the sdDNA-based novel platform technology contained in the present disclosure is, in one embodiment, achieved through a sense strand of oligonucleotide monomers and an antisense strand of oligonucleotide monomers that is substantially complementary to a targeted ribonucleotide sequence. Our data have shown that sdDNA molecules of the present invention, with their unique and novel compositions, can trigger gene silencing at pico molar concentrations, which are more potent than ASO, siRNA and other existing gene silencing technologies, and therefore enabling reduction of dose-dependent toxicities. The sdDNA molecules of the present invention are also expected to have at least one of the following advantages over existing gene silencing technologies including better tissue penetration; enabling gene silencing in in nucleus, in mitochondria *etc.*, in contrast siRNA-based gene silencing only occurs in cytoplasm; reduced off-target effects; better stability; elimination or reduction of undesired competition with endogenous microRNA pathways associated with siRNAs; lower synthesis cost and other improved pharmaceutical properties. Therefore, the sdDNA molecules of the present invention have great potential for addressing a variety of challenges facing existing technologies. The sdDNA molecules of the present invention can be used in all areas that current oligonucleotides are being applied or contemplated for use, including research, diagnosis, disease prevention and therapies as well as other applications in biological fields, including agriculture and veterinary medicine.

[00013] In a first aspect, the present invention provides a composition comprising a short duplex DNA (sdDNA) molecule that has a first strand and a second strand. The length of the second strand is at least equal to the length of the first strand, more specifically, the second strand is longer than the first strand or equal to the first strand in length. The first strand is substantially complementary to a targeted segment of a targeted RNA through at least one

targeting region, and can therefore be considered an antisense strand or an antisense oligonucleotide. Further, the second strand, which can be considered a sense strand or a sense oligonucleotide, is substantially complementary to the first strand, and forms at least one double-stranded region with the first strand. The sdDNA molecule includes at least one interspersed segment of ribonucleotide monomer(s) (ISR). In a feature, the ISR in the sdDNA molecule includes at least one ribonucleotide monomer(s), which can be in either or both strands.

[00014] The composition provided by the present invention is used for modulating gene expression or function in a eukaryotic cell, wherein the sdDNA is caused to contact a cell or administered to a subject.

[00015] In some embodiments, the sdDNA molecule includes at least one or at least two interspersed segments of ribonucleotide monomer(s) (ISR). In a feature of the sdDNA molecule of the invention, the first strand of the molecule includes at least one ISR or the second strand of the molecule may include at least one ISR. In an embodiment, the first strand includes at least one ISR and the second strand also includes at least one ISR. In a feature, each ISR, independently of each other, either consists of 1 ribonucleotide monomer, or comprises at least 2, 3, 4, 5 or 6 contiguous ribonucleotide monomers. In another feature, the ISR includes at least 2 ribonucleotide monomers, whether they are contiguous or spaced apart with at least one (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) intervening monomer of a different kind. In yet another feature, the total number of ribonucleotide monomers of all ISR(s) comprised in the first strand is at least 2.

[00016] In one feature, at least one ISR is disposed in at least one targeting region of the first (antisense) strand. In another feature, at least one ISR is disposed in at least one double-stranded region of the second (sense) strand. In yet another feature, at least one ISR is disposed in at least one targeting region of the first strand and at least one ISR is disposed in at least one double-stranded region of the second (sense) strand. In some embodiments, at least one ISR can be deposited at any position of the first strand. In some embodiments, at least one ISR is positioned at or near (within 7 nucleobases or within 33% of the total number of nucleobases in the said strand from a terminal counting said terminal, e.g., starting from the terminal, position no. 1, 2, 3, 4, 5, 6, or 7 for a strand that is about 21 nucleobases long) the 5' end of the first strand; and/or at or near (within 7 nucleobases or within 33% of the total number of nucleobases in the said strand from a terminal counting said terminal) the 3' end of the first strand; and/or at a more central part of the first strand. In some embodiments, at least one ISR disposed in the first strand

is positioned at only overhang region of the first strand. In some embodiments, at least one ISR disposed in the first strand is positioned at both overhang region and double-stranded region of the first strand. In some embodiments, ISR(s) in the first strand comprise at least one ribonucleotide monomer positioned at the 5’ end of the first strand or the 3’ end of the first strand. In some embodiments, at least one ISR is positioned at or near (within 7 nucleobases or within 33% of the total number of nucleobases in the said strand from a terminal counting said terminal) the 5’ end of the second strand; and/or at or near (within 7 nucleobases or within 33% of the total number of nucleobases in the said strand from a terminal counting said terminal) the 3’ end of the second strand; and/or at a more central part of the second strand.

[00017] In one feature, the first strand or the antisense strand includes multiple linked nucleotide monomers forming a nucleobase sequence, and is at least 70%, 80%, 85%, 90%, 95% complementary or fully complementary to the targeted segment of the targeted gene’s RNA. In certain embodiments, the targeted RNA is selected from mRNA or non-coding RNA where the RNA either encodes a protein or regulates a part of a biological pathway implicated in a disease, e.g., a mammalian disease. The terms “target” and “targeted” are used interchangeably in the present disclosure and share the same meaning.

[00018] In various embodiments, the first strand (antisense strand) has a backbone length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 linked nucleotide monomers, or equivalents thereof, or of a range bracketed by any two of the above values (both range endpoints included). For example, some of the ranges of the length of first strand include: (a) 8-36 nucleotide monomers; (b) 8-33 nucleotide monomers; (c) 10-30 nucleotide monomers; (d) 10-29 nucleotide monomers; (e) 12-29 nucleotide monomers; (f) 12-28 nucleotide monomers; (g) 12-26 nucleotide monomers; (h) 12-25 nucleotide monomers; (i) 13-25 nucleotide monomers; (j) 13-24 nucleotide monomers; (k) 13-23 nucleotide monomers; (l) 14-24 nucleotide monomers; (m) 15-23 nucleotide monomers; and (n) 8-50 nucleotide monomers; (o) 16-23 nucleotide monomers; (p) 10-36 nucleotide monomers; and (p) at least 8 nucleotide monomers.

[00019] In one feature, the second strand or the sense strand includes multiple linked nucleotide monomers forming a nucleobase sequence, and is at least 70%, 75%, 80%, 85%, 90%, 95% complementary or fully complementary to the linked region of the first strand or the antisense strand. As a result, the two strands form a double-stranded region that includes 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36,

37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 base pairs. In some embodiments, the sense strand is fully complementary to at least one linked region of the first/antisense strand, and forms the at least one double-stranded region without any mismatch. In some embodiments, the sense strand is complementary to at least one linked region of the first/antisense strand, and forms the at least one double-stranded region with 1, 2, 3 or more mismatches. In one feature, the mismatched monomer(s) in the sense strand has a nucleobase selected from the group consisting of A, G, C, and T or a modified nucleobase.

[00020] In one feature, the second strand or the sense strand has a backbone length equal to the first strand or the antisense strand. In an embodiment, the two strands of the sdDNA molecule form a symmetric duplex without any overhangs. In another embodiment, both the first strand and the second strand have a 3'-overhang or a 5'-overhang. In certain embodiments, the 3'-overhang and the 5'-overhang of the two strands have same length of at least 1, 2, 3, 4 or 5 nucleotide monomers.

[00021] In other feature, the second strand or the sense strand has a backbone length longer than the first strand or the antisense strand by or at least a number of nucleotide monomers as follows: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In certain embodiments, the 3'-overhang of the second strand has a length of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotide monomers, or of a range bracketed by any two of the above values (both range endpoints included). In certain embodiments, the 5'-overhang of the second strand has a length of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotide monomers, or of a range bracketed by any two of the above values (both range endpoints included). In an embodiment of the invention, the second strand has a 3'-overhang of 1-5 nucleotide monomers and a 5'-overhang of 1-5 nucleotide monomers (both range endpoints included). In another embodiment, the second strand has a 3'-overhang of 1-8 nucleotide monomers (both range endpoints included) and a 5’ blunt end. In yet another embodiment, the second strand has a 5'-overhang of 1-8 nucleotide monomers (both range endpoints included) and a 3’ blunt end. In yet another embodiment, the second strand has a 3'-overhang of 1-10 nucleotide monomers (both range endpoints included) and a 5’ recessed end, or a 5'-overhang of 1-10 nucleotide monomers ((both range endpoints included) and a 3’ recessed end.

[00022] In various embodiments, the second or sense strand has a backbone length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 linked nucleotide monomers, or

equivalents thereof, or of a range bracketed by any two of the above values (both range endpoints included). In certain embodiments, for example, some of the ranges of the length of the second sense strand include: (a) 8-36 nucleotide monomers; (b) 8-33 nucleotide monomers; (c) 10-30 nucleotide monomers; (d) 10-29 nucleotide monomers; (e) 12-29 nucleotide monomers; (f) 12-28 nucleotide monomers; (g) 12-26 nucleotide monomers; (h) 12-25 nucleotide monomers; (i) 12-24 nucleotide monomers; (j) 13-25 nucleotide monomers; (k) 13-24 nucleotide monomers; (l) 13-23 nucleotide monomers; (m) 14-24 nucleotide monomers; (n) 15-23 nucleotide monomers; (o) 8-50 nucleotide monomers; (p) 16-23 nucleotide monomers tides; (q) 10-36 nucleotide monomers; and (r) at least 8 nucleotide monomers.

[00023] In a feature of the sdDNA molecule of the invention, at least one nucleotide monomer in the first strand and/or the second strand is a modified nucleotide or nucleotide analogue, e.g., a sugar-, backbone-, and/or base-modified nucleotide. In an embodiment, such a backbone-modified nucleotide has at least a modification in an internucleoside linkage, e.g., to include at least one of a nitrogen or sulphur heteroatom. In some embodiments, the modified internucleoside linkage is or includes: phosphorothioate (P=S) group, phosphotriesters, methylphosphonates, or phosphoramidate.

[00024] In certain embodiments, the first strand and/or the second strand comprises at least one modified internucleoside linkage, where the modified internucleoside linkage is a phosphorothioate internucleoside linkage. In some embodiments, at least one internucleoside linkage between the deoxyribonucleotide monomers of the second strand is a phosphorothioate internucleoside linkage. In some embodiments, each internucleoside linkage of the first strand and/or the second strand is a phosphorothioate internucleoside linkage. In various embodiments, the internucleoside linkages are a mixture of phosphorothioate and phosphodiester linkages of the first strand and/or the second strand.

[00025] In a feature, the first strand and/or the second strand of the molecule of the invention comprises at least one modified nucleotide or nucleotide analogue that includes a modified sugar moiety. In certain embodiments, the 2′ position of the modified sugar moiety is replaced by a group selected from OR, R, halo, SH, SR, NH_2, NHR, NR_2, or CN, where each R is independently C_1-C_6 alkyl, alkenyl or alkynyl, and halo is F, Cl, Br or I. In some embodiments, the 2′ position of the modified sugar moiety is replaced by a group selected from allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF_3, OCH_2F, $O(CH2)_2SCH_3$, $O(CH_2)_2$-O-$N(R_m)(R_n)$, O-CH_2-C(=O)-$N(R_m)(R_n)$, or O-CH_2-C(=O)-$N(R_l)$-$(CH_2)_2$-$N(R_m)(R_n)$, where each R_l, R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl. In some embodiments, the

modified sugar moiety has substituent group(s) selected from the group of 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH_3, 2'-OCH_2CH_3, 2'-OCH_2CH_2F, 2'-O-aminopropylation (2'-AP) and 2'-$O(CH2)_2OCH_3$. In some embodiments, the modified sugar moiety is substituted by bicyclic sugar selected from the group of 4′-(CH_2)—O-2′ (LNA); 4′-(CH_2)—S-2; 4′-(CH_2)2—O-2′ (ENA); 4′-CH(CH_3)—O-2′ (cEt) and 4′-CH(CH_2OCH_3)—O-2′, 4′-C(CH_3)(CH_3)—O-2′, 4′-CH_2—N(OCH_3)-2′, 4′-CH_2—O—N(CH_3)-2′, 4′-CH_2—N(R)—O-2′, where R is H, C1-C12 alkyl, or a protecting group, 4′-CH_2—C(H)(CH_3)-2′, and 4′-CH_2—C—(=CH_2)-2′. In some embodiments, the modified sugar moiety is selected from the group of 2'-O-methoxyethyl modified sugar (MOE), a 4′-(CH_2)—O-2′ bicyclic sugar (LNA), 2'-deoxy-2'-fluoroarabinose (a 2'-F-arabino FANA), and a methyl(methyleneoxy) (4′-CH(CH_3)—O-2) bicyclic sugar (cEt).

[00026] In a feature of the sdDNA molecule of the invention, the sugar moiety of the deoxyribonucleotide monomer is either the sugar moiety of a naturally occurring deoxyribonucleotide (2-H) or 2'-deoxy-2'-fluoroarabinose (FA).

[00027] In a feature of the sdDNA molecule of the invention, the sugar moiety of the ribonucleotide monomer is selected from a naturally occurring ribonucleotide (2-OH), 2'-F modified sugar, 2'-OMe modified sugar, 2'-O-methoxyethyl modified sugar (MOE), a 4′-(CH_2)—O-2′ bicyclic sugar (LNA) and a methyl(methyleneoxy) (4′-CH(CH_3)—O-2) bicyclic sugar (cEt).

[00028] In another feature, the first strand and/or the second strand of the molecule of the invention includes at least one nucleotide monomer comprising a modified nucleobase. In some embodiments, the modified nucleobase is selected from the group of: 5-methylcytosine (5-Me-C), inosine base, a tritylated base, 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 (-C≡C-CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 1-methyl-pseudo-uracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl, 5-methyl uridine and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-aminoadenine, 8-azaguanine and 8-azaadenine, and 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. In a particular embodiment, the modified nucleobase is a 5-methylcytosine. In an embodiment, each cytosine base in the molecule of the invention is 5-methylcytosine. In an embodiment, each uridine base in the ISR of the sdDNA

molecule of the invention is 5-methyluridine.

[00029] In a feature, the sdDNA molecule of the present invention may include at least one CpG motif that can be recognized by the pattern recognition receptors (PRR), e.g., Toll-like receptors.

[00030] In one feature, the first strand and/or the second strand of the molecule of the invention is conjugated to a ligand or a moiety. In certain embodiments, the ligand or moiety is selected from the group of: peptide/protein, antibody, polymer, polysaccharide, lipid, hydrophobic moiety or molecule, cationic moiety or molecule, lipophilic compound or moiety oligonucleotide, cholesterol, GalNAc and aptamer.

[00031] In a feature of the invention, the sdDNA molecule is used for modulating gene expression or function in a cell, e.g., a eukaryotic cell such as a mammalian cell.

[00032] In certain embodiments, the targeted RNA, which dictates at least part of the nucleotide monomer sequence of the sdDNA molecule according to principles of the invention, is selected from mRNA or non-coding RNA wherein such RNA either encodes a protein or regulates a part of a biological pathway implicated in a disease. Such target RNA, in various embodiments, can be selected from: an mRNA of a gene implicated in human or animal diseases or conditions; an mRNA of a gene of a pathogenic microorganism; a viral RNA, and a RNA implicated in a disease or disorder selected from the group consisting of autoimmune diseases, inflammatory diseases, degenerative diseases, infectious diseases, proliferative diseases, metabolic diseases, immune-mediated disorders, allergic diseases, dermatological diseases, malignant diseases, gastrointestinal disorders, respiratory disorders, cardiovascular disorders, renal disorders, rheumatoid disorders, neurological disorders, endocrine disorders, and aging-related disorders or diseases.

[00033] In an embodiment, the invention provides a short duplex DNA (sdDNA) molecule comprising a first strand and a second strand each comprising linked nucleotide monomers selected from the group of nucleotides, analogs thereof, and modified nucleotides, where: (a) the first strand is equal in length to the second strand or is shorter than the second strand by a number of monomers selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 monomers; (b) the first strand is substantially complementary to a targeted segment of a targeted RNA through at least one targeting region, and wherein the first strand consists of 8-36 (both range endpoints included) nucleotide monomers linked through a linkage selected from the group consisting of a phosphorothioate linkage, a phosphodiester linkage, and a mixture of phosphorothioate and phosphodiester linkages between adjacent monomers; (c) the second strand is substantially complementary to the first strand, and forms at least one double-stranded

region with the first strand, and wherein the second strand consists of 10-36 (both range endpoints included) nucleotide monomers linked through a linkage selected from the group consisting of a phosphorothioate linkage, a phosphodiester linkage, and a mixture of phosphorothioate and phosphodiester linkages between adjacent monomers; (d) the sdDNA molecule comprises at least one interspersed segment of ribonucleotide monomers (ISR) linked to at least one deoxyribonucleotide monomer selected from the group consisting of a deoxyribonucleotide, an analog thereof, and a modified deoxyribonucleotide; (e) the ISR in the sdDNA molecule comprises at least one ribonucleotide monomer selected from the group consisting of a ribonucleotide, an analog thereof, and a modified ribonucleotide. In a feature, the sdDNA molecule is used for modulating a target gene expression or function in a cell, e.g., a eukaryotic cell such as a mammalian cell. In a further feature, the sdDNA molecule is more potent or more efficacious at silencing the expression of the target gene than a corresponding ASO in a cell.

[00034] In a second aspect, the present invention provides a pharmaceutical composition comprising the composition in the first aspect as active agent, and a pharmaceutically acceptable excipient, carrier, or diluent. Examples of such carriers include and are not limited to: a pharmaceutical carrier, a positive-charge carrier, a liposome, a lipid nanoparticle, a protein carrier, a hydrophobic moiety or molecule, a cationic moiety or molecule, GalNAc, a polysaccharide a polymer, a nanoparticle, a nanoemulsion, a cholesterol, a lipid, a lipophilic compound or moiety, and a lipoid.

[00035] In a third aspect, the present invention provides a method of using the composition in the first aspect or the pharmaceutical composition in the second aspect for treating or preventing a disease or a condition by administering a therapeutically effective amount of an sdDNA molecule of the invention or a pharmaceutical composition containing such a molecule. The administration method is a route selected from the group of intravenous injection (iv), subcutaneous injection (sc), per os (po), intramuscular (im) injection, oral administration, inhalation, topical, intrathecal, and other regional administrations.

[00036] In a feature, the disease or condition being prophylactically or therapeutically treated is selected from the group of cancer, autoimmune disease, inflammatory diseases, degenerative diseases, infectious diseases, proliferative diseases, metabolic diseases, immune-mediated disorders, allergic diseases, dermatological diseases, malignant diseases, gastrointestinal disorders, hepatic disorders, respiratory disorders, cardiovascular disorders, dermatological disorders, renal disorders, rheumatoid disorders, neurological disorders, psychiatric disorders,

endocrine disorders, and aging-related disorders or diseases.

[00037] In a fourth aspect, the present invention provides a method of using the composition in the first aspect or the pharmaceutical composition in the second aspect for regulating or modulating a gene expression or gene function in a eukaryotic cell. The method comprises the step of contacting the cell with an effective amount of any sdDNA molecule of the invention or a pharmaceutical composition containing such a molecule.

[00038] In one embodiment, said contacting step comprises the step of introducing a composition comprising said sdDNA molecule into a target cell in culture or in an organism in which the selective gene silencing can occur. In a further embodiment, the introducing step is selected from the group consisting of simple mixing, transfection, lipofection, electroporation, infection, injection, oral administration, intravenous injection (iv), subcutaneous injection (sc), per os (po), intramuscular (im) injection, inhalation, topical, intrathecal, and other regional administrations. In another embodiment, the introducing step comprises using a pharmaceutically acceptable excipient, carrier, or diluent selected from the group that includes a pharmaceutical carrier, a lipid nanoparticle, a positive-charge carrier, a liposome, a protein carrier, a hydrophobic moiety or molecule, a cationic moiety or molecule, GalNAc, a polysaccharide a polymer, a nanoparticle, a nanoemulsion, a cholesterol, a lipid, a lipophilic compound or moiety, and a lipoid.

[00039] In certain embodiments, the target gene is mRNA. In certain embodiments, the target gene is non-coding RNA, such as microRNA and lncRNA.

[00040] In an embodiment, the target gene is associated with a disease, a pathological condition, or an undesirable condition in a mammal. In a further embodiment, the target gene is a gene of a pathogenic microorganism. In an even further embodiment, the target gene is a viral gene. In another embodiment, the target gene is a tumor-associated gene. In yet another embodiment, the target gene is a gene associated with a disease selected from the group listed with respect to the third aspect.

[00041] In another aspect, the invention provides an oligomeric duplex comprising (a) one or more deoxyribonucleosides, analogs thereof or modified deoxyribonucleosides, and (b) one or more ISR comprising ribonucleosides, analogs thereof or modified ribonucleosides, linked into an antisense sequence of at least 8 nucleobases in length. The antisense sequence is at least 70% complementary to a target sequence.

[00042] Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate

different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention. While several embodiments have been shown and described, any modifications may be made without departing from the spirit and scope of the present invention.

BRIEF DESCRIPTION OF FIGURES

[00043] Figure 1A illustrate exemplary structures of some embodiments of short duplex DNAs (sdDNAs) according to principles of the present invention with at least one interspersed segment of ribonucleotide (ISR) in antisense strand (first strand, AS) (and sense strand (second strand, SS)) and exemplary sequences of sdDNAs having the designed structures for targeting APOCIII gene. In each duplex depicted here, the sense strand is listed on top of the antisense strand. Figure 1B shows gene silencing potency of sdDNAs shown in Figure 1A and their corresponding ASO (having the identical structure and sequence as the single-stranded AS of sdDNAs in FIG. 1A) as comparison. Relative mRNA levels of the APOCIII gene were analyzed after transfecting HepaRG cells with these sdDNAs and corresponding ASO at 100 pM.

[00044] Figure 2A shows exemplary structures and sequence of some embodiments of symmetric sdDNAs of various lengths. Figure 2B shows the gene silencing potency for targeting the APOCIII gene by sdDNAs shown in FIG. 2A. Figure 2C shows the gene silencing potency for targeting the APOCIII gene bycorresponding single-stranded AS oligonucleotide which has the identical sequence as the antisense strand of each sdDNAs shown in FIG. 2A. The relative mRNA levels of the APOCIII gene were detected after transfecting HepaRG cells with the sdDNAs and corresponding single-stranded AS oligonucleotide at 100 pM .

[00045] Figure 3A shows exemplary structure and sequence of some embodiments of symmetric sdDNAs of various lengths. Figure 3B shows the gene silencing potency for targeting the APOCIII gene bysdDNAs shown in FIG. 3A. Relative mRNA levels of the APOCIII gene were detected after transfecting HepaRG cells with the sdDNAs at 100 pM.

[00046] Figure 4A shows exemplary structure and sequence of some embodiments of sdDNAs with different PS modification motifs in SS. Figure 4B shows the gene silencing potency for targeting the APOCIII gene by sdDNAs shown in FIG. 4A and their corresponding ASO (having the same structure and sequence the single-stranded AS of sdDNAs in FIG. 4A) as comparison. Relative mRNA levels of the APOCIII gene were detected after transfecting HepaRG cells with the sdDNAs and corresponding ASO at 100 pM.

[00047] Figure 5A shows exemplary structure and sequence of some embodiments of sdDNAs

with various ISR motifs in antisense strand. Figure 5B shows the gene silencing potency for targeting the APOCIII gene by sdDNAs shown in FIG. 5A. Relative mRNA levels of the APOCIII gene were detected after transfecting HepaRG cells with sdDNAs at 100 pM.

[00048] Figure 6A shows exemplary structure and sequence of some embodiments of sdDNAs with at least one mismatch in the antisense strand upon binding to target RNA. Figure 6B shows the gene silencing potency for targeting the APOCIII gene by sdDNAs shown in FIG. 6A. Relative mRNA levels of the APOCIII gene were detected after transfecting HepaRG cells with the sdDNAs and at 100 pM.

[00049] Figure 7A shows exemplary structure and sequence of some embodiments of sdDNAs with SS longer than AS. Figure 7B shows the gene silencing potency for targeting the APOCIII gene by sdDNAs shown in FIG. 7A and their corresponding ASO (having the identical structure and sequence as the single-stranded AS of sdDNAs in FIG. 7A) as comparison. Relative mRNA levels of the APOCIII gene were detected after transfecting HepaRG cells with the sdDNAs and corresponding ASO at 100 pM.

DETAILED DESCRIPTION OF THE INVENTION

[00050] The present invention refers to gene or RNA modulation/silencing technology using short duplex DNAs. This new technology is used for modulation of gene expression or function in vitro and in vivo by using a short duplex DNA (sdDNA) composition. The present invention also provides methods of using the compositions for modulating expression or function of a target gene, or for treatment or prevention of diseases as well as for other medical and biological applications. These composition and methods provide high potency in regulating gene expression or gene function, but also reduces dose-dependent toxicities.

1. Definitions

[00051] As used herein, the singular form “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes a plurality of cells including mixtures thereof.

[00052] When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below those numerical values. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 20%, 10%, 5%, or 1%. In some embodiments, the term “about” is used to modify a numerical value above and below the stated value by a variance of 10%. In some embodiments, the term “about” is used to modify a numerical value above and below the stated value by a

variance of 5%. In some embodiments, the term "about" is used to modify a numerical value above and below the stated value by a variance of 1%.

[00053] As used herein, the term "analog" or "analogue," interchangeably, means a functional or structural equivalent. For instance, nucleoside and nucleotide analogues have been used in clinical treatment of cancer and viral infections for decades and new compounds are continually synthesized and evaluated by the researchers and the pharmaceutical industry, *see, e.g.*, Jordheim L.P. et al., *Nat Rev Drug Discov* 12, 447-464 (2013).

[00054] As used herein, the term "deoxyribonucleoside monomer" means a nucleoside monomer that includes a naturally occurring deoxyribonucleoside, an analog thereof, and a modified deoxyribonucleoside. The term "deoxyribonucleotide monomer" means a nucleotide monomer that includes a naturally occurring deoxyribonucleotide, an analog thereof, and a modified deoxyribonucleotide.

[00055] As used herein, the term "ribonucleoside monomer" means a nucleoside monomer that includes a naturally occurring ribonucleoside, an analog thereof, and a modified ribonucleoside. The term "ribonucleotide monomer" means a nucleotide monomer that includes a naturally occurring ribonucleotide, an analog thereof, and a modified ribonucleotide.

[00056] As used herein, the term "nucleoside" means a compound comprising a nucleobase moiety and a sugar moiety. Nucleoside monomers include, but are not limited to, naturally occurring nucleosides (e.g., deoxyribonucleosides and ribonucleosides as found in DNA and RNA, respectively), analogs thereof and modified nucleosides. A nucleoside monomer can be either a deoxyribonucleoside monomer or a ribonucleoside monomer. Nucleoside monomers may be linked to a phosphate moiety to become, for example, nucleotide monomers.

[00057] As used herein, the term "nucleotide" means a nucleoside further comprising a phosphate linking group. Nucleotide monomers include, but are not limited to, naturally occurring nucleotides (e.g., deoxyribonucleotides and ribonucleotides as found in DNA and RNA, respectively), analogs thereof and modified nucleotides. A nucleotide monomer can be either a deoxyribonucleotide monomer or a ribonucleotide monomer. A modified nucleotide may be modified at one of more of the following: its nitrogen-containing nucleobase moiety, its five-carbon sugar moiety, and its phosphate linking group that results in changes in the internucleoside linkage.

[00058] As used herein, the term "oligo" or "oligonucleotide" refers to a compound comprising a plurality of linked nucleoside monomers. In certain embodiments, one or more of nucleoside monomers or one or more of the internucleoside linkages are modified.

[00059] The terms “deoxynucleoside” and “deoxyribonucleoside” are used interchangeably herein. The terms “deoxynucleotide” and “deoxyribonucleotide” are also used interchangeably herein. As used herein, a “deoxynucleoside” or “deoxynucleotide” is a nucleoside or nucleotide, respectively, that contains a deoxy sugar moiety.

[00060] As used herein, the term “duplex DNA” as in “short duplex DNA (sdDNA)” means a molecule composed of two strands or chains of nucleotide monomers that hybridize with each other to form as duplex oligonucleotides and are caused to contact a cell or administered to a subject, and where the majority, i.e., 50% or more of the linked nucleotide monomers of the key RNA-targeting motifs are deoxyribonucleotide monomers including modified deoxyribonucleotides.

[00061] As used herein, the term “motif” means the pattern of chemically distinct regions, e.g., in an antisense strand or a sense strand.

[00062] As used herein, the term “immediately adjacent” means there are no intervening elements in between two elements, for example, between regions, segments, nucleotides and/or nucleosides.

[00063] As used herein, the term “modified nucleotide” means a nucleotide having at least one modified sugar moiety, modified internucleoside linkage, and/or modified nucleobase.

[00064] As used herein, the term “modified nucleoside” means a nucleoside having at least one modified sugar moiety, and/or modified nucleobase.

[00065] As used herein, the term “modified oligonucleotide” means an oligonucleotide comprising at least one modified nucleotide.

[00066] As used herein, the term “naturally occurring internucleoside linkage” means a 3’ to 5’ phosphodiester linkage.

[00067] As used herein, the term “modified internucleoside linkage” refers to a substitution or any change from a naturally occurring internucleoside bond. For example, a phosphorothioate linkage is a modified internucleoside linkage.

[00068] As used herein, the term “natural sugar moiety” means a sugar naturally found in DNA (2-H) or RNA (2-OH).

[00069] As used herein, the term “modified sugar” refers to a substitution or change from a natural sugar. For example, a 2’-O-methoxyethyl modified sugar is a modified sugar.

[00070] As used herein, the term “bicyclic sugar” means a furosyl ring modified by the bridging of two non-geminal ring atoms. A bicyclic sugar is a modified sugar.

[00071] As used herein, the term “bicyclic nucleic acid,” “BNA,” “bicyclic nucleoside,” or

"bicyclic nucleotide" refers to a nucleoside or nucleotide where the furanose portion of the nucleoside or nucleotide includes a bridge connecting two carbon atoms on the furanose ring, thereby forming a bicyclic ring system.

[00072] As used herein, the term "2'-O-methoxyethyl" (also 2'-MOE, 2'-O$(CH_2)_2$—OCH_3 and 2'-O-(2-methoxyethyl)) refers to an O-methoxy-ethyl modification of the 2' position of a furosyl ring. A 2'-O-methoxyethyl modified sugar is a modified sugar. As used herein, the term "2'-O-methoxyethyl nucleotide" (also 2'-MOE RNA) means a modified nucleotide comprising a 2'-O-methoxyethyl modified sugar moiety.

[00073] As used herein, the term "modified nucleobase" refers to any nucleobase other than adenine, cytosine, guanine, thymidine, or uracil. For example, 5-methylcytosine is a modified nucleobase. In contrast, an "unmodified nucleobase," as used herein, means the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U).

[00074] As used herein, the term "5-methylcytosine" means a cytosine modified with a methyl group attached to the 5 position. A 5-methylcytosine is a modified nucleobase.

[00075] As used herein, "RNA-like nucleotide" means a modified nucleotide that adopts a northern configuration and functions like RNA when incorporated into an oligonucleotide. RNA-like nucleotides include but are not limited to 2'-endo furanosyl nucleotides, bridged nucleic acid (BNA), LNA, cEt, 2'-O-methylated nucleic acid, 2'-O-methoxyethylated (2'-MOE) nucleic acid, 2'-fluorinated nucleic acid, 2'-O-aminopropylated (2'-AP) nucleic acid, hexitol nucleic acid (HNA), cyclohexane nucleic acid (CeNA), peptide nucleic acid (PNA), glycol nucleic acid (GNA), threose nucleic acid (TNA), morpholino nucleic acid, tricyclo-DNA (tcDNA) and RNA surrogates.

[00076] As used herein, "DNA-like nucleotide" means a modified nucleotide that functions like DNA when incorporated into an oligonucleotide. DNA-like nucleotides include but are not limited to 2'-deoxy-2'-fluoroarabinose (FANA) nucleotides and DNA surrogates.

[00077] As used herein, "non-coding RNA" means an RNA molecule that is not translated into a protein. Examples of non-coding RNAs include transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), as well as small non-coding RNAs and the long ncRNAs (lncRNAs). As used herein, examples of "small non-coding RNA" includes, but are not limited to, microRNAs (miRNAs), asRNA, pre-miRNAs, pri-miRNAs, piRNAs, snoRNAs, snRNAs, exRNAs, scaRNAs and mimics of any of the foregoing. As used herein, "lncRNA", "long non-coding RNA" are transcribed RNA molecules containing greater than 200 nucleotides that do not code for protein.

LncRNAs can also be subjected to common post-transcriptional modifications, including 5'-capping, 3'-polyadenylation, and splicing. Generally, IncRNA are a diverse class of molecules that play a variety of roles in modulation of gene and genome function. For example, lncRNAs are known to regulate gene transcription, translation, and epigenetic regulation. Examples of IncRNAs include, but are not limited to Kcnqlotl, Xlsirt, Xist, ANRIL, NEAT1, NRON, DANCR, OIP5-AS1, TUG1, CasC7, HOTAIR and MALAT1. As used herein, "splice" or "splicing" refers to a natural process that removes unnecessary regions of RNA and reforms the RNA. An example of modulation of RNA target function by oligonucleotides including duplex thereof is modulation of non-coding RNA function. In some embodiments, an oligonucleotide or an oligonucleotide duplex is designed to target one of the foregoing small non-coding RNAs. In some embodiments, the oligonucleotide or oligonucleotide duplex is designed to target miRNA. In some embodiments, the oligonucleotide or oligonucleotide duplex is designed to target pre-miRNA. In some embodiments, the oligonucleotide or oligonucleotide duplex is designed to target pri-miRNA. In some embodiments, the oligonucleotide or oligonucleotide duplex is designed to target lncRNA. In some embodiments, the oligonucleotide or oligonucleotide duplex is designed to target splice.

[00078] The term "isolated" or "purified" as used herein refers to a material being substantially or essentially free from components that normally accompany it in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high-performance liquid chromatography.

[00079] The term "interspersed" as used herein refers to having a different kind of moiety at an adjacent space, for instance, by a different kind of nucleotide or nucleotide analogue, a different modification on the same kind of nucleotide or nucleotide analogue. In various embodiments of the invention, an "interspersed segment of ribonucleotide monomer(s) (ISR)" refers to a section in an oligonucleotide strand where one or multiple ribonucleotide(s) are connected to at least one moiety that is a different kind from said ribonucleotide(s). For example: if said ribonucleotide(s) are unmodified, then a different kind of moiety may be a deoxynucleotide or an analog thereof, a modified deoxynucleotide, a modified ribonucleotide, or a ribonucleotide analog. if said ribonucleotide(s) are modified, then a different kind of moiety may be a deoxynucleotide or an analogue thereof, a modified deoxynucleotide, an unmodified ribonucleotide, a differently modified ribonucleotide, or a different kind of ribonucleotide analog.

[00080] As used herein, "modulating", "regulating" and its grammatical equivalents refer to

either increasing or decreasing (e.g., silencing), in other words, either up-regulating or down-regulating. As used herein, “gene silencing” refers to reduction of gene expression and may refer to a reduction of gene expression about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of the targeted gene.

[00081] As used herein, the terms “inhibiting”, “to inhibit” and their grammatical equivalents, when used in the context of a bioactivity, refer to a down-regulation of the bioactivity, which may reduce or eliminate the targeted function, such as the production of a protein or the phosphorylation of a molecule. In particular embodiments, inhibition may refer to a reduction of about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of the targeted activity. When used in the context of a disorder or disease, the terms refer to success at preventing the onset of symptoms, alleviating symptoms, or eliminating the disease, condition or disorder.

[00082] As used herein, the term “substantially complementary” or “complementary” refers to complementarity in a base-paired, double-stranded region between two chains of linked nucleosides and not any single-stranded region such as a terminal overhang or a gap region between two double-stranded regions. The complementarity does not need to be perfect; there may be any number of base pair mismatches, for example, between the two chains of linked nucleosides. However, if the number of mismatches is so great that no hybridization can occur under even the least stringent hybridization conditions, the sequence is not a substantially complementary sequence. Specifically, when two sequences are referred to as “substantially complementary” herein, it means that the sequences are sufficiently complementary to each other to hybridize under the selected reaction conditions. The relationship of nucleic acid complementarity and stringency of hybridization sufficient to achieve specificity is well known in the art. Two substantially complementary strands can be, for example, perfectly complementary or can contain from 1 to many mismatches so long as the hybridization conditions are sufficient to allow, for example discrimination between a pairing sequence and a non-pairing sequence. Accordingly, substantially complementary sequences can refer to sequences with base-pair complementarity of at least, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or any number in between, in a double-stranded region.

[00083] As used herein, “fully complementary” or “100% complementary” means each nucleobase of a nucleobase sequence of a first strand of linked nucleosides has a complementary nucleobase in a second nucleobase sequence of a second strand of linked nucleosides. In certain embodiments, a first strand of linked nucleosides is an antisense compound and a second strand

of linked nucleosides is a target nucleic acid. In certain embodiments, a first strand of linked nucleosides is a sense compound and a second strand of linked nucleosides is an antisense compound or vice versa.

[00084] As used herein, the term “targeting region” refers to a region in an oligonucleotide strand that is substantially or fully complementary to another oligonucleotide strand such that the two strands, under the right conditions, hybridize or anneal to each other at such targeting region. For example, an antisense strand can include a targeting region through which it can hybridize with a targeted mRNA.

[00085] The terms “administer,” “administering,” or “administration” are used herein in their broadest sense. These terms refer to any method of introducing to a subject a compound or pharmaceutical composition described herein and can include, for example, introducing the compound systemically, locally, or in situ to the subject. Thus, a compound of the present disclosure produced in a subject from a composition (whether or not it includes the compound) is encompassed in these terms. When these terms are used in connection with the term “systemic” or “systemically,” they generally refer to in vivo systemic absorption or accumulation of the compound or composition in the blood stream followed by distribution throughout the entire body.

[00086] The terms “effective amount” and “therapeutically effective amount” refer to that amount of a compound or pharmaceutical composition described herein that is sufficient to affect the intended result including, but not limited to, disease treatment, as illustrated below. In some embodiments, the “therapeutically effective amount” is the amount that is effective for detectable killing or inhibition of the growth or spread of cancer cells, the size or number of tumors, and/or other measure of the level, stage, progression and/or severity of the cancer. In some embodiments, the “therapeutically effective amount” refers to the amount that is administered systemically, locally, or in situ (e.g., the amount of compound that is produced in situ in a subject). The therapeutically effective amount can vary depending upon the intended application (in vitro or in vivo), or the subject and disease condition being treated, e.g., the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term also applies to a dose that will induce a particular response in target cells, e.g., reduction of cell migration. The specific dose may vary depending on, for example, the particular pharmaceutical composition, subject and their age and existing health conditions or risk for health conditions, the dosing regimen to be followed, the severity of the disease, whether it is administered in

combination with other agents, timing of administration, the tissue to which it is administered, and the physical delivery system in which it is carried.

[00087] The term “cancer” in a subject refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain morphological features. Often, cancer cells will be in the form of a tumor or mass, but such cells may exist alone within a subject, or may circulate in the blood stream as independent cells, such as leukemic or lymphoma cells. Examples of cancer as used herein include, but are not limited to, lung cancer, pancreatic cancer, bone cancer, skin cancer, head or neck cancer, cutaneous or intraocular melanoma, breast cancer, uterine cancer, ovarian cancer, peritoneal cancer, colon cancer, rectal cancer, colorectal adenocarcinoma, cancer of the anal region, stomach cancer, gastric cancer, gastrointestinal cancer, gastric adenocarcinoma, adrenocorticoid carcinoma, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, esophageal cancer, gastroesophageal junction cancer, gastroesophageal adenocarcinoma, chondrosarcoma, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, Ewing’s sarcoma, cancer of the urethra, cancer of the penis, prostate cancer, bladder cancer, testicular cancer, cancer of the ureter, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, kidney cancer, renal cell carcinoma, chronic or acute leukemia, lymphocytic lymphomas, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwannomas, ependymomas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenomas, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers. Some of the exemplified cancers are included in general terms and are included in this term. For example, urological cancer, a general term, includes bladder cancer, prostate cancer, kidney cancer, testicular cancer, and the like; and hepatobiliary cancer, another general term, includes liver cancers (itself a general term that includes hepatocellular carcinoma or cholangiocarcinoma), gallbladder cancer, biliary cancer, or pancreatic cancer. Both urological cancer and hepatobiliary cancer are contemplated by the present disclosure and included in the term “cancer.”

[00088] The term "pharmaceutical composition" is a formulation containing the active ingredient, e.g., the molecule or composition disclosed herein, in a form suitable for administration to a subject, often in mixture with other substances, e.g., a pharmaceutical carrier

such as a sterile aqueous solution. In one embodiment, the pharmaceutical composition is in bulk or in unit dosage form. The unit dosage form is any of a variety of forms, including, for example, a capsule, an IV bag, a tablet, a single pump on an aerosol inhaler, or a vial. The quantity of active ingredient in a unit dose of composition is an effect the amount and is varied according to the particular treatment involved. One skilled in the art will appreciate that it is sometimes necessary to make routine variations to the dosage depending on the age and condition of the patient. The dosage will also depend on the route of administration. A variety of routes are contemplated, including oral, pulmonary, rectal, parenteral, transdermal, subcutaneous, intravenous, intramuscular, intraperitoneal, intranasal, and the like. Dosage forms for the topical or transdermal administration of a sdDNA of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants.

[00089] The term “pharmaceutical agent” means a substance that provides a therapeutic benefit when administered to an individual.

[00090] The term “pharmaceutically acceptable carrier” means a medium or diluent that does not interfere with the structure of the compound. Certain of such carriers enable pharmaceutical compositions to be formulated as, for example, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspension and lozenges for the oral ingestion by a subject. Certain of such carriers enable pharmaceutical compositions to be formulated for injection, infusion or topical administration. For example, a pharmaceutically acceptable carrier can be a sterile aqueous solution.

[00091] The term “pharmaceutically acceptable derivative” encompasses derivatives of the compounds described herein such as solvates, hydrates, esters, prodrugs, polymorphs, isomers, isotopically labelled variants, pharmaceutically acceptable salts and other derivatives known in the art.

[00092] The term “pharmaceutically acceptable salts” means physiologically and pharmaceutically acceptable salts of compounds, i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. The term “pharmaceutically acceptable salt” or “salt” includes a salt prepared from reacting the parent compound with pharmaceutically acceptable non-toxic acids or bases, including inorganic or organic acids and bases. Pharmaceutically acceptable salts of the compounds described herein may be prepared by methods well-known in the art. For a review of pharmaceutically acceptable salts, see Stahl and Wermuth, *Handbook of Pharmaceutical Salts: Properties, Selection and Use* (Wiley-VCH, Weinheim, Germany, 2002). Pharmaceutically

acceptable salt can include, but is not limited to, acid addition salts including hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates. citrates, maleales, fumarates, succinates, lactates, and tartrates; alkali metal cations such as Na, K, Li, alkali earth metal salts such as Mg or Ca, or organic amine salts. In particular, sodium salts of oligonucleotides have proven to be useful and are well accepted for therapeutic administration to humans. Accordingly, in one embodiment, the compounds described herein are in the form of a sodium salt.

[00093] As used herein, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[00094] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" as used herein refer to both (1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and (2) prophylactic or preventative measures that prevent or slow the development of a targeted pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. A subject is successfully "treated" according to the methods of the present invention if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition of or an absence of tumor metastasis; inhibition or an absence of tumor growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; and improvement in quality of life.

[00095] The term "carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as, for example, a liquid or solid filler, diluent, excipient, solvent or encapsulating material involved in or capable of carrying or transporting the subject pharmaceutical 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 patient. Non-limiting examples of pharmaceutically acceptable carriers, carriers, and/or diluents include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes;

oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, emulsifiers, and lubricants, such as sodium lauryl sulfate, magnesium stearate, and polyethylene oxide-polypropylene oxide copolymer as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

2. Certain Embodiments-sdDNA structural scaffold

[00096] Certain embodiments of the present invention provide a duplex composition where the antisense and sense strands are both made of linked nucleoside monomers. Fifty percent or more of nucleoside monomers in the key RNA-targeting motifs are deoxyribonucleoside monomers, or fifty percent or more nucleobasesin one strand of the double-stranded region of the sdDNA molecule comprises deoxyribonucleoside monomer, and some of the deoxyribonucleoside monomers contained therein and/or the internucleoside linkage(s) may be modified from those found in natural DNAs. The duplex DNA molecule of the invention further includes ribonucleoside monomers in one or more interspersed segments of ribonucleotide monomer(s) (“ISRs”). One or more ISRs may be found in either the antisense or the sense strand, or both. In some embodiments, each ISR independently consists of 1 ribonucleotide monomer, or 2, 3, 4, 5 or 6 contiguous ribonucleotide monomers. In some embodiments, an ISR has at least two contiguous and linked ribonucleotide monomers.

[00097] Both the antisense and sense strands of the short duplex DNA (sdDNA) of the invention are relatively short, with the sense strand at least equal to the antisense strand in length, hence a “short duplex DNA (sdDNA).” Further, in certain embodiments, with same length between the two strands, the duplex molecule of invention can be more specifically called a “symmetric short duplex DNA”.

[00098] Exemplary structures and sequences of the duplex molecule of the invention are shown in **FIG. 1A, 2A, 4A, 5A, 6A and** **7A** where ISRs are found in both strands, or only in the antisense strand.

[00099] In some embodiments, the antisense and sense strands of the same length form a

symmetric structure without any overhangs.

[000100] The composition of the invention is used for modulating gene expression or function in eukaryotic cell in at least three ways: (i) one kind of sdDNA molecules are caused to contact a cell or administered to a subject; (ii) different kinds of sdDNA molecules are caused to contact a cell or administered to a subject separately at different times; (ii) different kinds of sdDNA molecules are caused to contact a cell or administered to a subject simultaneously.

[000101] In certain embodiments, the antisense oligonucleotide strand includes a nucleobase sequence region, called a "targeting region," that is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to the target segment of a target gene to which it is targeted, including an mRNA and a non-coding RNA. In certain embodiments, the antisense oligonucleotide has a nucleobase sequence comprising a fully complementary sequence of the target segment of a target gene to which it is targeted. In certain embodiments, the antisense oligonucleotide has a nucleobase sequence comprising no more than 1, 2 or 3 mismatch(es) when hybridized to the target segment of a target RNA to which it is targeted. In certain embodiments, the target gene is selected from mRNA or non-coding RNA that are implicated in a mammalian disease. In some embodiments, at least one ISR is disposed in a targeting region of the antisense strand. In certain embodiments, an ISR is positioned at or near (i.e., within one third of the length of strand, which means, e.g., for a strand that is about 21 nucleobases long, within 7 nucleobases counting the terminal) the 5' end of the antisense strand. Alternately, the ISR is at or near (i.e., within one third of the length of strand, which means, e.g., for a strand that is about 21 nucleobases long, within 7 nucleobases counting the terminal) the 3' end of the antisense strand. In some embodiments, an ISR or at least part of the ISR is also positioned at a more central part of the antisense strand, i.e., in the middle third of the length, which means, e.g., for a strand that is about 21 nucleobases long, more than 7 nucleobases away from both terminals of the antisense strand.

[000102] In various embodiments, the first or antisense strand has a backbone length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 linked nucleotide monomers, or equivalents thereof, or of a range bracketed by any two of the above values (both range endpoints included). For example, some of the ranges of the length of first antisense strand include: 8-50 nucleotide monomers; 8-36 nucleotide monomers; 8-33 nucleotide monomers; 10-36 nucleotide monomers; 10-30 nucleotide monomers; 10-29 nucleotide monomers; 12-36 nucleotide monomers;12-29 nucleotide monomers; 12-28 nucleotide monomers; 12-26

nucleotide monomers; 12-25 nucleotide monomers; 13-25 nucleotide monomers; 13-24 nucleotide monomers; 13-23 nucleotide monomers; 14-24 nucleotide monomers; 15-23 nucleotide monomers; and 16-23 nucleotide monomers; and at least 8 nucleotide monomers.

[000103] In certain embodiments, the antisense oligonucleotide strand is 10 to 36 (both range endpoints included) nucleotide monomers in length. In other words, antisense strands are from 10 to 36 (both range endpoints included) linked nucleobase monomers. In other embodiments, the antisense strand comprises a modified oligonucleotide consisting of 8 to 100, 10 to 80, 12 to 50, 14 to 30, 15 to 23, 16 to 22, 16 to 21, or 20 (both range endpoints included) linked nucleobases.

[000104] In certain embodiments, the antisense oligonucleotide consists of 14-23 (both range endpoints included) linked nucleoside monomers. In certain embodiments, the antisense oligonucleotide consists of 20 linked nucleoside monomers. In certain embodiments, the antisense oligonucleotide consists of 16 linked nucleoside monomers.

[000105] In certain embodiments, the sense strand includes a nucleobase sequence that is substantially complementary to the antisense strand and is at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a sequence of a linked region of the antisense oligonucleotide, as measured over the entire nucleobase sequence of the antisense strand. These substantially complementary sequences from both strands form one or more double-stranded regions. In certain embodiments, the sense strand has a nucleobase sequence comprising fully complementary sequence of the linked region of the antisense strand. As a result, the two strands form a double-stranded region that includes 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 base pairs. In some embodiments, at least one ISR is disposed in a double-stranded region of the sense strand.

[000106] In certain embodiments, an ISR is positioned at or near (within 33% of total number of nucleobases from a terminal counting said terminal) the 5' end of the sense strand, or at or near (within 33% of total number of nucleobases from a terminal counting said terminal) the 3' end of the sense strand. In some embodiments, an ISR or at least a part of the ISR is also positioned at a more central part of the sense strand, i.e., more than 33% of total number of nucleobases away from both terminals of the sense strand. In some embodiments, an ISR is not necessary to be deposited in the sense strand.

[000107] In a feature, the sense oligonucleotide strand has a length equal to or longer than the antisense oligonucleotide strand. In certain embodiments, the sense strand has a length from

full length to 16 linked nucleobases length longer than the antisense strand. In certain embodiments, the sense strand is 6 to 66 (both range endpoints included) nucleotide monomers in length. In other words, those sense strands are from 6 to 66 (both range endpoints included) linked nucleobases. In other embodiments, the sense strand comprises an oligonucleotide consisting of 20 to 21, 8 to 36, 10 to 30, 12 to 25, 14 to 24 or 16 to 23 (both range endpoints included) linked nucleobases. In certain such embodiments, the sense strand comprises an oligonucleotide consisting of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65 or 66 linked nucleobases in length, or a range defined by any two of the above values (both range endpoints included). In some embodiments, the sense strand is a sense oligonucleotide.

[000108] In one feature, the length of the second sense strand is equal to that of the first antisense strand. In certain embodiments, both ends of the duplex are blunt ends. In other feature, the second or sense strand has a backbone length longer than the first strand or the antisense strand by at least a number of nucleotide monomers as follows: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19 and 20. In various embodiments, the second or sense strand has a backbone length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65 or 66 linked nucleotide monomers, or equivalents thereof, or of a range bracketed by any two of the above values (both range endpoints included). In certain embodiments, for example, some of the ranges of the second, sense strand include: 6-66 nucleotide monomers; 8-50 nucleotide monomers; 8-40 nucleotide monomers; 8-36 nucleotide monomers; 8-33 nucleotide monomers; 10-36 nucleotide monomers; 10-30 nucleotide monomers; 10-29 nucleotide monomers; 12-29 nucleotide monomers; 12-28 nucleotide monomers; 12-26 nucleotide monomers; 12-25 nucleotide monomers; 12-24 nucleotide monomers; 13-25 nucleotide monomers; 13-24 nucleotide monomers; 13-23 nucleotide monomers; 14-24 nucleotide monomers; 15-23 nucleotide monomers; 16-23 nucleotide monomers tides; and at least 8 nucleotide monomers.

[000109] In certain embodiments, the sense strand is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 nucleotide monomers longer than the antisense strand. In certain embodiments, the sense strand consists of 8-36 (both range endpoints included) linked nucleoside monomers. In certain embodiments, the sense strand consists of 13 linked nucleoside monomers. In certain embodiments, the sense strand consists of 14 linked nucleoside monomers.

[000110] In certain embodiments of the invention, the two strands of the sdDNA molecule form a symmetric duplex without any overhangs. In various some other embodiments, the two ends of the first strand (antisense strand) are one of the following configurations: a 3'-overhang and a 5' blunt-end; a 5'-overhang and a 3' blunt-end; a 3' overhang and a 5' recessed-end; a 3' recessed end and a 5' overhang; or a 3' recessed-end and a 5' recessed end.

[000111] In certain embodiments, the 3'-overhang of the sense strand has a length of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotide monomers. In various embodiments, the 3'-overhang of the sense strand has a length of 1-8, 1-5, 1-3, or 1-2 nucleotide monomers (both range endpoints included).

[000112] In certain embodiments, the 5'-overhang of the sense strand has a length of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotide monomers. In various embodiments, the 3'-overhang of the sense strand has a length of 1-8, 1-5, 1-3, or 1-2 nucleotide monomers (both range endpoints included).

[000113] In the sdDNA molecule of the invention, at least one nucleotide monomer in the first strand and/or the second strand can be a modified nucleotide or nucleotide analogue, e.g., a sugar-, backbone-, and/or base-modified nucleotide. In an embodiment, such a backbone-modified nucleotide has at least a modification in an internucleoside linkage, e.g., to include at least one of a nitrogen or sulphur heteroatom. In some embodiments, the modified internucleoside linkage is or includes: phosphorothioate (P=S) group, phosphotriesters, methylphosphonates, or phosphoramidate.

[000114] In certain embodiments, the antisense strand and/or the sense strand comprises at least one modified internucleoside linkage. Such modified internucleoside linkage may be between two deoxyribonucleoside monomers, two ribonucleoside monomers, or one deoxyribonucleoside monomer and one ribonucleoside monomer. Alternately, the phosphate group on at least one of the terminal nucleoside monomers may be modified. In certain embodiments, the internucleoside linkage is a phosphorothioate internucleoside linkage. In certain embodiments, the internucleoside linkage is a thio-phosphoramidate internucleoside linkage. In certain embodiments, each internucleoside linkage of the oligonucleotide strand is a phosphorothioate internucleoside linkage. In certain embodiments, all the internucleoside linkages in a strand, antisense or sense or both, are phosphorothioate internucleoside linkages, or a mixture of phosphorothioate and phosphodiester linkages.

[000115] In certain embodiments, the antisense strand and/or the sense strand comprises at least one nucleoside monomer having a modified sugar moiety. Such a nucleoside monomer can

be a deoxyribonucleoside monomer or a ribonucleoside monomer.

[000116] In certain embodiments, the 2′ position of the modified sugar moiety is replaced by a group selected from OR, R, halo, SH, SR, NH_2, NHR, NR_2, or CN, where each R is independently C_1-C_6 alkyl, alkenyl or alkynyl, and halo is F, Cl, Br or I. In some embodiments, the 2′ position of the modified sugar moiety is replaced by a group selected from allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF_3, OCH_2F, $O(CH2)_2SCH_3$, $O(CH_2)_2$-O-N(R_m)(R_n), O-CH_2-C(=O)-N(R_m)(R_n), or O-CH_2-C(=O)-N(R_l)-$(CH_2)_2$-N(R_m)(R_n), where each R_l, R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl. In some embodiments, the modified sugar moiety is selected from the group of 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH_3, 2'-OCH_2CH_3, 2'-OCH_2CH_2F and 2'-$O(CH2)_2OCH_3$ substituent groups. In some embodiments, the modified sugar moiety is substituted by a bicyclic sugar selected from the group of 4′-(CH_2)—O-2′ (LNA); 4′-(CH_2)—S-2; 4′-(CH_2)2—O-2′ (ENA); 4′-CH(CH_3)—O-2′ (cEt) and 4′-CH(CH_2OCH_3)—O-2′, 4′-C(CH_3)(CH_3)—O-2′, 4′-CH_2—N(OCH_3)-2′, 4′-CH_2—O—N(CH_3)-2′, 4′-CH_2—N(R)—O-2′ (where R is H, C1-C12 alkyl, or a protecting group), 4′-CH_2—C(H)(CH_3)-2′, and 4′-CH_2—C—(=CH_2)-2′.

[000117] In some embodiments, the modified sugar moiety is selected from the group of 2'-O-methoxyethyl modified sugar (MOE), a 4′-(CH_2)—O-2′ bicyclic sugar (LNA), 2'-deoxy-2'-fluoroarabinose (FANA), and a methyl(methyleneoxy) (4′-CH(CH_3)—O-2) bicyclic sugar (cEt).

[000118] In some embodiments, the antisense strand and/or the sense strand of the molecule of the invention includes at least one nucleotide monomer having a modified nucleobase. Such a nucleoside monomer can be a deoxyribonucleoside monomer or a ribonucleoside monomer.

[000119] In some embodiments, the modified nucleobase is selected from the group of: 5-methylcytosine (5-Me-C), inosine base, a tritylated base, 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 (-C≡C-CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and 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, 2-F-adenine, 2-aminoadenine, 8-azaguanine and 8-azaadenine, and 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

[000120] In a particular embodiment, the modified nucleobase in the molecule of the

invention is a 5-methylcytosine. In an embodiment, each cytosine base in the molecule of the invention is 5-methylcytosine. In certain embodiments, the modified nucleobase is a 5-methyluracil. In certain embodiments, each uracil is a 5-methyluracil.

[000121] In certain embodiments, either the antisense strand or the sense strand or both of the molecule of the invention comprise linked deoxynucleoside monomers. In certain embodiments, an entire sense strand consists exclusively of linked deoxynucleoside monomers. In a feature, either the antisense strand or both antisense and the sense strand, in addition to the linked deoxynucleoside monomers, further includes an ISR that consist of one or more linked ribonucleoside monomers. Further, there may be even more ISR segments. The ISR can be anywhere in either strand. In some embodiments, one or more ISRs include a terminal nucleoside monomer, or a penultimate terminal nucleoside monomer. In certain embodiments, each of the ISRs independently consists of 1 ribonucleoside monomer, or 2, 3, 4, or 5 linked ribonucleoside monomers.

[000122] In certain embodiments, at least half of the nucelobases in at least one strand of the double-stranded region are deoxyribonucleotide monomer.

[000123] In certain embodiments, at least 50% of nucleotides in the first strand in the RNA-targeting part of the double-stranded region are deoxyribonucleotide monomer.

[000124] In certain embodiments, the total number of ribonucleotide monomer(s) in the sdDNA molecule is no more than the total number of deoxyribonucleotide monomers in the same sdDNA molecule.

[000125] In certain embodiments, at least one or each of the linked ribonucleoside monomers of the ISRs is a modified ribonucleotide or ribonucleotide analog. The ribonucleotide may be modified in the same or a similar way as follows: have a modified internucleoside linkage, a modified sugar moiety and/or a modified nucleobase.

[000126] In some embodiments, the sugar moiety of the deoxyribonucleotide monomer is either the sugar moiety of a naturally occurring deoxyribonucleotide (2-H) or 2’-deoxy-2’-fluoroarabinose (FANA).

[000127] In some embodiments, the sugar moiety of the ribonucleotide monomer is selected from the group consisting of a naturally occurring ribonucleotide (2-OH), 2’-F modified sugar, 2’-OMe modified sugar, 2’-O-methoxyethyl modified sugar (MOE), a 4′-(CH_2)—O-2′ bicyclic sugar (LNA) and a methyl(methyleneoxy) (4′-CH(CH_3)—O-2) bicyclic sugar (cEt).

[000128] In certain embodiments, in the antisense strand, the sense strand or both strands, at least one or each ribonucleoside monomer of each ISR therein has a modified sugar moiety

selected from the group of 2'-O-methoxyethyl modified sugar (MOE), a 4′-(CH_2)—O-2′ bicyclic sugar (LNA), and a methyl(methyleneoxy) (4′-CH(CH_3)—O-2) bicyclic sugar (cEt). In certain embodiments, in the antisense strand, the sense strand or both strands, at least one deoxyribonucleoside monomer has a modified sugar moiety of 2'-deoxy-2'-fluoroarabinose (FANA). In certain embodiments, each ribonucleoside monomer of each ISR has a 2'-O-methoxyethyl modified sugar, a 4′-(CH_2)—O-2′ bicyclic sugar, or a methyl(methyleneoxy) (4′-CH(CH_3)—O-2) bicyclic sugar (cEt), where each cytosine is a 5-methylcytosine, where each uracil is a 5-methyluracil, or methyl-pseudouracil, and where each internucleoside linkage is a phosphorothioate linkage.

[000129] In certain embodiments, the molecule of the invention has either an antisense strand or a sense strand consisting of deoxynucleoside monomers where each internucleoside linkage is a phosphorothioate linkage. In certain embodiments, the molecule of the invention has either an antisense strand or a sense strand consisting of deoxynucleoside monomers wherein each internucleoside linkage is a natural phosphate linkage without the phosphorothioate modification.

[000130] In certain embodiments, the molecule of the invention comprises a sense strand, wherein each nucleotide monomer of the sense strand comprising the same modification as the complementary nucleotide monomer of the antisense strand.

[000131] Exemplary structures and exemplary sequences of exemplary molecules of the invention with an antisense oligonucleotide strand and a sense oligonucleotide strand are showed in **FIG. 1A, 2A, 4A, 5A, 6A and 7A**.

[000132] In certain embodiments, short DNA duplex and at least one ISR in the antisense strand of the duplex molecular enable potent gene silencing. Data shown in all Examples below suggest that a new platform technology based on the duplex with an antisense oligodeoxyribonucleotide and at least one ISR in the antisense oligodeoxyribonucleotide enabled extremely potent gene silencing. Further studies on SAR (structure -activity relationship) features of the sdDNA, including various ISR motifs, various lengths, complementary and mismatches, various modifications, *etc.*, were carried out, which help to define various structural factors or scaffold features that may influence gene silencing activities. Such SAR factors and design features are important for designing optimized gene silencers to target various sequences and structures of more than 100,000 different mRNAs in a typical mammalian cell as well as much more non-coding RNAs. Our data on the gene silencing activities and the SAR of sdDNAs suggest that gene silencing features of sdDNA are vastly

different from siRNA and ASO, indicating a novel and distinct mechanism of gene silencing mechanism which is yet to be identified.

[000133] In certain embodiments, the molecule of the invention can be stabilized against degradation, either through at least one chemical modification or a secondary structure. The sense oligonucleotide strand and antisense oligonucleotide strand can have unmatched or imperfectly matched nucleotide monomers. The sense oligonucleotide strand and/or antisense oligonucleotide strand may have one or more nicks (a cut in the nucleic acid backbone), gaps (a fragmented strand with one or more missing nucleotides), and modified nucleotides or nucleotide analogues. Not only can any or all of the nucleotide monomers in the sense and antisense oligonucleotide strands chemically modified, each strand may be conjugated to one or more moieties or ligands to enhance its functionality, for example, with moieties or ligands selected from: peptide, antibody, antibody fragment, polymer, polysaccharide, lipid, hydrophobic moiety or molecule, cationic moiety or molecule, lipophilic compound or moiety oligonucleotide, cholesterol, GalNAc and aptamer.

[000134] In certain embodiments, the double-stranded region of the duplex molecule of the invention does not contain any mismatch or bulge, and the two strands are perfectly complementary to each other in the double-stranded region. In another embodiment, the double-stranded region of the duplex contains mismatch and/or bulge.

[000135] In certain embodiments, the target is mRNA or non-coding RNA implicated in a mammalian disease. In certain embodiments, the target is mRNA. In certain embodiments, the target is non-coding RNA, such as microRNA and lncRNA. The antisense strand can occupy the target by hybridizing to the target sequence as long as they are substantially complementary to each other, and inactive the target gene.

3. Unmatched or Mismatched Regions

[000136] The complementary region between the antisense strand and the sense strand of the present invention can have at least one unmatched or imperfectly matched region containing, e.g., one or more mismatches. In some embodiments, the sense strand of the sdDNA provided in this invention can contain three or more (at least 15% of the targeting region) mismatches without any effect on gene silencing activities of the sdDNA. Mismatches in sense strand are sometimes desired for reducing off-target effects or enable other features to the sdDNAs.

[000137] As is well known to one skilled in the art, it is possible to introduce mismatch bases without eliminating activity. Similarly, the antisense oligonucleotides strands of sdDNA of

the present invention can include unmatched or mismatched region(s). In some embodiments, antisense oligonucleotides strands of the sdDNA of the present invention can tolerate at least three (at least 15% of the targeting region) mismatches while maintaining gene silencing activities. Mismatches in antisense strand are sometimes desired for reducing off-target effects or enable other features to the sdDNAs.

4. Modifications

[000138] A nucleoside monomer is a base-sugar composition. The nucleobase (also known as base) portion of the nucleoside monomer is normally a heterocyclic base moiety. Nucleotide monomers are nucleoside monomers that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleoside monomers that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleoside monomers to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

[000139] Modifications to the sdDNA molecule, antisense strand and/or sense strand of the invention encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified sdDNA, antisense strand and/or sense strands are in some cases preferred over native forms because of desirable properties such as, for example, increased inhibitory activity, enhanced cellular uptake, enhanced strand affinity, solubility, reduce the non-specific interaction and resistance to RNase degradation or enhanced stability otherwise. Consequently, comparable results can often be obtained with shorter antisense strands that have such chemically modified nucleoside monomers. One or more of the natural nucleotides in the antisense and the sense strands of the invention can be substituted with modified nucleotides or nucleotide analogues. The substitution can take place anywhere in the antisense strand and the sense strand.

[000140] The modifications of oligonucleotide molecules have been investigated to improve the stability of various oligonucleotide molecules, including antisense oligonucleotide, ribozyme, aptamer, and RNAi (*Chiu and Rana, 2003; Czauderna et al., 2003; de Fougerolles et al., 2007; Kim and Rossi, 2007; Mack, 2007; Zhang et al., 2006; Schrnidt, 2007; Setten RL et al., 2020; Crooke ST et al., 2018; and Roberts TC et al., 2020*).

[000141] Any stabilizing modification known to a person skilled in the art can be used to

improve the stability of the oligonucleotide molecules. Within the oligonucleotide molecules, chemical modifications can be introduced to the phosphate backbone (e.g., phosphorothioate linkages), the sugar (e.g., locked nucleic acids, glycerol nucleic acid, cEt, 2'-MOE, 2'-fluorouridine, 2'-O-methyl), and/or the base (e.g., 2'-fluoropyrimidines).

[000142] Several examples of such chemical modifications are summarized in the sections that follow.

[000143] In various embodiment, the modified nucleotide or a nucleotide analogue is sugar-, backbone- and/or base-modified nucleotide.

4.1 Modified Internucleoside Linkages or Backbone-Modified Nucleotide

[000144] The naturally occurring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. The sdDNA molecule of the invention having one or more modified, i.e., non-naturally occurring, internucleoside linkages in one or both of its strands are sometimes selected over a corresponding molecule with only naturally occurring internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

[000145] Oligonucleotide strands having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. In an embodiment, the phosphodiester internucleoside linkage is modified to include at least a nitrogen and/or sulphur heteroatom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, thio-phosphoramidate and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

[000146] In one embodiment, a modified nucleotide or nucleotide analogue is a backbone-modified nucleotide. The backbone-modified nucleotide may have a modification in a phosphodiester internucleoside linkage. In a further embodiment, the backbone-modified nucleotide is phosphorothioate internucleoside linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

4.2 Modified Sugar Moieties

[000147] The antisense and/or the sense strand of the invention can optionally contain one or more nucleoside monomers where the sugar group has been modified. Such sugar-modified nucleoside monomers may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the strand. In certain embodiments, nucleoside

monomers comprise chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include without limitation, addition of substitut groups (including 5' and 2' substituent groups, bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or $C(R_1)(R_2)$ (R, R_1 and R_2 are each independently H, C_1-C_{12} alkyl or a protecting group) and combinations thereof. Examples of chemically modified sugars include 2'-F-5'-methyl substituted nucleoside (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) or replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a BNA (see PCT International Application WO 2007/134181 Published on 11/22/07 wherein LNA is substituted with for example a 5'-methyl or a 5'-vinyl group).

[000148] Examples of nucleoside monomers having modified sugar moieties include without limitation nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH_3, 2'-OCH_2CH_3, 2'-OCH_2CH_2F and 2'-$O(CH2)_2OCH_3$ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF_3, OCH_2F, $O(CH2)_2SCH_3$, $O(CH_2)_2$-O-N(Rm)(Rn), O-CH_2-C(=O)-N(Rm)(Rn), and O-CH_2-C(=O)-N(R1)-$(CH2)_2$-N(Rm)(Rn), where each Rl, Rm and Rn is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl.

[000149] Bicyclic nucleosides are modified nucleosides having a bicyclic sugar moiety. Examples of bicyclic nucleic acids (BNAs) include without limitation nucleosides comprising a bridge between the 4′ and the 2′ ribosyl ring atoms. In certain embodiments, sdDNA, the antisense strand and/or the sense strand provided herein include one or more BNA nucleosides wherein the bridge comprises one of the formulas: 4′-(CH_2)—O-2′ (LNA); 4′-(CH_2)—S-2; 4′-(CH_2)2—O-2′ (ENA); 4′-CH(CH_3)—O-2′ and 4′-CH(CH_2OCH_3)—O-2′ (and analogs thereof see U.S. Pat. No. 7,399,845, issued on Jul. 15, 2008); 4′-C(CH_3)(CH_3)—O-2′ (and analogs thereof see PCT/US2008/068922 published as WO/2009/006478, published Jan. 8, 2009); 4′-CH_2—N(OCH_3)-2′ (and analogs thereof see PCT/US2008/064591 published as WO/2008/150729, published Dec. 11, 2008); 4′-CH_2—O—N(CH_3)-2′ (see published U.S. Patent Application US2004-0171570, published Sep. 2, 2004); 4′-CH_2—N(R)—O-2′, wherein R is H, C_1-C_{12} alkyl, or a protecting group (see U.S. Pat. No. 7,427,672, issued on Sep. 23, 2008); 4′-CH_2—C(H)(CH3)-2′ (see Chattopadhyaya et al., J. Org. Chem., 2009, 74, 118-134); and 4′-CH_2—C—(=CH_2)-2′ (and analogs thereof see PCT/US2008/066154 published as WO 2008/154401,

published on Dec. 8, 2008).

[000150] In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-methyleneoxy (4′-CH_2—O-2) BNA (B) β-D-methyleneoxy (4′-CH_2—O-2) BNA (C) ethyleneoxy (4′-$(CH_2)_2$—O-2′) BNA, (D) aminooxy (4′-CH_2—O—N(R)-2′) BNA, (E) oxyamino (4′-CH_2—N(R)—O-2) BNA, (F) methyl(methyleneoxy) (4′-CH(CH3)—O-2) BNA (also referred to as constrained ethyl or cEt), (G) methylene-thio (4′-CH_2—S-2′) BNA, (H) methylene-amino (4′-CH_2—N(R)-2′) BNA, (I) methyl carbocyclic (4′-CH_2—$CH(CH_3)$-2) BNA, (J) propylene carbocyclic (4′-$(CH_2)_3$-2′) BNA, and (K) vinyl BNA.

[000151] In certain embodiments, a modified nucleotide or a nucleotide analogue is a sugar-modified ribonucleotide, in which the 2'-OH group is replaced by a group selected from: H, OR, R, halo, SH, SR, NH2, NHR, NR2, and CN, where each R is independently selected from the group consisting of: C_1-C_6 alkyl, alkenyl and alkynyl, and halo is selected from the group of F, Cl, Br and I. In certain embodiments, the sugar-modified ribonucleotide is selected from the group of 2’-OMe modified nucleotide, 2’-F modified nucleotide, 2’-O-methoxyethyl (2’MOE) modified nucleotide, LNA (Locked nucleic acid) modified nucleotide, GNA (Glycerol nucleic acid) modified nucleotide, and cEt (Constrained ethyl) modified nucleotide.

[000152] Chemical modifications at the 2’ position of the ribose, such as 2’-O-methylpurines and 2’-fluoropyrimidines, which increases resistance to endonuclease activity in serum, can be adopted to stabilize the molecules of the present invention. The position for the introduction of the modification should be carefully selected to avoid significantly reducing the silencing/regulating of potency of the molecule. In certain embodiments, the first nucleotide monomer adjacent to the 5’-terminal nucleotide monomer of the antisense strand is a 2’-flouro-ribonucleotide.

4.3 Modified Nucleobases

[000153] The antisense strand and/or the sense strand in the sdDNA molecule can also have nucleobase (or base) modifications or substitutions. Nucleobase (or base) modifications or substitutions are structurally distinguishable from, yet functionally interchangeable with, naturally occurring or synthetic unmodified nucleobases. Both natural and modified nucleobases are capable of participating in hydrogen bonding. Such nucleobase modifications may impart nuclease stability, binding affinity or some other beneficial biological property to the sdDNA molecule. Modified nucleobases include synthetic and natural nucleobases such as, for example, 5-methylcytosine (5-Me-C). Certain nucleobase substitutions, including 5-methylcytosine substitutions, are particularly useful for increasing the binding affinity of the antisense and the

sense strands. For example, 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., *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278).

[000154] Additional modified nucleobases include and are not limited to: 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, 1-methyl pseudouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and 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, 2-F-adenine, 2-aminoadenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

[000155] Heterocyclic base moieties may include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Nucleobases that are particularly useful for increasing the binding affinity of antisense and the sense strands include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2 aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

[000156] In certain embodiments, a modified nucleotide or a nucleotide analogue is a base-modified nucleotide. In an embodiment, a modified nucleotide or a nucleotide analogue has an unusual base or a modified base. In certain embodiments, the modified base is a 5-methylcytosine (5'-Me-C). In certain embodiments, each cytosine is a 5-methylcytosine. In certain embodiments, the modified base is a 5-methyluracil (5'-Me-U). In certain embodiments, each uracil is a 5-methyluracil.

[000157] Any modified nucleotide or analogue that may benefit the stability or affinity can be made without departing from the spirit and scope of the present invention. Several examples of such chemical modifications are same as summarized above.

5. Pharmaceutical Composition

[000158] In some embodiments, the present invention also provides pharmaceutical formulations comprising the sdDNA of the present invention, or a pharmaceutically acceptable derivative thereof and at least one pharmaceutically acceptable excipient or carrier. As used

herein, "pharmaceutically acceptable excipient" or "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. Suitable carriers are described in "Remington: The Science and Practice of Pharmacy, Twentieth Edition," Lippincott Williams & Wilkins, Philadelphia, PA., which is incorporated herein by reference. Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. 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 sdDNA molecule, use thereof in the compositions is contemplated.

[000159] Examples of the pharmaceutically acceptable carrier that can be used with the molecule of the invention include but are not limited to: a pharmaceutical carrier, a positive-charge carrier, a liposome, a lipid nanoparticle, a protein carrier, a hydrophobic moiety or molecule, a cationic moiety or molecule, GalNAc, a polysaccharide a polymer, a nanoparticle, a nanoemulsion, a cholesterol, a lipid, a lipophilic compound or moiety, and a lipoid.

[000160] In a certain embodiment, the present invention provides a method of treatment comprising administering a therapeutically effective amount of the pharmaceutical composition to a subject in need thereof. In an embodiment, the pharmaceutical composition is administered via a route selected from the group of: intravenous injection (iv), subcutaneous injection (sc), per os (po), intramuscular (im) injection, oral administration, inhalation, topical, intrathecal, and other regional administrations. In another embodiment, the therapeutically effective amount is 1 ng to 1 g per day, 100 ng to l g per day, or 1 μg to l000 mg per day.

[000161] Methods for formulation are disclosed in PCT International Application PCT/US02/24262 (WO03/01 1224), U.S. Patent Application Publication No. 2003/0091639 and U.S. Patent Application Publication No. 2004/0071775, each of which is incorporated by reference herein.

[000162] A sdDNA molecule of the present invention is administered in a suitable dosage form prepared by combining a therapeutically effective amount (e.g., an efficacious level sufficient to achieve the desired therapeutic effect through inhibition of tumor growth, killing of tumor cells, treatment or prevention of cell proliferative disorders, etc.) of the sdDNA molecule of the present invention (as an active ingredient) with standard pharmaceutical carriers or diluents according to conventional procedures (i.e., by producing a pharmaceutical composition

of the invention).

[000163] These procedures may involve mixing, granulating, and compressing or dissolving the ingredients as appropriate to attain the desired preparation. In another embodiment, a therapeutically effective amount of sdDNA molecules is administered in a suitable dosage form without standard pharmaceutical carriers or diluents. In some embodiments, a therapeutically effective amount of the duplex molecule of the invention is administered in a suitable dosage form. Pharmaceutically acceptable carriers include solid carriers such as lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary liquid carriers include syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time-delay material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or with a wax, eihylcellulose, hydroxypropylmethylcellulose, methylmethacrylate or the like. Other fillers, excipients, flavorants, and other additives such as are known in the art may also be included in a pharmaceutical composition according to this invention.

[000164] The pharmaceutical compositions of the present invention may be manufactured in a manner that is generally known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. Pharmaceutical compositions may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and/or auxiliaries which facilitate processing of the sense oligonucleotide and the antisense oligonucleotide into preparations that can be used pharmaceutically. Of course, the appropriate formulation is dependent upon the route of administration chosen.

[000165] The composition, compound, combination or the pharmaceutical composition of the invention can be administered to a subject in many of the well-known methods currently used for chemotherapeutic treatment. For example, for treatment of cancers, the sdDNA molecule of the invention may be injected directly into tumors, injected into the blood stream or body cavities or taken orally or applied through the skin with patches. For treatment of psoriatic conditions, systemic administration (e.g., oral administration), or topical administration to affected areas of the skin, are preferred routes of administration. The dose chosen should be sufficient to constitute effective treatment but not as high as to cause unacceptable side effects. The state of the disease condition (e.g., cancer, psoriasis, and the like) and the health of the patient should be closely monitored during and for a reasonable period after treatment.

6. Utility

6.1 Method of Use

[000166] The present invention provides a method of modulating gene expression or function in a cell or an organism. The cell may be a eukaryotic cell, e.g., a mammalian cell. The method comprises the steps of contacting said cell or organism with the sdDNA molecule disclosed herein, under conditions wherein selective gene silencing can occur, and mediating a selective gene silencing effected by the sdDNA molecule towards a target nucleic acid having a sequence portion substantially complementary to the antisense strand. The target nucleic acid may be an RNA such as a mRNA or non-coding RNA where such RNA either encodes a protein or regulates a part of a biological pathway implicated in a disease.

[000167] In an embodiment, the contacting step comprises the step of introducing sdDNA molecule into a target cell in culture or in an organism in which the selective gene silencing can occur. In a further embodiment, the introducing step comprises a mixing, transfection, lipofection, infection, electroporation, or other delivery technologies. In another embodiment, the introducing step comprises using a pharmaceutically acceptable excipient, carrier, or diluent selected from the group of a pharmaceutical carrier, a positive-charge carrier, a liposome, a lipid nanoparticle, a protein carrier, a polymer, a nanoparticle, a nanoemulsion, a lipid, N-Acetyl-Galactosamine (GalNAc), a lipophilic compound or moiety and a lipoid to be administered via iv, sc, intrathecal, po, inhalation, topical or other clinically acceptable administration methods.

[000168] In an embodiment, the silencing method is used for determining the function or utility of a gene in a cell or an organism.

[000169] In an embodiment, the gene or RNA targeted by the composition of the invention is associated with or implicated in a disease, e.g., a human disease or an animal disease, a pathological condition, or an undesirable condition. In a further embodiment, the target gene or RNA is that of a pathogenic microorganism. In an even further embodiment, the target gene or RNA is of a viral origin. In another embodiment, the target gene or RNA is tumor-associated.

[000170] In an alternative embodiment, the gene or RNA targeted by the composition of the invention is a gene or a RNA associated with, or more specifically, implicated with cancer, autoimmune disease, inflammatory diseases, degenerative diseases, infectious diseases, proliferative diseases, metabolic diseases, immune-mediated disorders, allergic diseases, dermatological diseases, malignant diseases, gastrointestinal disorders, hepatic disorders, respiratory disorders, cardiovascular disorders, dermatological disorders, renal disorders, rheumatoid disorders, neurological disorders, psychiatric disorders, endocrine disorders, or aging-related disorders or diseases.

6.2 Treatment Method

[000171] The present invention also provides a method of treating or preventing various diseases or conditions, including those summarized for ASO and siRNAs (*Czech, 2006; de Fougerolles et al., 2007; Dykxhoorn et al., 2003; Kim and Rossi, 2007; Mack, 2007; Crooke ST et al., 2018; Setten RL et al., 2019*; *Roberts TC et al., 2020*). The method comprises administering an effective amount of the sdDNA molecule to a subject in need thereof under conditions wherein desired gene inhibition described in the section immediately above can occur.

[000172] In an exemplary embodiment, a pharmaceutical composition having the sdDNA molecule and a pharmaceutically acceptable excipient, carrier, or diluent is administered to a patient in need thereof for treating or preventing a disease or an undesirable condition in a therapeutically effective amount.

[000173] In some embodiments, the present invention can be used as a cancer therapy or to prevent cancer. The composition of the sdDNA can be used to silence or knock down genes involved with cell proliferation disorders or a malignant disease. Examples of these genes are k-Ras, β-catenin, Stat3. These oncogenes are active and relevant in a large number of human cancer.

[000174] The novel composition of the invention can also be used to treat or prevent ocular disease, (e.g., age-related macular degeneration (AMD) and diabetic retinopathy (DR)); infectious diseases (e.g., HIV/AIDS, hepatitis B virus (HBV), hepatitis C virus (HCV), human papillomavirus (HPV), herpes simplex virus (HSV), RCV, cytomegalvirus (CMV), dengue fever, west Nile virus); respiratory disease (e.g., respiratory syncytial virus (RSC), asthma, cystic fibrosis); neurological diseases (e.g., Huntingdon's disease (HD), amyotrophic lateral sclerosis (ALS), spinal cord injury, Parkinson's disease, Alzheimer's disease, pain); cardiovascular diseases; metabolic disorders (e.g., hyperlipidemia, hypercholesterolemia, and diabetes); genetic disorders; and inflammatory conditions (e.g., inflammatory bowel disease (IBD), arthritis, rheumatoid disease, autoimmune disorders), dermatological diseases.

[000175] In an alternative embodiment, the administration method is a route selected from the group of intravenous injection (iv), subcutaneous injection (sc), per os (po), intrathecal, inhalation, topical, and regional administration.

EXAMPLES

[000176] Examples are provided below to further illustrate different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These

examples do not limit the claimed invention.

Methods and Materials

Cell culture

[000177] HepaRG cells were grown in William's Medium supplemented with 10% FBS, 10mg/ml Hydrocortisone, and 4 mg/ml human recombinant insulin.

Transfection of sdDNAs to HepaRG cells

[000178] 24 hours before transfection, the HepaRG cells were seeded to 6-well plates (1 x 105 cells/2 mL/well). The sdDNAs were transfected by Lipofectamine® RNAiMAX (Thermo Fisher, USA) at 100 pM final concentrations as described the manufacture methods, briefly sdDNAs and RNAiMAX were incubate for 20 minutes in serum free OPTI-MEM (Thermo Fisher), then added to the cell with culture medium.

Quantitative PCR

[000179] Cells transfected with the indicated sdDNAs were harvested at 48 hours after transfection. RNA was isolated with TRIZOL, and qRT-PCR performed using TaqMan one-step RT-PCR reagents and APOCIII assay for APOCIII mRNA detection and the gene GAPDH mRNA levels were used as internal control.

[000180] Target Sequences

[000181] To demonstrate the gene silencing effects of sdDNAs disclosed in the present invention, sdDNAs designed and made to target the APOCIII gene (APOCIII mRNA) were used in below examples. The target sequences are segments from Accession # NM_000040 that can bind to the antisense stand of sdDNA or a corresponding single-stranded antisense oligonucleotide.

Example 1: Gene silencing activity of sdDNAs with various ISR motifs

[000182] **FIG. 1A** shows structures of embodiments of sdDNAs (sdDNA1-3) provided by the present invention with different ISR motifs, where ISR is positioned in both AS (antisense strand) and SS (sense strand) or only in AS, and the sequences of the designed sdDNAs (sdDNA1-3) for targeting APOCIII gene. The single-stranded antisense oligonucleotide with identical structure and sequence as the antisense strand of the sdDNAs (sdDNA1-3) are also used as corresponding single-stranded AS oligonucleotide (corresponding ASO) for comparison. The corresponding ASO (SEQ ID No.: 1) used here is ISIS304801, a typical ASO product optimized with the most advanced state-of-art ASO technology and knowhows. Gene silencing activities for targeting APOCIII by sdDNA1-3 and corresponding ASOs were tested in HepaRG Cells (results are shown in **FIG. 1B**).

[000183] In **FIG. 1A,** all Letters "D" in the illustrated structures represent DNA residues or deoxyribonucleotide monomers; all Letters "R" in the illustrated structures represent RNA residues or ribonucleotide monomers; all lowercase Letters "**a, c, g, t**" in the sequences represent DNA residues; all uppercase Letters "**A, C, G, U**" in the sequences represent 2'-MOE modified RNA residues, wherein all "**U**" is 5-Methyl Uridine 2'-MOE RNA residues; wherein all "**C**" and "**c**" are 5-Me-C; all "*" in the illustrated structures represent PS (phosphorothioate internucleoside linkage).

[000184] The results suggest that all the designed sdDNAs have great potency for gene silencing activity against APOCIII at very low concentrations (pico molar concentration) and are significantly more potent as well as more efficacious than the corresponding single-stranded antisense oligonucleotide (ASO).

Example 2: sdDNA of various lengths

[000185] **FIG. 2A** shows some embodiments of sdDNAs. In these sdDNAs, AS and SS are symmetric in length. Various sdDNA duplex lengths (8 to 36 bp), were designed (sdDNA_1-10 structures and sequences of the sdDNAs for targeting the APOCIII gene, shown in **FIG. 2A**). Single-stranded antisense oligonucleotides with identical structure and sequence as the antisense strand of sdDNA_1-10 were also made as corresponding single-stranded AS of each sdDNA. Gene silencing activities of sdDNA_1-10 and each corresponding single-stranded ASO designed to target APOCIII were tested in HepaRG Cells (results of sdDNAs are shown in **FIG. 2B**, corresponding single-stranded AS are shown in **FIG. 2C)**.

[000186] In **FIG. 2A,** all lowercase Letters "**a, c, g, t**", uppercase Letters "**A, C, G, U**" and "*" in the illustrated sequences represent the same as in **FIG. 1A**.

[000187] The results suggest that symmetric blunt-ended sdDNAs with 10 bp have highly potent gene silencing activity at very low concentrations (pico molar concentration) and are significantly more potent as well as more efficacious than the corresponding single-stranded ASO. While corresponding single-stranded ASOs here show generally low activities at pico molar level, they can show gene silencing activity at nano molar level (about 10 nM-30 nM), which is consistent with known oligonucleotides developed under well-known the current state-of-art ASO technology. It is also a surprising discovery here that single-stranded AS Oligonucleotide with much longer length than typical ASO (generally has length of 16 nt to 20 nt) shows more potent gene silencing activity than the typical ASO. However, the sdDNAs in present invention can always show relatively better activities than a corresponding single-stranded AS Oligonucleotide, including known typical well-optimized ASO (such as SEQ ID

No.: 1) by the state-of-art ASO technology.

Example 3: Symmetric sdDNA of various lengths

[000188] **FIG. 3A** shows different structural designs of embodiments of sdDNAs. In these sdDNAs, AS and SS are symmetric in length. Further, various lengths of the AS and the SS duplex, from 8 to 14bp, were designed and made (sdDNA_1-3 structures and sequences of the sdDNAs for targeting the APOC gene shown in **FIG. 3A**). Gene silencing activities of sdDNA_1-3 designed to target APOCIII were tested in HepaRG Cells (results of sdDNAs are shown in **FIG. 2B)**.

[000189] In **FIG. 3A,** all lowercase Letters "**a, c, g, t**" and "*" in the illustrated sequences represent the same as in **FIG. 1A**, all uppercase Letters underlined "***A, C, G, U***" in the illustrated sequences are represent LNA modified RNA residues, wherein all "***U***" is 5-Methyl Uridine LNA RNA residues and all "***C***" are 5-Me-C LNA RNA residues.

[000190] The results suggest that all designed sdDNAs have highly potent gene silencing activity at very low concentrations (pico molar concentration).

Example 4: sdDNA with various modification motifs in SS

[000191] **FIG. 4A** shows different structural designs of embodiments of sdDNAs with different PS modification motifs in SS. Specifically, in these sdDNAs, the AS was kept constant, and the SS was kept the same except for internucleoside linkage(s): specifically, while sdDNA_SS:PS has an entire SS with each internucleoside linkage being PS modified, sdDNA_SS:PO has an entire SS with each internucleoside linkage being naturally PO internucleoside linkage and sdDNA_SS:PS/PO has a mixed SS with PS modified internucleoside linkage only in some but not all of the internucleoside linkage of the linked deoxyribonucleoside monomers. The single-stranded antisense oligonucleotide with identical structure and sequence as the antisense strand of the sdDNAs (sdDNA1-3) are also used as corresponding single-stranded AS oligonucleotide (corresponding ASO) for comparison. The corresponding ASO (SEQ ID No.: 1) used here is ISIS304801, a typical ASO product optimized with the most advanced state-of-art ASO technology and knowhows. Gene silencing activities to target APOCIII by sdDNA (PS, PO, PS/PO) and corresponding ASO designed were tested in HepaRG Cells (results are shown in **FIG. 4B)**.

[000192] In **FIG. 4A,** all lowercase Letters "**a, c, g, t**", uppercase Letters "**A, C, G, U**" and "*" in the illustrated sequences represent the same as in **FIG. 1A**.

[000193] All designed sdDNAs with different PS modification motifs in SS show highly potent gene silencing activity against APOCIII at very low concentrations (pico molar

concentration) and are significantly more potent as well as more efficacious than the corresponding ASO. In other words, various modification motifs in the internucleoside linkage of the linked deoxyribonucleoside monomers/DNA residues can maintain the advantage of significant improvement in gene silencing activities by the sdDNA-based gene silencing technology provided in present invention, at least compared to the conventional ASO technology. sdDNAs having SS partially or entirely modified with PS internucleoside linkage showed potent gene silencing activities that are at least as good as sdDNAs with entirely natural PO internucleotide linkage. Yet it is well known in the field that PS modification is needed for in vivo applications.

[000194] These results also suggest that the SS is removed or degraded from the sdDNA duplex through an unknown mechanism other than typical DNase since sdDNAs with entirely PS modified SS are highly or at least equally potent as sdDNAs with a non-modified SS. Furthermore, it is also expected that using at least one modification (such as PS modified internucleoside linkage) in the linked deoxyribonucleoside monomers/DNA residues in SS may help to design sdDNAs with improved pharmaceutical properties for in vivo applications, including better stability and tissue distributions.

Example 5: sdDNA with Various ISR motifs disposed at various positions in AS

[000195] **FIG. 5A** shows different structural designs of a further series of sdDNAs. In these sdDNAs, the sense strand was kept constant while changing the positions and total number of ribonucleotide monomers of ISR(s) in the AS (ISR_0-5, structures and sequences shown in **FIG. 5A**). The various ISR motifs in the antisense strand in **FIG. 5A** shows that each ISR have a number of ribonucleotide monomers as low as 1 or 2 and each ISR is spaced apart with at least one intervening deoxyribonucleotide monomers. In **FIG. 5A,** all lowercase Letters "**a, c, g, t**", uppercase Letters "**A, C, G, U**" and "*" in the illustrated sequences represent the same as in **FIG. 1A**. Gene silencing activities of ISR_0-5 designed to target APOCIII were tested in HepaRG Cells (results are shown in **FIG. 5B)**.

[000196] All designed sdDNAs have highly potent gene silencing activity at very low concentrations (pico molar level). The results further suggest that the at least one ISR(s) in AS having various numbers of ribonucleotide monomers and disposed at various positions in AS can enhance or enable highly potent gene silencing activities of the sdDNAs provided in the present invention.

Example 6: sdDNA with mismatch in AS

[000197] **FIG. 6A** shows different structural designs of a further series of sdDNAs. In these

sdDNAs, the antisense strand was design to comprise at least one mismatch when hybridize to a target RNA (Mis1-2, structures and sequences shown in **FIG. 6A**) and antisense strand has no mismatch (Mis0) was designed as comparison. In **FIG. 6A,** all lowercase Letters "**a, c, g, t**", uppercase Letters "**A, C, G, U**" and "*" in the illustrated sequences represent the same as in **FIG. 1A**. Gene silencing activities of Mis_0-3 designed to target APOCIII were tested in HepaRG Cells (results are shown in **FIG. 6B)**.

[000198] All designed sdDNAs have highly potent gene silencing activity at very low concentrations (pico molar level). The results further suggest that the antisense strand of the sdDNA provided in present invention can have at least two (at least 10% of the targeting region) mismatches while maintaining gene silencing activities of the sdDNA provided in this invention. Some mismatch or multiple mismatches in certain position in AS may reduce the gene silencing activity of the sdDNA.

Example 7: sdDNA with SS longer than AS

[000199] **FIG. 7A** shows different structural designs of a further series of sdDNAs with a longer SS than AS. In these sdDNAs, the antisense strand was kept constant while changing the length of the sense strand (sdDNA_1-2, structures and sequences shown in **FIG. 7A**). In **FIG. 7A,** all lowercase Letters "**a, c, g, t**", uppercase Letters "**A, C, G, U**" and "*" in the illustrated sequences represent the same as in **FIG. 1A**. The single-stranded antisense oligonucleotide with identical structure and sequence as the antisense strand of the sdDNAs (sdDNA_1-2) are also used as corresponding single-stranded AS oligonucleotide (corresponding ASO) for comparison. Gene silencing activities to target APOCIII bysdDNA1-2 and corresponding ASO designed were tested in HepaRG Cells (results are shown in **FIG. 7B)**.

[000200] All designed sdDNAs have highly potent gene silencing activity at very low concentrations (pico molar level) and are more potent as well as more efficacious than the corresponding ASO. The results suggest that the sense strand of sdDNAs can be longer than the antisense strand. As shown in the example, the SS can be longer than AS by at least 16 monomers.

Equivalents

[000201] The representative examples are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples and the references to the scientific and patent

literature included herein. The examples contain important additional information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

[000202] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. Methods recited herein may be carried out in any order that is logically possible, in addition to a particular order disclosed.

Incorporation by Reference

[000203] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made in this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes. Any material, or portion thereof, that is said to be incorporated by reference herein, but which conflicts with existing definitions, statements, or other disclosure material explicitly set forth herein is only incorporated to the extent that no conflict arises between that incorporated material and the present disclosure material. In the event of a conflict, the conflict is to be resolved in favor of the present disclosure as the preferred disclosure.

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CLAIMS

1. A short duplex DNA (sdDNA) molecule comprising a first strand and a second strand each comprising linked nucleotide monomers,

wherein the first strand is substantially complementary to a targeted segment of a targeted RNA;

wherein the second strand is substantially complementary to the first strand, and forms at least one double-stranded region with the first strand;

wherein the length of the second strand is equal to or longer than the length of the first strand; and

wherein the sdDNA molecule comprises at least one interspersed segment of ribonucleotide monomer(s) (ISR) that comprises at least one ribonucleotide monomer.

2. The sdDNA molecule of claim 1, wherein the first strand comprises at least one ISR.

3. The sdDNA molecule of claim 1, wherein the second strand comprises at least one ISR.

4. The sdDNA molecule of claim 1, wherein the first strand comprises at least one ISR, and the second strand also comprises at least one ISR.

5. The sdDNA molecule of claim 2 or 4, wherein the at least one ISR is disposed in at least one targeting region of the first strand.

6. The sdDNA molecule of claim 5, wherein the total number of ribonucleotide monomers of all ISR(s) in the first strand is at least 2.

7. The sdDNA molecule of claim 3 or 4, wherein the at least one ISR is disposed in at least one double-stranded region of the second strand.

8. The sdDNA molecule of any one of claims 1 to 7, wherein the sdDNA molecule comprises at least two or more ISRs, wherein each ISR, independently of each other, either consists of one ribonucleotide monomer, or comprises at least 2, 3, 4 or 5 contiguous ribonucleotide monomers.

9. The sdDNA molecule of any one of claims 1 to 7, wherein the at least one ISR comprises at least 2, 3, 4, 5 or 6 contiguous ribonucleotide monomers.

10. The sdDNA molecule of claim 1, wherein the first strand is at least 70%, 80%, 85%, 90%, 95% complementary or fully complementary to the targeted segment of the targeted RNA.

11. The sdDNA molecule of any one of claims 1-10, wherein the first strand comprises no more than 1, 2 or 3 mismatch(es) when hybridized to the targeted RNA.

12. The sdDNA molecule of claim 1, wherein the second strand is at least 70%, 75%, 80%, 85%, 90%, 95% complementary or fully complementary to the first strand.

13. The sdDNA molecule of any one of claims 1-12, wherein the second strand comprises 1, 2, 3 or more mismatch(es) upon forming a complementary duplex to the at least one region of the first

strand.

14. The sdDNA molecule of claim 1, wherein the first strand has a length selected from the group consisting of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 and 50 nucleotide monomers.

15. The sdDNA molecule of claim 1, wherein the first strand has a length selected from the group consisting of:

a) 6-50 nucleotide monomers,
b) 8-36 nucleotide monomers,
c) 8-33 nucleotide monomers,
d) 8-36 nucleotide monomers,
e) 10-30 nucleotide monomers, and
f) 8-29 nucleotide monomers.

16. The sdDNA molecule of claim 1, wherein the second strand is equal to the first strand in length.

17. The sdDNA molecule of claim 1, wherein the second strand is longer than the first strand by at least a number of nucleotide monomers selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20.

18. The sdDNA molecule of claim 1, wherein the second strand has a length selected from the group consisting of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 and 50 nucleotide monomers.

19. The sdDNA molecule of claim 1, wherein the second strand has a length selected from the group consisting of:

a) 6-50 nucleotide monomers,
b) 8-36 nucleotide monomers,
c) 8-33 nucleotide monomers,
d) 8-36 nucleotide monomers,
e) 8-32 nucleotide monomers,
f) 8-30 nucleotide monomers,
g) 8-29 nucleotide monomers, and
h) 8-25 nucleotide monomers.

20. The sdDNA molecule of claim 1, wherein the double-stranded region consists of a number of

base pairs selected from the group consisting of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 base pairs.

21. The sdDNA molecule of claim 1, wherein both ends of the duplex are blunt ends.

22. The sdDNA molecule of claim 1, wherein both the first strand and the second strand have a 3'-overhang, or both the first strand and the second strand have a 5'-overhang.

23. The sdDNA molecule of claim 17, wherein the second strand has one of the following configurations: a 3'-overhang and a 5'-overhang, a 3'-overhang and a 5'-blunt end, a 3'-blunt end and a 5'-overhang, 3’ overhang and a 5’ recessed end, or 5’ overhang and a 3’ recessed end.

24. The sdDNA of claim 16 or 17, wherein the first strand has one of the following configurations: a 3'-overhang and a 5'-blunt end, a 3'-blunt end and a 5'-overhang, 3’ overhang and a 5’ recessed end, a 5’ overhang and a 3’ recessed, a 3’ blunt-end and a 5’ blunt-end, or a 3’ recessed-end and a 5’ recessed end.

25. The sdDNA molecule of claim 23 or 24, wherein the 3'-overhangs or the 5'-overhangs have no more than 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotide monomers.

26. The sdDNA molecule of any one of claims 1 to 25, wherein at least one nucleotide monomer is a modified nucleotide or nucleotide analogue.

27. The sdDNA molecule of claim 26, wherein the modified nucleotide or nucleotide analogue is sugar-, backbone-, and/or base-modified nucleotide.

28. The sdDNA molecule of claim 27, wherein the backbone-modified nucleotide has a modification in an internucleoside linkage.

29. The sdDNA molecule of claim 28, wherein the internucleoside linkage is modified to include at least one of a nitrogen or sulphur heteroatom.

30. The sdDNA molecule of claim 29, wherein the modified internucleoside linkage is selected from the group consisting of phosphorothioate (P=S) group, phosphotriesters, methylphosphonates, and phosphoramidate.

31. The sdDNA molecule of claim 26, wherein the first strand and/or the second strand comprises at least one modified internucleoside linkage, and wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

32. The sdDNA molecule of claim 26, wherein the second strand comprise at least one modified internucleoside linkage in the linked deoxyribonucleotide monomer region, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

33. The sdDNA molecule of claim 31, wherein each internucleoside linkage of the first strand is

a phosphorothioate internucleoside linkage.

34. The sdDNA molecule of claim 31, wherein each internucleoside linkage of the second strand is a phosphorothioate internucleoside linkage.

35. The sdDNA molecule of claim 26, wherein the modified nucleotide or nucleotide analogue comprises a modified sugar moiety.

36. The sdDNA molecule of claim 35, wherein the 2′ position of the modified sugar moiety is replaced by a group selected from the group consisting of OR, R, halo, SH, SR, NH_2, NHR, NR_2, and CN, where each R is independently C_1-C_6 alkyl, alkenyl or alkynyl, and halo is F, Cl, Br or I.

37. The sdDNA molecule of claim 35, wherein the 2′ position of the modified sugar moiety is replaced by a group selected from the group consisting of allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF_3, OCH_2F, $O(CH2)_2SCH_3$, $O(CH_2)_2$-O-N(R_m)(R_n), O-CH_2-C(=O)-N(R_m)(R_n), and O-CH_2-C(=O)-N(R_1)-$(CH_2)_2$-N(R_m)(R_n), where each of R_l, R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl.

38. The sdDNA molecule of claim 35, wherein the modified sugar moiety is selected from the group consisting of 5’-vinyl, 5’-methyl (R or S), 4’-S, 2’-F, 2’-OCH_3, 2’-OCH_2CH_3, 2’-OCH_2CH_2F and 2’-$O(CH2)_2OCH_3$ substituent groups.

39. The sdDNA molecule of claim 35, wherein the modified sugar moiety is substituted by a bicyclic sugar selected from the group consisting of 4′-(CH_2)—O-2′ (LNA); 4′-(CH_2)—S-2; 4′-(CH_2)2—O-2′ (ENA); 4′-CH(CH_3)—O-2′ (cEt) and 4′-CH(CH_2OCH_3)—O-2′, 4′-C(CH_3)(CH_3)—O-2′, 4′-CH_2—N(OCH_3)-2′, 4′-CH_2—O—N(CH_3)-2′, 4′-CH_2—N(R)—O-2′(where R is H, C1-C12 alkyl, or a protecting group), 4′-CH_2—C(H)(CH_3)-2′, and 4′-CH_2—C—(=CH_2)-2′.

40. The sdDNA molecule of claim 35, wherein the modified sugar moiety is selected from the group consisting of 2’-O-methoxyethyl modified sugar (MOE), a 4′-(CH_2)—O-2′ bicyclic sugar (LNA), 2’-deoxy-2’-fluoroarabinose (FANA), and a methyl(methyleneoxy) (4′-CH(CH_3)—O-2) bicyclic sugar (cEt).

41. The sdDNA molecule of claim 26, wherein the modified nucleotide or nucleotide analogue comprises a modified nucleobase.

42. The sdDNA molecule of claim 41, wherein the modified nucleobase is selected from the group consisting of 5-methylcytosine (5-Me-C), inosine base, a tritylated base, 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, 1-methyl-pseudo-uracil, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil,

cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and 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, 2-F-adenine, 2-aminoadenine, 8-azaguanine and 8-azaadenine, and 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

43. The sdDNA molecule of claim 37, wherein the modified nucleobase is a 5-methylcytosine.

44. The sdDNA molecule of claim 41, wherein each cytosine base is 5-methylcytosine.

45. The sdDNA molecule of any one of claims 1 to 44, wherein the sdDNA has capability of modulating gene expression or function in a cell.

46. The sdDNA molecule of any one of claims 1 to 45, wherein the sdDNA molecule is more potent or more efficacious at silencing the target RNA than a corresponding single-stranded antisense oligonucleotide.

47. The sdDNA molecule of any one of claims 1 to 46 is used for modulating gene expression or function in a cell.

48. The sdDNA molecule of claim 47 wherein the cell is a eukaryotic cell.

49. The sdDNA molecule of claim 48 wherein the eukaryotic cell is a mammalian cell.

50. The sdDNA molecule of claim 1, wherein the targeted RNA is either mRNA or non-coding RNA, where such RNA either encodes a protein or regulates a part of a biological pathway implicated in a disease or conditions.

51. The sdDNA molecule of claim 1, wherein the targeted RNA is selected from the group consisting of:

a) an mRNA of a gene implicated in human or animal diseases or disorder,

b) an mRNA of a gene of a pathogenic microorganism,

c) a viral RNA, and

d) an RNA implicated in a disease selected from the group consisting of autoimmune diseases, inflammatory diseases, degenerative diseases, infectious diseases, proliferative diseases, metabolic diseases, immune-mediated disorders, allergic diseases, dermatological diseases, malignant diseases, gastrointestinal disorders, respiratory disorders, cardiovascular disorders, renal disorders, rheumatoid disorders, neurological disorders, endocrine disorders, and aging-related disorders.

52. The sdDNA molecule of any one of claims 1 to 51 wherein the first strand and/or the second strand is conjugated to a ligand or a moiety.

53. The sdDNA molecule of claim 52 wherein the ligand or moiety selected from the group

consisting of peptide, antibody, polymer, polysaccharide, lipid, hydrophobic moiety or molecule, cationic moiety or molecule, lipophilic compound or moiety oligonucleotide, cholesterol, GalNAc and aptamer.

54. A pharmaceutical composition comprises an sdDNA molecule of any of claims 1-53 as active agent and a pharmaceutically acceptable excipient, carrier, or diluent.

55. The pharmaceutical composition of claim 54 wherein the carrier is selected from the group consisting of a pharmaceutical carrier, a positive-charge carrier, a lipid nanoparticle, a liposome, a protein carrier, a hydrophobic moiety or molecule, a cationic moiety or molecule, GalNAc, a polysaccharide a polymer, a nanoparticle, a nanoemulsion, a cholesterol, a lipid, a lipophilic compound or moiety and a lipoid.

56. A method for treating or preventing a disease or a condition, wherein the method comprises administering a therapeutically effective amount of the sdDNA molecule of any one of claims 1-53 or the pharmaceutical composition of either claim 54 or claim 55 to a subject in need thereof.

57. The method of claim 50, wherein the disease or condition is selected from the group consisting of cancer, autoimmune disease, inflammatory diseases, degenerative diseases, infectious diseases, proliferative diseases, metabolic diseases, immune-mediated disorders, allergic diseases, dermatological diseases, malignant diseases, gastrointestinal disorders, hepatic disorders, respiratory disorders, cardiovascular disorders, dermatological disorders, renal disorders, rheumatoid disorders, neurological disorders, psychiatric disorders, endocrine disorders, and aging-related disorders or diseases.

58. The method of claim 57, wherein the sdDNA molecule or pharmaceutical composition is administered via a route selected from the group consisting of intravenous injection (iv), subcutaneous injection (sc), per os (po), intramuscular (im) injection, oral administration, inhalation, topical, intrathecal, and other regional administrations.

59. A method for modulating a gene expression or gene function in a eukaryotic cell, wherein the method comprises contacting the cell with an effective amount of the sdDNA molecule of any one of claims 1-53 or the pharmaceutical composition of either claim 54 or claim 55.

60. A short duplex DNA (sdDNA) molecule comprising a first strand and a second strand each comprising linked nucleotide monomers selected from the group consisting of nucleotides, analogs thereof, and modified nucleotides,

wherein the first strand is equal to or shorter than the second strand in length by a number of monomers selected from the group consisting of 1, 2, 3, 4, 5, 6, 7 and 8 monomers,

wherein the first strand is substantially complementary to a targeted segment of a targeted

RNA through at least one targeting region, and wherein the first strand consists of 8-36 (both range endpoints included) nucleoside monomers linked through a linkage selected from the group consisting of a phosphorothioate linkage, a phosphodiester linkage, or a mixture of phosphorothioate and phosphodiester linkages between adjacent monomers,

wherein the second strand is substantially complementary to the first strand, and forms at least one double-stranded region with the first strand, and wherein the second strand consists of 10-36 (both range endpoints included) nucleoside monomers linked through a linkage selected from the group consisting of a phosphorothioate linkage, a phosphodiester linkage, and a mixture of phosphorothioate and phosphodiester linkages between adjacent monomers,

wherein the sdDNA molecule comprises at least one interspersed segment of ribonucleotide monomers (ISR) linked to at least one deoxyribonucleotide monomer selected from the group consisting of a deoxyribonucleotide, an analog thereof, and a modified deoxyribonucleotide,

wherein the ISR in the sdDNA molecule comprises at least one ribonucleotide monomer selected from the group consisting of a ribonucleotide, an analog thereof, and a modified ribonucleotide,

wherein the sdDNA molecule is used for modulating a target gene expression or function in a cell,

and wherein the sdDNA molecule is more potent or more efficacious at silencing the expression of the target gene than a corresponding ASO in a cell.

#	sdDNA Structures	Sequence of sdDNAs targeting APOCIII SS 5' to 3' AS 3' to 5'	SEQ ID No.:
sdDNA1	5'R*R*R*R*R*D*D*D*D*D*D*D*D*D*D*R*R*R*R*R3' 3'R*R*R*R*R*D*D*D*D*D*D*D*D*D*D*R*R*R*R*R5'	A*U*A*A*A*g*c*t *g*g*a*c*a*a*g*A*A*G*C*U U*A*U*U*U*c*g*a*c*c*t *g*t *t *c*U*U*C*G*A	2 1
sdDNA2	5'D*D*D*D*D*D*D*D*D*D*D*D*D*D*D*D*D*D*D*D3' 3'R*R*R*R*R*D*D*D*D*D*D*D*D*D*D*R*R*R*R*R5'	a *t *a *a *a *g*c*t *g*g*a*c*a*a*g*a *a *g *c *t U*A*U*U*U*c*g*a*c*c*t *g*t *t *c*U*U*C*G*A	3 1
sdDNA3	5'D D D D D D D D D D D D D D D D D D D D3' 3'R*R*R*R*R*D*D*D*D*D*D*D*D*D*D*R*R*R*R*R5'	a t a a a g c t g g a c a a g a a g c t U*A*U*U*U*c*g*a*c*c*t *g*t *t *c*U*U*C*G*A	4 1

FIG. 1A

FIG. 1B

FIG. 2A

sdDNA#	Length SS/AS	Structure and Sequence of asdDNAs_APOCIII SS 5' to 3' AS 3' to 5'	SEQ ID No.:
sdDNA_1	**8/8**	**c t g g a c a a** **G*a*c*c*t *g*t *U**	5 6
sdDNA_2	**10/10**	**g c t g g a c a a g** **C*g*a*c*c*t *g*t *t *C**	7 8
sdDNA_3	**12/12**	**a g c t g g a c a a g a** **U*C*g*a*c*c*t *g*t *t *C*U**	9 10
sdDNA_4	**14/14**	**a a g c t g g a c a a g a a** **U*U*c*g*a*c*c*t *g*t *t *c*U*U**	11 12
sdDNA_5	**16/16**	**a a a g c t g g a c a a g a a g** **U*U*U*c*g*a*c*c*t *g*t *t *c*U*U*C**	13 14
sdDNA_6	**20/20**	**a t a a a g c t g g a c a a g a a g c t** **U*A*U*U*U*c*g*a*c*c*t*g*t*t*c*U*U*C*G*A**	15 1
sdDNA_7	**24/24**	**c a a t a a a g c t g g a c a a g a a g c t g c** **G*U*U*A*U*U*t *c*g*a*c*c*t *g*t *t *c*t *U*C*G*A*C*G**	17 16
sdDNA_8	**28/28**	**c c c a a t a a a g c t g g a c a a g a a g c t g c t a** **G*G*G*U*U*A*U*t *t *c*g*a*c*c*t *g*t *t *c*t *t *C*G*A*C*G*A*U**	19 18
sdDNA_9	**32/32**	**c t c c c a a t a a a g c t g g a c a a g a a g c t g c t a t g** **G*A*G*G*G*U*U*A*t *t *t *c*g*a*c*c*t *g*t *t *c*t *t *c*G*A*C*G*A*U*A*C**	21 20
sdDNA_10	**36/36**	**g g c c t c c c a a t a a a g c t g g a c a a g a a g c t g c t a t g a** **C*C*G*G*A*G*G*G*U*U*a*t *t *t *c*g*a*c*c*t *g*t *t *c*t *t *c*g*A*C*G*A*U*A*C*U**	23 22

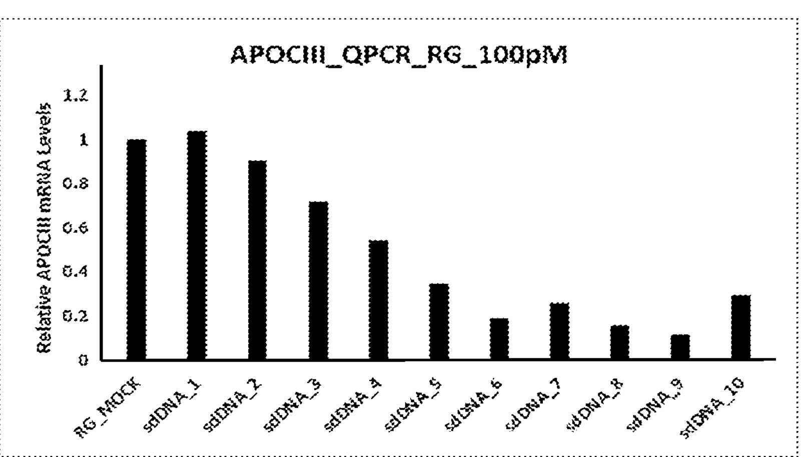

FIG. 2B

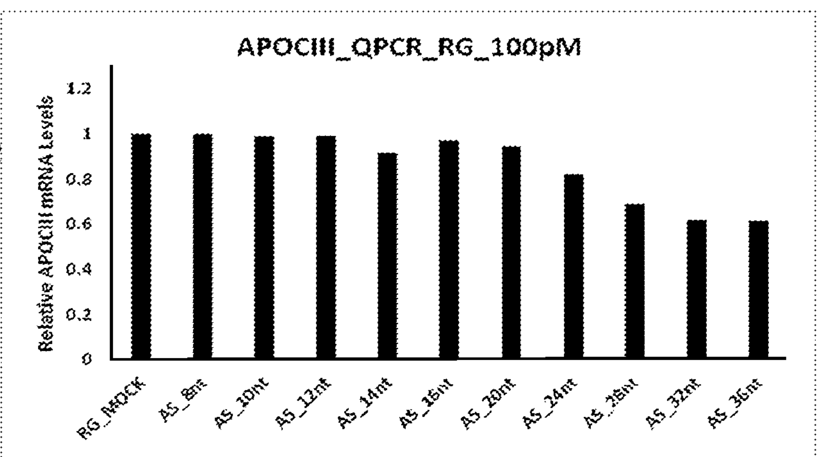

FIG. 2C

sdDNA#	Length SS/AS	Structure and Sequence of asdDNAs_APOCIII SS 5' to 3' AS 3' to 5'	SEQ ID No.:
sdDNA1	**8/8**	**c t g g a c a a** ***G****a*c*c*t*g*t****U***	5 33
sdDNA2	**10/10**	**g c t g g a c a a g** ***C****g*a*c*c*t*g*t*t****C***	7 34
SdDNA3	**14/14**	**a a g c t g g a c a a g a a** ***U****U****c*g*a*c*c*t*g*t*t*c****U*U***	11 25

FIG. 3A

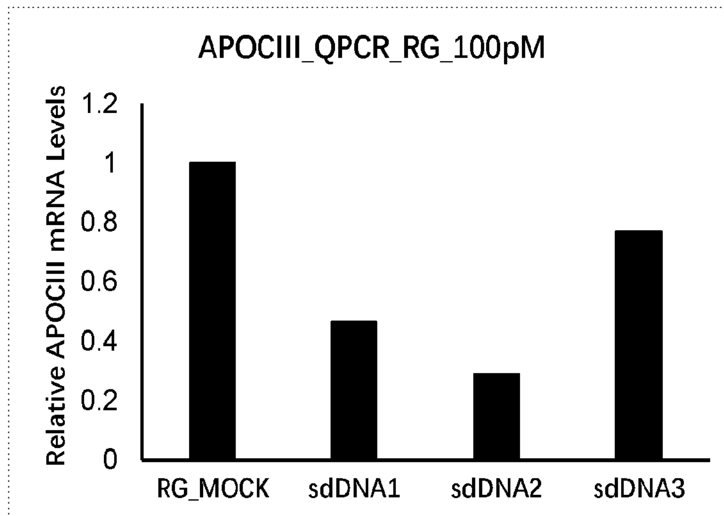

FIG. 3B

#	Structure and Sequence of asdDNAs_APOCIII SS 5' to 3' AS 3' to 5'	SEQ ID No.:
sdDNA_SS:PS	a*t *a*a*a*g*c*t*g*g*a*c*a*a*g*a*a*g*c*t U*A*U*U*U*c*g*a*c*c*t*g*t*t*c*U*U*C*G*A	3 1
SdDNA_SS:PO	a t a a a g c t g g a c a a g a a g c t U*A*U*U*U*c*g*a*c*c*t*g*t*t*c*U*U*C*G*A	4 1
SdDNA_SS:PS/PO	a*t a*a a*g c*t g*g a*c a*a g*a a*g c*t U*A*U*U*U*c*g*a*c*c*t*g*t*t*c*U*U*C*G*A	24 1

FIG.4A

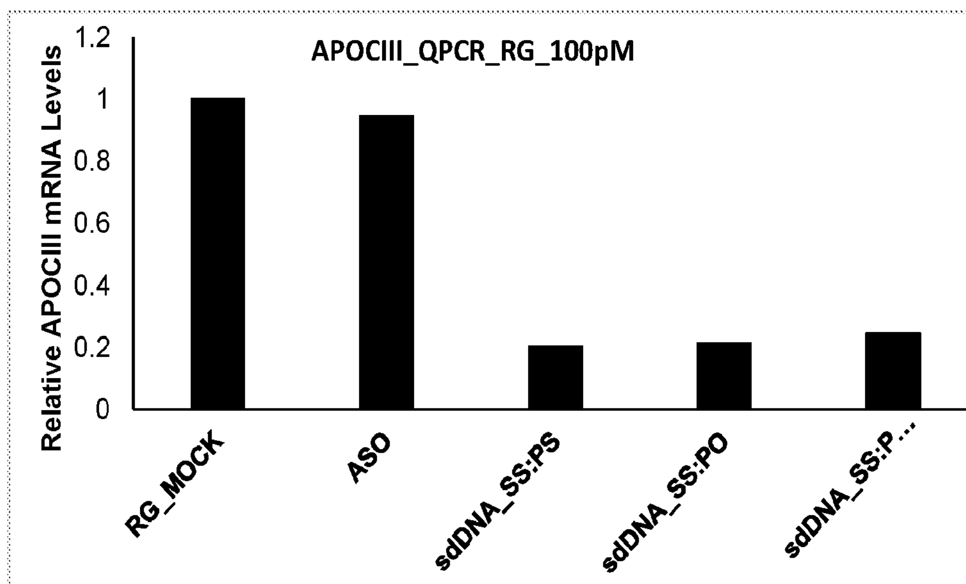

FIG. 4B

#	Structure and Sequence of asdDNAs_APOCIII SS 5' to 3' AS 3' to 5'	SEQ ID No.:
ISR0	a t a a a g c t g g a c a a g a a g c t U* A* U* U* U* c * g * a * c * c * t * g * t * t * c * U* U* C* G* A	4 1
ISR1	a t a a a g c t g g a c a a g a a g c t U* a * U* t * t * c * g * a * c * c * t * g * t * t * c * t * t * C* g * A	4 26
ISR2	a t a a a g c t g g a c a a g a a g c t U* a * U* t * U* c * g * a * c * c * t * g * t * t * c * U* t * C* g * A	4 27
ISR3	a t a a a g c t g g a c a a g a a g c t U* a * t * U* t * c * g * a * c * c * t * g * t * t * c * U* U* c * g * A	4 28
ISR4	a t a a a g c t g g a c a a g a a g c t U* a * t * U* t * c * G* a * c * c * t * g * t * U* c * U* U* c * g * A	4 29
ISR5	a t a a a g c t g g a c a a g a a g c t U* a * t * t * t * c * g * a * c * c * t * g * t * t * c * t * t * c * g * A	4 30

FIG.5A

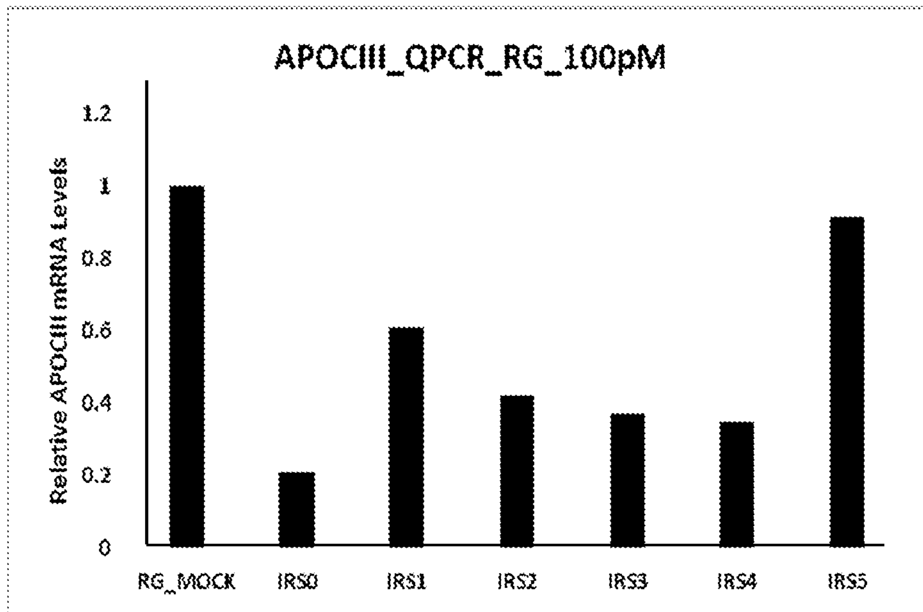

FIG. 5B

#	Structure and Sequence of asdDNAs_APOCIII SS 5' to 3' AS 3' to 5'	SEQ ID No.:
Mis0	a t a a a g c t g g a c a a g a a g c t U*A*U*U*U*c*g*a*c*c*t*g*t*t*c*U*U*C*G*A	4 1
Mis1	a t a a a g c t g g a c a a g a a g c t U*A*U*U*U*c*g*a*c*c*t*c*t*t*c*U*U*C*G*A	4 31
Mis2	a t a a a g c t g g a c a a g a a g c t U*A*U*A*U*c*g*a*c*c*t*g*t*t*g*U*U*C*G*A	4 32

FIG. 6A

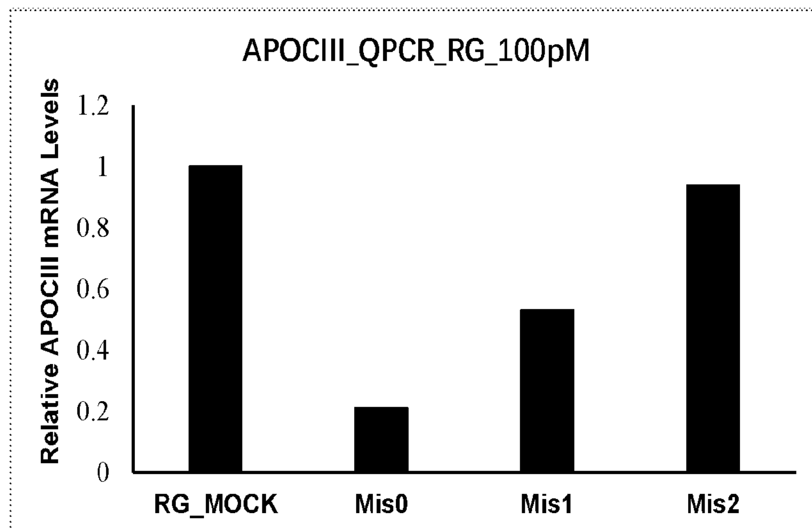

FIG. 6B

sdDNA#	Length SS/AS	Structure and Sequence of asdDNAs_APOCIII SS 5' to 3' AS 3' to 5'	SEQ ID No.:
sdDNA_1	**32/20**	**c t c c c a a t a a a g c t g g a c a a g a a g c t g c t a t g** **U*A*U*U*U*c*g*a*c*c*t *g*t *t *c*U*U*C*G*A**	21 1
sdDNA_2	**36/20**	**g g c c t c c c a a t a a a g c t g g a c a a g a a g c t g c t a t g a** **U*A*U*U*U*c*g*a*c*c*t *g*t *t *c*U*U*C*G*A**	23 1

FIG. 7A

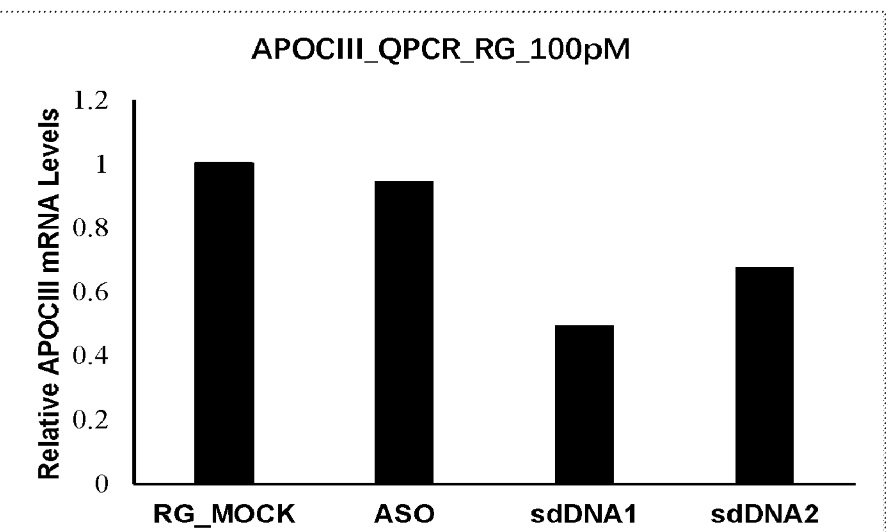

FIG. 7B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/031666

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE, EMBL, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOIZUMI MAKOTO ET AL: "Design of 2'-O-methyl RNA and DNAdouble-stranded oligonucleotides: naturally-occurring nucleotide components with strong RNAinterference gene expression inhibitory activity", NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, TAYLOR & FRANCIS, US, [Online] vol. 39, no. 1-3, 11 September 2019 (2019-09-11), pages 292-309, XP009539132, ISSN: 1525-7770, DOI: 10.1080/15257770.2019.1663384 Retrieved from the Internet: URL:https://www.tandfonline.com/doi/full/10.1080/15257770.2019.1663384> [retrieved on 2019-09-11] page 295; figures 1,2,4 -----	1-8, 10-12, 14-27, 35-39, 45-51, 54,55, 59,60

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 September 2022	30/09/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bucka, Alexander

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 3 521 430 A1 (UNIV NAT CORP TOKYO MEDICAL & DENTAL [JP]) 7 August 2019 (2019-08-07) paragraphs [0033] - [0042], [0069], [0082] - [0085]; figures 7,9,11,13,15,17	1,3, 7-20, 26-60
X	EP 3 366 773 A1 (RENA THERAPEUTICS INC [JP]) 29 August 2018 (2018-08-29) paragraphs [0054] - [0056], [0186] - [0206]; claims 1-28, 49-68; example 9	1-16, 18-21, 26-32, 35-60
A	HOSHIKO TOSHIMI ET AL: "Naked antisense double-stranded DNA oligonucleotide efficiently suppresses BCR-ABL positive leukemic cells", INVESTIGATIONAL NEW DRUGS, SPRINGER US, NEW YORK, vol. 38, no. 4, 24 October 2019 (2019-10-24), pages 1012-1019, XP037185489, ISSN: 0167-6997, DOI: 10.1007/S10637-019-00862-9 [retrieved on 2019-10-24] figure 1	1-60
A	ASAMI YUTARO ET AL: "Efficient Gene Suppression by DNA/DNA Double-Stranded Oligonucleotide In Vivo", MOLECULAR THERAPY, vol. 29, no. 2, 7 December 2020 (2020-12-07), pages 838-847, XP055834158, US ISSN: 1525-0016, DOI: 10.1016/j.ymthe.2020.10.017 figures 1-3,5	1-60
A	WO 2014/192310 A1 (NAT UNIV CORP TOKYO MED & DENT [JP]) 4 December 2014 (2014-12-04) paragraphs [0030], [0039] - [0044], [0069]; figures 9,11,13,17,19,21	1-60
A	SU SU LEI MON ET AL: "Highly efficient gene silencing in mouse brain by overhanging-duplex oligonucleotides via intraventricular route", FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 594, no. 9, 28 January 2020 (2020-01-28), pages 1413-1423, XP071257332, ISSN: 0014-5793, DOI: 10.1002/1873-3468.13742 figures 2,3	1-60

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MARKOV OLEG V. ET AL: "Transport Oligonucleotides-A Novel System for Intracellular Delivery of Antisense Therapeutics", MOLECULES, vol. 25, no. 16, 11 August 2020 (2020-08-11), page 3663, XP055961370, DE ISSN: 1433-1373, DOI: 10.3390/molecules25163663 page 14 - page 15; table 1 -----	1-60
A	LU ZHENG ET AL: "RNase HIII from Chlamydophila pneumoniae can efficiently cleave double-stranded DNA carrying a chimeric ribonucleotide in the presence of manganese : RNase HIII can substitute for RNase HII", MOLECULAR MICROBIOLOGY, vol. 83, no. 5, 14 February 2012 (2012-02-14), pages 1080-1093, XP055961353, GB ISSN: 0950-382X, DOI: 10.1111/j.1365-2958.2012.07990.x figure 1; table 1 -----	1-60
A	ROBERTS THOMAS C ET AL: "Advances in oligonucleotide drug delivery", NATURE REVIEWS DRUG DISCOVERY, NATURE PUBLISHING GROUP, GB, vol. 19, no. 10, 11 August 2020 (2020-08-11), pages 673-694, XP037256878, ISSN: 1474-1776, DOI: 10.1038/S41573-020-0075-7 [retrieved on 2020-08-11] figures 1,3 -----	1-60

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/031666

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/031666

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
EP 3521430	A1	07-08-2019	EP	3521430	A1	07-08-2019
			JP	7129702	B2	02-09-2022
			JP	WO2018062510	A1	18-07-2019
			US	2019240352	A1	08-08-2019
			WO	2018062510	A1	05-04-2018
EP 3366773	A1	29-08-2018	EP	3366773	A1	29-08-2018
			JP	WO2017068790	A1	09-08-2018
			US	2018223280	A1	09-08-2018
			WO	2017068790	A1	27-04-2017
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			EP	3004347	A1	13-04-2016
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			JP	6472087	B2	27-02-2019
			JP	2016524588	A	18-08-2016
			US	2016145614	A1	26-05-2016
			WO	2014192310	A1	04-12-2014