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(54) **Title**: MODULATORS OF KRAS EXPRESSION

(57) **Abstract**: The present embodiments provide methods, compounds, and compositions for inhibiting KRAS expression, which can be useful for treating, preventing, or ameliorating a disease associated with KRAS.

MODULATORS OF KRAS EXPRESSION

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0276WOSEQ_ST25.txt created August 30, 2016, which is 567 kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field

The present embodiments provide methods, compounds, and compositions for inhibiting KRAS expression, which can be useful for treating, preventing, or ameliorating a disease associated with KRAS.

Background

Kirsten Rat Sarcoma Viral Oncogene Homologue (KRAS) is one of three RAS protein family members (N, H and K-RAS) that are small membrane bound intracellular GTPase proteins. KRAS cycles between an inactive guanosine diphosphate (GDP)-bound state and an active guanosine triphosphate (GTP)-bound state. The process of exchanging the bound nucleotide is facilitated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). GEFs promote release of GDP from KRAS in exchange for GTP, resulting in active GTP-bound KRAS. GAPs promote hydrolysis of GTP to GDP, resulting in inactive GDP-bound KRAS. Active GTP-bound KRAS interacts with numerous effector proteins to stimulate signaling pathways regulating various cellular processes including proliferation and survival. Activating mutations render KRAS resistant to GAP-catalyzed hydrolysis of GTP and therefore lock the protein in an activated state.

KRAS is the most commonly mutated oncogene in human cancer. Approximately 30% of all human cancers have activating KRAS mutations with the highest incidence in colon, lung and pancreatic tumors, where KRAS mutation is also associated with poor prognosis.

Summary

Despite the prevalent role of KRAS in several types of cancer, KRAS is considered an "undruggable" target and no inhibitors directly targeting KRAS have yet entered clinical development.

The present embodiments provided herein are directed to potent and tolerable compounds and compositions for inhibiting KRAS expression, which can be useful for treating, preventing, ameliorating, or slowing progression of cancer.

Detailed Description

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including" as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, treatises, and GenBank and NCBI reference sequence records are hereby expressly incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

It is understood that the sequence set forth in each SEQ ID NO in the examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Compounds described by ISIS number (ISIS #) indicate a combination of nucleobase sequence, chemical modification, and motif.

Unless otherwise indicated, the following terms have the following meanings:

"2'-deoxynucleoside" means a nucleoside comprising 2'-H(H) furanosyl sugar moiety, as found in naturally occurring deoxyribonucleic acids (DNA). In certain embodiments, a 2'-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (*e.g.*, uracil).

"2'-O-methoxyethyl" (also 2'-MOE and 2'-O$(CH_2)_2$-OCH_3) refers to an O-methoxy-ethyl modification at the 2' position of a sugar ring, e.g. a furanose ring. A 2'-O-methoxyethyl modified sugar is a modified sugar.

"2'-MOE nucleoside" (also 2'-O-methoxyethyl nucleoside) means a nucleoside comprising a 2'-MOE modified sugar moiety.

"2'-substituted nucleoside" or "2-modified nucleoside" means a nucleoside comprising a 2'-substituted or 2'-modified sugar moiety. As used herein, "2'-substituted" or "2-modified" in reference to a sugar moiety means a sugar moiety comprising a 2'-substituent group other than H or OH. "3' target site" refers to the nucleotide of a target nucleic acid which is complementary to the 3'-most nucleotide of a particular compound.

"5' target site" refers to the nucleotide of a target nucleic acid which is complementary to the 5'-most nucleotide of a particular compound.

"5-methylcytosine" means a cytosine with a methyl group attached to the 5 position.

"About" means within ±10% of a value. For example, if it is stated, "the compounds affected at least about 70% inhibition of KRAS", it is implied that KRAS levels are inhibited within a range of 60% and 80%.

"Administration" or "administering" refers to routes of introducing a compound or composition provided herein to an individual to perform its intended function. An example of a route of administration that can be used includes, but is not limited to parenteral administration, such as subcutaneous, intravenous, or intramuscular injection or infusion.

"Administered concomitantly" or "co-administration" means administration of two or more compounds in any manner in which the pharmacological effects of both are manifest in the patient. Concomitant administration does not require that both compounds be administered in a single pharmaceutical composition, in the same dosage form, by the same route of administration, or at the same time. The effects of both compounds need not manifest themselves at the same time. The effects need only be overlapping for a period of time and need not be coextensive. Concomitant administration or co-administration encompasses administration in parallel or sequentially.

"Amelioration" refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. In certain embodiments, amelioration includes a delay or slowing in the progression of one or more indicators of a condition or disease. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

"Animal" refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

"Antisense activity" means any detectable or measurable activity attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity is a

decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid compared to target nucleic acid levels or target protein levels in the absence of the antisense compound to the target.

"Antisense compound" means a compound comprising an antisense oligonucleotide and optionally one or more additional features, such as a conjugate group or terminal group. Examples of antisense compounds include single-stranded and double-stranded compounds, such as, antisense oligonucleotides, ribozymes, siRNAs, shRNAs, ssRNAs, and occupancy-based compounds.

"Antisense inhibition" means reduction of target nucleic acid levels in the presence of an antisense compound complementary to a target nucleic acid compared to target nucleic acid levels in the absence of the antisense compound.

"Antisense mechanisms" are all those mechanisms involving hybridization of a compound with target nucleic acid, wherein the outcome or effect of the hybridization is either target degradation or target occupancy with concomitant stalling of the cellular machinery involving, for example, transcription or splicing.

"Antisense oligonucleotide" means an oligonucleotide having a nucleobase sequence that is complementary to a target nucleic acid or region or segment thereof. In certain embodiments, an antisense oligonucleotide is specifically hybridizable to a target nucleic acid or region or segment thereof.

"Bicyclic nucleoside" or "BNA" means a nucleoside comprising a bicyclic sugar moiety. As used herein, "bicyclic sugar" or "bicyclic sugar moiety" means a modified sugar moiety comprising two rings, wherein the second ring is formed via a bridge connecting two of the atoms in the first ring thereby forming a bicyclic structure. In certain embodiments, the first ring of the bicyclic sugar moiety is a furanosyl moiety. In certain embodiments, the bicyclic sugar moiety does not comprise a furanosyl moiety.

"Branching group" means a group of atoms having at least 3 positions that are capable of forming covalent linkages to at least 3 groups. In certain embodiments, a branching group provides a plurality of reactive sites for connecting tethered ligands to an oligonucleotide via a conjugate linker and/or a cleavable moiety.

"Cell-targeting moiety" means a conjugate group or portion of a conjugate group that is capable of binding to a particular cell type or particular cell types.

"cEt" or "constrained ethyl" means a bicyclic furanosyl sugar moiety comprising a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-$CH(CH_3)$-O-2'.

"Chemical modification" in a compound describes the substitutions or changes through chemical reaction, of any of the units in the compound. "Modified nucleoside" means a nucleoside having, independently, a modified sugar moiety and/or modified nucleobase. "Modified oligonucleotide" means an oligonucleotide comprising at least one modified internucleoside linkage, a modified sugar, and/or a modified nucleobase.

"Chemically distinct region" refers to a region of an antisense compound that is in some way chemically different than another region of the same antisense compound. For example, a region having 2'-O-methoxyethyl nucleotides is chemically distinct from a region having nucleotides without 2'-O-methoxyethyl modifications.

"Chimeric antisense compounds" means antisense compounds that have at least 2 chemically distinct regions, each position having a plurality of subunits.

"Cleavable bond" means any chemical bond capable of being split. In certain embodiments, a cleavable bond is selected from among: an amide, a polyamide, an ester, an ether, one or both esters of a phosphodiester, a phosphate ester, a carbamate, a di-sulfide, or a peptide.

"Cleavable moiety" means a bond or group of atoms that is cleaved under physiological conditions, for example, inside a cell, an animal, or a human.

"Constrained ethyl nucleoside" (also cEt nucleoside) means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-$CH(CH_3)$-O-2' bridge.

"Complementary" in reference to an oligonucleotide means the nucleobase sequence of such oligonucleotide or one or more regions thereof matches the nucleobase sequence of another oligonucleotide or nucleic acid or one or more regions thereof when the two nucleobase sequences are aligned in opposing directions. Nucleobase matches or complementary nucleobases, as described herein, are limited to adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), and 5-methyl cytosine (^{m}C) and guanine (G) unless otherwise specified. Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside and may include one or more nucleobase mismatches. By contrast, "fully complementary" or "100% complementary" in reference to oligonucleotides means that such oligonucleotides have nucleobase matches at each nucleoside without any nucleobase mismatches.

"Conjugate group" means a group of atoms that is attached to a parent compound, e.g., an oligonucleotide.

"Conjugate linker" means a group of atoms that connects a conjugate group to a parent compound, e.g., an oligonucleotide.

"Contiguous" in the context of an oligonucleotide refers to nucleosides, nucleobases, sugar moieties, or internucleoside linkages that are immediately adjacent to each other. For example, "contiguous nucleobases" means nucleobases that are immediately adjacent to each other.

"Designing" or "Designed to" refer to the process of designing an oligomeric compound that specifically hybridizes with a selected nucleic acid molecule.

"Differently modified" mean chemical modifications or chemical substituents that are different from one another, including absence of modifications. Thus, for example, a MOE nucleoside and an unmodified DNA nucleoside are "differently modified," even though the DNA nucleoside is unmodified. Likewise, DNA and RNA are "differently modified," even though both are naturally-occurring unmodified nucleosides. Nucleosides that are the same but for comprising different nucleobases are not differently modified. For example, a nucleoside comprising a 2'-OMe modified sugar and an unmodified adenine nucleobase and a nucleoside comprising a 2'-OMe modified sugar and an unmodified thymine nucleobase are not differently modified.

"Dose" means a specified quantity of a pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose may be administered in two or more boluses, tablets, or injections. For example, in certain embodiments, where subcutaneous administration is desired, the desired dose may require a volume not easily accommodated by a single injection. In such embodiments, two or more injections may be used to achieve the desired dose. In certain embodiments, a dose may be administered in two or more injections to minimize injection site reaction in an individual. In other embodiments, the pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses may be stated as the amount of pharmaceutical agent per hour, day, week or month.

"Dosing regimen" is a combination of doses designed to achieve one or more desired effects.

"Double-stranded antisense compound" means an antisense compound comprising two oligomeric compounds that are complementary to each other and form a duplex, and wherein one of the two said oligomeric compounds comprises an antisense oligonucleotide.

"Effective amount" means the amount of compound sufficient to effectuate a desired physiological outcome in an individual in need of the agent. The effective amount may vary among individuals depending on the health and physical condition of the individual to be treated, the taxonomic

group of the individuals to be treated, the formulation of the composition, assessment of the individual's medical condition, and other relevant factors.

"Efficacy" means the ability to produce a desired effect.

"Expression" includes all the functions by which a gene's coded information is converted into structures present and operating in a cell. Such structures include, but are not limited to the products of transcription and translation.

"Fully modified" in reference to an oligonucleotide means a modified oligonucleotide in which each nucleoside is modified. "Uniformly modified" in reference to an oligonucleotide means a fully modified oligonucleotide in which at least one modification of each nucleoside is the same. For example, the nucleosides of a uniformly modified oligonucleotide can each have a 2'-MOE modification but different nucleobase modifications, and the internucleoside linkages may be different.

"Gapmer" means a chimeric antisense compound in which an internal region having a plurality of nucleosides that support RNase H cleavage is positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as the "gap" and the external regions may be referred to as the "wings."

"Hybridization" means the annealing of complementary oligonucleotides and/or nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense compound and a nucleic acid target. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense oligonucleotide and a nucleic acid target.

"Immediately adjacent" means there are no intervening elements between the immediately adjacent elements of the same kind (e.g. no intervening nucleobases between the immediately adjacent nucleobases).

"Individual" means a human or non-human animal selected for treatment or therapy.

"Inhibiting the expression or activity" refers to a reduction or blockade of the expression or activity relative to the expression of activity in an untreated or control sample and does not necessarily indicate a total elimination of expression or activity.

"Internucleoside linkage" means a group or bond that forms a covalent linkage between adjacent nucleosides in an oligonucleotide. As used herein "modified internucleoside linkage" means any internucleoside linkage other than a naturally occurring, phosphate internucleoside linkage.

"KRAS" means any nucleic acid or protein of KRAS. "KRAS nucleic acid" means any nucleic acid encoding KRAS. For example, in certain embodiments, a KRAS nucleic acid includes a DNA sequence encoding KRAS, an RNA sequence transcribed from DNA encoding KRAS (including genomic DNA comprising introns and exons), including a non-protein encoding (*i.e.* non-coding) RNA sequence, and an mRNA sequence encoding KRAS. "KRAS mRNA" means an mRNA encoding a KRAS protein. "KRAS," "K-ras," "kras," "k-ras," "Ki-ras," and "ki-ras" can be used interchangably without capitalizaiton or italicization of their spelling referring to nucleic acid or protein in a mutually exclusive manner unless specifically indicated to the contrary.

"KRAS specific inhibitor" refers to any agent capable of specifically inhibiting KRAS RNA and/or KRAS protein expression or activity at the molecular level. For example, KRAS specific inhibitors include nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of KRAS RNA and/or KRAS protein.

"Lengthened antisense oligonucleotides" are those that have one or more additional nucleosides relative to an antisense oligonucleotide disclosed herein, e.g. a parent oligonucleotide.

"Linearly modified sugar" or "linearly modified sugar moiety" means a modified sugar moiety that comprises an acyclic or non-bridging modification. Such linear modifications are distinct from bicyclic sugar modifications.

"Linked nucleosides" means adjacent nucleosides linked together by an internucleoside linkage.

"Mismatch" or "non-complementary" means a nucleobase of a first oligonucleotide that is not complementary to the corresponding nucleobase of a second oligonucleotide or target nucleic acid when the first and second oligonucleotides are aligned. For example, nucleobases including but not limited to a universal nucleobase, inosine, and hypoxanthine, are capable of hybridizing with at least one nucleobase but are still mismatched or non-complementary with respect to nucleobase to which it hybridized. As another example, a nucleobase of a first oligonucleotide that is not capable of hybridizing to the corresponding nucleobase of a second oligonucleotide or target nucleic acid when the first and second oligonucleotides are aligned is a mismatch or non-complementary nucleobase.

"Modulating" refers to changing or adjusting a feature in a cell, tissue, organ or organism. For example, modulating KRAS RNA can mean to increase or decrease the level of KRAS RNA and/or KRAS protein in a cell, tissue, organ or organism. A "modulator" effects the change in the cell, tissue, organ or organism. For example, a KRAS antisense compound can be a modulator that decreases the amount of KRAS RNA and/or KRAS protein in a cell, tissue, organ or organism.

"Monomer" refers to a single unit of an oligomer. Monomers include, but are not limited to, nucleosides and nucleotides.

"Motif" means the pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages, in an oligonucleotide.

"Natural" or "naturally occurring" means found in nature.

"Nucleic acid" refers to molecules composed of monomeric nucleotides. A nucleic acid includes, but is not limited to, ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, and double-stranded nucleic acids.

"Nucleobase" means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

"Nucleobase sequence" means the order of contiguous nucleobases independent of any sugar, linkage, and/or nucleobase modification.

"Nucleoside" means a compound comprising a nucleobase and a sugar moiety. The nucleobase and sugar moiety are each, independently, unmodified or modified.

"Oligomeric compound" means a compound comprising a single oligonucleotide and optionally one or more additional features, such as a conjugate group or terminal group.

"Oligonucleotide" means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another.

"Parent oligonucleotide" means an oligonucleotide whose sequence is used as the basis of design for more oligonucleotides of similar sequence but with different lengths, motifs, and/or chemistries. The newly designed oligonucleotides may have the same or overlapping sequence as the parent oligonucleotide.

"Parenteral administration" means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intrathecal or intracerebroventricular administration.

"Pharmaceutically acceptable carrier or diluent" means any substance suitable for use in administering to an animal. For example, a pharmaceutically acceptable carrier can be a sterile aqueous solution, such as PBS or water-for-injection. As used herein "pharmaceutically acceptable salts" means physiologically and pharmaceutically acceptable salts of compounds, such as oligomeric compounds, i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

"Pharmaceutical agent" means a compound that provides a therapeutic benefit when administered to an individual.

"Pharmaceutical composition" means a mixture of substances suitable for administering to an individual. For example, a pharmaceutical composition may comprise one or more compounds or salt thereof and a sterile aqueous solution.

"Phosphorothioate linkage" means a modified internucleoside linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom.

"Phosphorus moiety" means a group of atoms comprising a phosphorus atom. In certain embodiments, a phosphorus moiety comprises a mono-, di-, or tri-phosphate, or phosphorothioate.

"Portion" means a defined number of contiguous (i.e., linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an oligomeric compound.

"Prodrug" means a form of a compound which, when administered to an individual, is metabolized to another form. In certain embodiments, the metabolized form is the active, or more active, form of the compound (e.g., drug).

"Prophylactically effective amount" refers to an amount of a pharmaceutical agent that provides a prophylactic or preventative benefit to an animal.

"Region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic.

"RNAi compound" means a compound that acts, at least in part, through RISC or Ago2, but not through RNase H, to modulate a target nucleic acid and/or protein encoded by a target nucleic acid. RNAi compounds include, but are not limited to double-stranded siRNA, single-stranded RNA (ssRNA), and microRNA, including microRNA mimics.

"Segments" are defined as smaller or sub-portions of regions within a nucleic acid.

"Side effects" means physiological disease and/or conditions attributable to a treatment other than the desired effects. In certain embodiments, side effects include injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, myopathies, and malaise. For example, increased aminotransferase levels in serum may

indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

"Single-stranded" in reference to a compound means the compound has only one oligonucleotide. "Self-complementary" means an oligonucleotide that at least partially hybridizes to itself. A compound consisting of one oligonucleotide, wherein the oligonucleotide of the compound is self-complementary, is a single-stranded compound. A single-stranded antisense compound may be capable of binding to a complementary compound to form a duplex.

"Sites," as used herein, are defined as unique nucleobase positions within a target nucleic acid.

"Specifically hybridizable" refers to an antisense compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids. In certain embodiments, specific hybridization occurs under physiological conditions.

"Specifically inhibit" a target nucleic acid means to reduce or block expression of the target nucleic acid while exhibiting fewer, minimal, or no effects on non-target nucleic acids reduction and does not necessarily indicate a total elimination of the target nucleic acid's expression.

"Sugar moiety" means a group of atoms that can link a nucleobase to another group, such as an internucleoside linkage, conjugate group, or terminal group. In certain embodiments, a sugar moiety is attached to a nucleobase to form a nucleoside. As used herein, "unmodified sugar moiety" or "unmodified sugar" means a 2'-OH(H) furanosyl moiety, as found in RNA, or a 2'-H(H) moiety, as found in DNA. Unmodified sugar moieties have one hydrogen at each of the 1', 3', and 4' positions, an oxygen at the 3' position, and two hydrogens at the 5' position. As used herein, "modified sugar moiety" or "modified sugar" means a modified furanosyl moiety comprising a non-hydrogen substituent in place of at least one hydrogen of an unmodified sugar moiety, or a sugar surrogate. In certain embodiments, a modified sugar moiety is a 2'-substituted sugar moiety. Such modified sugar moieties include bicyclic sugars and linearly modified sugars.

"Sugar surrogate" means a modified sugar moiety having other than a furanosyl moiety that can link a nucleobase to another group, such as an internucleoside linkage, conjugate group, or terminal group. Modified nucleosides comprising sugar surrogates can be incorporated into one or more positions within an oligonucleotide. In certain embodiments, such oligonucleotides are capable of hybridizing to complementary oligomeric compounds or nucleic acids.

"Target gene" refers to a gene encoding a target.

"Target nucleic acid," "target RNA," "target RNA transcript" and "nucleic acid target" all mean a nucleic acid capable of being targeted by antisense compounds.

"Target region" means a portion of a target nucleic acid to which one or more antisense compounds is targeted.

"Target segment" means the sequence of nucleotides of a target nucleic acid to which an antisense compound is targeted. "5' target site" refers to the 5'-most nucleotide of a target segment. "3' target site" refers to the 3'-most nucleotide of a target segment.

"Terminal group" means a chemical group or group of atoms that is covalently linked to a terminus of an oligonucleotide.

"Therapeutically effective amount" means an amount of a compound, pharmaceutical agent, or composition that provides a therapeutic benefit to an individual.

"Treat" refers to administering a compound or pharmaceutical composition to an animal in order to effect an alteration or improvement of a disease, disorder, or condition in the animal.

Certain Embodiments

Certain embodiments provide methods, compounds and compositions for inhibiting KRAS expression.

Certain embodiments provide compounds targeted to a KRAS nucleic acid. In certain embodiments, the KRAS nucleic acid has the sequence set forth in GENBANK Accession No. NM_004985.4 (herein incorporated by reference, disclosed herein as SEQ ID NO: 1); GENBANK Accession No. NT_009714.17_TRUNC_18116000_18166000_COMP (herein incorporated by reference, disclosed herein as SEQ ID NO: 2), or GENBANK Accession No. NM_033360.3 (herein incorporated by reference, disclosed herein as SEQ ID NO: 3). In certain embodiments, the compound is a single-stranded oligonucleotide. In certain embodiments, the compound is double-stranded.

Certain embodiments provide a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the compound is a single-stranded oligonucleotide. In certain embodiments, the compound is double-stranded. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides.

Certain embodiments provide a compound comprising a modified oligonucleotide consisting of 9 to 80 linked nucleosides and having a nucleobase sequence comprising at least 9 contiguous nucleobases

of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the compound is a single-stranded oligonucleotide. In certain embodiments, the compound is double-stranded. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides.

Certain embodiments provide a compound comprising a modified oligonucleotide consisting of 10 to 80 linked nucleosides and having a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the compound is a single-stranded oligonucleotide. In certain embodiments, the compound is double-stranded. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides.

Certain embodiments provide a compound comprising a modified oligonucleotide consisting of 11 to 80 linked nucleosides and having a nucleobase sequence comprising at least 11 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the compound is a single-stranded oligonucleotide. In certain embodiments, the compound is double-stranded. In certain embodiments, the modified oligonucleotide consists of 11 to 30 linked nucleosides.

Certain embodiments provide a compound comprising a modified oligonucleotide consisting of 12 to 80 linked nucleosides and having a nucleobase sequence comprising at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the compound is a single-stranded oligonucleotide. In certain embodiments, the compound is double-stranded. In certain embodiments, the modified oligonucleotide consists of 12 to 30 linked nucleosides.

Certain embodiments provide a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the compound is a single-stranded oligonucleotide. In certain embodiments, the compound is double-stranded. In certain embodiments, the modified oligonucleotide consists of 16 to 30 linked nucleosides.

Certain embodiments provide a compound comprising a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the compound is a single-stranded oligonucleotide. In certain embodiments, the compound is double-stranded.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 8 to 80 linked nucleosides having at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion complementary to an equal length portion within nucleotides 463-478, 877-892, 1129-1144, 1313-1328, 1447-1462, 1686-1701, 1690-1705, 1778-1793, 1915-1930, 1919-1934, 1920-1935, 2114-2129, 2115-2130, 2461-2476, 2462-2477, 2463-2478, 4035-4050 of SEQ ID NO: 1. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleotides 463-478, 877-892, 1129-1144, 1313-1328, 1447-1462, 1686-1701, 1690-1705, 1778-1793, 1915-1930, 1919-1934, 1920-1935, 2114-2129, 2115-2130, 2461-2476, 2462-2477, 2463-2478, 4035-4050 of SEQ ID NO: 1. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 8 to 80 linked nucleosides having a nucleobase sequence comprising at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion of any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 8 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the modified oligonucleotide consists of 10 to 30 linked nucleosides.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide having a nucleobase sequence consisting of any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158.

In certain embodiments, a compound comprises or consists of ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, or 740233. Out of over 2,000 antisense oligonucleotides that were screened as described in the Examples section below, ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, and 740233 emerged as the top lead compounds in terms of potency and/or tolerability.

In certain embodiments, any of the foregoing oligonucleotides comprises at least one modified internucleoside linkage, at least one modified sugar, and/or at least one modified nucleobase.

In certain embodiments, any of the foregoing oligonucleotides comprises at least one modified sugar. In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl group. In certain embodiments, at least one modified sugar is a bicyclic sugar, such as a 4'-$CH(CH_3)$-O-2' group, a 4'-CH_2-O-2' group, or a 4'-$(CH_2)_2$-O-2'group.

In certain embodiments, the modified oligonucleotide comprises at least one modified internucleoside linkage, such as a phosphorothioate internucleoside linkage.

In certain embodiments, any of the foregoing oligonucleotides comprises at least one modified nucleobase, such as 5-methylcytosine.

In certain embodiments, any of the foregoing oligonucleotides comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar. In certain embodiments, the oligonucleotide consists of 16 to 80 linked nucleosides having a nucleobase sequence comprising the sequence recited in any one of SEQ ID NOs: 13-2190. In certain embodiments, the oligonucleotide consists of 16 to 80 linked nucleosides having a nucleobase sequence comprising the sequence recited in any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the oligonucleotide consists of 16 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the oligonucleotide consists of 16 linked nucleosides having a nucleobase sequence consisting of the sequence recited in any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16-80 linked nucleobases having a nucleobase sequence comprising or consisting of the sequence recited in any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, and 854, wherein the modified oligonucleotide comprises

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a constrained ethyl (cEt) nucleoside; wherein each internucleoside linkage is a phosphorothioate linkage and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16-80 linked nucleobases having a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 2130, wherein the modified oligonucleotide comprises

a gap segment consisting of nine linked deoxynucleosides;

a 5' wing segment consisting of one linked nucleoside; and

a 3' wing segment consisting of six linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, and 2'-O-methoxyethyl nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16-80 linked nucleobases having a nucleobase sequence comprising or consisting of the sequence recited in any one of SEQ ID NOs: 804, 1028, and 2136, wherein the modified oligonucleotide comprises

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, and a 2'-O-methoxyethyl nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16-80 linked nucleobases having a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 2142, wherein the modified oligonucleotide comprises

a gap segment consisting of eight linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of six linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16-80 linked nucleobases having a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 2154, wherein the modified oligonucleotide comprises

a gap segment consisting of nine linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In certain embodiments, a compound comprises or consists of a modified oligonucleotide consisting of 16-80 linked nucleobases having a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 2158, wherein the modified oligonucleotide comprises

a gap segment consisting of eight linked deoxynucleosides;

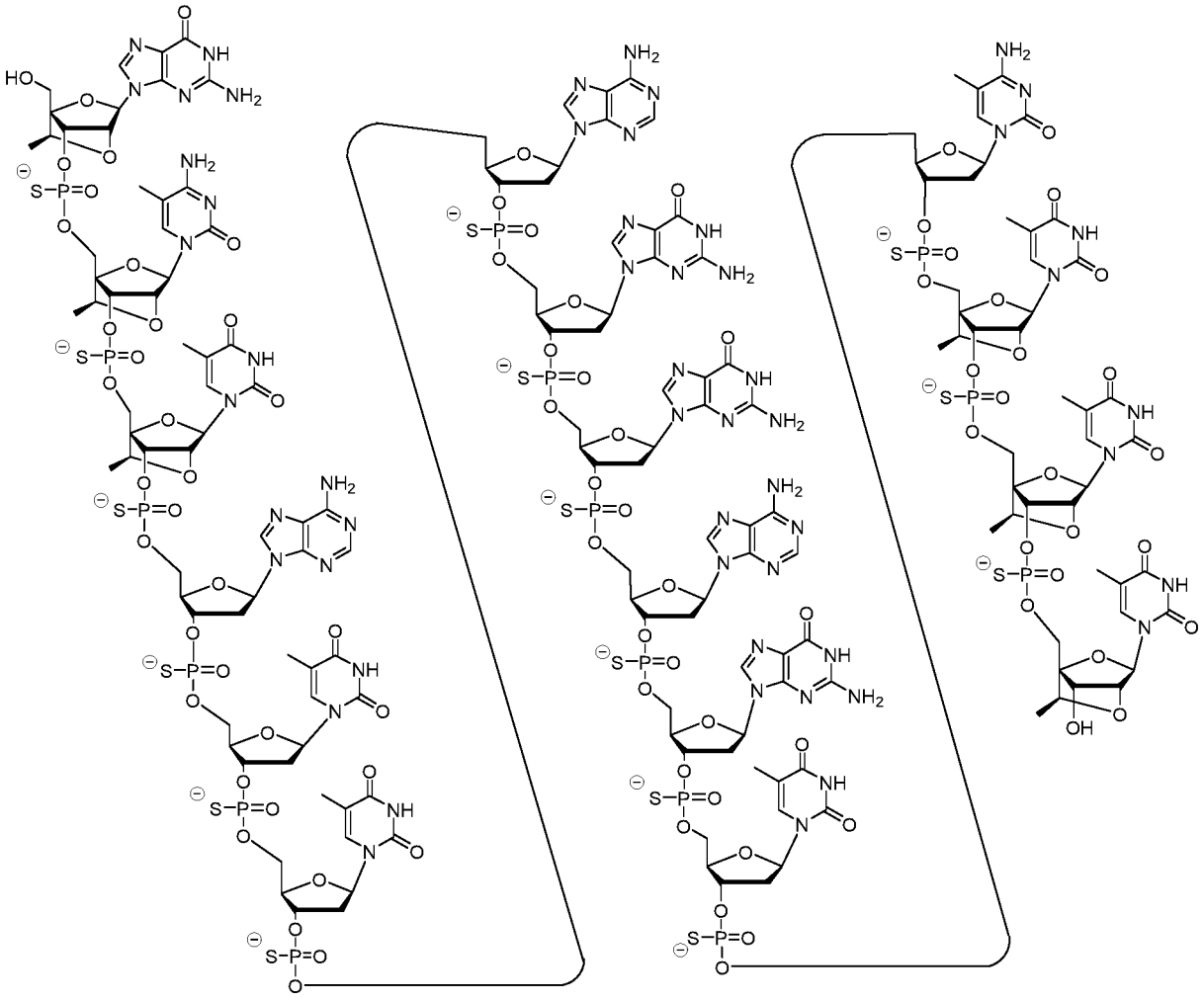
a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

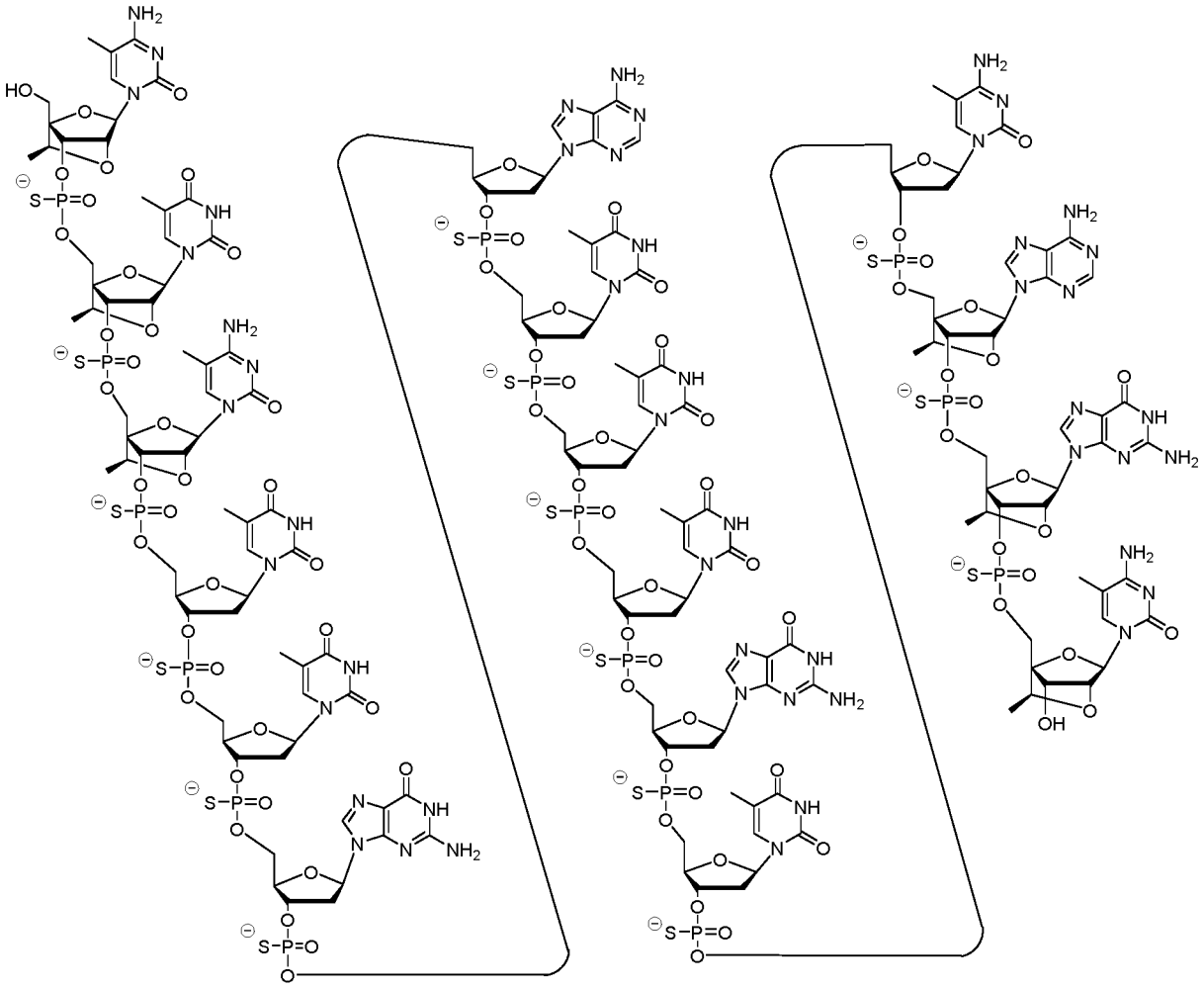
wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside, a cEt nucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a deoxynucleoside, a cEt nucleoside, a deoxynucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

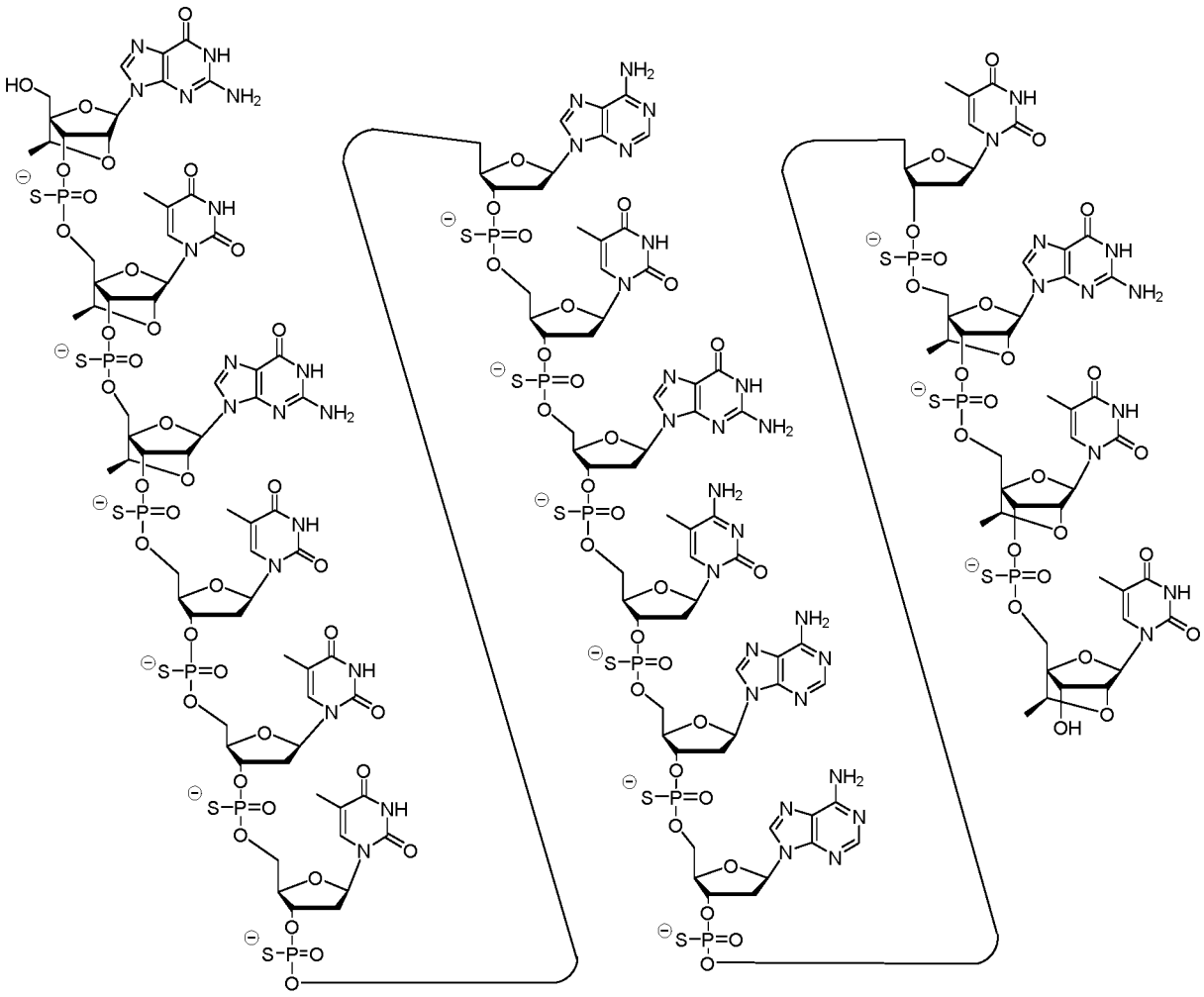
In certain embodiments, a compound comprises or consists of ISIS 651987, or a salt thereof, which has the following chemical structure:



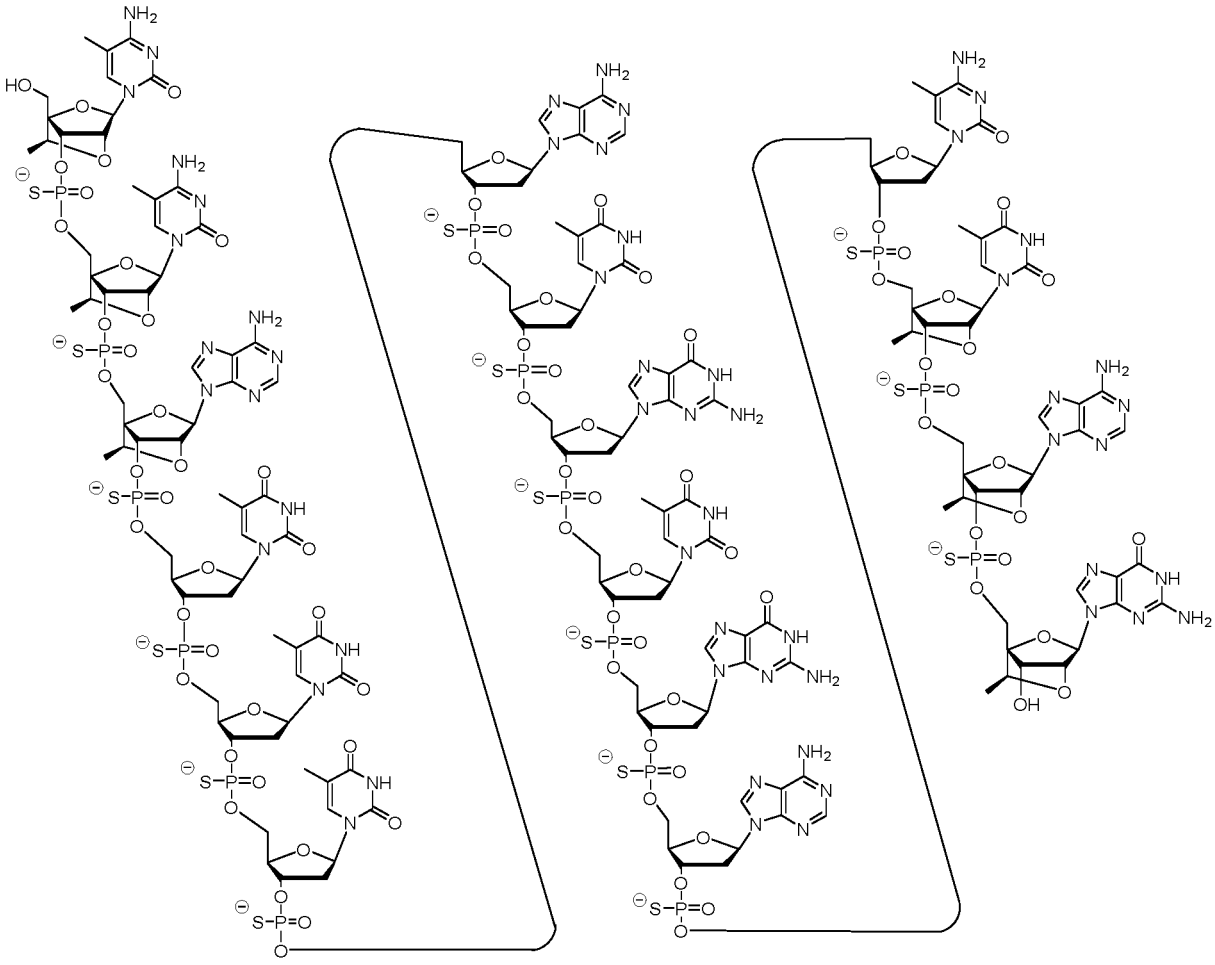
In certain embodiments, a compound comprises or consists of ISIS 696018, or a salt thereof, which has the following chemical structure:



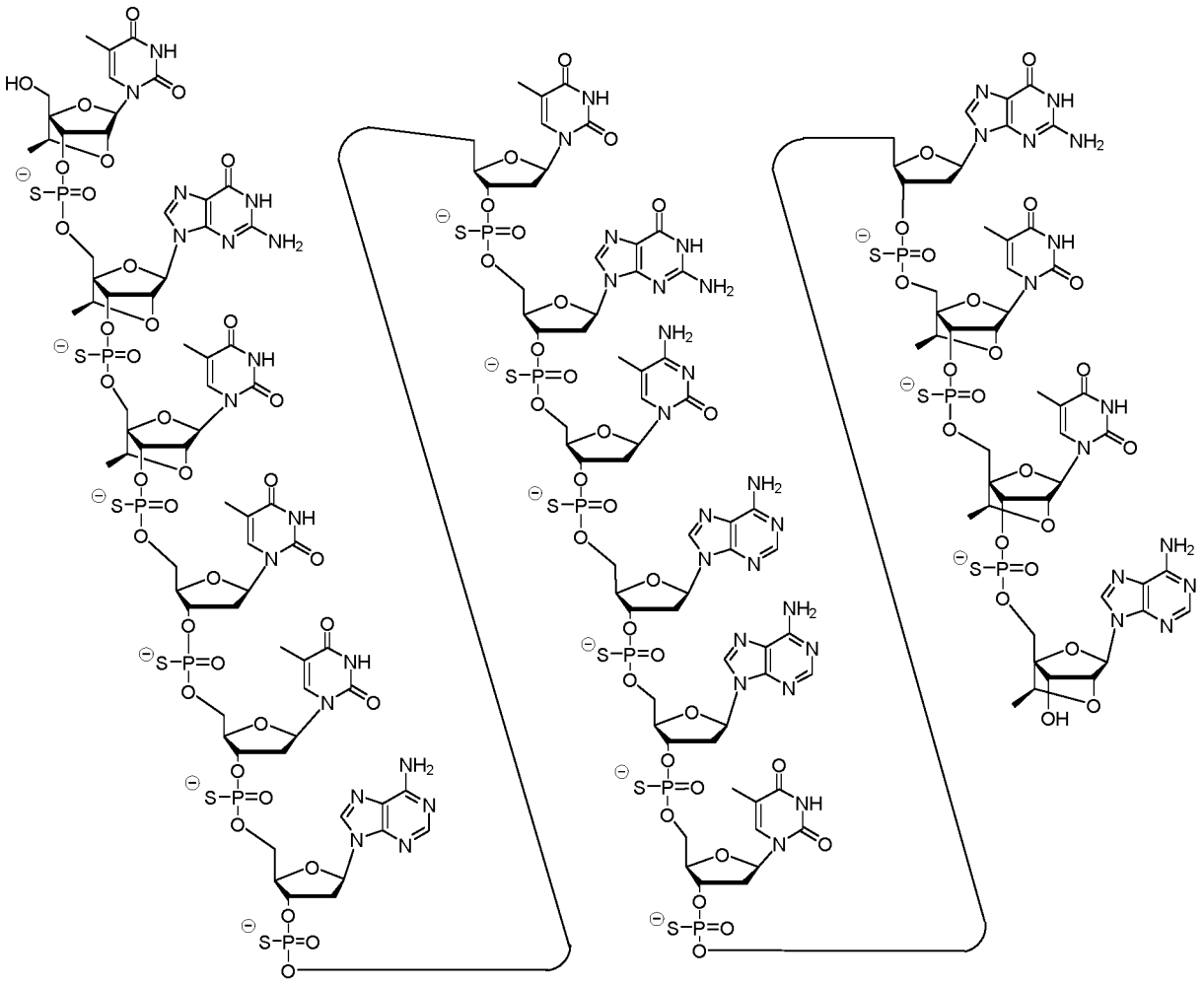
In certain embodiments, a compound comprises or consists of ISIS 696044, or a salt thereof, which has the following chemical structure:



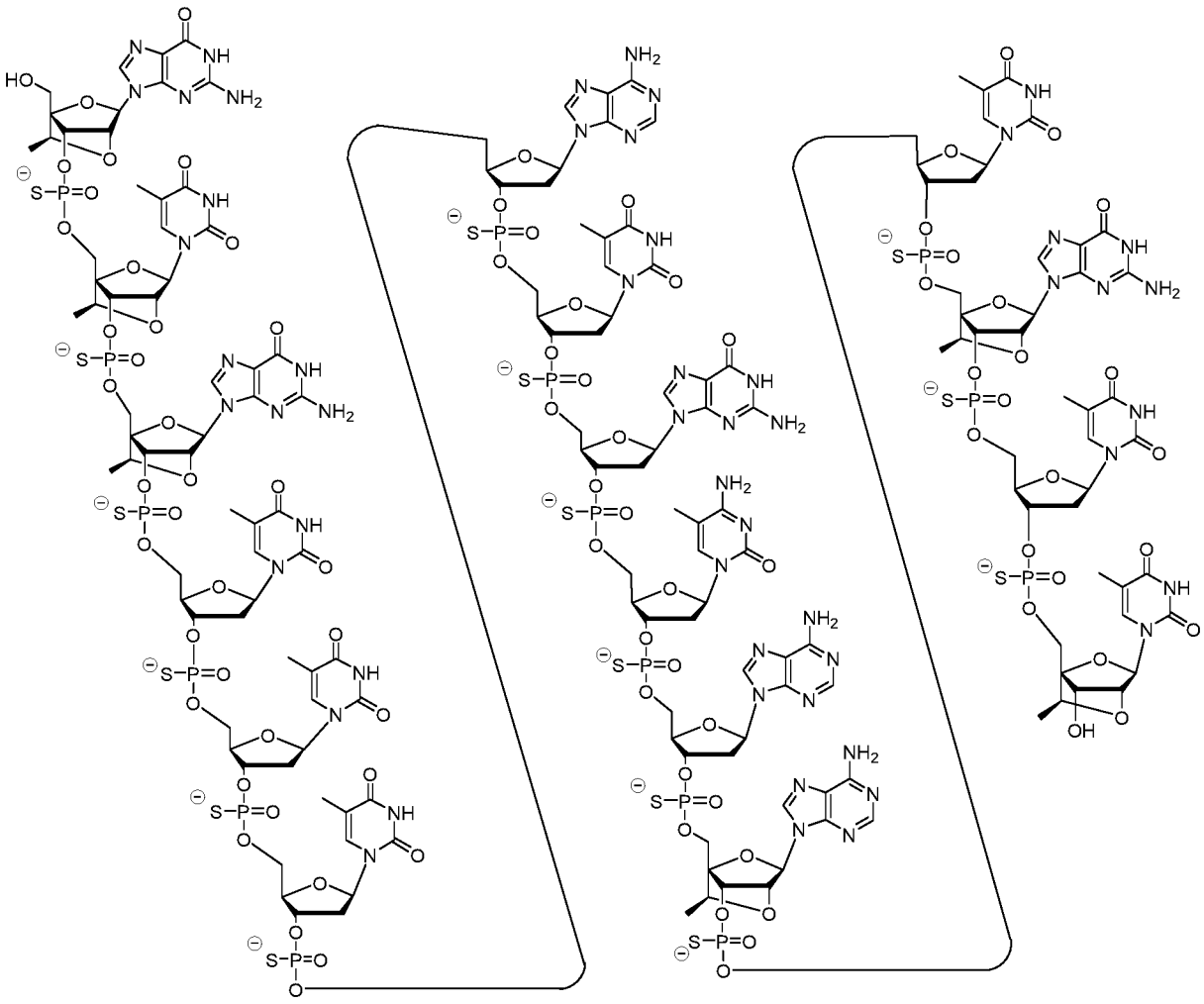
In certain embodiments, a compound comprises or consists of ISIS 716600, or a salt thereof, which has the following chemical structure:



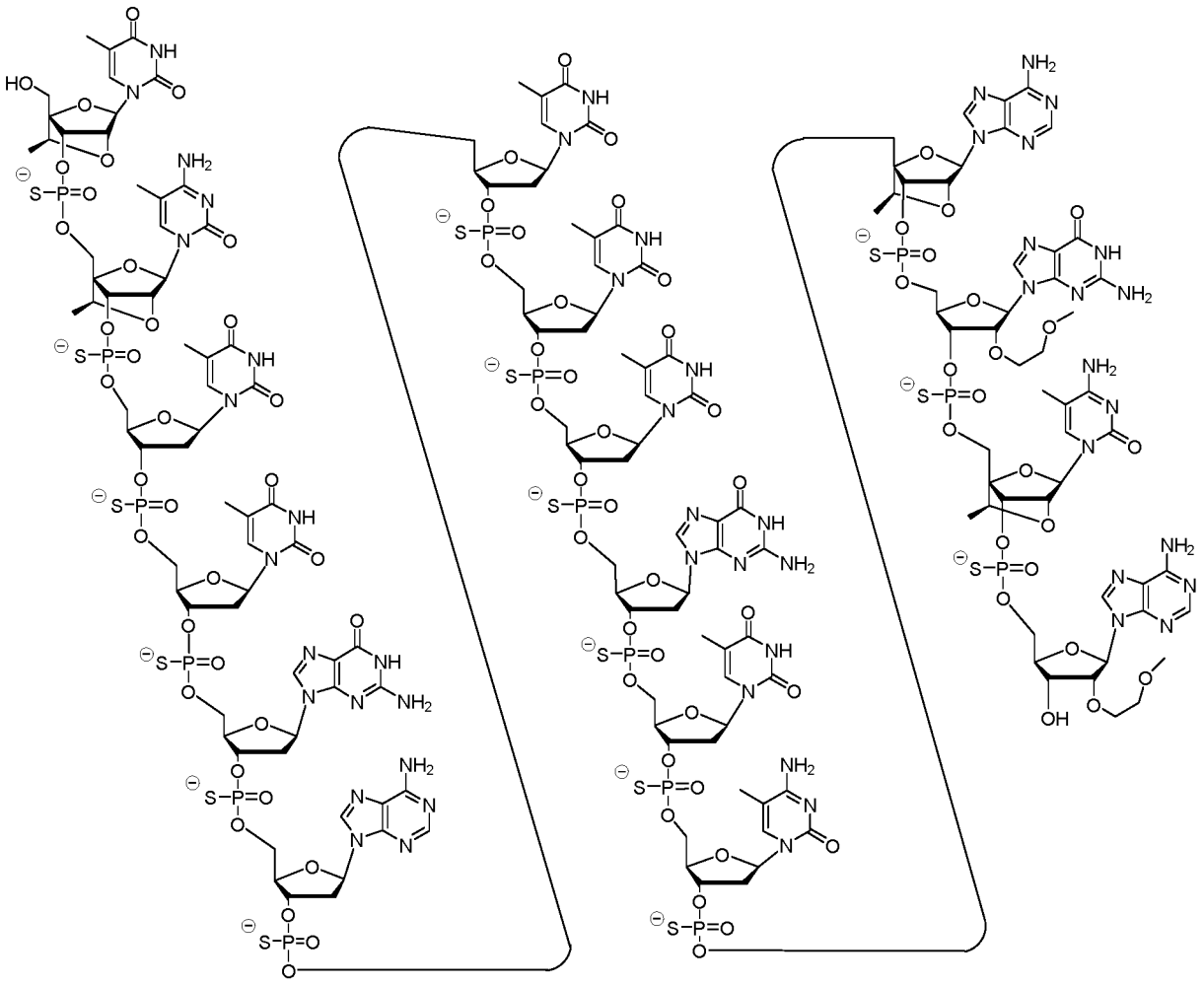
In certain embodiments, a compound comprises or consists of ISIS 716655, or a salt thereof, which has the following chemical structure:



In certain embodiments, a compound comprises or consists of ISIS 740233, or a salt thereof, which has the following chemical structure:



In certain embodiments, a compound comprises or consists of ISIS 746275, or a salt thereof, which has the following chemical structure:



In any of the foregoing embodiments, the compound or oligonucleotide can be at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% complementary to a nucleic acid encoding KRAS.

In any of the foregoing embodiments, the compound can be a single-stranded oligonucleotide. In certain embodiments, the compound comprises deoxyribonucleotides. In certain embodiments, the compound is double-stranded. In certain embodiments, the compound is double-stranded and comprises ribonucleotides.

In any of the foregoing embodiments, the oligonucleotide can consist of 8 to 80, 16 to 80, 10 to 30, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, or 16 to 50 linked nucleosides.

In certain embodiments, a compound comprises a modified oligonucleotide described herein and a conjugate group. In certain embodiments, the conjugate group is linked to the modified oligonucleotide

at the 5' end of the modified oligonucleotide. In certain embodiments, the conjugate group is linked to the modified oligonucleotide at the 3' end of the modified oligonucleotide. In certain embodiments, the conjugate group comprises at least one N- Acetylgalactosamine (GalNAc), at least two N-Acetylgalactosamines (GalNAcs), or at least three N- Acetylgalactosamines (GalNAcs).

In certain embodiments, compounds or compositions provided herein comprise a salt of the modified oligonucleotide. In certain embodiments, the salt is a sodium salt. In certain embodiments, the salt is a potassium salt.

In certain embodiments, the compounds or compositions as described herein are active by virtue of having at least one of an *in vitro* IC_{50} of less than 250 nM, less than 200 nM, less than 150 nM, less than 100 nM, less than 90 nM, less than 80 nM, less than 70 nM, less than 65 nM, less than 60 nM, less than 55 nM, less than 50 nM, less than 45 nM, less than 40 nM, less than 35 nM, less than 30 nM, less than 25 nM, or less than 20 nM.

In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having at least one of an increase an alanine transaminase (ALT) or aspartate transaminase (AST) value of no more than 4 fold, 3 fold, or 2 fold over control treated animals or an increase in liver, spleen, or kidney weight of no more than 30%, 20%, 15%, 12%, 10%, 5%, or 2% compared to control treated animals. In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having no increase of ALT or AST over control treated animals. In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having no increase in liver, spleen, or kidney weight over control treated animals.

Certain Indications

Certain embodiments provided herein relate to methods of inhibiting KRAS expression by administration of a KRAS specific inhibitor, such as a compound targeted to KRAS, which can be useful for treating, preventing, or ameliorating cancer in an individual. Examples of types of cancer include but are not limited to lung cancer (e.g. non-small cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC)), gastrointestinal cancer (e.g. large intestinal cancer, small intestinal cancer, and stomach cancer), colon cancer, colorectal cancer, bladder cancer, liver cancer, esophageal cancer, pancreatic cancer, biliary tract cancer, breast cancer, ovarian cancer, endometrial cancer, cervical cancer, prostate cancer, hematopoetic cancer (e.g. leukemia, myeloid leukemia, and lymphoma), brain cancer (e.g. glioblastoma), malignant peripheral nerve sheath tumor (MPNST), neurofibromatosis type 1 (NF1)

mutant MPNST, or neurofibroma. In certain embodiments, the cancer has cancer cells expressing mutant KRAS.

In certain embodiments, a method of treating, preventing, or ameliorating cancer comprises administering to the individual a KRAS specific inhibitor, thereby treating, preventing, or ameliorating cancer. In certain embodiments, the cancer is lung cancer (e.g. non-small cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC)), gastrointestinal cancer (e.g. large intestinal cancer, small intestinal cancer, and stomach cancer), colon cancer, colorectal cancer, bladder cancer, liver cancer, esophageal cancer, pancreatic cancer, biliary tract cancer, breast cancer, ovarian cancer, endometrial cancer, cervical cancer, prostate cancer, hematopoetic cancer (e.g. leukemia, myeloid leukemia, and lymphoma), brain cancer (e.g. glioblastoma), malignant peripheral nerve sheath tumor (MPNST), neurofibromatosis type 1 (NF1) mutant MPNST, or neurofibroma. In certain embodiments, the cancer has cancer cells expressing mutant KRAS. In certain embodiments, the KRAS specific inhibitor is a compound targeted to KRAS, such as an antisense oligonucleotide targeted to KRAS. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, or 740233. In certain embodiments, the KRAS specific inhibitor is ISIS # 651987. In certain embodiments, the KRAS specific inhibitor is ISIS # 746275. In any of the foregoing embodiments, the compound can be a single-stranded oligonucleotide. In any of the foregoing embodiments, the modified oligonucleotide can consist of 10 to 30 linked nucleosides. In certain

embodiments, the compound is administered to the individual parenterally. In certain embodiments, administering the compound reduces the number of cancer cells in an individual, reduces the size of a tumor in an individual, reduces or inhibits growth or proliferation of a tumor in an individual, prevents metastasis or reduces the extent of metastasis, and/or extends the survival of an individual having cancer, including but not limited to progression free survival (PFS) or overall survival.

In certain embodiments, a method of inhibiting expression of KRAS in an individual having, or at risk of having, cancer comprises administering a KRAS specific inhibitor to the individual, thereby inhibiting expression of KRAS in the individual. In certain embodiments, the cancer expresses mutant KRAS. In certain embodiments, administering the inhibitor inhibits expression of KRAS in a tumor, such as a tumor in the lung, gastrointestinal system, bladder, liver, esophagus, pancreas, biliary tract, breast, ovary, endometrium, cervix, prostate, or brain. In certain embodiments, administering the KRAS specific inhibitor inhibits expression of mutant KRAS. In certain embodiments, administering the KRAS specific inhibitor selectively inhibits expression of mutant KRAS relative to wildtype KRAS. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, or 740233. In certain embodiments, the KRAS specific inhibitor is ISIS # 651987. In certain embodiments, the KRAS specific inhibitor is ISIS # 746275. In any of the foregoing embodiments, the compound can be a single-stranded oligonucleotide. In any of the foregoing embodiments, the modified oligonucleotide can consist of 10 to 30 linked nucleosides.

In certain embodiments, a method of inhibiting expression of KRAS in a cell comprises contacting the cell with a KRAS specific inhibitor, thereby inhibiting expression of KRAS in the cell. In certain embodiments, the cell is a cancer cell. In certain embodiments, the cell is in the lung, gastrointestinal system, bladder, liver, esophagus, pancreas, biliary tract, breast, ovary, endometrium, cervix, prostate, or brain. In certain embodiments, the cell is in the lung, gastrointestinal system, bladder, liver, esophagus, pancreas, biliary tract, breast, ovary, endometrium, cervix, prostate, or brain of an individual who has, or is at risk of having cancer. In certain embodiments, the cancer cell expresses mutant KRAS and contacting the cancer cell with the KRAS specific inhibitor inhibits expression of mutant KRAS in the cancer cell. In certain embodiments, contacting the cancer cell with the KRAS specific inhibitor selectively inhibits expression of mutant KRAS. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, or 740233. In certain embodiments, the KRAS specific inhibitor is ISIS # 651987. In certain embodiments, the KRAS specific inhibitor is ISIS # 746275. In any of the foregoing embodiments, the compound can be a single-stranded oligonucleotide. In any of the foregoing embodiments, the modified oligonucleotide can consist of 10 to 30 linked nucleosides.

In certain embodiments, a method of reducing the number of cancer cells in an individual, reducing the size of a tumor in an individual, reducing or inhibiting growth or proliferation of a tumor in an individual, preventing metastasis or reducing the extent of metastasis, and/or extending the survival (including but not limited to progression free survival (PFS) or overall survival) of an individual having

cancer comprises administering a KRAS specific inhibitor to the individual. In certain embodiments, the inhibitor is a compound targeted to KRAS. In certain embodiments, the inhibitor is a compound targeted to mutant KRAS. In certain embodiments, the inhibitor is a compound selectively targeted to mutant KRAS. In certain embodiments, the cancer cells or tumor expresses mutant KRAS. In certain embodiments, administering the KRAS specific inhibitor to the individual selectively reduces the number of mutant KRAS expressing cancer cells, selectively reduces the size of a mutant KRAS expressing tumor, selectively reduces or inhibits growth or proliferation of a mutant KRAS expressing tumor, selectively prevents metastasis or reduces the extent of metastasis of a mutant KRAS expressing tumor, and/or selectively extends the survival of an individual having a mutant KRAS expressing cancer relative to cells, tumors, and cancer expressing wildtype KRAS. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, or 740233. In certain embodiments, the KRAS specific inhibitor is ISIS # 651987. In certain embodiments, the KRAS specific inhibitor is ISIS # 746275. In any of the foregoing embodiments, the compound can be a single-stranded oligonucleotide. In any of the foregoing embodiments, the modified oligonucleotide can consist of 10 to 30 linked nucleosides. In certain embodiments, the compound is administered to the individual parenterally.

Certain embodiments are drawn to a KRAS specific inhibitor for use in treating cancer. In certain embodiments, the cancer is lung cancer (e.g. non-small cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC)), gastrointestinal cancer (e.g. large intestinal cancer, small intestinal cancer, and

stomach cancer), colon cancer, colorectal cancer, bladder cancer, liver cancer, esophageal cancer, pancreatic cancer, biliary tract cancer, breast cancer, ovarian cancer, endometrial cancer, cervical cancer, prostate cancer, hematopoetic cancer (e.g. leukemia, myeloid leukemia, and lymphoma), brain cancer (e.g. glioblastoma), malignant peripheral nerve sheath tumor (MPNST), neurofibromatosis type 1 (NF1) mutant MPNST, or neurofibroma. In certain embodiments, the cancer expresses mutant KRAS. In certain embodiments, the inhibitor is a compound targeted to KRAS. In certain embodiments, the inhibitor is a compound targeted to mutant KRAS. In certain embodiments, the inhibitor is a compound selectively targeted to mutant KRAS. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, or 740233. In certain embodiments, the KRAS specific inhibitor is ISIS # 651987. In certain embodiments, the KRAS specific inhibitor is ISIS # 746275. In any of the foregoing embodiments, the compound can be a single-stranded oligonucleotide. In any of the foregoing embodiments, the modified oligonucleotide can consist of 10 to 30 linked nucleosides. In certain embodiments, the compound is administered to the individual parenterally.

Certain embodiments are drawn to a KRAS specific inhibitor for use in reducing the number of cancer cells in an individual, reducing the size of a tumor in an individual, reducing or inhibiting growth or proliferation of a tumor in an individual, preventing metastasis or reducing the extent of metastasis, and/or extending the survival (including but not limited to progression free survival (PFS) or overall survival) of an individual having or at risk of having cancer. In certain embodiments, the cancer cells or

tumor express mutant KRAS. In certain embodiments, the inhibitor is a compound targeted to KRAS. In certain embodiments, the inhibitor is a compound targeted to mutant KRAS. In certain embodiments, the inhibitor is a compound selectively targeted to mutant KRAS for use in selectively reducing the number of cancer cells in an individual, selectively reducing the size of a tumor in an individual, selectively reducing or inhibiting growth or proliferation of a tumor in an individual, selectively preventing metastasis or reducing the extent of metastasis, and/or selectively extending the survival (including but not limited to progression free survival (PFS) or overall survival) of an individual having or at risk of having cancer expressing mutant KRAS. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, or 740233. In certain embodiments, the KRAS specific inhibitor is ISIS # 651987. In certain embodiments, the KRAS specific inhibitor is ISIS # 746275. In any of the foregoing embodiments, the compound can be a single-stranded oligonucleotide. In any of the foregoing embodiments, the modified oligonucleotide can consist of 10 to 30 linked nucleosides. In certain embodiments, the compound is administered to the individual parenterally.

Certain embodiments are drawn to use of a KRAS specific inhibitor for the manufacture of a medicament for treating cancer. Certain embodiments are drawn to use of a KRAS specific inhibitor for the preparation of a medicament for treating cancer. In certain embodiments, the cancer expresses mutant KRAS. In certain embodiments, the cancer is lung cancer (e.g. non-small cell lung carcinoma (NSCLC) and small-cell lung carcinoma (SCLC)), gastrointestinal cancer (e.g. large intestinal cancer, small

intestinal cancer, and stomach cancer), colon cancer, colorectal cancer, bladder cancer, liver cancer, esophageal cancer, pancreatic cancer, biliary tract cancer, breast cancer, ovarian cancer, endometrial cancer, cervical cancer, prostate cancer, hematopoetic cancer (e.g. leukemia, myeloid leukemia, and lymphoma), brain cancer (e.g. glioblastoma), malignant peripheral nerve sheath tumor (MPNST), neurofibromatosis type 1 (NF1) mutant MPNST, or neurofibroma. In certain embodiments, the inhibitor is a compound targeted to KRAS. In certain embodiments, the inhibitor is a compound targeted to mutant KRAS. In certain embodiments, the inhibitor is a compound selectively targeted to mutant KRAS. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, or 740233. In certain embodiments, the KRAS specific inhibitor is ISIS # 651987. In certain embodiments, the KRAS specific inhibitor is ISIS # 746275. In any of the foregoing embodiments, the compound can be a single-stranded oligonucleotide. In any of the foregoing embodiments, the modified oligonucleotide can consist of 10 to 30 linked nucleosides. In certain embodiments, the compound is administered to the individual parenterally.

Certain embodiments are drawn to use of a KRAS specific inhibitor for the manufacture or preparation of a medicament for use in reducing the number of cancer cells in an individual, reducing the size of a tumor in an individual, reducing or inhibiting growth or proliferation of a tumor in an individual, preventing metastasis or reducing the extent of metastasis, and/or extending the survival (including but not limited to progression free survival (PFS) or overall survival) in an individual having or at risk of having cancer. In certain embodiments, the cancer cells or tumor expresses mutant KRAS. In certain

embodiments, the inhibitor is a compound targeted to KRAS. In certain embodiments, the inhibitor is a compound targeted to KRAS. In certain embodiments, the inhibitor is a compound targeted to mutant KRAS. In certain embodiments, the inhibitor is a compound selectively targeted to mutant KRAS for the manufacture or preparation of a medicament for use in selectively reducing the number of cancer cells in an individual, selectively reducing the size of a tumor in an individual, selectively reducing or inhibiting growth or proliferation of a tumor in an individual, selectively preventing metastasis or reducing the extent of metastasis, and/or selectively extending the survival (including but not limited to progression free survival (PFS) or overall survival) of an individual having or at risk of having cancer expressing mutant KRAS. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is a compound comprising a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, 804, 854, 1028, 2130, 2136, 2142, 2154, and 2158. In certain embodiments, the KRAS specific inhibitor is ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, or 740233. In certain embodiments, the KRAS specific inhibitor is ISIS # 651987. In certain embodiments, the KRAS specific inhibitor is ISIS # 746275. In any of the foregoing embodiments, the compound can be a single-stranded oligonucleotide. In any of the foregoing embodiments, the modified oligonucleotide can consist of 10 to 30 linked nucleosides. In certain embodiments, the compound is administered to the individual parenterally.

In any of the foregoing methods or uses, the KRAS specific inhibitor can be a compound targeted to KRAS, a compound targeted to mutant KRAS, or a compound selectively targeted to mutant KRAS. In certain embodiments, the compound is an antisense oligonucleotide, for example an antisense oligonucleotide consisting of 8 to 80 linked nucleosides, 10 to 30 linked nucleosides, 12 to 30 linked

nucleosides, or 16 linked nucleosides. In certain embodiments, the antisense oligonucleotide is at least 80%, 85%, 90%, 95% or 100% complementary to any of the nucleobase sequences recited in SEQ ID NOs: 1-3. In certain embodiments, the antisense oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar and/or at least one modified nucleobase. In certain embodiments, the modified internucleoside linkage is a phosphorothioate internucleoside linkage, the modified sugar is a bicyclic sugar or a 2'-O-methoxyethyl, and the modified nucleobase is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide comprises a gap segment consisting of linked deoxynucleosides; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

In any of the foregoing embodiments, the antisense oligonucleotide consists of 12 to 30, 15 to 30, 15 to 25, 15 to 24, 16 to 24, 17 to 24, 18 to 24, 19 to 24, 20 to 24, 19 to 22, 20 to 22, 16 to 20, or 17 or 20 linked nucleosides. In certain aspects, the antisense oligonucleotide is at least 80%, 85%, 90%, 95% or 100% complementary to any of the nucleobase sequences recited in SEQ ID NOs: 1-3. In certain aspects, the antisense oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar and/or at least one modified nucleobase. In certain aspects, the modified internucleoside linkage is a phosphorothioate internucleoside linkage, the modified sugar is a bicyclic sugar or a 2'-O-methoxyethyl, and the modified nucleobase is a 5-methylcytosine. In certain aspects, the modified oligonucleotide comprises a gap segment consisting of linked 2'-deoxynucleosides; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides, wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

In any of the foregoing methods or uses, the KRAS specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide consisting of 16 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 13-2190, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

In any of the foregoing methods or uses, the KRAS specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence comprising or consisting of the sequence recited in any one of SEQ ID NOs: 239, 272, 569, 607, 615, 621, 640, 655, 678, 715, 790, and 854, wherein the modified oligonucleotide comprises

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a constrained ethyl (cEt) nucleoside; wherein each internucleoside linkage is a phosphorothioate linkage and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-80 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In any of the foregoing methods or uses, the KRAS specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 2130, wherein the modified oligonucleotide comprises

a gap segment consisting of nine linked deoxynucleosides;

a 5' wing segment consisting of one linked nucleoside; and

a 3' wing segment consisting of six linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, and 2'-O-methoxyethyl nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-80 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In any of the foregoing methods or uses, the KRAS specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence comprising or consisting of the sequence recited in any one of SEQ ID NOs: 804, 1028, and 2136, wherein the modified oligonucleotide comprises

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, and a 2'-O-methoxyethyl nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-80 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In any of the foregoing methods or uses, the KRAS specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 2142, wherein the modified oligonucleotide comprises

a gap segment consisting of eight linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of six linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-80 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In any of the foregoing methods or uses, the KRAS specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 2154, wherein the modified oligonucleotide comprises

a gap segment consisting of nine linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-80 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In any of the foregoing methods or uses, the KRAS specific inhibitor can be a compound comprising or consisting of a modified oligonucleotide having a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 2158, wherein the modified oligonucleotide comprises

a gap segment consisting of eight linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside, a cEt nucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a deoxynucleoside, a cEt nucleoside, a deoxynucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide consists of 16-80 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16-30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In any of the foregoing methods or uses, the KRAS specific inhibitor can be administered parenterally. For example, in certain embodiments the KRAS specific inhibitor can be administered through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intrathecal or intracerebroventricular administration.

Antisense compounds

Antisense compounds are provided in certain embodiments. In certain embodiments, antisense compounds comprise at least one oligonucleotide. In certain embodiments, antisense compounds consist

of an oligonucleotide. In certain embodiments, antisense compounds consist of an oligonucleotide attached to one or more conjugate groups. In certain embodiments, antisense compounds consist of an oligonucleotide attached to one or more conjugate groups via one or more conjugate linkers and/or a cleavable moiety. In certain embodiments, the oligonucleotide of an antisense compound is modified. In certain embodiments, the oligonucleotide of an antisense compound may have any nucleobase sequence. In certain embodiments, the oligonucleotide of an antisense compound is an antisense oligonucleotide having a nucleobase sequence that is complementary to a target nucleic acid. In certain embodiments, antisense oligonucleotides are complementary to a messenger RNA (mRNA).

In certain embodiments, an antisense compound has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted.

In certain embodiments, an antisense compound is 10 to 30 subunits in length. In certain embodiments, an antisense compound is 12 to 30 subunits in length. In certain embodiments, an antisense compound is 12 to 22 subunits in length. In certain embodiments, an antisense compound is 14 to 30 subunits in length. In certain embodiments, an antisense compound is 14 to 20 subunits in length. In certain embodiments, an antisense compoun is 15 to 30 subunits in length. In certain embodiments, an antisense compound is 15 to 20 subunits in length. In certain embodiments, an antisense compound is 16 to 30 subunits in length. In certain embodiments, an antisense compound is 16 to 20 subunits in length. In certain embodiments, an antisense compound is 17 to 30 subunits in length. In certain embodiments, an antisense compound is 17 to 20 subunits in length. In certain embodiments, an antisense compound is 18 to 30 subunits in length. In certain embodiments, an antisense compound is 18 to 21 subunits in length. In certain embodiments, an antisense compound is 18 to 20 subunits in length. In certain embodiments, an antisense compound is 20 to 30 subunits in length. In other words, such antisense compounds are from 12 to 30 linked subunits, 14 to 30 linked subunits, 14 to 20 subunits, 15 to 30 subunits, 15 to 20 subunits, 16 to 30 subunits, 16 to 20 subunits, 17 to 30 subunits, 17 to 20 subunits, 18 to 30 subunits, 18 to 20 subunits, 18 to 21 subunits, 20 to 30 subunits, or 12 to 22 linked subunits, respectively. In certain embodiments, an antisense compound is 14 subunits in length. In certain embodiments, an antisense compound is 16 subunits in length. In certain embodiments, an antisense compound is 17 subunits in length. In certain embodiments, an antisense compound is 18 subunits in length. In certain embodiments, an antisense compound is 19 subunits in length. In certain embodiments, an antisense compound is 20 subunits in length. In other embodiments, the antisense compound is 8 to 80, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked subunits. In certain such embodiments, the antisense compounds are 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29,

30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked subunits in length, or a range defined by any two of the above values. In some embodiments the antisense compound is an antisense oligonucleotide, and the linked subunits are nucleotides, nucleosides, or nucleobases.

In certain embodiments, the antisense compound or oligomeric compound may further comprise additional features or elements, such as a conjugate group, that are attached to the oligonucleotide. In embodiments where a conjugate group comprises a nucleoside (*i.e.* a nucleoside that links the conjugate group to the oligonucleotide), the nucleoside of the conjugate group is not counted in the length of the oligonucleotide.

In certain embodiments antisense compounds may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to an KRAS nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound.

When a single additional subunit is present in a lengthened antisense compound, the additional subunit may be located at the 5' or 3' end of the antisense compound. When two or more additional subunits are present, the added subunits may be adjacent to each other, for example, in an antisense compound having two subunits added to the 5' end (5' addition), or alternatively to the 3' end (3' addition), of the antisense compound. Alternatively, the added subunits may be dispersed throughout the antisense compound, for example, in an antisense compound having one subunit added to the 5' end and one subunit added to the 3' end.

It is possible to increase or decrease the length of an antisense compound, such as an antisense oligonucleotide, and/or introduce mismatch bases without eliminating activity (Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7305-7309, 1992; Gautschi *et al. J. Natl. Cancer Inst.* 93:463-471, March 2001; Maher and Dolnick *Nuc. Acid. Res.* 16:3341-3358,1988). However, seemingly small changes in oligonucleotide sequence, chemistry and motif can make large differences in one or more of the many properties required for clinical development (Seth et al. *J. Med. Chem.* **2009**, *52*, 10; Egli et al. *J. Am. Chem. Soc.* **2011**, *133*, 16642).

In certain embodiments, antisense compounds are single-stranded, consisting of one oligomeric compound. The oligonucleotide of such single-stranded antisense compounds is an antisense oligonucleotide. In certain embodiments, the antisense oligonucleotide of a single-stranded antisense compound is modified. In certain embodiments, the oligonucleotide of a single-stranded antisense compound or oligomeric compound comprises a self-complementary nucleobase sequence. In certain

embodiments, antisense compounds are double-stranded, comprising two oligomeric compounds that form a duplex. In certain such embodiments, one oligomeric compound of a double-stranded antisense compound comprises one or more conjugate groups. In certain embodiments, each oligomeric compound of a double-stranded antisense compound comprises one or more conjugate groups. In certain embodiments, each oligonucleotide of a double-stranded antisense compound is a modified oligonucleotide. In certain embodiments, one oligonucleotide of a double-stranded antisense compound is a modified oligonucleotide. In certain embodiments, one oligonucleotide of a double-stranded antisense compound is an antisense oligonucleotide. In certain such embodiments, the antisense oligonucleotide is a modified oligonucleotide. Examples of single-stranded and double-stranded antisense compounds include but are not limited to antisense oligonucleotides, siRNAs, microRNA targeting oligonucleotides, and single-stranded RNAi compounds, such as small hairpin RNAs (shRNAs), single-stranded siRNAs (ssRNAs), and microRNA mimics.

In certain embodiments, antisense compounds are interfering RNA compounds (RNAi), which include double-stranded RNA compounds (also referred to as short-interfering RNA or siRNA) and single-stranded RNAi compounds (or ssRNA). Such compounds work at least in part through the RISC pathway to degrade and/or sequester a target nucleic acid (thus, include microRNA/microRNA-mimic compounds). As used herein, the term siRNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics.

In certain embodiments, a double-stranded compound can comprise any of the oligonucleotide sequences targeted to KRAS described herein. In certain embodiments, a double-stranded compound comprises a first strand comprising at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobase portion of any one of SEQ ID NOs: 13-2190 and a second strand. In certain embodiments, a double-stranded compound comprises a first strand comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190 and a second strand. In certain embodiments, the double-stranded compound comprises ribonucleotides in which the first strand has uracil (U) in place of thymine (T) in any one of SEQ ID NOs: 13-2190. In certain embodiments, a double-stranded compound comprises (i) a first strand comprising a nucleobase sequence complementary to the site on KRAS to which any of SEQ ID NOs: 13-2190 is targeted, and (ii) a second strand. In certain embodiments, the double-stranded compound

comprises one or more modified nucleotides in which the 2' position in the sugar contains a halogen (such as fluorine group; 2'-F) or contains an alkoxy group (such as a methoxy group; 2'-OMe). In certain embodiments, the double-stranded compound comprises at least one 2'-F sugar modification and at least one 2'-OMe sugar modification. In certain embodiments, the at least one 2'-F sugar modification and at least one 2'-OMe sugar modification are arranged in an alternating pattern for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases along a strand of the dsRNA compound. In certain embodiments, the double-stranded compound comprises one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. The double-stranded compounds may also be chemically modified nucleic acid molecules as taught in U.S. Pat. No. 6,673,661. In other embodiments, the dsRNA contains one or two capped strands, as disclosed, for example, by WO 00/63364, filed Apr. 19, 2000. In certain embodiments, the first strand of the double-stranded compound is an siRNA guide strand and the second strand of the double-stranded compound is an siRNA passenger strand. In certain embodiments, the second strand of the double-stranded compound is complementary to the first strand. In certain embodiments, each strand of the double-stranded compound consists of 16, 17, 18, 19, 20, 21, 22, or 23 linked nucleosides. In certain embodiments, the first or second strand of the double-stranded compound can comprise a conjugate group.

In certain embodiments, a single-stranded RNAi (ssRNAi) compound can comprise any of the oligonucleotide sequences targeted to KRAS described herein. In certain embodiments, an ssRNAi compound comprises at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobase portion of any one of SEQ ID NOs: 13-2190. In certain embodiments, an ssRNAi compound comprises the nucleobase sequence of any one of SEQ ID NOs: 13-2190. In certain embodiments, the ssRNAi compound comprises ribonucleotides in which uracil (U) is in place of thymine (T) in any one of SEQ ID NOs: 13-2190. In certain embodiments, an ssRNAi compound comprises a nucleobase sequence complementary to the site on KRAS to which any of SEQ ID NOs: 13-2190 is targeted. In certain embodiments, an ssRNAi compound comprises one or more modified nucleotides in which the 2' position in the sugar contains a halogen (such as fluorine group; 2'-F) or contains an alkoxy group (such as a methoxy group; 2'-OMe). In certain embodiments, an ssRNAi compound comprises at least one 2'-F sugar modification and at least one 2'-OMe sugar modification. In certain embodiments, the at least one 2'-F sugar modification and at least one 2'-OMe sugar modification are arranged in an alternating pattern for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases along a strand of the ssRNAi compound. In certain embodiments, the ssRNAi compound comprises one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. The ssRNAi

compounds may also be chemically modified nucleic acid molecules as taught in U.S. Pat. No. 6,673,661. In other embodiments, the ssRNAi contains a capped strand, as disclosed, for example, by WO 00/63364, filed Apr. 19, 2000. In certain embodiments, the ssRNAi compound consists of 16, 17, 18, 19, 20, 21, 22, or 23 linked nucleosides. In certain embodiments, the ssRNAi compound can comprise a conjugate group.

In certain embodiments, antisense compounds comprise modified oligonucleotides. Certain modified oligonucleotides have one or more asymmetric center and thus give rise to enantiomers, diastereomers, and other stereoisomeric configurations that may be defined, in terms of absolute stereochemistry, as (R) or (S), as α or β such as for sugar anomers, or as (D) or (L) such as for amino acids etc. Included in the modified oligonucleotides provided herein are all such possible isomers, including their racemic and optically pure forms, unless specified otherwise. Likewise, all cis- and trans-isomers and tautomeric forms are also included.

Certain Antisense Compound Mechanisms

In certain embodiments, antisense compounds are capable of hybridizing to a target nucleic acid, resulting in at least one antisense activity. In certain embodiments, antisense compounds specifically affect one or more target nucleic acid. Such specific antisense compounds comprises a nucleobase sequence that hybridizes to one or more target nucleic acid, resulting in one or more desired antisense activity and does not hybridize to one or more non-target nucleic acid or does not hybridize to one or more non-target nucleic acid in such a way that results in an undesired antisense activity.

In certain antisense activities, hybridization of an antisense compound to a target nucleic acid results in recruitment of a protein that cleaves the target nucleic acid. For example, certain antisense compounds result in RNase H mediated cleavage of the target nucleic acid. RNase H is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex. The DNA in such an RNA:DNA duplex need not be unmodified DNA. In certain embodiments, the invention provides antisense compounds that are sufficiently "DNA-like" to elicit RNase H activity. Further, in certain embodiments, one or more non-DNA-like nucleoside in the gap of a gapmer is tolerated.

In certain antisense activities, an antisense compound or a portion of an antisense compound is loaded into an RNA-induced silencing complex (RISC), ultimately resulting in cleavage of the target nucleic acid. For example, certain antisense compounds result in cleavage of the target nucleic acid by Argonaute. In certain embodiments, antisense compounds that are loaded into RISC are RNAi compounds.

In certain embodiments, hybridization of an antisense compound to a target nucleic acid does not result in recruitment of a protein that cleaves that target nucleic acid. In certain such embodiments, hybridization of the antisense compound to the target nucleic acid results in alteration of splicing of the

target nucleic acid. In certain embodiments, hybridization of an antisense compound to a target nucleic acid results in inhibition of a binding interaction between the target nucleic acid and a protein or other nucleic acid. In certain such embodiments, hybridization of an antisense compound to a target nucleic acid results in alteration of translation of the target nucleic acid.

Antisense activities may be observed directly or indirectly. In certain embodiments, observation or detection of an antisense activity involves observation or detection of a change in an amount of a target nucleic acid or protein encoded by such target nucleic acid, a change in the ratio of splice variants of a nucleic acid or protein, and/or a phenotypic change in a cell or animal.

In certain embodiments, modified oligonucleotides having a gapmer sugar motif described herein have desirable properties compared to non-gapmer oligonucleotides or to gapmers having other sugar motifs. In certain circumstances, it is desirable to identify motifs resulting in a favorable combination of potent antisense activity and relatively low toxicity. In certain embodiments, compounds of the present invention have a favorable therapeutic index (measure of activity divided by measure of toxicity).

Target Nucleic Acids, Target Regions and Nucleotide Sequences

In certain embodiments, antisense compounds comprise or consist of an oligonucleotide comprising a region that is complementary to a target nucleic acid. In certain embodiments, the target nucleic acid is an endogenous RNA molecule. In certain embodiments, the target nucleic acid encodes a protein. In certain such embodiments, the target nucleic acid is selected from: an mRNA and a pre-mRNA, including intronic, exonic and untranslated regions. In certain embodiments, the target RNA is an mRNA. In certain embodiments, the target nucleic acid is a pre-mRNA. In certain such embodiments, the target region is entirely within an intron. In certain embodiments, the target region spans an intron/exon junction. In certain embodiments, the target region is at least 50% within an intron.

Nucleotide sequences that encode KRAS include, without limitation, GENBANK Accession No. NM_004985.4 (incorporated by reference, disclosed herein as SEQ ID NO: 1); GENBANK Accession No. NT_009714.17_TRUNC_18116000_18166000_COMP (incorporated by reference, disclosed herein as SEQ ID NO: 2), and GENBANK Accession No. NM_033360.3 (incorporated by reference, disclosed herein as SEQ ID NO: 3).

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a KRAS nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g.,

Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

Hybridization can occur under varying conditions. Hybridization conditions are sequence-dependent and are determined by the nature and composition of the nucleic acid molecules to be hybridized.

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a KRAS nucleic acid.

Complementarity

An oligonucleotide is said to be complementary to another nucleic acid when the nucleobase sequence of such oligonucleotide or one or more regions thereof matches the nucleobase sequence of another oligonucleotide or nucleic acid or one or more regions thereof when the two nucleobase sequences are aligned in opposing directions. Nucleobase matches or complementary nucleobases, as described herein, are limited to adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), and 5-methyl cytosine (mC) and guanine (G) unless otherwise specified. Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside and may include one or more nucleobase mismatches. An oligonucleotide is fully complementary or 100% complementary when such oligonucleotides have nucleobase matches at each nucleoside without any nucleobase mismatches.

Non-complementary nucleobases between an antisense compound and a KRAS nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or more segments of a KRAS nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a KRAS nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining non-complementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to

complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having four non-complementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403 410; Zhang and Madden, Genome Res., 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482 489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to a KRAS nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

In certain embodiments, antisense compounds comprise one or more mismatched nucleobases relative to the target nucleic acid. In certain such embodiments, antisense activity against the target is reduced by such mismatch, but activity against a non-target is reduced by a greater amount. Thus, in certain such embodiments selectivity of the antisense compound is improved. In certain embodiments, the mismatch is specifically positioned within an oligonucleotide having a gapmer motif. In certain such embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, or 8 from the 5'-end of the gap region. In certain such embodiments, the mismatch is at position 9, 8, 7, 6, 5, 4, 3, 2, 1 from the 3'-end of the gap

region. In certain such embodiments, the mismatch is at position 1, 2, 3, or 4 from the 5'-end of the wing region. In certain such embodiments, the mismatch is at position 4, 3, 2, or 1 from the 3'-end of the wing region.

The location of a non-complementary nucleobase may be at the 5' end or 3' end of the antisense compound. Alternatively, the non-complementary nucleobase or nucleobases may be at an internal position of the antisense compound. When two or more non-complementary nucleobases are present, they may be contiguous (i.e. linked) or non-contiguous. In one embodiment, a non-complementary nucleobase is located in the wing segment of a gapmer antisense oligonucleotide.

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a KRAS nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a KRAS nucleic acid, or specified portion thereof.

The antisense compounds provided also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 9 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 10 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least an 11 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 13 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 15 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 16 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof. As used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

In certain embodiments, the antisense compounds, or portions thereof, are, or are at least, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

In certain embodiments, a portion of the antisense compound is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

In certain embodiments, a portion of the antisense oligonucleotide is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

Modifications

Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

Chemically modified nucleosides may also be employed to increase the binding affinity of a shortened or truncated antisense oligonucleotide for its target nucleic acid. Consequently, comparable results can often be obtained with shorter antisense compounds that have such chemically modified nucleosides.

Modified Internucleoside Linkages

The naturally occuring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having 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.

Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, nucleosides of modified oligonucleotides may be linked together using any internucleoside linkage. The two main classes of internucleoside linking groups are defined by the presence or absence of a phosphorus atom. Representative phosphorus-containing internucleoside linkages include but are not limited to phosphates, which contain a phosphodiester bond ("P=O") (also referred to as unmodified or naturally occurring linkages), phosphotriesters, methylphosphonates, phosphoramidates, and phosphorothioates ("P=S"), and phosphorodithioates ("HS-P=S"). Representative non-phosphorus containing internucleoside linking groups include but are not limited to methylenemethylimino (-CH2-N(CH3)-O-CH2-), thiodiester (-O-C(=O)-S-), thionocarbamate (-O-C(=O)(NH)-S-); siloxane (-O-SiH2-O-); and N,N'-dimethylhydrazine (-CH2-N(CH3)-N(CH3)-). Modified internucleoside linkages, compared to naturally occurring phosphate linkages, can be used to alter, typically increase, nuclease resistance of the oligonucleotide. In certain embodiments, internucleoside linkages having a chiral atom can be prepared as a racemic mixture, or as separate enantiomers. Representative chiral internucleoside linkages include but are not limited to alkylphosphonates and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing internucleoside linkages are well known to those skilled in the art.

Neutral internucleoside linkages include, without limitation, phosphotriesters, methylphosphonates, MMI (3'-CH2-N(CH3)-O-5'), amide-3 (3'-CH2-C(=O)-N(H)-5'), amide-4 (3'-CH2-N(H)-C(=O)-5'), formacetal (3'-O-CH2-O-5'), methoxypropyl, and thioformacetal (3'-S-CH2-O-5'). Further neutral internucleoside linkages include nonionic linkages comprising siloxane (dialkylsiloxane), carboxylate ester, carboxamide, sulfide, sulfonate ester and amides (See for example: Carbohydrate Modifications in Antisense Research; Y.S. Sanghvi and P.D. Cook, Eds., ACS Symposium Series 580;

Chapters 3 and 4, 40-65). Further neutral internucleoside linkages include nonionic linkages comprising mixed N, O, S and CH2 component parts.

In certain embodiments, antisense compounds targeted to a KRAS nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

In certain embodiments, oligonucleotides comprise modified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or modified internucleoside linkage motif. In certain embodiments, internucleoside linkages are arranged in a gapped motif. In such embodiments, the internucleoside linkages in each of two wing regions are different from the internucleoside linkages in the gap region. In certain embodiments the internucleoside linkages in the wings are phosphodiester and the internucleoside linkages in the gap are phosphorothioate. The nucleoside motif is independently selected, so such oligonucleotides having a gapped internucleoside linkage motif may or may not have a gapped nucleoside motif and if it does have a gapped nucleoside motif, the wing and gap lengths may or may not be the same.

In certain embodiments, oligonucleotides comprise a region having an alternating internucleoside linkage motif. In certain embodiments, oligonucleotides of the present invention comprise a region of uniformly modified internucleoside linkages. In certain such embodiments, the oligonucleotide comprises a region that is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide is uniformly linked by phosphorothioate. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate and at least one internucleoside linkage is phosphorothioate.

In certain embodiments, the oligonucleotide comprises at least 6 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 8 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 10 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 6 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 8 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 10 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 12 consecutive phosphorothioate internucleoside linkages. In certain such embodiments, at least one such block is located at the 3'

end of the oligonucleotide. In certain such embodiments, at least one such block is located within 3 nucleosides of the 3' end of the oligonucleotide.

In certain embodiments, oligonucleotides comprise one or more methylphosponate linkages. In certain embodiments, oligonucleotides having a gapmer nucleoside motif comprise a linkage motif comprising all phosphorothioate linkages except for one or two methylphosponate linkages. In certain embodiments, one methylphosponate linkage is in the central gap of an oligonucleotide having a gapmer nucleoside motif.

In certain embodiments, it is desirable to arrange the number of phosphorothioate internucleoside linkages and phosphodiester internucleoside linkages to maintain nuclease resistance. In certain embodiments, it is desirable to arrange the number and position of phosphorothioate internucleoside linkages and the number and position of phosphodiester internucleoside linkages to maintain nuclease resistance. In certain embodiments, the number of phosphorothioate internucleoside linkages may be decreased and the number of phosphodiester internucleoside linkages may be increased. In certain embodiments, the number of phosphorothioate internucleoside linkages may be decreased and the number of phosphodiester internucleoside linkages may be increased while still maintaining nuclease resistance. In certain embodiments it is desirable to decrease the number of phosphorothioate internucleoside linkages while retaining nuclease resistance. In certain embodiments it is desirable to increase the number of phosphodiester internucleoside linkages while retaining nuclease resistance.

Modified Sugar Moieties

Antisense compounds can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds.

In certain embodiments, modified oligonucleotides comprise one or more modified nucleosides comprising a modified sugar moiety. Such modified oligonucleotides comprising one or more sugar-modified nucleosides may have desirable properties, such as enhanced nuclease stability or increased binding affinity with a target nucleic acid relative to oligonucleotides lacking such sugar-modified nucleosides. In certain embodiments, modified sugar moieties are linearly modified sugar moieties. In certain embodiments, modified sugar moieties are bicyclic or tricyclic sugar moieties. In certain embodiments, modified sugar moieties are sugar surrogates. Such sugar surrogates may comprise one or more substitutions corresponding to those of substituted sugar moieties.

In certain embodiments, modified sugar moieties are linearly modified sugar moieties comprising a furanosyl ring with one or more acyclic substituent, including but not limited to substituents

at the 2' and/or 5' positions. Examples of 2'-substituent groups suitable for linearly modified sugar moieties include but are not limited to: 2'-F, 2'-OCH_3 ("OMe" or "O-methyl"), and 2'-$O(CH_2)_2OCH_3$ ("MOE"). In certain embodiments, 2'-substituent groups are selected from among: halo, allyl, amino, azido, SH, CN, OCN, CF_3, OCF_3, O-C_1-C_{10} alkoxy, O-C_1-C_{10} substituted alkoxy, O-C_1-C_{10} alkyl, O-C_1-C_{10} substituted alkyl, S-alkyl, N(R_m)-alkyl, O-alkenyl, S-alkenyl, N(R_m)-alkenyl, O-alkynyl, S-alkynyl, N(R_m)-alkynyl, O-alkylenyl-O-alkyl, alkynyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$ or $OCH_2C(=O)$-N(R_m)(R_n), where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_1-C_{10} alkyl. Certain embodiments of these 2'-substituent groups can be further substituted with one or more substituent groups independently selected from among: hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro (NO_2), thiol, thioalkoxy, thioalkyl, halogen, alkyl, aryl, alkenyl and alkynyl. Examples of 5'-substituent groups suitable for linearly modified sugar moieties include but are not limited to: 5'-methyl (R or S), 5'-vinyl, and 5'-methoxy. In certain embodiments, linearly modified sugars comprise more than one non-bridging sugar substituent, for example, 2'-F-5'-methyl sugar moieties (*see*, e.g., PCT International Application WO 2008/101157, for additional 2', 5'-bis substituted sugar moieties and nucleosides).

In certain embodiments, a 2'-substituted nucleoside or 2'-linearly modified nucleoside comprises a sugar moiety comprising a linear 2'-substituent group selected from: F, NH_2, N_3, OCF_3, OCH_3, $O(CH_2)_3NH_2$, $CH_2CH{=}CH_2$, $OCH_2CH{=}CH_2$, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and N-substituted acetamide ($OCH_2C(=O)$-N(R_m)(R_n)), where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_1-C_{10} alkyl.

In certain embodiments, a 2'-substituted nucleoside or 2'-linearly modified nucleoside comprises a sugar moiety comprising a linear 2'-substituent group selected from: F, OCF_3, OCH_3, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(CH_3)_2$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and $OCH_2C(=O)$-N(H)CH_3 ("NMA").

In certain embodiments, a 2'-substituted nucleoside or 2'-linearly modified nucleoside comprises a sugar moiety comprising a linear 2'-substituent group selected from: F, OCH_3, and $OCH_2CH_2OCH_3$.

Nucleosides comprising modified sugar moieties, such as linearly modified sugar moieties, are referred to by the position(s) of the substitution(s) on the sugar moiety of the nucleoside. For example, nucleosides comprising 2'-substituted or 2-modified sugar moieties are referred to as 2'-substituted nucleosides or 2-modified nucleosides.

Certain modifed sugar moieties comprise a bridging sugar substituent that forms a second ring resulting in a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety comprises a

bridge between the 4' and the 2' furanose ring atoms. Examples of such 4' to 2' bridging sugar substituents include but are not limited to: 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2' ("LNA"), 4'-CH_2-S-2', 4'-$(CH_2)_2$-O-2' ("ENA"), 4'-$CH(CH_3)$-O-2' (referred to as "constrained ethyl" or "cEt" when in the *S* configuration), 4'-CH_2-O-CH_2-2', 4'-CH_2-N(R)-2', 4'-$CH(CH_2OCH_3)$-O-2' ("constrained MOE" or "cMOE") and analogs thereof (*see, e.g.,* U.S. Patent 7,399,845), 4'-$C(CH_3)(CH_3)$-O-2' and analogs thereof (*see, e.g.,* WO2009/006478), 4'-CH_2-N(OCH_3)-2' and analogs thereof (*see, e.g.,* WO2008/150729), 4'-CH_2-O-N(CH_3)-2' (*see, e.g.,* US2004/0171570), 4'-CH_2-C(H)(CH_3)-2' (*see, e.g.,* Chattopadhyaya, *et al., J. Org. Chem.,*2009, *74*, 118-134), 4'-CH_2-C(=CH_2)-2' and analogs thereof (*see*, published PCT International Application WO 2008/154401), 4'-$C(R_aR_b)$-N(R)-O-2', 4'-$C(R_aR_b)$-O-N(R)-2', 4'-CH_2-O-N(R)-2', and 4'-CH_2-N(R)-O-2', wherein each R, R_a, and R_b is, independently, H, a protecting group, or C_1-C_{12} alkyl (*see, e.g.* U.S. Patent 7,427,672).

In certain embodiments, such 4' to 2' bridges independently comprise from 1 to 4 linked groups independently selected from: -$[C(R_a)(R_b)]_n$-, -$[C(R_a)(R_b)]_n$-O-, -$C(R_a)$=$C(R_b)$-, -$C(R_a)$=N-, -C(=NR_a)-, -C(=O)-, -C(=S)-, -O-, -$Si(R_a)_2$-, -$S(=O)_x$-, and -$N(R_a)$-;

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

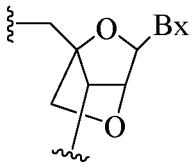
each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)-H), substituted acyl, CN, sulfonyl ($S(=O)_2$-J_1), or sulfoxyl (S(=O)-J_1); and

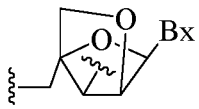
each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl, or a protecting group.

Additional bicyclic sugar moieties are known in the art, for example: Freier *et al., Nucleic Acids Research*, 1997, *25*(*22*), 4429-4443, Albaek *et al., J. Org. Chem.*, 2006, *71*, 7731-7740, Singh et al., *Chem. Commun.*, 1998, *4*, 455-456; Koshkin et al., *Tetrahedron*, 1998, *54*, 3607-3630; Wahlestedt et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2000, *97*, 5633-5638; Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222; Singh et al., *J. Org. Chem.*, 1998, *63*, 10035-10039; Srivastava et al., *J. Am. Chem. Soc.*, 20017, *129,* 8362-8379; Elayadi *et al., Curr. Opinion Invens. Drugs,* 2001, *2*, 558-561; Braasch *et al., Chem. Biol.,* 2001, *8*, 1-7; Orum *et al., Curr. Opinion Mol. Ther.,* 2001, *3*, 239-243; U.S. Patent Nos. 7,053,207, 6,268,490, 6,770,748, 6,794,499, 7,034,133, 6,525,191, 6,670,461, and 7,399,845; WO

2004/106356, WO 1994/14226, WO 2005/021570, and WO 2007/134181; U.S. Patent Publication Nos. US2004/0171570, US2007/0287831, and US2008/0039618; U.S. Patent Serial Nos. 12/129,154, 60/989,574, 61/026,995, 61/026,998, 61/056,564, 61/086,231, 61/097,787, and 61/099,844; and PCT International Applications Nos. PCT/US2008/064591, PCT/US2008/066154, and PCT/US2008/068922.

In certain embodiments, bicyclic sugar moieties and nucleosides incorporating such bicyclic sugar moieties are further defined by isomeric configuration. For example, an LNA nucleoside (described above) may be in the α-L configuration or in the β-D configuration.





LNA (β-D-configuration) bridge = 4'-CH_2-O-2'

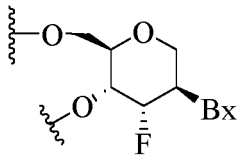
α-*L*-LNA (α-*L*-configuration) bridge = 4'-CH_2-O-2'

α-L-methyleneoxy (4'-CH_2-O-2') or α-L-LNA bicyclic nucleosides have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden et al., *Nucleic Acids Research,* 2003, *21*, 6365-6372). Herein, general descriptions of bicyclic nucleosides include both isomeric configurations. When the positions of specific bicyclic nucleosides (*e.g.*, LNA or cEt) are identified in exemplified embodiments herein, they are in the β-D configuration, unless otherwise specified.

In certain embodiments, modified sugar moieties comprise one or more non-bridging sugar substituent and one or more bridging sugar substituent (e.g., 5'-substituted and 4'-2' bridged sugars). (*see, e.g.,* WO 2007/134181, wherein LNA nucleosides are further substituted with, for example, a 5'-methyl or a 5'-vinyl group, and *see, e.g.,* U.S. Patents 7,547,684; 7,750,131; 8,030,467; 8,268,980; 7,666,854; and 8,088,746).

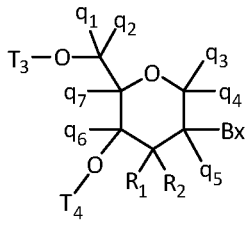
In certain embodiments, modified sugar moieties are sugar surrogates. In certain such embodiments, the oxygen atom of the sugar moiety is replaced, *e.g.,* with a sulfur, carbon or nitrogen atom. In certain such embodiments, such modified sugar moieties also comprise bridging and/or non-bridging substituents as described above. For example, certain sugar surrogates comprise a 4'-sulfur atom and a substitution at the 2'-position (*see*, *e.g.,* US2005/0130923) and/or the 5' position.

In certain embodiments, sugar surrogates comprise rings having other than 5 atoms. For example, in certain embodiments, a sugar surrogate comprises a six-membered tetrahydropyran ("THP"). Such tetrahydropyrans may be further modified or substituted. Nucleosides comprising such modified tetrahydropyrans include but are not limited to hexitol nucleic acid ("HNA"), anitol nucleic acid ("ANA"), manitol nucleic acid ("MNA") (*see* Leumann, CJ. *Bioorg. & Med. Chem.* 2002, *10*, 841-854), fluoro HNA:



F-HNA

("F-HNA", *see e.g.,* US Patents 8,088,904; 8,440,803; and 8,796,437, F-HNA can also be referred to as a F-THP or 3'-fluoro tetrahydropyran), and nucleosides comprising additional modified THP compounds having the formula:



wherein, independently, for each of said modified THP nucleoside:

Bx is a nucleobase moiety;

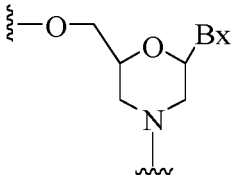
T_3 and T_4 are each, independently, an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide or one of T_3 and T_4 is an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide and the other of T_3 and T_4 is H, a hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group;

q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each, independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, or substituted C_2-C_6 alkynyl; and

each of R_1 and R_2 is independently selected from among: hydrogen, halogen, substituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$, and CN, wherein X is O, S or NJ_1, and each J_1, J_2, and J_3 is, independently, H or C_1-C_6 alkyl.

In certain embodiments, modified THP nucleosides are provided wherein q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is other than H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is methyl. In certain embodiments, modified THP nucleosides are provided wherein one of R_1 and R_2 is F. In certain embodiments, R_1 is F and R_2 is H, in certain embodiments, R_1 is methoxy and R_2 is H, and in certain embodiments, R_1 is methoxyethoxy and R_2 is H.

In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example, nucleosides comprising morpholino sugar moieties and their use in oligonucleotides have been reported (*see, e.g.,* Braasch et al., Biochemistry, 2002, *41*, 4503-4510 and U.S. Patents 5,698,685; 5,166,315; 5,185,444; and 5,034,506). As used here, the term "morpholino" means a sugar surrogate having the following structure:



In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are refered to herein as "modifed morpholinos."

In certain embodiments, sugar surrogates comprise acyclic moieites. Examples of nucleosides and oligonucleotieds comprising such acyclic sugar surrogates include but are not limited to: peptide nucleic acid ("PNA"), acyclic butyl nucleic acid (*see, e.g.,* Kumar et al., *Org. Biomol. Chem.*, 2013, *11*, 5853-5865), and nucleosides and oligonucleotides described in WO2011/133876.

Many other bicyclic and tricyclic sugar and sugar surrogate ring systems are known in the art that can be used in modified nucleosides (*see, e.g.,* Leumann, J. C, *Bioorganic & Medicinal Chemistry*, 2002, *10*, 841-854).

Modified Nucleobases

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 can impart nuclease stability, binding affinity or some other beneficial biological property to antisense compounds.

In certain embodiments, modified oligonucleotides comprise one or more nucleoside comprising an unmodified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleoside comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleoside that does not comprise a nucleobase, referred to as an abasic nucleoside.

In certain embodiments, modified nucleobases are selected from: 5-substituted pyrimidines, 6-azapyrimi¬dines, alkyl or alkynyl substituted pyrimidines, alkyl substituted purines, and N-2, N-6 and O-6 substituted purines. In certain embodiments, modified nucleobases are selected from: 2-aminopropyladenine, 5-hydroxymethyl cytosine, 5-methylcytosine, xanthine, hypoxanthine, 2-

aminoadenine, 6-N-methylguanine, 6-N-methyladenine, 2-propyladenine , 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl (C≡C-CH3) uracil, 5-propynylcytosine, 6-azouracil, 6-azocytosine, 6-azothymine, 5-ribosyluracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-aza and other 8-substituted purines, 5-halo, particularly 5-bromo, 5-trifluoromethyl, 5-halouracil, and 5-halocytosine, 7-methylguanine, 7-methyladenine, 2-F-adenine, 2-aminoadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 6-N-benzoyladenine, 2-N-isobutyrylguanine, 4-N-benzoylcytosine, 4-N-benzoyluracil, 5-methyl 4-N-benzoylcytosine, 5-methyl 4-N-benzoyluracil, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. Further modified nucleobases include tricyclic pyrimidines, such as 1,3-diazaphenoxazine-2-one, 1,3-diazaphenothiazine-2-one and 9-(2-aminoethoxy)-1,3-diazaphenoxazine-2-one (G-clamp). Modified nucleobases may also 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. Further nucleobases include those disclosed in United States Patent No. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613; Sanghvi, Y.S., Chapter 15, Antisense Research and Applications, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288; and those disclosed in Chapters 6 and 15, Antisense Drug Technology, Crooke S.T., Ed., CRC Press, 2008, 163-166 and 442-443.

Publications that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include without limitation, Manoharan et al., US2003/0158403, Manoharan et al., US2003/0175906; Dinh et al., U.S. 4,845,205; Spielvogel et al., U.S. 5,130,302; Rogers et al., U.S. 5,134,066; Bischofberger et al., U.S. 5,175,273; Urdea et al., U.S. 5,367,066; Benner et al., U.S. 5,432,272; Matteucci et al., U.S. 5,434,257; Gmeiner et al., U.S. 5,457,187; Cook et al., U.S. 5,459,255; Froehler et al., U.S. 5,484,908; Matteucci et al., U.S. 5,502,177; Hawkins et al., U.S. 5,525,711; Haralambidis et al., U.S. 5,552,540; Cook et al., U.S. 5,587,469; Froehler et al., U.S. 5,594,121; Switzer et al., U.S. 5,596,091; Cook et al., U.S. 5,614,617; Froehler et al., U.S. 5,645,985; Cook et al., U.S. 5,681,941; Cook et al., U.S. 5,811,534; Cook et al., U.S. 5,750,692; Cook et al., U.S. 5,948,903; Cook et al., U.S. 5,587,470; Cook et al., U.S. 5,457,191; Matteucci et al., U.S. 5,763,588; Froehler et al., U.S. 5,830,653; Cook et al., U.S. 5,808,027; Cook et al., 6,166,199; and Matteucci et al., U.S. 6,005,096.

In certain embodiments, antisense compounds targeted to a KRAS nucleic acid comprise one or more modified nucleobases. In certain embodiments, the modified nucleobase is 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

Certain Motifs

Oligonucleotides can have a motif, *e.g.* a pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages. In certain embodiments, modified oligonucleotides comprise one or more modified nucleoside comprising a modified sugar. In certain embodiments, modified oligonucleotides comprise one or more modified nucleosides comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more modified internucleoside linkage. In such embodiments, the modified, unmodified, and differently modified sugar moieties, nucleobases, and/or internucleoside linkages of a modified oligonucleotide define a pattern or motif. In certain embodiments, the patterns of sugar moieties, nucleobases, and internucleoside linkages are each independent of one another. Thus, a modified oligonucleotide may be described by its sugar motif, nucleobase motif and/or internucleoside linkage motif (as used herein, nucleobase motif describes the modifications to the nucleobases independent of the sequence of nucleobases).

1. Certain Sugar Motifs

In certain embodiments, oligonucleotides comprise one or more type of modified sugar and/or unmodified sugar moiety arranged along the oligonucleotide or region thereof in a defined pattern or sugar motif. In certain instances, such sugar motifs include but are not limited to any of the sugar modifications discussed herein.

In certain embodiments, modified oligonucleotides comprise or consist of a region having a gapmer motif, which comprises two external regions or "wings" and a central or internal region or "gap." The three regions of a gapmer motif (the 5'-wing, the gap, and the 3'-wing) form a contiguous sequence of nucleosides wherein at least some of the sugar moieties of the nucleosides of each of the wings differ from at least some of the sugar moieties of the nucleosides of the gap. Specifically, at least the sugar moieties of the nucleosides of each wing that are closest to the gap (the 3'-most nucleoside of the 5'-wing and the 5'-most nucleoside of the 3'-wing) differ from the sugar moiety of the neighboring gap nucleosides, thus defining the boundary between the wings and the gap (i.e., the wing/gap junction). In certain embodiments, the sugar moieties within the gap are the same as one another. In certain embodiments, the gap includes one or more nucleoside having a sugar moiety that differs from the sugar moiety of one or more other nucleosides of the gap. In certain embodiments, the sugar motifs of the two wings are the same as one another (symmetric gapmer). In certain embodiments, the sugar motif of the 5'-wing differs from the sugar motif of the 3'-wing (asymmetric gapmer).

In certain embodiments, the wings of a gapmer comprise 1-5 nucleosides. In certain embodiments, the wings of a gapmer comprise 2-5 nucleosides. In certain embodiments, the wings of a gapmer comprise 3-5 nucleosides. In certain embodiments, the nucleosides of a gapmer are all modified nucleosides.

In certain embodiments, the gap of a gapmer comprises 7-12 nucleosides. In certain embodiments, the gap of a gapmer comprises 7-10 nucleosides. In certain embodiments, the gap of a gapmer comprises 8-10 nucleosides. In certain embodiments, the gap of a gapmer comprises 10 nucleosides. In certain embodiment, each nucleoside of the gap of a gapmer is an unmodified 2'-deoxy nucleoside.

In certain embodiments, the gapmer is a deoxy gapmer. In such embodiments, the nucleosides on the gap side of each wing/gap junction are unmodified 2'-deoxy nucleosides and the nucleosides on the wing sides of each wing/gap junction are modified nucleosides. In certain such embodiments, each nucleoside of the gap is an unmodified 2'-deoxy nucleoside. In certain such embodiments, each nucleoside of each wing is a modified nucleoside.

In certain embodiments, modified oligonucleotides comprise or consist of a region having a fully modified sugar motif. In such embodiments, each nucleoside of the fully modified region of the modified oligonucleotide comprises a modified sugar moiety. In certain such embodiments, each nucleoside to the entire modified oligonucleotide comprises a modified sugar moiety. In certain embodiments, modified oligonucleotides comprise or consist of a region having a fully modified sugar motif, wherein each nucleoside within the fully modified region comprises the same modified sugar moiety, referred to herein as a uniformly modified sugar motif. In certain embodiments, a fully modified oligonucleotide is a uniformly modified oligonucleotide. In certain embodiments, each nucleoside of a uniformly modified comprises the same 2'-modification.

2. Certain Nucleobase Motifs

In certain embodiments, oligonucleotides comprise modified and/or unmodified nucleobases arranged along the oligonucleotide or region thereof in a defined pattern or motif. In certain embodiments, each nucleobase is modified. In certain embodiments, none of the nucleobases are modified. In certain embodiments, each purine or each pyrimidine is modified. In certain embodiments, each adenine is modified. In certain embodiments, each guanine is modified. In certain embodiments, each thymine is modified. In certain embodiments, each uracil is modified. In certain embodiments, each cytosine is modified. In certain embodiments, some or all of the cytosine nucleobases in a modified oligonucleotide are 5-methylcytosines.

In certain embodiments, modified oligonucleotides comprise a block of modified nucleobases. In certain such embodiments, the block is at the 3'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleosides of the 3'-end of the oligonucleotide. In certain embodiments, the block is at the 5'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleosides of the 5'-end of the oligonucleotide.

In certain embodiments, oligonucleotides having a gapmer motif comprise a nucleoside

comprising a modified nucleobase. In certain such embodiments, one nucleoside comprising a modified nucleobase is in the central gap of an oligonucleotide having a gapmer motif. In certain such embodiments, the sugar moiety of said nucleoside is a 2'-deoxyribosyl moiety. In certain embodiments, the modified nucleobase is selected from: a 2-thiopyrimidine and a 5-propynepyrimidine.

3. Certain Internucleoside Linkage Motifs

In certain embodiments, oligonucleotides comprise modified and/or unmodified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or motif. In certain embodiments, essentially each internucleoside linking group is a phosphate internucleoside linkage (P=O). In certain embodiments, each internucleoside linking group of a modified oligonucleotide is a phosphorothioate (P=S). In certain embodiments, each internucleoside linking group of a modified oligonucleotide is independently selected from a phosphorothioate and phosphate internucleoside linkage. In certain embodiments, the sugar motif of a modified oligonucleotide is a gapmer and the internucleoside linkages within the gap are all modified. In certain such embodiments, some or all of the internucleoside linkages in the wings are unmodified phosphate linkages. In certain embodiments, the terminal internucleoside linkages are modified.

Certain Oligonucleotides

In certain embodiments, oligonucleotides are characterized by their motifs and overall lengths. In certain embodiments, such parameters are each independent of one another. Thus, unless otherwise indicated, each internucleoside linkage of an oligonucleotide having a gapmer motif may be modified or unmodified and may or may not follow the gapmer modification pattern of the sugar modifications. For example, the internucleoside linkages within the wing regions of a gapmer may be the same or different from one another and may be the same or different from the internucleoside linkages of the gap region. Likewise, such gapmer oligonucleotides may comprise one or more modified nucleobase independent of the gapmer pattern of the sugar modifications. Furthermore, unless otherwise indicated, each internucleoside linkage and each nucleobase of a fully modified oligonucleotide may be modified or unmodified. One of skill in the art will appreciate that such motifs may be combined to create a variety of oligonucleotides. Herein, if a description of an oligonucleotide is silent with respect to one or more parameter, such parameter is not limited. Thus, a modified oligonucleotide described only as having a gapmer motif without further description may have any length, internucleoside linkage motif, and nucleobase motif. Unless otherwise indicated, all modifications are independent of nucleobase sequence.

In certain embodiments, oligonucleotides have a nucleobase sequence that is complementary to a second oligonucleotide or a target nucleic acid. In certain such embodiments, a region of an oligonucleotide has a nucleobase sequence that is complementary to a second oligonucleotide or a target

nucleic acid. In certain embodiments, the nucleobase sequence of a region or entire length of an oligonucleotide is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% complementary to the second oligonucleotide or target nucleic acid. In certain embodiments, antisense compounds comprise two oligomeric compounds, wherein the two oligonucleotides of the oligomeric compounds are at least 80%, at least 90%, or 100% complementary to each other. In certain embodiments, one or both oligonucleotides of a double-stranded antisense compound comprise two nucleosides that are not complementary to the other oligonucleotide.

Certain Conjugate Groups and Terminal Groups

In certain embodiments, antisense compounds and oligomeric compounds comprise conjugate groups and/or terminal groups. In certain such embodiments, oligonucleotides are covalently attached to one or more conjugate group. In certain embodiments, conjugate groups modify one or more properties of the attached oligonucleotide, including but not limited to pharmacodynamics, pharmacokinetics, stability, binding, absorption, cellular distribution, cellular uptake, charge and clearance. In certain embodiments, conjugate groups impart a new property on the attached oligonucleotide, *e.g.*, fluorophores or reporter groups that enable detection of the oligonucleotide. Conjugate groups and/or terminal groups may be added to oligonucleotides having any of the modifications or motifs described above. Thus, for example, an antisense compound or oligomeric compound comprising an oligonucleotide having a gapmer motif may also comprise a conjugate group.

Conjugate groups include, without limitation, intercalators, reporter molecules, polyamines, polyamides, peptides, carbohydrates, vitamin moieties, polyethylene glycols, thioethers, polyethers, cholesterols, thiocholesterols, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins, fluorophores, and dyes. Certain conjugate groups have been described previously, for example: cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, *4*, 1053-1060), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, *660*, 306-309; Manoharan et al., *Bioorg. Med. Chem. Let.*, 1993, *3*, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, *20*, 533-538), an aliphatic chain, *e.g.*, do-decan-diol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, *10*, 1111-1118; Kabanov et al., *FEBS Lett.*, 1990, *259*, 327-330; Svinarchuk et al., *Biochimie*, 1993, *75*, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, *36*, 3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, *18*, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, *14*, 969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, *36*, 3651-3654), a

palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, *1264,* 229-237), an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.,* 1996, *277*, 923-937), a tocopherol group (Nishina et al., *Molecular Therapy Nucleic Acids*, 2015, *4*, e220; doi:10.1038/mtna.2014.72 and Nishina et al., *Molecular Therapy,* 2008, *16*, 734-740), or a GalNAc cluster (*e.g.*, WO2014/179620).

In certain embodiments, a conjugate group comprises an active drug substance, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fen-bufen, ketoprofen, (*S*)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, fingolimod, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indo-methicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

Conjugate groups are attached directly or via an optional conjugate linker to a parent compound, such as an oligonucleotide. In certain embodiments, conjugate groups are directly attached to oligonucleotides. In certain embodiments, conjugate groups are indirectly attached to oligonucleotides via conjugate linkers. In certain embodiments, the conjugate linker comprises a chain structure, such as a hydrocarbyl chain, or an oligomer of repeating units such as ethylene glycol or amino acid units. In certain embodiments, conjugate groups comprise a cleavable moiety. In certain embodiments, conjugate groups are attached to oligonucleotides via a cleavable moiety. In certain embodiments, conjugate linkers comprise a cleavable moiety. In certain such embodiments, conjugate linkers are attached to oligonucleotides via a cleavable moiety. In certain embodiments, oligonucleotides comprise a cleavable moiety, wherein the cleavable moiety is a nucleoside is attached to a cleavable internucleoside linkage, such as a phosphate internucleoside linakge. In certain embodiments, a conjugate group comprises a nucleoside or oligonucleotide, wherein the nucleoside or oligonucleotide of the conjugate group is indirectly attached to a parent oligonucleotide.

In certain embodiments, a conjugate linker comprises one or more groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether, and hydroxylamino. In certain such embodiments, the conjugate linker comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and amide groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and ether groups. In certain embodiments, the conjugate linker comprises at least one phosphorus moiety. In certain embodiments, the conjugate linker comprises at least one phosphate group. In certain embodiments, the conjugate linker includes at least one neutral linking group.

In certain embodiments, conjugate linkers, including the conjugate linkers described above, are bifunctional linking moieties, *e.g.,* those known in the art to be useful for attaching conjugate groups to parent compounds, such as the oligonucleotides provided herein. In general, a bifunctional linking moiety

comprises at least two functional groups. One of the functional groups is selected to bind to a particular site on a parent compound and the other is selected to bind to a conjugate group. Examples of functional groups used in a bifunctional linking moiety include but are not limited to electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In certain embodiments, bifunctional linking moieties comprise one or more groups selected from amino, hydroxyl, carboxylic acid, thiol, alkyl, alkenyl, and alkynyl.

Examples of conjugate linkers include but are not limited to pyrrolidine, 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and 6-aminohexanoic acid (AHEX or AHA). Other conjugate linkers include but are not limited to substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl or substituted or unsubstituted C_2-C_{10} alkynyl, wherein a nonlimiting list of preferred substituent groups includes hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

In certain embodiments, a cleavable moiety is a cleavable bond. In certain embodiments, a cleavable moiety comprises a cleavable bond. In certain embodiments, a cleavable moiety is a group of atoms comprising at least one cleavable bond. In certain embodiments, a cleavable moiety comprises a group of atoms having one, two, three, four, or more than four cleavable bonds. In certain embodiments, a cleavable moiety is selectively cleaved inside a cell or subcellular compartment, such as a lysosome. In certain embodiments, a cleavable moiety is selectively cleaved by endogenous enzymes, such as nucleases.

In certain embodiments, a cleavable bond is selected from among: an amide, an ester, an ether, one or both esters of a phosphodiester, a phosphate ester, a carbamate, or a disulfide. In certain embodiments, a cleavable bond is one or both of the esters of a phosphodiester. In certain embodiments, a cleavable moiety comprises a phosphate or phosphodiester. In certain embodiments, the cleavable moiety is a phosphate linkage between an oligonucleotide and a conjugate linker or conjugate group.

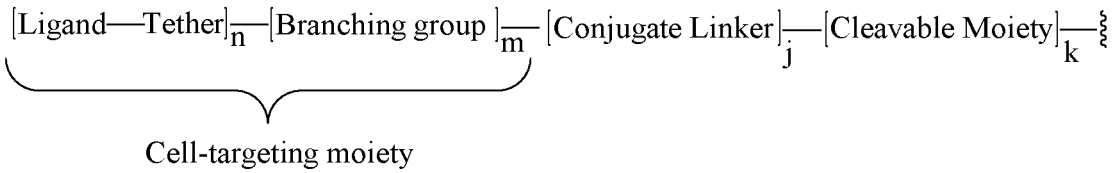
In certain embodiments, a cleavable moiety is a nucleoside. In certain such embodiments, the unmodified or modified nucleoside comprises an optionally protected heterocyclic base selected from a purine, substituted purine, pyrimidine or substituted pyrimidine. In certain embodiments, a cleavable moiety is a nucleoside selected from uracil, thymine, cytosine, 4-N-benzoylcytosine, 5-methylcytosine, 4-N-benzoyl-5-methylcytosine, adenine, 6-N-benzoyladenine, guanine and 2-N-isobutyrylguanine. In certain embodiments, a cleavable moiety is 2'-deoxy nucleoside that is attached to either the 3' or 5'-terminal nucleoside of an oligonucleotide by a phosphate internucleoside linkage and covalently attached to the conjugate linker or conjugate group by a phosphate or phosphorothioate linkage. In certain such embodiments, the cleavable moiety is 2'-deoxyadenosine.

Conjugate groups may be attached to either or both ends of an oligonucleotide and/or at any

internal position. In certain embodiments, conjugate groups are attached to the 2'-position of a nucleoside of a modified oligonucleotide. In certain embodiments, conjugate groups that are attached to either or both ends of an oligonucleotide are terminal groups. In certain such embodiments, conjugate groups or terminal groups are attached at the 3' and/or 5'-end of oligonucleotides. In certain such embodiments, conjugate groups (or terminal groups) are attached at the 3'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 3'-end of oligonucleotides. In certain embodiments, conjugate groups (or terminal groups) are attached at the 5'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 5'-end of oligonucleotides.

Examples of terminal groups include but are not limited to conjugate groups, capping groups, phosphate moieties, protecting groups, modified or unmodified nucleosides, and two or more nucleosides that are independently modified or unmodified.

In certain embodiments, a conjugate group is a cell-targeting moiety. In certain embodiments, a conjugate group, optional conjugate linker, and optional cleavable moiety have the general formula:



wherein n is from 1 to about 3, m is 0 when n is 1, m is 1 when n is 2 or greater, j is 1 or 0, and k is 1 or 0.

In certain embodiments, n is 1, j is 1 and k is 0. In certain embodiments, n is 1, j is 0 and k is 1. In certain embodiments, n is 1, j is 1 and k is 1. In certain embodiments, n is 2, j is 1 and k is 0. In certain embodiments, n is 2, j is 0 and k is 1. In certain embodiments, n is 2, j is 1 and k is 1. In certain embodiments, n is 3, j is 1 and k is 0. In certain embodiments, n is 3, j is 0 and k is 1. In certain embodiments, n is 3, j is 1 and k is 1.

In certain embodiments, conjugate groups comprise cell-targeting moieties that have at least one tethered ligand. In certain embodiments, cell-targeting moieties comprise two tethered ligands covalently attached to a branching group. In certain embodiments, cell-targeting moieties comprise three tethered ligands covalently attached to a branching group.

In certain embodiments, the cell-targeting moiety comprises a branching group comprising one or more groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether and hydroxylamino groups. In certain embodiments, the branching group comprises a branched aliphatic group comprising groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether,

thioether and hydroxylamino groups. In certain such embodiments, the branched aliphatic group comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain such embodiments, the branched aliphatic group comprises groups selected from alkyl, amino and ether groups. In certain such embodiments, the branched aliphatic group comprises groups selected from alkyl and ether groups. In certain embodiments, the branching group comprises a mono or polycyclic ring system.

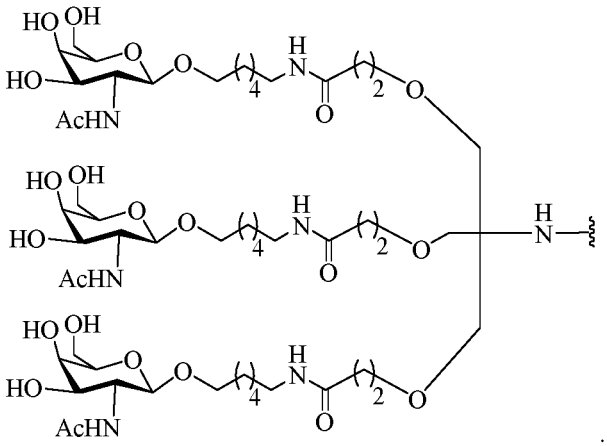
In certain embodiments, each tether of a cell-targeting moiety comprises one or more groups selected from alkyl, substituted alkyl, ether, thioether, disulfide, amino, oxo, amide, phosphodiester, and polyethylene glycol, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether, thioether, disulfide, amino, oxo, amide, and polyethylene glycol, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, phosphodiester, ether, amino, oxo, and amide, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether, amino, oxo, and amid, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, amino, and oxo, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl and oxo, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl and phosphodiester, in any combination. In certain embodiments, each tether comprises at least one phosphorus linking group or neutral linking group. In certain embodiments, each tether comprises a chain from about 6 to about 20 atoms in length. In certain embodiments, each tether comprises a chain from about 10 to about 18 atoms in length. In certain embodiments, each tether comprises about 10 atoms in chain length.

In certain embodiments, each ligand of a cell-targeting moiety has an affinity for at least one type of receptor on a target cell. In certain embodiments, each ligand has an affinity for at least one type of receptor on the surface of a mammalian liver cell. In certain embodiments, each ligand has an affinity for the hepatic asialoglycoprotein receptor (ASGP-R). In certain embodiments, each ligand is a carbohydrate. In certain embodiments, each ligand is, independently selected from galactose, N-acetyl galactoseamine (GalNAc), mannose, glucose, glucoseamine and fucose. In certain embodiments, each ligand is N-acetyl galactoseamine (GalNAc). In certain embodiments, the cell-targeting moiety comprises 3 GalNAc ligands. In certain embodiments, the cell-targeting moiety comprises 2 GalNAc ligands. In certain embodiments, the cell-targeting moiety comprises 1 GalNAc ligand.

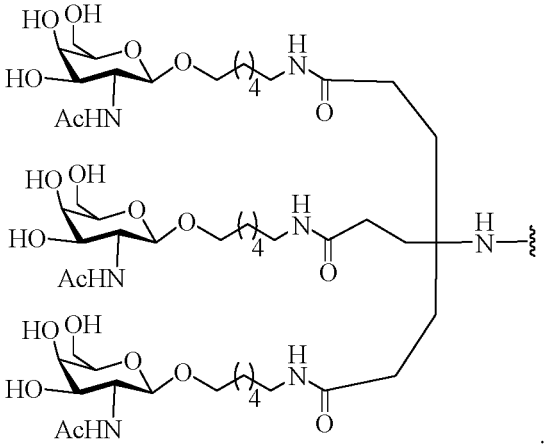
In certain embodiments, each ligand of a cell-targeting moiety is a carbohydrate, carbohydrate derivative, modified carbohydrate, polysaccharide, modified polysaccharide, or polysaccharide derivative. In certain such embodiments, the conjugate group comprises a carbohydrate cluster (*see*, e.g., Maier et al., "Synthesis of Antisense Oligonucleotides Conjugated to a Multivalent Carbohydrate Cluster for Cellular

Targeting," *Bioconjugate Chemistry*, 2003, *14*, 18-29, or Rensen et al., "Design and Synthesis of Novel *N*-Acetylgalactosamine-Terminated Glycolipids for Targeting of Lipoproteins to the Hepatic Asiaglycoprotein Receptor," *J. Med. Chem.* 2004, *47*, 5798-5808, which are incorporated herein by reference in their entirety). In certain such embodiments, each ligand is an amino sugar or a thio sugar. For example, amino sugars may be selected from any number of compounds known in the art, such as sialic acid, α-D-galactosamine, β-muramic acid, 2-deoxy-2-methylamino-L-glucopyranose, 4,6-dideoxy-4-formamido-2,3-di-*O*-methyl-D-mannopyranose, 2-deoxy-2-sulfoamino-D-glucopyranose and *N*-sulfo-D-glucosamine, and *N*-glycoloyl-α-neuraminic acid. For example, thio sugars may be selected from 5-Thio-β-D-glucopyranose, methyl 2,3,4-tri-*O*-acetyl-1-thio-6-*O*-trityl-α-D-glucopyranoside, 4-thio-β-D-galactopyranose, and ethyl 3,4,6,7-tetra-*O*-acetyl-2-deoxy-1,5-dithio-α-D-*gluco*-heptopyranoside.

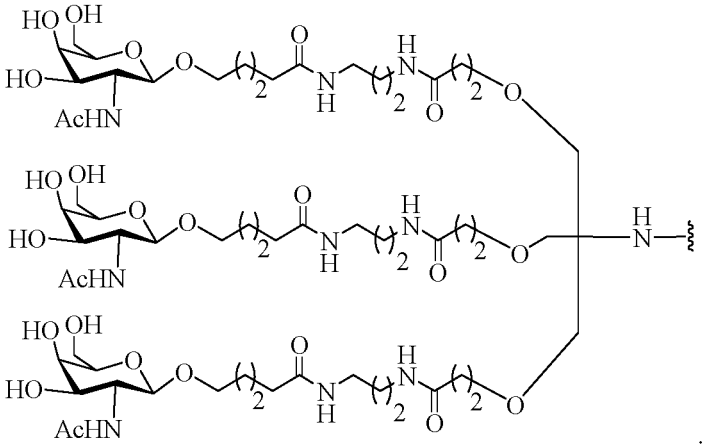
In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:



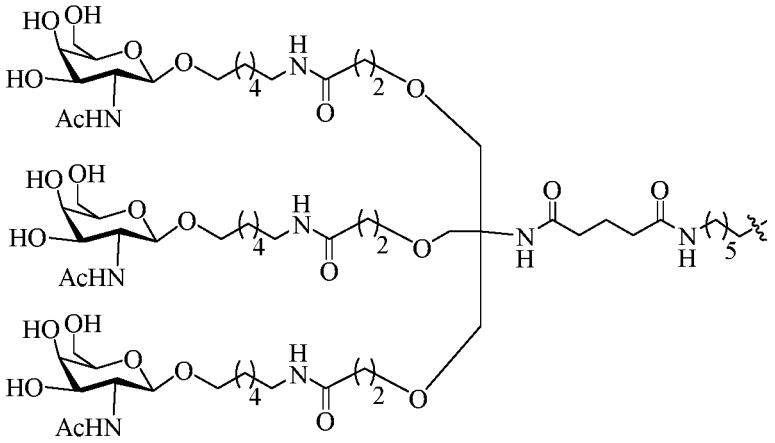
In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:



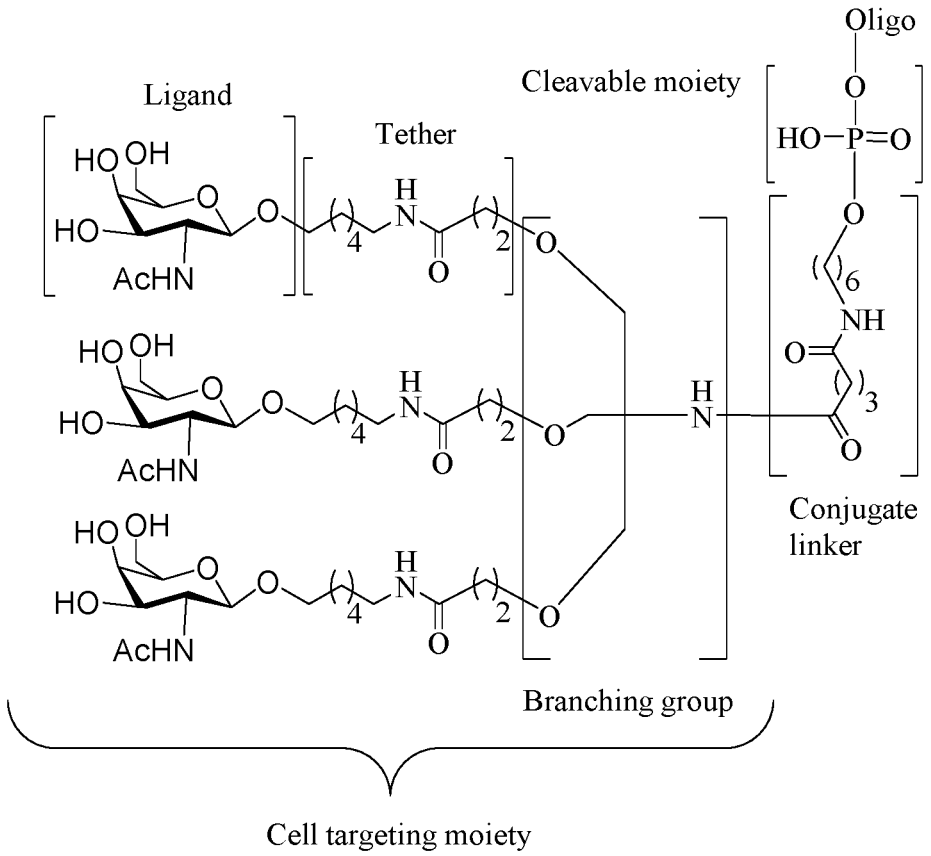
In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:



In certain embodiments, antisense compounds and oligomeric compounds comprise a conjugate group and conjugate linker described herein as "LICA-1". LICA-1 has the formula:



In certain embodiments, antisense compounds and oligomeric compounds comprising LICA-1 have the formula:



wherein oligo is an oligonucleotide.

Representative publications that teach the preparation of certain of the above noted conjugate groups, oligomeric compounds and antisense compounds comprising conjugate groups, tethers, conjugate linkers, branching groups, ligands, cleavable moieties as well as other modifications include without limitation, US 5,994,517, US 6,300,319, US 6,660,720, US 6,906,182, US 7,262,177, US 7,491,805, US 8,106,022, US 7,723,509, US 2006/0148740, US 2011/0123520, WO 2013/033230 and WO 2012/037254, Biessen et al., *J. Med. Chem.* 1995, *38*, 1846-1852, Lee et al., *Bioorganic & Medicinal Chemistry* 2011,*19*, 2494-2500, Rensen et al., *J. Biol. Chem.* 2001, *276*, 37577-37584, Rensen et al., *J. Med. Chem.* 2004, *47*, 5798-5808, Sliedregt et al., *J. Med. Chem.* 1999, *42*, 609-618, and Valentijn et al., *Tetrahedron,* 1997, *53,* 759-770, each of which is incorporated by reference herein in its entirety.

In certain embodiments, antisense compounds and oligomeric compounds comprise modified oligonucleotides comprising a gapmer or fully modified motif and a conjugate group comprising at least one, two, or three GalNAc ligands. In certain embodiments antisense compounds and oligomeric

compounds comprise a conjugate group found in any of the following references: Lee, *Carbohydr Res*, 1978, 67, 509-514; Connolly et al., *J Biol Chem*, 1982, 257, 939-945; Pavia et al., *Int J Pep Protein Res*, 1983, 22, 539-548; Lee et al., *Biochem*, 1984, 23, 4255-4261; Lee et al., *Glycoconjugate J*, 1987, 4, 317-328; Toyokuni et al., *Tetrahedron Lett*, 1990, 31, 2673-2676; Biessen et al., *J Med Chem*, 1995, 38, 1538-1546; Valentijn et al., *Tetrahedron*, 1997, 53, 759-770; Kim et al., *Tetrahedron Lett*, 1997, 38, 3487-3490; Lee et al., *Bioconjug Chem*, 1997, 8, 762-765; Kato et al., *Glycobiol*, 2001, 11, 821-829; Rensen et al., *J Biol Chem*, 2001, 276, 37577-37584; Lee et al., *Methods Enzymol*, 2003, 362, 38-43; Westerlind et al., *Glycoconj J*, 2004, 21, 227-241; Lee et al., *Bioorg Med Chem Lett*, 2006, 16(19), 5132-5135; Maierhofer et al., *Bioorg Med Chem*, 2007, 15, 7661-7676; Khorev et al., *Bioorg Med Chem*, 2008, 16, 5216-5231; Lee et al., *Bioorg Med Chem*, 2011, 19, 2494-2500; Kornilova et al., Analyt Biochem, 2012, 425, 43-46; Pujol et al., *Angew Chemie Int Ed Engl*, 2012, 51, 7445-7448; Biessen et al., *J Med Chem*, 1995, 38, 1846-1852; Sliedregt et al., *J Med Chem*, 1999, 42, 609-618; Rensen et al., *J Med Chem*, 2004, 47, 5798-5808; Rensen et al., *Arterioscler Thromb Vasc Biol*, 2006, 26, 169-175; van Rossenberg et al., *Gene Ther*, 2004, 11, 457-464; Sato et al., *J Am Chem Soc*, 2004, 126, 14013-14022; Lee et al., *J Org Chem*, 2012, 77, 7564-7571; Biessen et al., *FASEB J*, 2000, 14, 1784-1792; Rajur et al., *Bioconjug Chem*, 1997, 8, 935-940; Duff et al., *Methods Enzymol*, 2000, 313, 297-321; Maier et al., *Bioconjug Chem*, 2003, 14, 18-29; Jayaprakash et al., *Org Lett*, 2010, 12, 5410-5413; Manoharan, *Antisense Nucleic Acid Drug Dev*, 2002, 12, 103-128; Merwin et al., *Bioconjug Chem*, 1994, 5, 612-620; Tomiya et al., Bioorg Med Chem, 2013, 21, 5275-5281; International applications WO1998/013381; WO2011/038356; WO1997/046098; WO2008/098788; WO2004/101619; WO2012/037254; WO2011/120053; WO2011/100131; WO2011/163121; WO2012/177947; WO2013/033230; WO2013/075035; WO2012/083185; WO2012/083046; WO2009/082607; WO2009/134487; WO2010/144740; WO2010/148013; WO1997/020563; WO2010/088537; WO2002/043771; WO2010/129709; WO2012/068187; WO2009/126933; WO2004/024757; WO2010/054406; WO2012/089352; WO2012/089602; WO2013/166121; WO2013/165816; U.S. Patents 4,751,219; 8,552,163; 6,908,903; 7,262,177; 5,994,517; 6,300,319; 8,106,022; 7,491,805; 7,491,805; 7,582,744; 8,137,695; 6,383,812; 6,525,031; 6,660,720; 7,723,509; 8,541,548; 8,344,125; 8,313,772; 8,349,308; 8,450,467; 8,501,930; 8,158,601; 7,262,177; 6,906,182; 6,620,916; 8,435,491; 8,404,862; 7,851,615; Published U.S. Patent Application Publications US2011/0097264; US2011/0097265; US2013/0004427; US2005/0164235; US2006/0148740; US2008/0281044; US2010/0240730; US2003/0119724; US2006/0183886; US2008/0206869; US2011/0269814; US2009/0286973; US2011/0207799; US2012/0136042; US2012/0165393; US2008/0281041; US2009/0203135; US2012/0035115; US2012/0095075; US2012/0101148; US2012/0128760; US2012/0157509; US2012/0230938; US2013/0109817;

US2013/0121954; US2013/0178512; US2013/0236968; US2011/0123520; US2003/0077829; US2008/0108801; and US2009/0203132; each of which is incorporated by reference in its entirety.

Compositions and Methods for Formulating Pharmaceutical Compositions

Compounds may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

In certain embodiments, the present invention provides pharmaceutical compositions comprising one or more compounds or a salt thereof. In certain such embodiments, the pharmaceutical composition comprises a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutical composition comprises a sterile saline solution and one or more compounds. In certain embodiments, such pharmaceutical composition consists of a sterile saline solution and one or more compounds. In certain embodiments, the sterile saline is pharmaceutical grade saline. In certain embodiments, a pharmaceutical composition comprises one or more antisense compound and sterile water. In certain embodiments, a pharmaceutical composition consists of one compounds and sterile water. In certain embodiments, the sterile water is pharmaceutical grade water. In certain embodiments, a pharmaceutical composition comprises one or more compounds and phosphate-buffered saline (PBS). In certain embodiments, a pharmaceutical composition consists of one or more compounds and sterile PBS. In certain embodiments, the sterile PBS is pharmaceutical grade PBS. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

A compound targeted to KRAS nucleic acid can be utilized in pharmaceutical compositions by combining the compound with a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutically acceptable diluent is water, such as sterile water suitable for injection. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising a compound targeted to KRAS nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is water. In certain embodiments, the compound is an antisense oligonucleotide provided herein.

Pharmaceutical compositions comprising compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other

bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A prodrug can include the incorporation of additional nucleosides at one or both ends of a compound which are cleaved by endogenous nucleases within the body, to form the active compound.

In certain embodiments, the compounds or compositions further comprise a pharmaceutically acceptable carrier or diluent.

EXAMPLES

The Examples below describe the screening process to identify lead compounds targeted to KRAS. Approximately 2,000 newly designed compounds were tested for their effect on human KRAS mRNA. New compounds were compared with a previously described compound, ISIS 6957, which was reported as one of the most potent antisense compounds in US Patent No. 6,784,290. Out of over 2,000 antisense oligonucleotides that were screened, ISIS # 651530, 651987, 695785, 695823, 651555, 651587, 695980, 695995, 696018, 696044, 716600, 746275, 716655, 716772, 740179, 740191, 740201, 740223, and 740233 emerged as the top lead compounds.

Non-limiting disclosure and incorporation by reference

Although the sequence listing accompanying this filing identifies each sequence as either "RNA" or "DNA" as required, in reality, those sequences may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as "RNA" or "DNA" to describe modified oligonucleotides is, in certain instances, arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) for natural uracil of RNA).

Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, an oligonucleotide having the nucleobase sequence "ATCGATCG" encompasses any oligonucleotides having such nucleobase sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence "AUCGAUCG" and those having some DNA bases and some RNA bases such as "AUCGATCG".

While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the

compounds described herein and are not intended to limit the same. Each of the references recited in the present application is incorporated herein by reference in its entirety.

Example 1: Antisense inhibition of human K-Ras in SKOV3 cells by cEt gapmers

Antisense oligonucleotides were designed targeting a K-Ras nucleic acid and were tested for their effects on K-Ras mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured SKOV3 cells at a density of 20,000 cells per well were transfected using electroporation with 2,500 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS246 (forward sequence CCCAGGTGCGGGAGAGA, designated herein as SEQ ID NO: 4; reverse sequence GCTGTATCGTCAAGGCACTCTTG; designated herein as SEQ ID NO: 5; probe sequence CTTGTGGTAGTTGGAGCTGGTGGCGTAG, designated herein as SEQ ID NO: 6) was used to measure mRNA levels. K-Ras mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either a human K-Ras mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_004985.4), the human K-Ras genomic sequence, designated herein as SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_009714.17 truncated from nucleotides 18116000 to 18166000), or a human K-Ras mRNA sequence, designated herein as SEQ ID NO: 3 (GENBANK Accession No. NM_033360.3). 'N/A' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 1

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1, 2, and 3

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO: 3 Start Site	SEQ ID NO: 3 Stop Site	Sequence	% Inhibition	SEQ ID NO
540729	N/A	N/A	N/A	N/A	754	769	ACCCAGATTACATTAT	0	13
540731	669	684	43058	43073	793	808	TCGAATTTCTCGAACT	41	14
540733	830	845	43219	43234	954	969	GCTAAAACAAATGCTA	54	15
540741	346	361	25573	25588	346	361	CGAGAATATCCAAGAG	48	16
540743	356	371	25583	25598	356	371	CCTGCTGTGTCGAGAA	66	17
540745	358	373	25585	25600	358	373	GACCTGCTGTGTCGAG	52	18
540747	466	481	25693	25708	466	481	TATAATGGTGAATATC	37	19
540749	476	491	N/A	N/A	476	491	ATTTGTTCTCTATAAT	21	20
540751	586	601	27273	27288	586	601	AACTTCTTGCTAAGTC	43	21
540753	658	673	43047	43062	782	797	GAACTAATGTATAGAA	44	22
540755	789	804	43178	43193	913	928	TACCACTTGTACTAGT	74	23
540757	868	883	43257	43272	992	1007	CTAACAGTCTGCATGG	63	24
540759	934	949	43323	43338	1058	1073	AATACTGGCACTTAGA	42	25
540761	1072	1087	43461	43476	1196	1211	TGTTTCACACCAACAT	61	26
540763	1228	1243	43617	43632	1352	1367	TGCCTAGAAGAATCAT	58	27
540765	1291	1306	43680	43695	1415	1430	GACAAAACCTTTGTGA	54	28
540767	1316	1331	43705	43720	1440	1455	CCATGACTAATAGCAG	88	29
540769	1473	1488	43862	43877	1597	1612	ATACTGGGTCTGCCTT	79	30
540771	1507	1522	43896	43911	1631	1646	GCCCCAAAATGGTTGC	55	31
540773	1526	1541	43915	43930	1650	1665	TTAGTAGCATGTAAAT	46	32
540775	1637	1652	44026	44041	1761	1776	GAAAAGATTTAAAGTT	0	33
540777	1709	1724	44098	44113	1833	1848	GCTATAACTGGCCCAA	83	34
540779	1898	1913	44287	44302	2022	2037	ACCACAGAGTGAGATT	79	35
540781	2102	2117	44491	44506	2226	2241	GTTAATTTAACCAGTG	80	36
540783	2223	2238	44612	44627	2347	2362	TGCCATCTCACTTCAT	57	37
540785	2318	2333	44707	44722	2442	2457	TAGTAAGTGATGTCCT	73	38
540787	2460	2475	44849	44864	2584	2599	GTGTAACATAGGTTAA	75	39
540789	2490	2505	44879	44894	2614	2629	CAATTTTGCCCAAGAC	42	40
540791	2542	2557	44931	44946	2666	2681	GAAGAGTCCTAAAACG	50	41
540793	2571	2586	44960	44975	2695	2710	TAGGGAGGCAAGATGA	56	42
540795	2599	2614	44988	45003	2723	2738	TGCATCAAGTCATGGG	83	43
540797	2694	2709	45083	45098	2818	2833	TAGGGCATTTCTGATG	38	44
540799	2794	2809	45183	45198	2918	2933	GAGATGTTCAAAGCAT	49	45
540801	2818	2833	45207	45222	2942	2957	GTCGCTAATGGATTGG	92	46
540803	2879	2894	45268	45283	3003	3018	TAAATTCTCCTTCCAC	49	47
540805	2957	2972	45346	45361	3081	3096	ACAATGGAATGTATTA	40	48

540807	3335	3350	45777	45792	3459	3474	CGGTGACTGGCATCTG	76	49
540809	3388	3403	N/A	N/A	3512	3527	AGGACCGGGATTATGT	70	50
540811	3428	3443	45817	45832	3552	3567	GGCCTTAGTAAGATAT	28	51
540813	3673	3688	46062	46077	3797	3812	TGAATATCTGACATAC	60	52
540815	3780	3795	46169	46184	3904	3919	CTAGTTCAGGCACCTG	65	53
540817	3871	3886	46260	46275	3995	4010	CCTACCTAAACAGTGT	21	54
540819	3896	3911	46285	46300	4020	4035	CGAGGTACTGTGTAAG	82	55
540821	3926	3941	46315	46330	4050	4065	AGTATGGCCATTTCTT	74	56
540823	3954	3969	46343	46358	4078	4093	ATCCCCTCATAAGCAC	61	57
540825	4107	4122	46496	46511	4231	4246	AATAATTAGGTAACAT	17	58
540827	4205	4220	46594	46609	4329	4344	GTCTGCTATATTCTTC	69	59
540829	4240	4255	46629	46644	4364	4379	TACTTGGGAACATTCA	63	60
540831	4276	4291	46665	46680	4400	4415	TGCAGTGTGACTCAGT	76	61
540833	4278	4293	46667	46682	4402	4417	TATGCAGTGTGACTCA	78	62
540835	4284	4299	46673	46688	4408	4423	AATTCCTATGCAGTGT	67	63
540839	4343	4358	46732	46747	4467	4482	TAGGACAAAATTGTGC	75	64
540842	4365	4380	46754	46769	4489	4504	CACAAAGTTTCTATGT	29	65
540844	4531	4546	46920	46935	4655	4670	ATCATTACTTTTTGAC	17	66
540846	4579	4594	46968	46983	4703	4718	AAGGTAACTGCTGGGT	86	67
540848	4642	4657	47031	47046	4766	4781	CTCAATGCAGAATTCA	75	68
540850	4872	4887	47261	47276	4996	5011	ACCCAGTTAGCTCTGT	51	69
540852	4910	4925	47299	47314	5034	5049	AGACAGTGGAATTGGA	63	70
540854	4964	4979	47353	47368	5088	5103	AAGAAATTGGCACTCA	64	71
540856	4966	4981	47355	47370	5090	5105	GTAAGAAATTGGCACT	66	72
540858	4998	5013	47387	47402	5122	5137	AGGTAAACATGTTACA	72	73
540860	5089	5104	47478	47493	5213	5228	TCACACTGCATATGTC	57	74
540862	5091	5106	47480	47495	5215	5230	GATCACACTGCATATG	31	75
540868	N/A	N/A	17921	17936	N/A	N/A	GCCCTTACTTATATGC	13	76
540870	N/A	N/A	20681	20696	N/A	N/A	ATCTTGCCCACTGTTT	15	77
540872	N/A	N/A	25497	25512	N/A	N/A	AGTCTGGATTATTACA	19	78
540874	N/A	N/A	25507	25522	N/A	N/A	GGAGAAACACAGTCTG	16	79
540876	N/A	N/A	25700	25715	N/A	N/A	ACCCACCTATAATGGT	13	80
540878	N/A	N/A	34485	34500	N/A	N/A	GAAGCCAATAATTAAA	27	81
540880	N/A	N/A	34495	34510	N/A	N/A	GAGAGAATTGGAAGCC	74	82
540882	N/A	N/A	35991	36006	N/A	N/A	TTAAAGCTGGTATATT	34	83
540884	N/A	N/A	37456	37471	716	731	CAGCCAGGAGTCTTTT	26	84
540886	N/A	N/A	43024	43039	N/A	N/A	TCAACACCCTGAAATA	16	85

Table 2
Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1, 2, and 3

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO: 3 Start Site	SEQ ID NO: 3 Stop Site	Sequence	% Inhibition	SEQ ID NO
540728	N/A	N/A	37402	37417	662	677	TCCCTCACCAATGTAT	0	86
540730	N/A	N/A	N/A	N/A	759	774	TCAACACCCAGATTAC	7	87
540732	673	688	43062	43077	797	812	GTTTTCGAATTTCTCG	65	88
540734	1924	1939	44313	44328	2048	2063	AATGCTCTTGATTTGT	74	89
540742	351	366	25578	25593	351	366	TGTGTCGAGAATATCC	76	90
540744	357	372	25584	25599	357	372	ACCTGCTGTGTCGAGA	65	91
540746	359	374	25586	25601	359	374	TGACCTGCTGTGTCGA	18	92
540748	471	486	N/A	N/A	471	486	TTCTCTATAATGGTGA	55	93
540750	495	510	27182	27197	495	510	AGAGTCCTTAACTCTT	24	94
540752	601	616	27288	27303	601	616	TAAAAGGAATTCCATA	19	95
540754	777	792	43166	43181	901	916	TAGTATGCCTTAAGAA	55	96
540756	810	825	43199	43214	934	949	TTTAGTGTAATGTACA	72	97
540758	933	948	43322	43337	1057	1072	ATACTGGCACTTAGAG	40	98
540760	996	1011	43385	43400	1120	1135	AAATCTTAGGTATTCA	47	99
540762	1096	1111	43485	43500	1220	1235	GATGATTCAAAAGCTT	78	100
540764	1240	1255	43629	43644	1364	1379	TATAGGACATGATGCC	67	101
540766	1304	1319	43693	43708	1428	1443	GCAGTGGAAAGGAGAC	85	102
540768	1462	1477	43851	43866	1586	1601	GCCTTAACAGGAAAAG	58	103
540770	1488	1503	43877	43892	1612	1627	AATAATCCCCATTTCA	24	104
540772	1520	1535	43909	43924	1644	1659	GCATGTAAATATAGCC	61	105
540774	1606	1621	43995	44010	1730	1745	AGTCTGACACAGGGAG	87	106
540776	1680	1695	44069	44084	1804	1819	GTCACAAGCAGAATTA	70	107
540778	1841	1856	44230	44245	1965	1980	TTTTGACTAACCAATG	33	108
540780	1910	1925	44299	44314	2034	2049	GTCAGCAGGACCACCA	79	109
540782	2132	2147	44521	44536	2256	2271	TGGATCAGACTTGAAA	80	110
540784	2263	2278	44652	44667	2387	2402	GTCACCTTCTTCCTAG	61	111
540786	2441	2456	44830	44845	2565	2580	TTTACAGATTGTGCTG	60	112
540788	2485	2500	44874	44889	2609	2624	TTGCCCAAGACTGGCA	0	113
540790	2502	2517	44891	44906	2626	2641	TCACCTCTTGCACAAT	60	114
540792	2556	2571	44945	44960	2680	2695	ACACTAATATGGAAGA	55	115
540794	2583	2598	44972	44987	2707	2722	GCATGTGGAAGGTAGG	73	116
540796	2681	2696	45070	45085	2805	2820	ATGTGACTCAGTGGGA	83	117
540798	2738	2753	45127	45142	2862	2877	TATGGTATCTGTCAGA	80	118
540800	2806	2821	45195	45210	2930	2945	TTGGGCAGCAAAGAGA	39	119
540802	2848	2863	45237	45252	2972	2987	CTATTCATACCAGGTT	74	120
540804	2944	2959	45333	45348	3068	3083	TTACTGTTACCAGGAG	81	121

540806	2981	2996	45370	45385	3105	3120	GCATGAAGATTTCTGG	91	122
540808	3376	3391	45765	45780	3500	3515	ATGTCTCTTGTTTGGG	83	123
540810	3416	3431	45805	45820	3540	3555	ATATTACAGACCACAC	52	124
540812	3627	3642	46016	46031	3751	3766	GAATCACAGTTATGCC	78	125
540814	3688	3703	46077	46092	3812	3827	ACATTTGGGTCAATAT	46	126
540816	3834	3849	46223	46238	3958	3973	ATAGCAATTCAGAAAT	18	127
540818	3883	3898	46272	46287	4007	4022	AAGTCTTAACACCCTA	68	128
540820	3908	3923	46297	46312	4032	4047	TCTGTGTAGAAACGAG	76	129
540822	3942	3957	46331	46346	4066	4081	GCACTGCAGTTCCTGA	82	130
540824	4013	4028	46402	46417	4137	4152	TAATTAACCACTACCT	6	131
540826	4167	4182	46556	46571	4291	4306	TCTATGTAATTTAGCT	65	132
540828	4220	4235	46609	46624	4344	4359	AATGATACAATATACG	33	133
540830	4261	4276	46650	46665	4385	4400	TTAAATAGAGCCTAGA	28	134
540832	4277	4292	46666	46681	4401	4416	ATGCAGTGTGACTCAG	79	135
540834	4279	4294	46668	46683	4403	4418	CTATGCAGTGTGACTC	73	136
540836	4338	4353	46727	46742	4462	4477	CAAAATTGTGCAATGG	74	137
540840	4351	4366	46740	46755	4475	4490	GTATATATTAGGACAA	37	138
540843	4455	4470	46844	46859	4579	4594	TACTGTTTGAAGAAAA	9	139
540845	4546	4561	46935	46950	4670	4685	CACAATTATCAAGAAA	18	140
540847	4641	4656	47030	47045	4765	4780	TCAATGCAGAATTCAT	67	141
540849	4655	4670	47044	47059	4779	4794	AGCTATTCAGTTTCTC	30	142
540851	4887	4902	47276	47291	5011	5026	GGATAAAACACTGTAA	58	143
540853	4956	4971	47345	47360	5080	5095	GGCACTCAAAGGAAAA	60	144
540855	4965	4980	47354	47369	5089	5104	TAAGAAATTGGCACTC	48	145
540857	4993	5008	47382	47397	5117	5132	AACATGTTACATTAAG	43	146
540859	5006	5021	47395	47410	5130	5145	TACATTCCAGGTAAAC	51	147
540861	5090	5105	47479	47494	5214	5229	ATCACACTGCATATGT	50	148
540863	5132	5147	47521	47536	5256	5271	ACATTCCTAGGTCAGC	76	149
540867	N/A	N/A	9205	9220	N/A	N/A	AAACTTCCTTTTACAT	19	150
540869	N/A	N/A	17927	17942	N/A	N/A	TACTGAGCCCTTACTT	15	151
540871	N/A	N/A	25492	25507	N/A	N/A	GGATTATTACAGTGCA	16	152
540873	N/A	N/A	25502	25517	N/A	N/A	AACACAGTCTGGATTA	6	153
540875	N/A	N/A	25695	25710	N/A	N/A	CCTATAATGGTGAATA	32	154
540877	N/A	N/A	32700	32715	N/A	N/A	GATAAATGTGAACTAG	33	155
540879	N/A	N/A	34490	34505	N/A	N/A	AATTGGAAGCCAATAA	29	156
540881	N/A	N/A	34511	34526	N/A	N/A	TGTTTCCAGCAATGCA	59	157
540883	N/A	N/A	37365	37380	N/A	N/A	GCATTGTAAAACACAA	16	158
540885	N/A	N/A	37494	37509	N/A	N/A	TACCAGATTACATTAT	0	159

Example 2: Antisense inhibition of human K-Ras in Hep3B cells by cEt gapmers

Antisense oligonucleotides were designed targeting a K-Ras nucleic acid and were tested for their effects on K-Ras mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3496_MGB (forward sequence GACACAAAACAGGCTCAGGACTT, designated herein as SEQ ID NO: 7; reverse sequence TCTTGTCTTTGCTGATGTTTCAATAA, designated herein as SEQ ID NO: 8; probe sequence AAGAAGTTATGGAATTCC, designated herein as SEQ ID NO: 9) was used to measure mRNA levels. K-Ras mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either SEQ ID NO: 1 or SEQ ID NO: 2. 'N/A' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 3

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	87	122
651467	148	163	2159	2174	AGCCGCTGAGCCTCTG	35	160
651470	228	243	7594	7609	ACTCTTGCCTACGCCA	73	161
651474	336	351	25563	25578	CAAGAGACAGGTTTCT	24	162
651475	348	363	25575	25590	GTCGAGAATATCCAAG	77	163

651476	354	369	25581	25596	TGCTGTGTCGAGAATA	58	164
651477	420	435	25647	25662	TACACAAAGAAAGCCC	76	165
651487	690	705	43079	43094	GCTCATCTTTTCTTTA	26	166
651490	786	801	43175	43190	CACTTGTACTAGTATG	63	167
651491	792	807	43181	43196	AATTACCACTTGTACT	21	168
651494	882	897	43271	43286	ATTTAAGGTAAAAGCT	4	169
651506	1093	1108	43482	43497	GATTCAAAAGCTTCAT	79	170
651507	1099	1114	43488	43503	AGGGATGATTCAAAAG	63	171
651512	1157	1172	43546	43561	AAGGTCTCAACTGAAA	39	172
651514	1175	1190	43564	43579	TCAGTAAAAACCAATT	24	173
651515	1184	1199	43573	43588	CTCAATGTTTCAGTAA	28	174
651524	1301	1316	43690	43705	GTGGAAAGGAGACAAA	48	175
651921	95	110	2106	2121	TCCCAGTCCGAAATGG	25	176
651922	159	174	2170	2185	CCGCACCTGGGAGCCG	32	177
651923	183	198	7549	7564	AGTCATTTTCAGCAGG	87	178
651924	195	210	7561	7576	AAGTTTATATTCAGTC	70	179
651925	204	219	7570	7585	AACTACCACAAGTTTA	43	180
651926	237	252	7603	7618	CGTCAAGGCACTCTTG	75	181
651927	252	267	7618	7633	CTGAATTAGCTGTATC	63	182
651928	312	327	25539	25554	TACTACTTGCTTCCTG	59	183
651929	322	337	25549	25564	CTCCATCAATTACTAC	48	184
651930	439	454	25666	25681	TAGTATTATTTATGGC	77	185
651931	448	463	25675	25690	CAAATGATTTAGTATT	8	186
651932	457	472	25684	25699	GAATATCTTCAAATGA	23	187
651933	485	500	27172	27187	ACTCTTTTAATTTGTT	27	188
651934	528	543	27215	27230	TTTATTTCCTACTAGG	45	189
651935	540	555	27227	27242	AGGCAAATCACATTTA	79	190
651936	551	566	27238	27253	ACTGTTCTAGAAGGCA	75	191
651938	648	663	43037	43052	ATAGAAGGCATCATCA	42	192
651939	679	694	43068	43083	CTTTATGTTTTCGAAT	5	193
651940	732	747	43121	43136	ACACTTTGTCTTTGAC	61	194
651941	741	756	43130	43145	CATAATTACACACTTT	32	195
651942	756	771	43145	43160	TACAAATTGTATTTAC	0	196
651943	771	786	43160	43175	GCCTTAAGAAAAAAGT	38	197
651944	816	831	43205	43220	TAATAATTTAGTGTAA	9	198
651945	835	850	43224	43239	GTAATGCTAAAACAAA	12	199
651946	844	859	43233	43248	AAAAATTAGGTAATGC	0	200
651947	860	875	43249	43264	CTGCATGGAGCAGGAA	31	201
651948	873	888	43262	43277	AAAAGCTAACAGTCTG	56	202

651949	907	922	43296	43311	CTTCCACTGTCATTTT	59	203
651950	927	942	43316	43331	GCACTTAGAGGAAAAA	44	204
651951	942	957	43331	43346	ACTCTGGGAATACTGG	82	205
651952	958	973	43347	43362	TAGTTCAAAAACCAAA	6	206
651953	967	982	43356	43371	AGGCATTGCTAGTTCA	83	207
651954	976	991	43365	43380	CTTTTTCACAGGCATT	75	208
651955	989	1004	43378	43393	AGGTATTCAGTTTCTT	79	209
651956	1001	1016	43390	43405	GACAGAAATCTTAGGT	51	210
651957	1010	1025	N/A	N/A	AAACCCCAAGACAGAA	16	211
651958	1019	1034	N/A	N/A	ATGCACCAAAAACCCC	69	212
651959	1028	1043	43417	43432	ATCAACTGCATGCACC	85	213
651960	1037	1052	43426	43441	TAAGAAGTAATCAACT	11	214
651961	1054	1069	43443	43458	ACAATTGGTAAGAAAA	4	215
651962	1063	1078	43452	43467	CCAACATTCACAATTG	53	216
651963	1078	1093	43467	43482	TTAATTTGTTTCACAC	34	217
651964	1110	1125	43499	43514	AAACACAGAATAGGGA	21	218
651965	1119	1134	43508	43523	GACTAGATAAAACACA	67	219
651966	1128	1143	43517	43532	CATTTATGTGACTAGA	79	220
651967	1138	1153	43527	43542	GTAATTAATCCATTTA	53	221
651968	1147	1162	43536	43551	CTGAAATTAGTAATTA	6	222
651969	1166	1181	43555	43570	ACCAATTAGAAGGTCT	38	223
651970	1195	1210	43584	43599	ATTTGTGTTCCCTCAA	68	224
651971	1204	1219	43593	43608	AGCCCATAAATTTGTG	42	225
651972	1213	1228	43602	43617	TCATCAGGAAGCCCAT	83	226
651973	1222	1237	43611	43626	GAAGAATCATCATCAG	78	227
651974	1233	1248	43622	43637	CATGATGCCTAGAAGA	41	228
651975	1245	1260	43634	43649	CAAACTATAGGACATG	56	229
651976	1254	1269	43643	43658	CAGGGATGACAAACTA	63	230
651977	1264	1279	43653	43668	TTACATTCATCAGGGA	9	231
651978	1273	1288	43662	43677	AGTGTAACTTTACATT	41	232
651979	1283	1298	43672	43687	CTTTGTGAACAGTGTA	79	233
651980	1296	1311	43685	43700	AAGGAGACAAAACCTT	3	234

Table 4

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540734	1924	1939	44313	44328	AATGCTCTTGATTTGT	71	89
540767	1316	1331	43705	43720	CCATGACTAATAGCAG	77	29

540777	1709	1724	44098	44113	GCTATAACTGGCCCAA	71	34
540779	1898	1913	44287	44302	ACCACAGAGTGAGATT	73	35
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	81	122
651526	1305	1320	43694	43709	AGCAGTGGAAAGGAGA	61	235
651527	1307	1322	43696	43711	ATAGCAGTGGAAAGGA	61	236
651528	1309	1324	43698	43713	TAATAGCAGTGGAAAG	24	237
651529	1311	1326	43700	43715	ACTAATAGCAGTGGAA	44	238
651530	1313	1328	43702	43717	TGACTAATAGCAGTGG	83	239
651531	1315	1330	43704	43719	CATGACTAATAGCAGT	56	240
651533	1319	1334	43708	43723	TGACCATGACTAATAG	52	241
651534	1321	1336	43710	43725	AGTGACCATGACTAAT	68	242
651537	1398	1413	43787	43802	CTTATAATAGTTTCCA	65	243
651542	1470	1485	43859	43874	CTGGGTCTGCCTTAAC	59	244
651543	1476	1491	43865	43880	TTCATACTGGGTCTGC	78	245
651547	1601	1616	43990	44005	GACACAGGGAGACTAC	67	246
651548	1603	1618	43992	44007	CTGACACAGGGAGACT	71	247
651551	1609	1624	43998	44013	AGCAGTCTGACACAGG	80	248
651557	1704	1719	44093	44108	AACTGGCCCAAATAAT	29	249
651558	1706	1721	44095	44110	ATAACTGGCCCAAATA	35	250
651559	1708	1723	44097	44112	CTATAACTGGCCCAAA	23	251
651560	1710	1725	44099	44114	AGCTATAACTGGCCCA	73	252
651561	1712	1727	44101	44116	TAAGCTATAACTGGCC	70	253
651562	1714	1729	44103	44118	AATAAGCTATAACTGG	30	254
651571	1816	1831	44205	44220	ACTGGATGACCGTGGG	63	255
651578	1897	1912	44286	44301	CCACAGAGTGAGATTG	67	256
651579	1899	1914	44288	44303	CACCACAGAGTGAGAT	48	257
651580	1901	1916	44290	44305	ACCACCACAGAGTGAG	72	258
651581	1903	1918	44292	44307	GGACCACCACAGAGTG	62	259
651583	1907	1922	44296	44311	AGCAGGACCACCACAG	52	260
651586	1913	1928	44302	44317	TTTGTCAGCAGGACCA	86	261
651589	1921	1936	44310	44325	GCTCTTGATTTGTCAG	68	262
651591	1927	1942	44316	44331	AGCAATGCTCTTGATT	49	263
651592	1929	1944	44318	44333	AAAGCAATGCTCTTGA	53	264
651595	2020	2035	44409	44424	ATGTCTTGGCACACCA	81	265
651981	1340	1355	43729	43744	AATATAATATTTTGGG	10	266
651982	1387	1402	43776	43791	TTCCATTGCCTTGTAA	49	267
651983	1408	1423	43797	43812	AGGAAATGGCCTTATA	50	268
651984	1419	1434	43808	43823	CTAATGTGAAAAGGAA	16	269
651985	1429	1444	43818	43833	AGTAATTTATCTAATG	5	270

651986	1438	1453	43827	43842	AGTCTTTATAGTAATT	42	271
651987	1447	1462	43836	43851	GCTATTAGGAGTCTTT	82	272
651988	1456	1471	43845	43860	ACAGGAAAAGCTATTA	51	273
651989	1481	1496	43870	43885	CCCATTTCATACTGGG	2	274
651990	1493	1508	43882	43897	GCTATAATAATCCCCA	86	275
651991	1502	1517	43891	43906	AAAATGGTTGCTATAA	0	276
651992	1513	1528	43902	43917	AATATAGCCCCAAAAT	22	277
651993	1531	1546	43920	43935	AAAATTTAGTAGCATG	13	278
651994	1561	1576	43950	43965	ATACTTGTTAAAATCT	23	279
651995	1570	1585	43959	43974	GAATTTTTTATACTTG	13	280
651996	1579	1594	43968	43983	TTCCTATGAGAATTTT	62	281
651997	1588	1603	43977	43992	TACATTTAATTCCTAT	4	282
651998	1620	1635	44009	44024	TACTATGAAAGAGCAG	78	283
651999	1629	1644	44018	44033	TTAAAGTTATACTATG	10	284
652000	1643	1658	44032	44047	GTTGAAGAAAAGATTT	15	285
652001	1652	1667	44041	44056	AAGACTCAAGTTGAAG	72	286
652002	1661	1676	44050	44065	CTATCTTCAAAGACTC	87	287
652003	1672	1687	44061	44076	CAGAATTAAAACTATC	15	288
652004	1685	1700	44074	44089	TTAATGTCACAAGCAG	88	289
652005	1699	1714	44088	44103	GCCCAAATAATCTTTT	47	290
652006	1723	1738	44112	44127	TCAACACCTAATAAGC	46	291
652007	1736	1751	44125	44140	AACCTTGGTCTCTTCA	77	292
652008	1758	1773	44147	44162	TTCACACAGGGCCTGG	65	293
652009	1767	1782	44156	44171	GCTCAAAGGTTCACAC	71	294
652010	1776	1791	44165	44180	TCTATGAAAGCTCAAA	51	295
652011	1785	1800	44174	44189	GTGAAACTCTCTATGA	55	296
652012	1794	1809	44183	44198	GTCCATGCTGTGAAAC	31	297
652013	1825	1840	44214	44229	CATGACAACACTGGAT	56	298
652014	1834	1849	44223	44238	TAACCAATGCATGACA	67	299
652015	1846	1861	44235	44250	CCCCATTTTGACTAAC	38	300
652016	1862	1877	44251	44266	AACTGCCCTAGTCCCT	61	301
652017	1871	1886	44260	44275	AGCTATCCAAACTGCC	44	302
652018	1880	1895	44269	44284	ATCTTGTTGAGCTATC	75	303
652019	1918	1933	44307	44322	CTTGATTTGTCAGCAG	80	304
652020	1990	2005	44379	44394	CAACTTTTGAGTTAAT	14	305
652021	2001	2016	44390	44405	CCCCAAAATCTCAACT	20	306
652022	2010	2025	44399	44414	ACACCACCACCCCAAA	46	307

Table 5
Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	83	122
651601	2099	2114	44488	44503	AATTTAACCAGTGTTA	40	308
651602	2105	2120	44494	44509	AATGTTAATTTAACCA	27	309
651604	2129	2144	44518	44533	ATCAGACTTGAAAAGT	58	310
651605	2135	2150	44524	44539	ATATGGATCAGACTTG	74	311
651623	2466	2481	44855	44870	AAGATGGTGTAACATA	68	312
651629	2600	2615	44989	45004	CTGCATCAAGTCATGG	73	313
651630	2602	2617	44991	45006	AACTGCATCAAGTCAT	64	314
651631	2604	2619	44993	45008	AAAACTGCATCAAGTC	72	315
651634	2634	2649	45023	45038	AATCTTATGGTTAGGG	85	316
651637	2676	2691	45065	45080	ACTCAGTGGGAAAACT	33	317
651638	2678	2693	45067	45082	TGACTCAGTGGGAAAA	59	318
651641	2684	2699	45073	45088	CTGATGTGACTCAGTG	76	319
651642	2686	2701	45075	45090	TTCTGATGTGACTCAG	65	320
651645	2733	2748	45122	45137	TATCTGTCAGATTCTC	83	321
651646	2735	2750	45124	45139	GGTATCTGTCAGATTC	89	322
651647	2737	2752	45126	45141	ATGGTATCTGTCAGAT	84	323
651649	2741	2756	45130	45145	CTTTATGGTATCTGTC	67	324
651650	2743	2758	45132	45147	CCCTTTATGGTATCTG	76	325
651659	2815	2830	45204	45219	GCTAATGGATTGGGCA	17	326
651660	2817	2832	45206	45221	TCGCTAATGGATTGGG	67	327
651662	2821	2836	45210	45225	ACTGTCGCTAATGGAT	43	328
652023	2047	2062	44436	44451	TCACTTCATTGTTTAA	70	329
652024	2056	2071	44445	44460	AAAACTTTTTCACTTC	62	330
652025	2065	2080	44454	44469	AGAGATTGTAAAACTT	21	331
652026	2074	2089	44463	44478	GCCAAACCTAGAGATT	42	332
652027	2083	2098	44472	44487	AGAGAACTAGCCAAAC	62	333
652028	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	78	334
652029	2118	2133	44507	44522	AAAGTGTTTATGCAAT	49	335
652030	2146	2161	44535	44550	AGCATTATTAAATATG	14	336
652031	2176	2191	44565	44580	TCAAAAGGATTGTTTT	2	337
652032	2228	2243	44617	44632	CACCATGCCATCTCAC	47	338
652033	2237	2252	44626	44641	CTTTCACCTCACCATG	38	339
652034	2248	2263	44637	44652	GTCCAGTGATACTTTC	77	340
652035	2258	2273	44647	44662	CTTCTTCCTAGTCCAG	72	341
652036	2268	2283	44657	44672	CCTAAGTCACCTTCTT	47	342

652037	2277	2292	44666	44681	TATCTAGAACCTAAGT	8	343
652038	2286	2301	44675	44690	AAAGACACCTATCTAG	1	344
652039	2297	2312	44686	44701	TCAGAGTCCTAAAAGA	2	345
652040	2306	2321	44695	44710	TCCTCAAAATCAGAGT	42	346
652041	2323	2338	44712	44727	ATGGATAGTAAGTGAT	51	347
652042	2333	2348	44722	44737	ACATGAAGAAATGGAT	12	348
652043	2342	2357	44731	44746	CTTCTTTTAACATGAA	9	349
652044	2352	2367	44741	44756	TTTGAGATGACTTCTT	51	350
652045	2361	2376	44750	44765	AACTAAGAGTTTGAGA	17	351
652046	2385	2400	44774	44789	AATTACATAGTTGTAA	0	352
652047	2405	2420	44794	44809	CCTTATGTAAATGGAA	33	353
652048	2414	2429	44803	44818	TAAGTGTATCCTTATG	56	354
652049	2423	2438	44812	44827	CTTGACAAATAAGTGT	48	355
652050	2434	2449	44823	44838	ATTGTGCTGAGCTTGA	78	356
652051	2450	2465	44839	44854	GGTTAAAAATTTACAG	22	357
652052	2478	2493	44867	44882	AGACTGGCACTGAAGA	54	358
652053	2507	2522	44896	44911	AAACTTCACCTCTTGC	62	359
652054	2522	2537	44911	44926	TGGATATTCAAATATA	12	360
652055	2531	2546	44920	44935	AAACGAGAATGGATAT	28	361
652056	2547	2562	44936	44951	TGGAAGAAGAGTCCTA	14	362
652057	2561	2576	44950	44965	AGATGACACTAATATG	49	363
652058	2576	2591	44965	44980	GAAGGTAGGGAGGCAA	38	364
652059	2613	2628	45002	45017	ACAAGTATTAAAACTG	17	365
652060	2643	2658	45032	45047	GCAGCAGTAAATCTTA	60	366
652061	2652	2667	45041	45056	ATATCCACAGCAGCAG	70	367
652062	2662	2677	45051	45066	CTTCATGGAGATATCC	68	368
652063	2699	2714	45088	45103	AGATGTAGGGCATTTC	54	369
652064	2708	2723	45097	45112	GAGGAAATAAGATGTA	15	370
652065	2723	2738	45112	45127	ATTCTCTTGAGCCCTG	65	371
652066	2755	2770	45144	45159	ATTAGGTCAAATCCCT	72	372
652067	2764	2779	45153	45168	AAATTAGTGATTAGGT	32	373
652068	2773	2788	45162	45177	ACCACCTGAAAATTAG	24	374
652069	2785	2800	45174	45189	AAAGCATCAGCCACCA	46	375
652070	2799	2814	45188	45203	GCAAAGAGATGTTCAA	43	376
652071	2832	2847	45221	45236	TGAAAAATCCTACTGT	12	377
652072	2841	2856	45230	45245	TACCAGGTTTGAAAAA	23	378
652073	2864	2879	45253	45268	CTGGATAGGGTTCTGT	40	379
652074	2873	2888	45262	45277	CTCCTTCCACTGGATA	31	380
652075	2885	2900	45274	45289	CTTTATTAAATTCTCC	26	381

652076	2894	2909	45283	45298	CAGCACTATCTTTATT	33	382
652077	2906	2921	45295	45310	AAGGAATTCTTTCAGC	58	383
652078	2915	2930	45304	45319	AGATTACCTAAGGAAT	23	384

Example 3: Antisense inhibition of human K-Ras in A431 cells by cEt gapmers

Antisense oligonucleotides were designed targeting a K-Ras nucleic acid and were tested for their effects on K-Ras mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured A431 cells at a density of 5,000 cells per well were treated with 1,000 nM antisense oligonucleotide by free uptake. After a treatment period of approximately 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3496_MGB was used to measure mRNA levels. K-Ras mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either SEQ ID NO: 1 or SEQ ID NO: 2. 'N/A' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 6

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	65	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	63	122
651530	1313	1328	43702	43717	TGACTAATAGCAGTGG	69	239
651538	1421	1436	43810	43825	ATCTAATGTGAAAAGG	8	385

651539	1427	1442	43816	43831	TAATTTATCTAATGTG	0	386
651540	1443	1458	43832	43847	TTAGGAGTCTTTATAG	33	387
651541	1449	1464	43838	43853	AAGCTATTAGGAGTCT	60	388
651544	1494	1509	43883	43898	TGCTATAATAATCCCC	47	389
651545	1582	1597	43971	43986	TAATTCCTATGAGAAT	18	390
651552	1611	1626	44000	44015	AGAGCAGTCTGACACA	40	391
651987	1447	1462	43836	43851	GCTATTAGGAGTCTTT	72	272
651990	1493	1508	43882	43897	GCTATAATAATCCCCA	53	275
663529	1522	1537	43911	43926	TAGCATGTAAATATAG	24	392
695897	1249	1264	43638	43653	ATGACAAACTATAGGA	34	393
695898	1251	1266	43640	43655	GGATGACAAACTATAG	35	394
695899	1256	1271	43645	43660	ATCAGGGATGACAAAC	15	395
695900	1258	1273	43647	43662	TCATCAGGGATGACAA	17	396
695901	1260	1275	43649	43664	ATTCATCAGGGATGAC	20	397
695902	1262	1277	43651	43666	ACATTCATCAGGGATG	0	398
695903	1269	1284	43658	43673	TAACTTTACATTCATC	21	399
695904	1275	1290	43664	43679	ACAGTGTAACTTTACA	38	400
695905	1277	1292	43666	43681	GAACAGTGTAACTTTA	26	401
695906	1279	1294	43668	43683	GTGAACAGTGTAACTT	44	402
695907	1281	1296	43670	43685	TTGTGAACAGTGTAAC	44	403
695908	1285	1300	43674	43689	ACCTTTGTGAACAGTG	48	404
695909	1287	1302	43676	43691	AAACCTTTGTGAACAG	48	405
695910	1289	1304	43678	43693	CAAAACCTTTGTGAAC	3	406
695911	1293	1308	43682	43697	GAGACAAAACCTTTGT	10	407
695912	1312	1327	43701	43716	GACTAATAGCAGTGGA	67	408
695913	1314	1329	43703	43718	ATGACTAATAGCAGTG	35	409
695914	1323	1338	43712	43727	AGAGTGACCATGACTA	42	410
695915	1385	1400	43774	43789	CCATTGCCTTGTAATT	37	411
695916	1391	1406	43780	43795	TAGTTTCCATTGCCTT	44	412
695917	1393	1408	43782	43797	AATAGTTTCCATTGCC	64	413
695918	1395	1410	43784	43799	ATAATAGTTTCCATTG	14	414
695919	1400	1415	43789	43804	GCCTTATAATAGTTTC	44	415
695920	1403	1418	43792	43807	ATGGCCTTATAATAGT	25	416
695921	1405	1420	43794	43809	AAATGGCCTTATAATA	0	417
695922	1439	1454	43828	43843	GAGTCTTTATAGTAAT	32	418
695923	1440	1455	43829	43844	GGAGTCTTTATAGTAA	45	419
695924	1441	1456	43830	43845	AGGAGTCTTTATAGTA	69	420
695925	1442	1457	43831	43846	TAGGAGTCTTTATAGT	48	421
695926	1444	1459	43833	43848	ATTAGGAGTCTTTATA	37	422

695927	1445	1460	43834	43849	TATTAGGAGTCTTTAT	18	423
695928	1446	1461	43835	43850	CTATTAGGAGTCTTTA	30	424
695929	1448	1463	43837	43852	AGCTATTAGGAGTCTT	29	425
695930	1450	1465	43839	43854	AAAGCTATTAGGAGTC	70	426
695931	1451	1466	43840	43855	AAAAGCTATTAGGAGT	29	427
695932	1452	1467	43841	43856	GAAAAGCTATTAGGAG	32	428
695933	1453	1468	43842	43857	GGAAAAGCTATTAGGA	43	429
695934	1454	1469	43843	43858	AGGAAAAGCTATTAGG	48	430
695935	1455	1470	43844	43859	CAGGAAAAGCTATTAG	47	431
695936	1464	1479	43853	43868	CTGCCTTAACAGGAAA	43	432
695937	1478	1493	43867	43882	ATTTCATACTGGGTCT	46	433
695938	1483	1498	43872	43887	TCCCCATTTCATACTG	14	434
695939	1492	1507	43881	43896	CTATAATAATCCCCAT	23	435
695940	1495	1510	43884	43899	TTGCTATAATAATCCC	57	436
695941	1496	1511	43885	43900	GTTGCTATAATAATCC	29	437
695942	1497	1512	43886	43901	GGTTGCTATAATAATC	35	438
695943	1498	1513	43887	43902	TGGTTGCTATAATAAT	0	439
695944	1499	1514	43888	43903	ATGGTTGCTATAATAA	26	440
695945	1500	1515	43889	43904	AATGGTTGCTATAATA	12	441
695946	1501	1516	43890	43905	AAATGGTTGCTATAAT	5	442
695947	1504	1519	43893	43908	CCAAAATGGTTGCTAT	18	443
695948	1516	1531	43905	43920	GTAAATATAGCCCCAA	45	444
695949	1518	1533	43907	43922	ATGTAAATATAGCCCC	36	445
695950	1524	1539	43913	43928	AGTAGCATGTAAATAT	28	446
695951	1528	1543	43917	43932	ATTTAGTAGCATGTAA	17	447
695952	1584	1599	43973	43988	TTTAATTCCTATGAGA	20	448
695953	1591	1606	43980	43995	GACTACATTTAATTCC	0	449
695954	1594	1609	43983	43998	GGAGACTACATTTAAT	12	450
695955	1597	1612	43986	44001	CAGGGAGACTACATTT	22	451
695956	1610	1625	43999	44014	GAGCAGTCTGACACAG	41	452
695957	1613	1628	44002	44017	AAAGAGCAGTCTGACA	36	453
695958	1614	1629	44003	44018	GAAAGAGCAGTCTGAC	49	454
695959	1615	1630	44004	44019	TGAAAGAGCAGTCTGA	36	455
695960	1616	1631	44005	44020	ATGAAAGAGCAGTCTG	41	456
695961	1617	1632	44006	44021	TATGAAAGAGCAGTCT	23	457
695962	1618	1633	44007	44022	CTATGAAAGAGCAGTC	33	458

Table 7
Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	58	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	63	122
651497	948	963	43337	43352	ACCAAAACTCTGGGAA	19	459
651498	959	974	43348	43363	CTAGTTCAAAAACCAA	0	460
651499	965	980	43354	43369	GCATTGCTAGTTCAAA	53	461
651501	1014	1029	N/A	N/A	CCAAAAACCCCAAGAC	33	462
651502	1026	1041	43415	43430	CAACTGCATGCACCAA	49	463
651503	1032	1047	43421	43436	AGTAATCAACTGCATG	35	464
651509	1124	1139	43513	43528	TATGTGACTAGATAAA	10	465
651510	1142	1157	43531	43546	ATTAGTAATTAATCCA	34	466
663502	1025	1040	43414	43429	AACTGCATGCACCAAA	30	467
695829	943	958	43332	43347	AACTCTGGGAATACTG	36	468
695830	944	959	43333	43348	AAACTCTGGGAATACT	6	469
695831	949	964	43338	43353	AACCAAAACTCTGGGA	5	470
695832	960	975	43349	43364	GCTAGTTCAAAAACCA	52	471
695833	962	977	43351	43366	TTGCTAGTTCAAAAAC	31	472
695834	963	978	43352	43367	ATTGCTAGTTCAAAAA	0	473
695835	964	979	43353	43368	CATTGCTAGTTCAAAA	32	474
695836	966	981	43355	43370	GGCATTGCTAGTTCAA	51	475
695837	970	985	43359	43374	CACAGGCATTGCTAGT	12	476
695838	971	986	43360	43375	TCACAGGCATTGCTAG	2	477
695839	972	987	43361	43376	TTCACAGGCATTGCTA	27	478
695840	994	1009	43383	43398	ATCTTAGGTATTCAGT	30	479
695841	999	1014	43388	43403	CAGAAATCTTAGGTAT	13	480
695842	1007	1022	43396	43411	CCCCAAGACAGAAATC	4	481
695843	1017	1032	N/A	N/A	GCACCAAAAACCCCAA	16	482
695844	1020	1035	N/A	N/A	CATGCACCAAAAACCC	29	483
695845	1023	1038	N/A	N/A	CTGCATGCACCAAAAA	12	484
695846	1024	1039	43413	43428	ACTGCATGCACCAAAA	14	485
695847	1027	1042	43416	43431	TCAACTGCATGCACCA	63	486
695848	1029	1044	43418	43433	AATCAACTGCATGCAC	14	487
695849	1030	1045	43419	43434	TAATCAACTGCATGCA	34	488
695850	1033	1048	43422	43437	AAGTAATCAACTGCAT	8	489
695851	1034	1049	43423	43438	GAAGTAATCAACTGCA	31	490
695852	1035	1050	43424	43439	AGAAGTAATCAACTGC	54	491
695853	1056	1071	43445	43460	TCACAATTGGTAAGAA	34	492

695854	1058	1073	43447	43462	ATTCACAATTGGTAAG	31	493
695855	1060	1075	43449	43464	ACATTCACAATTGGTA	13	494
695856	1065	1080	43454	43469	CACCAACATTCACAAT	32	495
695857	1068	1083	43457	43472	TCACACCAACATTCAC	20	496
695858	1070	1085	43459	43474	TTTCACACCAACATTC	10	497
695859	1074	1089	43463	43478	TTTGTTTCACACCAAC	36	498
695860	1076	1091	43465	43480	AATTTGTTTCACACCA	43	499
695861	1101	1116	43490	43505	ATAGGGATGATTCAAA	33	500
695862	1103	1118	43492	43507	GAATAGGGATGATTCA	6	501
695863	1105	1120	43494	43509	CAGAATAGGGATGATT	38	502
695864	1107	1122	43496	43511	CACAGAATAGGGATGA	29	503
695865	1121	1136	43510	43525	GTGACTAGATAAAACA	19	504
695866	1126	1141	43515	43530	TTTATGTGACTAGATA	26	505
695867	1130	1145	43519	43534	TCCATTTATGTGACTA	74	506
695868	1151	1166	43540	43555	TCAACTGAAATTAGTA	0	507
695869	1160	1175	43549	43564	TAGAAGGTCTCAACTG	28	508
695870	1162	1177	43551	43566	ATTAGAAGGTCTCAAC	0	509
695871	1164	1179	43553	43568	CAATTAGAAGGTCTCA	21	510
695872	1168	1183	43557	43572	AAACCAATTAGAAGGT	12	511
695873	1172	1187	43561	43576	GTAAAAACCAATTAGA	37	512
695874	1186	1201	43575	43590	CCCTCAATGTTTCAGT	10	513
695875	1188	1203	43577	43592	TTCCCTCAATGTTTCA	35	514
695876	1197	1212	43586	43601	AAATTTGTGTTCCCTC	39	515
695877	1199	1214	43588	43603	ATAAATTTGTGTTCCC	48	516
695878	1201	1216	43590	43605	CCATAAATTTGTGTTC	31	517
695879	1205	1220	43594	43609	AAGCCCATAAATTTGT	21	518
695880	1208	1223	43597	43612	AGGAAGCCCATAAATT	28	519
695881	1209	1224	43598	43613	CAGGAAGCCCATAAAT	37	520
695882	1210	1225	43599	43614	TCAGGAAGCCCATAAA	26	521
695883	1211	1226	43600	43615	ATCAGGAAGCCCATAA	55	522
695884	1212	1227	43601	43616	CATCAGGAAGCCCATA	48	523
695885	1214	1229	43603	43618	ATCATCAGGAAGCCCA	67	524
695886	1215	1230	43604	43619	CATCATCAGGAAGCCC	45	525
695887	1216	1231	43605	43620	TCATCATCAGGAAGCC	52	526
695888	1217	1232	43606	43621	ATCATCATCAGGAAGC	39	527
695889	1219	1234	43608	43623	GAATCATCATCAGGAA	50	528
695890	1220	1235	43609	43624	AGAATCATCATCAGGA	43	529
695891	1224	1239	43613	43628	TAGAAGAATCATCATC	27	530
695892	1226	1241	43615	43630	CCTAGAAGAATCATCA	40	531

695893	1235	1250	43624	43639	GACATGATGCCTAGAA	32	532
695894	1237	1252	43626	43641	AGGACATGATGCCTAG	4	533
695895	1242	1257	43631	43646	ACTATAGGACATGATG	27	534
695896	1247	1262	43636	43651	GACAAACTATAGGACA	14	535

Table 8

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	51	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	58	122
651468	201	216	7567	7582	TACCACAAGTTTATAT	0	536
651472	258	273	7624	7639	ATGATTCTGAATTAGC	39	537
651478	451	466	25678	25693	CTTCAAATGATTTAGT	0	538
651479	460	475	25687	25702	GGTGAATATCTTCAAA	18	539
651483	572	587	27259	27274	TCCTGAGCCTGTTTTG	97	540
651486	651	666	43040	43055	TGTATAGAAGGCATCA	0	541
651489	738	753	43127	43142	AATTACACACTTTGTC	0	542
651495	909	924	43298	43313	AACTTCCACTGTCATT	7	543
663453	184	199	7550	7565	CAGTCATTTTCAGCAG	35	544
663455	255	270	7621	7636	ATTCTGAATTAGCTGT	41	545
663469	544	559	27231	27246	TAGAAGGCAAATCACA	13	546
663470	547	562	27234	27249	TTCTAGAAGGCAAATC	7	547
663472	553	568	27240	27255	CTACTGTTCTAGAAGG	5	548
663479	676	691	43065	43080	TATGTTTTCGAATTTC	0	549
667550	224	239	7590	7605	TTGCCTACGCCACCAG	5	550
695767	48	63	2059	2074	CACCTTCGCCGCCGCC	4	551
695768	67	82	2078	2093	GTACTGGCCGAGCCGC	0	552
695769	91	106	2102	2117	AGTCCGAAATGGCGGG	0	553
695770	120	135	2131	2146	CCTTCAGTGCCTGCGC	23	554
695771	123	138	2134	2149	CCGCCTTCAGTGCCTG	0	555
695772	156	171	2167	2182	CACCTGGGAGCCGCTG	0	556
695773	167	182	2178	2193	CCTCTCTCCCGCACCT	11	557
695774	180	195	7546	7561	CATTTTCAGCAGGCCT	0	558
695775	182	197	7548	7563	GTCATTTTCAGCAGGC	22	559
695776	232	247	7598	7613	AGGCACTCTTGCCTAC	0	560
695777	314	329	25541	25556	ATTACTACTTGCTTCC	17	561
695778	316	331	25543	25558	CAATTACTACTTGCTT	0	562
695779	318	333	25545	25560	ATCAATTACTACTTGC	9	563

695780	320	335	25547	25562	CCATCAATTACTACTT	4	564
695781	339	354	25566	25581	ATCCAAGAGACAGGTT	15	565
695782	343	358	25570	25585	GAATATCCAAGAGACA	6	566
695783	363	378	25590	25605	CTCTTGACCTGCTGTG	15	567
695784	367	382	25594	25609	ACTCCTCTTGACCTGC	18	568
695785	463	478	25690	25705	AATGGTGAATATCTTC	63	569
695786	469	484	N/A	N/A	CTCTATAATGGTGAAT	10	570
695787	492	507	27179	27194	GTCCTTAACTCTTTTA	0	571
695788	498	513	27185	27200	TTCAGAGTCCTTAACT	0	572
695789	542	557	27229	27244	GAAGGCAAATCACATT	16	573
695790	555	570	27242	27257	GTCTACTGTTCTAGAA	2	574
695791	580	595	27267	27282	TTGCTAAGTCCTGAGC	86	575
695792	583	598	27270	27285	TTCTTGCTAAGTCCTG	96	576
695793	588	603	27275	27290	ATAACTTCTTGCTAAG	18	577
695794	590	605	27277	27292	CCATAACTTCTTGCTA	48	578
695795	597	612	27284	27299	AGGAATTCCATAACTT	31	579
695796	599	614	27286	27301	AAAGGAATTCCATAAC	4	580
695797	615	630	27302	27317	TGCTGATGTTTCAATA	6	581
695798	654	669	43043	43058	TAATGTATAGAAGGCA	7	582
695799	656	671	43045	43060	ACTAATGTATAGAAGG	0	583
695800	660	675	43049	43064	TCGAACTAATGTATAG	2	584
695801	662	677	43051	43066	TCTCGAACTAATGTAT	0	585
695802	665	680	43054	43069	ATTTCTCGAACTAATG	0	586
695803	667	682	43056	43071	GAATTTCTCGAACTAA	0	587
695804	671	686	43060	43075	TTTCGAATTTCTCGAA	0	588
695805	681	696	43070	43085	TTCTTTATGTTTTCGA	7	589
695806	734	749	43123	43138	ACACACTTTGTCTTTG	8	590
695807	779	794	43168	43183	ACTAGTATGCCTTAAG	7	591
695808	781	796	43170	43185	GTACTAGTATGCCTTA	0	592
695809	783	798	43172	43187	TTGTACTAGTATGCCT	51	593
695810	794	809	43183	43198	AAAATTACCACTTGTA	1	594
695811	799	814	43188	43203	GTACAAAAATTACCAC	0	595
695812	807	822	43196	43211	AGTGTAATGTACAAAA	12	596
695813	814	829	43203	43218	ATAATTTAGTGTAATG	0	597
695814	818	833	43207	43222	GCTAATAATTTAGTGT	0	598
695815	820	835	43209	43224	ATGCTAATAATTTAGT	39	599
695816	837	852	43226	43241	AGGTAATGCTAAAACA	17	600
695817	839	854	43228	43243	TTAGGTAATGCTAAAA	0	601
695818	841	856	43230	43245	AATTAGGTAATGCTAA	0	602

695819	862	877	43251	43266	GTCTGCATGGAGCAGG	39	603
695820	866	881	43255	43270	AACAGTCTGCATGGAG	4	604
695821	870	885	43259	43274	AGCTAACAGTCTGCAT	10	605
695822	875	890	43264	43279	GTAAAAGCTAACAGTC	0	606
695823	877	892	43266	43281	AGGTAAAAGCTAACAG	52	607
695824	879	894	43268	43283	TAAGGTAAAAGCTAAC	4	608
695825	884	899	43273	43288	GCATTTAAGGTAAAAG	21	609
695826	912	927	43301	43316	AAAAACTTCCACTGTC	17	610
695827	929	944	43318	43333	TGGCACTTAGAGGAAA	1	611
695828	935	950	43324	43339	GAATACTGGCACTTAG	22	612

Table 9

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	65	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	66	122
651553	1656	1671	44045	44060	TTCAAAGACTCAAGTT	27	613
651554	1662	1677	44051	44066	ACTATCTTCAAAGACT	33	614
651555	1686	1701	44075	44090	TTTAATGTCACAAGCA	68	615
651565	1740	1755	44129	44144	TTGCAACCTTGGTCTC	24	616
651568	1771	1786	44160	44175	GAAAGCTCAAAGGTTC	12	617
651572	1822	1837	44211	44226	GACAACACTGGATGAC	18	618
651573	1828	1843	44217	44232	ATGCATGACAACACTG	14	619
651585	1911	1926	44300	44315	TGTCAGCAGGACCACC	39	620
651587	1915	1930	44304	44319	GATTTGTCAGCAGGAC	63	621
651593	1992	2007	44381	44396	CTCAACTTTTGAGTTA	22	622
652004	1685	1700	44074	44089	TTAATGTCACAAGCAG	63	289
695963	1619	1634	44008	44023	ACTATGAAAGAGCAGT	2	623
695964	1622	1637	44011	44026	TATACTATGAAAGAGC	26	624
695965	1624	1639	44013	44028	GTTATACTATGAAAGA	48	625
695966	1633	1648	44022	44037	AGATTTAAAGTTATAC	6	626
695967	1650	1665	44039	44054	GACTCAAGTTGAAGAA	17	627
695968	1654	1669	44043	44058	CAAAGACTCAAGTTGA	20	628
695969	1655	1670	44044	44059	TCAAAGACTCAAGTTG	33	629
695970	1657	1672	44046	44061	CTTCAAAGACTCAAGT	26	630
695971	1658	1673	44047	44062	TCTTCAAAGACTCAAG	25	631
695972	1663	1678	44052	44067	AACTATCTTCAAAGAC	9	632
695973	1681	1696	44070	44085	TGTCACAAGCAGAATT	39	633

695974	1682	1697	44071	44086	ATGTCACAAGCAGAAT	25	634
695975	1683	1698	44072	44087	AATGTCACAAGCAGAA	53	635
695976	1684	1699	44073	44088	TAATGTCACAAGCAGA	61	636
695977	1687	1702	44076	44091	TTTTAATGTCACAAGC	58	637
695978	1688	1703	44077	44092	CTTTTAATGTCACAAG	2	638
695979	1689	1704	44078	44093	TCTTTTAATGTCACAA	38	639
695980	1690	1705	44079	44094	ATCTTTTAATGTCACA	64	640
695981	1691	1706	44080	44095	AATCTTTTAATGTCAC	57	641
695982	1692	1707	44081	44096	TAATCTTTTAATGTCA	0	642
695983	1693	1708	44082	44097	ATAATCTTTTAATGTC	6	643
695984	1716	1731	44105	44120	CTAATAAGCTATAACT	16	644
695985	1718	1733	44107	44122	ACCTAATAAGCTATAA	9	645
695986	1720	1735	44109	44124	ACACCTAATAAGCTAT	1	646
695987	1725	1740	44114	44129	CTTCAACACCTAATAA	4	647
695988	1727	1742	44116	44131	CTCTTCAACACCTAAT	27	648
695989	1729	1744	44118	44133	GTCTCTTCAACACCTA	52	649
695990	1744	1759	44133	44148	GGCCTTGCAACCTTGG	36	650
695991	1763	1778	44152	44167	AAAGGTTCACACAGGG	43	651
695992	1765	1780	44154	44169	TCAAAGGTTCACACAG	30	652
695993	1769	1784	44158	44173	AAGCTCAAAGGTTCAC	48	653
695994	1773	1788	44162	44177	ATGAAAGCTCAAAGGT	18	654
695995	1778	1793	44167	44182	TCTCTATGAAAGCTCA	60	655
695996	1780	1795	44169	44184	ACTCTCTATGAAAGCT	30	656
695997	1782	1797	44171	44186	AAACTCTCTATGAAAG	20	657
695998	1790	1805	44179	44194	ATGCTGTGAAACTCTC	64	658
695999	1792	1807	44181	44196	CCATGCTGTGAAACTC	35	659
696000	1798	1813	44187	44202	CACAGTCCATGCTGTG	17	660
696001	1800	1815	44189	44204	GACACAGTCCATGCTG	19	661
696002	1818	1833	44207	44222	ACACTGGATGACCGTG	6	662
696003	1820	1835	44209	44224	CAACACTGGATGACCG	35	663
696004	1830	1845	44219	44234	CAATGCATGACAACAC	31	664
696005	1832	1847	44221	44236	ACCAATGCATGACAAC	38	665
696006	1836	1851	44225	44240	ACTAACCAATGCATGA	25	666
696007	1838	1853	44227	44242	TGACTAACCAATGCAT	0	667
696008	1843	1858	44232	44247	CATTTTGACTAACCAA	7	668
696009	1865	1880	44254	44269	CCAAACTGCCCTAGTC	18	669
696010	1867	1882	44256	44271	ATCCAAACTGCCCTAG	19	670
696011	1875	1890	44264	44279	GTTGAGCTATCCAAAC	10	671
696012	1878	1893	44267	44282	CTTGTTGAGCTATCCA	74	672

696013	1882	1897	44271	44286	GTATCTTGTTGAGCTA	73	673
696014	1884	1899	44273	44288	TTGTATCTTGTTGAGC	52	674
696015	1912	1927	44301	44316	TTGTCAGCAGGACCAC	39	675
696016	1914	1929	44303	44318	ATTTGTCAGCAGGACC	56	676
696017	1917	1932	44306	44321	TTGATTTGTCAGCAGG	74	677
696018	1920	1935	44309	44324	CTCTTGATTTGTCAGC	67	678
696019	1994	2009	44383	44398	ATCTCAACTTTTGAGT	17	679
696020	2005	2020	44394	44409	ACCACCCCAAAATCTC	23	680
696021	2021	2036	44410	44425	AATGTCTTGGCACACC	46	681
696022	2022	2037	44411	44426	TAATGTCTTGGCACAC	49	682
696023	2023	2038	44412	44427	TTAATGTCTTGGCACA	23	683
696024	2024	2039	44413	44428	ATTAATGTCTTGGCAC	35	684
696025	2025	2040	44414	44429	AATTAATGTCTTGGCA	25	685
696026	2026	2041	44415	44430	AAATTAATGTCTTGGC	64	686
696027	2067	2082	44456	44471	CTAGAGATTGTAAAAC	11	687
696028	2069	2084	44458	44473	ACCTAGAGATTGTAAA	4	688

Table 10

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	61	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	69	122
651597	2076	2091	44465	44480	TAGCCAAACCTAGAGA	7	689
651599	2088	2103	44477	44492	TGTTAAGAGAACTAGC	0	690
651600	2095	2110	44484	44499	TAACCAGTGTTAAGAG	37	691
651603	2120	2135	44509	44524	GAAAAGTGTTTATGCA	75	692
651610	2253	2268	44642	44657	TCCTAGTCCAGTGATA	12	693
651613	2281	2296	44670	44685	CACCTATCTAGAACCT	14	694
651616	2302	2317	44691	44706	CAAAATCAGAGTCCTA	44	695
651620	2418	2433	44807	44822	CAAATAAGTGTATCCT	45	696
651621	2424	2439	44813	44828	GCTTGACAAATAAGTG	24	697
651622	2452	2467	44841	44856	TAGGTTAAAAATTTAC	14	698
663561	2440	2455	44829	44844	TTACAGATTGTGCTGA	29	699
696029	2071	2086	44460	44475	AAACCTAGAGATTGTA	26	700
696030	2078	2093	44467	44482	ACTAGCCAAACCTAGA	14	701
696031	2080	2095	44469	44484	GAACTAGCCAAACCTA	17	702
696032	2084	2099	44473	44488	AAGAGAACTAGCCAAA	33	703
696033	2085	2100	44474	44489	TAAGAGAACTAGCCAA	21	704

696034	2086	2101	44475	44490	TTAAGAGAACTAGCCA	26	705
696035	2087	2102	44476	44491	GTTAAGAGAACTAGCC	22	706
696036	2089	2104	44478	44493	GTGTTAAGAGAACTAG	39	707
696037	2090	2105	44479	44494	AGTGTTAAGAGAACTA	0	708
696038	2091	2106	44480	44495	CAGTGTTAAGAGAACT	17	709
696039	2092	2107	44481	44496	CCAGTGTTAAGAGAAC	47	710
696040	2096	2111	44485	44500	TTAACCAGTGTTAAGA	19	711
696041	2097	2112	44486	44501	TTTAACCAGTGTTAAG	15	712
696042	2107	2122	44496	44511	GCAATGTTAATTTAAC	0	713
696043	2113	2128	44502	44517	GTTTATGCAATGTTAA	60	714
696044	2115	2130	44504	44519	GTGTTTATGCAATGTT	83	715
696045	2127	2142	44516	44531	CAGACTTGAAAAGTGT	0	716
696046	2137	2152	44526	44541	AAATATGGATCAGACT	11	717
696047	2139	2154	44528	44543	TTAAATATGGATCAGA	32	718
696048	2141	2156	44530	44545	TATTAAATATGGATCA	28	719
696049	2178	2193	44567	44582	TATCAAAAGGATTGTT	23	720
696050	2180	2195	44569	44584	TTTATCAAAAGGATTG	9	721
696051	2232	2247	44621	44636	ACCTCACCATGCCATC	41	722
696052	2239	2254	44628	44643	TACTTTCACCTCACCA	22	723
696053	2241	2256	44630	44645	GATACTTTCACCTCAC	36	724
696054	2246	2261	44635	44650	CCAGTGATACTTTCAC	35	725
696055	2249	2264	44638	44653	AGTCCAGTGATACTTT	15	726
696056	2250	2265	44639	44654	TAGTCCAGTGATACTT	22	727
696057	2251	2266	44640	44655	CTAGTCCAGTGATACT	20	728
696058	2254	2269	44643	44658	TTCCTAGTCCAGTGAT	12	729
696059	2261	2276	44650	44665	CACCTTCTTCCTAGTC	30	730
696060	2270	2285	44659	44674	AACCTAAGTCACCTTC	16	731
696061	2272	2287	44661	44676	AGAACCTAAGTCACCT	32	732
696062	2274	2289	44663	44678	CTAGAACCTAAGTCAC	24	733
696063	2279	2294	44668	44683	CCTATCTAGAACCTAA	21	734
696064	2284	2299	44673	44688	AGACACCTATCTAGAA	12	735
696065	2288	2303	44677	44692	TAAAAGACACCTATCT	36	736
696066	2290	2305	44679	44694	CCTAAAAGACACCTAT	16	737
696067	2292	2307	44681	44696	GTCCTAAAAGACACCT	18	738
696068	2295	2310	44684	44699	AGAGTCCTAAAAGACA	21	739
696069	2304	2319	44693	44708	CTCAAAATCAGAGTCC	38	740
696070	2308	2323	44697	44712	TGTCCTCAAAATCAGA	29	741
696071	2315	2330	44704	44719	TAAGTGATGTCCTCAA	38	742
696072	2320	2335	44709	44724	GATAGTAAGTGATGTC	31	743

696073	2325	2340	44714	44729	AAATGGATAGTAAGTG	14	744
696074	2329	2344	44718	44733	GAAGAAATGGATAGTA	52	745
696075	2344	2359	44733	44748	GACTTCTTTTAACATG	44	746
696076	2347	2362	44736	44751	GATGACTTCTTTTAAC	20	747
696077	2354	2369	44743	44758	AGTTTGAGATGACTTC	35	748
696078	2356	2371	44745	44760	AGAGTTTGAGATGACT	23	749
696079	2359	2374	44748	44763	CTAAGAGTTTGAGATG	41	750
696080	2387	2402	44776	44791	TAAATTACATAGTTGT	12	751
696081	2407	2422	44796	44811	ATCCTTATGTAAATGG	2	752
696082	2409	2424	44798	44813	GTATCCTTATGTAAAT	27	753
696083	2411	2426	44800	44815	GTGTATCCTTATGTAA	50	754
696084	2416	2431	44805	44820	AATAAGTGTATCCTTA	12	755
696085	2420	2435	44809	44824	GACAAATAAGTGTATC	52	756
696086	2425	2440	44814	44829	AGCTTGACAAATAAGT	31	757
696087	2426	2441	44815	44830	GAGCTTGACAAATAAG	21	758
696088	2429	2444	44818	44833	GCTGAGCTTGACAAAT	22	759
696089	2430	2445	44819	44834	TGCTGAGCTTGACAAA	27	760
696090	2435	2450	44824	44839	GATTGTGCTGAGCTTG	68	761
696091	2436	2451	44825	44840	AGATTGTGCTGAGCTT	59	762
696092	2438	2453	44827	44842	ACAGATTGTGCTGAGC	48	763
696093	2439	2454	44828	44843	TACAGATTGTGCTGAG	42	764
696094	2443	2458	44832	44847	AATTTACAGATTGTGC	22	765

Example 4: Antisense inhibition of human K-Ras in A431 cells by cEt gapmers

Antisense oligonucleotides were designed targeting a K-Ras nucleic acid and were tested for their effects on K-Ras mRNA in vitro. Cultured A431 cells at a density of 5,000 cells per well were with 1,000 nM antisense oligonucleotide by free uptake. After a treatment period of approximately 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS132 (forward sequence CAAGTAGTAATTGATGGAGAAACCTGTCT, designated herein as SEQ ID NO: 10; reverse sequence CTGGTCCCTCATTGCACTGTAC; designated herein as SEQ ID NO: 11; probe sequence TGGATATTCTCGACACAGCAGGTCAAGAGG, designated herein as SEQ ID NO: 12) was used to measure mRNA levels. K-Ras mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

The newly designed chimeric antisense oligonucleotides in the Table below were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises

of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Table below is targeted to either SEQ ID NO: 1 or SEQ ID NO: 2. 'N/A' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 11

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	50	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	53	122
651468	201	216	7567	7582	TACCACAAGTTTATAT	11	536
651472	258	273	7624	7639	ATGATTCTGAATTAGC	13	537
651478	451	466	25678	25693	CTTCAAATGATTTAGT	24	538
651479	460	475	25687	25702	GGTGAATATCTTCAAA	47	539
651483	572	587	27259	27274	TCCTGAGCCTGTTTTG	44	540
651486	651	666	43040	43055	TGTATAGAAGGCATCA	0	541
651489	738	753	43127	43142	AATTACACACTTTGTC	0	542
651495	909	924	43298	43313	AACTTCCACTGTCATT	14	543
663453	184	199	7550	7565	CAGTCATTTTCAGCAG	0	544
663455	255	270	7621	7636	ATTCTGAATTAGCTGT	31	545
663469	544	559	27231	27246	TAGAAGGCAAATCACA	18	546
663470	547	562	27234	27249	TTCTAGAAGGCAAATC	3	547
663472	553	568	27240	27255	CTACTGTTCTAGAAGG	16	548
663479	676	691	43065	43080	TATGTTTTCGAATTTC	0	549
667550	224	239	7590	7605	TTGCCTACGCCACCAG	0	550
695767	48	63	2059	2074	CACCTTCGCCGCCGCC	0	551
695768	67	82	2078	2093	GTACTGGCCGAGCCGC	5	552
695769	91	106	2102	2117	AGTCCGAAATGGCGGG	0	553
695770	120	135	2131	2146	CCTTCAGTGCCTGCGC	45	554
695771	123	138	2134	2149	CCGCCTTCAGTGCCTG	0	555
695772	156	171	2167	2182	CACCTGGGAGCCGCTG	0	556
695773	167	182	2178	2193	CCTCTCTCCCGCACCT	0	557

695774	180	195	7546	7561	CATTTTCAGCAGGCCT	0	558
695775	182	197	7548	7563	GTCATTTTCAGCAGGC	0	559
695776	232	247	7598	7613	AGGCACTCTTGCCTAC	0	560
695777	314	329	25541	25556	ATTACTACTTGCTTCC	0	561
695778	316	331	25543	25558	CAATTACTACTTGCTT	5	562
695779	318	333	25545	25560	ATCAATTACTACTTGC	13	563
695780	320	335	25547	25562	CCATCAATTACTACTT	17	564
695781	339	354	25566	25581	ATCCAAGAGACAGGTT	70	565
695782	343	358	25570	25585	GAATATCCAAGAGACA	52	566
695783	363	378	25590	25605	CTCTTGACCTGCTGTG	80	567
695784	367	382	25594	25609	ACTCCTCTTGACCTGC	96	568
695785	463	478	25690	25705	AATGGTGAATATCTTC	54	569
695786	469	484	N/A	N/A	CTCTATAATGGTGAAT	3	570
695787	492	507	27179	27194	GTCCTTAACTCTTTTA	6	571
695788	498	513	27185	27200	TTCAGAGTCCTTAACT	0	572
695789	542	557	27229	27244	GAAGGCAAATCACATT	18	573
695790	555	570	27242	27257	GTCTACTGTTCTAGAA	0	574
695791	580	595	27267	27282	TTGCTAAGTCCTGAGC	0	575
695792	583	598	27270	27285	TTCTTGCTAAGTCCTG	46	576
695793	588	603	27275	27290	ATAACTTCTTGCTAAG	20	577
695794	590	605	27277	27292	CCATAACTTCTTGCTA	0	578
695795	597	612	27284	27299	AGGAATTCCATAACTT	6	579
695796	599	614	27286	27301	AAAGGAATTCCATAAC	1	580
695797	615	630	27302	27317	TGCTGATGTTTCAATA	0	581
695798	654	669	43043	43058	TAATGTATAGAAGGCA	0	582
695799	656	671	43045	43060	ACTAATGTATAGAAGG	10	583
695800	660	675	43049	43064	TCGAACTAATGTATAG	0	584
695801	662	677	43051	43066	TCTCGAACTAATGTAT	0	585
695802	665	680	43054	43069	ATTTCTCGAACTAATG	0	586
695803	667	682	43056	43071	GAATTTCTCGAACTAA	4	587
695804	671	686	43060	43075	TTTCGAATTTCTCGAA	0	588
695805	681	696	43070	43085	TTCTTTATGTTTTCGA	0	589
695806	734	749	43123	43138	ACACACTTTGTCTTTG	8	590
695807	779	794	43168	43183	ACTAGTATGCCTTAAG	3	591
695808	781	796	43170	43185	GTACTAGTATGCCTTA	0	592
695809	783	798	43172	43187	TTGTACTAGTATGCCT	43	593
695810	794	809	43183	43198	AAAATTACCACTTGTA	2	594
695811	799	814	43188	43203	GTACAAAAATTACCAC	0	595
695812	807	822	43196	43211	AGTGTAATGTACAAAA	0	596

695813	814	829	43203	43218	ATAATTTAGTGTAATG	0	597
695814	818	833	43207	43222	GCTAATAATTTAGTGT	0	598
695815	820	835	43209	43224	ATGCTAATAATTTAGT	0	599
695816	837	852	43226	43241	AGGTAATGCTAAAACA	12	600
695817	839	854	43228	43243	TTAGGTAATGCTAAAA	0	601
695818	841	856	43230	43245	AATTAGGTAATGCTAA	0	602
695819	862	877	43251	43266	GTCTGCATGGAGCAGG	2	603
695820	866	881	43255	43270	AACAGTCTGCATGGAG	9	604
695821	870	885	43259	43274	AGCTAACAGTCTGCAT	0	605
695822	875	890	43264	43279	GTAAAAGCTAACAGTC	45	606
695823	877	892	43266	43281	AGGTAAAAGCTAACAG	52	607
695824	879	894	43268	43283	TAAGGTAAAAGCTAAC	0	608
695825	884	899	43273	43288	GCATTTAAGGTAAAAG	10	609
695826	912	927	43301	43316	AAAAACTTCCACTGTC	14	610
695827	929	944	43318	43333	TGGCACTTAGAGGAAA	19	611
695828	935	950	43324	43339	GAATACTGGCACTTAG	13	612

Example 5: Antisense inhibition of human K-Ras in A431 cells by cEt gapmers

Antisense oligonucleotides were designed targeting a K-Ras nucleic acid and were tested for their effects on K-Ras mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured A431cells at a density of 5,000 cells per well were treated with 2,000 nM antisense oligonucleotide by free uptake. After a treatment period of approximately 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3496_MGB was used to measure mRNA levels. K-Ras mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each

gapmer listed in the Tables below is targeted to either SEQ ID NO: 1 or SEQ ID NO: 2. 'N/A' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 12

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540733	830	845	43219	43234	GCTAAAACAAATGCTA	33	15
540747	466	481	25693	25708	TATAATGGTGAATATC	7	19
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	62	122
651479	460	475	25687	25702	GGTGAATATCTTCAAA	28	539
651490	786	801	43175	43190	CACTTGTACTAGTATG	36	167
651499	965	980	43354	43369	GCATTGCTAGTTCAAA	46	461
651502	1026	1041	43415	43430	CAACTGCATGCACCAA	31	463
651529	1311	1326	43700	43715	ACTAATAGCAGTGGAA	27	238
651530	1313	1328	43702	43717	TGACTAATAGCAGTGG	58	239
651540	1443	1458	43832	43847	TTAGGAGTCTTTATAG	30	387
651541	1449	1464	43838	43853	AAGCTATTAGGAGTCT	65	388
651953	967	982	43356	43371	AGGCATTGCTAGTTCA	55	207
651959	1028	1043	43417	43432	ATCAACTGCATGCACC	42	213
651966	1128	1143	43517	43532	CATTTATGTGACTAGA	56	220
651967	1138	1153	43527	43542	GTAATTAATCCATTTA	15	221
651987	1447	1462	43836	43851	GCTATTAGGAGTCTTT	76	272
663467	461	476	25688	25703	TGGTGAATATCTTCAA	9	766
663485	819	834	43208	43223	TGCTAATAATTTAGTG	6	767
663486	823	838	43212	43227	CAAATGCTAATAATTT	0	768
695785	463	478	25690	25705	AATGGTGAATATCTTC	50	569
695808	781	796	43170	43185	GTACTAGTATGCCTTA	50	592
695809	783	798	43172	43187	TTGTACTAGTATGCCT	55	593
695814	818	833	43207	43222	GCTAATAATTTAGTGT	18	598
695815	820	835	43209	43224	ATGCTAATAATTTAGT	11	599
695822	875	890	43264	43279	GTAAAAGCTAACAGTC	21	606
695823	877	892	43266	43281	AGGTAAAAGCTAACAG	41	607
695824	879	894	43268	43283	TAAGGTAAAAGCTAAC	20	608
695835	964	979	43353	43368	CATTGCTAGTTCAAAA	9	474
695836	966	981	43355	43370	GGCATTGCTAGTTCAA	43	475
695847	1027	1042	43416	43431	TCAACTGCATGCACCA	52	486
695867	1130	1145	43519	43534	TCCATTTATGTGACTA	68	506

695912	1312	1327	43701	43716	GACTAATAGCAGTGGA	62	408
695913	1314	1329	43703	43718	ATGACTAATAGCAGTG	49	409
695916	1391	1406	43780	43795	TAGTTTCCATTGCCTT	16	412
695917	1393	1408	43782	43797	AATAGTTTCCATTGCC	56	413
695918	1395	1410	43784	43799	ATAATAGTTTCCATTG	8	414
695923	1440	1455	43829	43844	GGAGTCTTTATAGTAA	38	419
695924	1441	1456	43830	43845	AGGAGTCTTTATAGTA	66	420
695925	1442	1457	43831	43846	TAGGAGTCTTTATAGT	41	421
695926	1444	1459	43833	43848	ATTAGGAGTCTTTATA	20	422
695927	1445	1460	43834	43849	TATTAGGAGTCTTTAT	13	423
695928	1446	1461	43835	43850	CTATTAGGAGTCTTTA	34	424
695929	1448	1463	43837	43852	AGCTATTAGGAGTCTT	35	425
695930	1450	1465	43839	43854	AAAGCTATTAGGAGTC	65	426
695931	1451	1466	43840	43855	AAAAGCTATTAGGAGT	25	427
696286	3841	3856	46230	46245	GTTTCACATAGCAATT	29	769
696287	3844	3859	46233	46248	GTAGTTTCACATAGCA	46	770
696288	3846	3861	46235	46250	CTGTAGTTTCACATAG	25	771
716582	459	474	25686	25701	GTGAATATCTTCAAAT	0	772
716583	462	477	25689	25704	ATGGTGAATATCTTCA	64	773
716584	464	479	25691	25706	TAATGGTGAATATCTT	11	774
716585	465	480	25692	25707	ATAATGGTGAATATCT	41	775
716586	780	795	43169	43184	TACTAGTATGCCTTAA	16	776
716587	782	797	43171	43186	TGTACTAGTATGCCTT	66	777
716588	784	799	43173	43188	CTTGTACTAGTATGCC	59	778
716589	785	800	43174	43189	ACTTGTACTAGTATGC	51	779
716590	821	836	43210	43225	AATGCTAATAATTTAG	0	780
716591	822	837	43211	43226	AAATGCTAATAATTTA	13	781
716592	824	839	43213	43228	ACAAATGCTAATAATT	1	782
716593	825	840	43214	43229	AACAAATGCTAATAAT	0	783
716594	826	841	43215	43230	AAACAAATGCTAATAA	9	784
716595	827	842	43216	43231	AAAACAAATGCTAATA	12	785
716596	828	843	43217	43232	TAAAACAAATGCTAAT	5	786
716597	829	844	43218	43233	CTAAAACAAATGCTAA	15	787
716598	876	891	43265	43280	GGTAAAAGCTAACAGT	49	788
716599	878	893	43267	43282	AAGGTAAAAGCTAACA	21	789
716600	1129	1144	43518	43533	CCATTTATGTGACTAG	75	790
716601	1131	1146	43520	43535	ATCCATTTATGTGACT	38	791
716602	1132	1147	43521	43536	AATCCATTTATGTGAC	26	792
716603	1133	1148	43522	43537	TAATCCATTTATGTGA	31	793

716604	1134	1149	43523	43538	TTAATCCATTTATGTG	34	794
716605	1135	1150	43524	43539	ATTAATCCATTTATGT	3	795
716606	1136	1151	43525	43540	AATTAATCCATTTATG	16	796
716607	1137	1152	43526	43541	TAATTAATCCATTTAT	0	797
716608	1392	1407	43781	43796	ATAGTTTCCATTGCCT	65	798
716609	1394	1409	43783	43798	TAATAGTTTCCATTGC	53	799
716610	3842	3857	46231	46246	AGTTTCACATAGCAAT	38	800
716611	3843	3858	46232	46247	TAGTTTCACATAGCAA	51	801
716612	3845	3860	46234	46249	TGTAGTTTCACATAGC	59	802

Table 13

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540770	1488	1503	43877	43892	AATAATCCCCATTTCA	16	104
540772	1520	1535	43909	43924	GCATGTAAATATAGCC	11	105
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	60	122
651544	1494	1509	43883	43898	TGCTATAATAATCCCC	43	389
651555	1686	1701	44075	44090	TTTAATGTCACAAGCA	57	615
651569	1789	1804	44178	44193	TGCTGTGAAACTCTCT	27	803
651587	1915	1930	44304	44319	GATTTGTCAGCAGGAC	47	621
651588	1919	1934	44308	44323	TCTTGATTTGTCAGCA	62	804
651589	1921	1936	44310	44325	GCTCTTGATTTGTCAG	23	262
651990	1493	1508	43882	43897	GCTATAATAATCCCCA	56	275
652004	1685	1700	44074	44089	TTAATGTCACAAGCAG	52	289
652010	1776	1791	44165	44180	TCTATGAAAGCTCAAA	18	295
652011	1785	1800	44174	44189	GTGAAACTCTCTATGA	28	296
652018	1880	1895	44269	44284	ATCTTGTTGAGCTATC	40	303
652019	1918	1933	44307	44322	CTTGATTTGTCAGCAG	44	304
652023	2047	2062	44436	44451	TCACTTCATTGTTTAA	32	329
695867	1130	1145	43519	43534	TCCATTTATGTGACTA	63	506
695939	1492	1507	43881	43896	CTATAATAATCCCCAT	6	435
695940	1495	1510	43884	43899	TTGCTATAATAATCCC	39	436
695948	1516	1531	43905	43920	GTAAATATAGCCCCAA	29	444
695949	1518	1533	43907	43922	ATGTAAATATAGCCCC	28	445
695975	1683	1698	44072	44087	AATGTCACAAGCAGAA	49	635
695976	1684	1699	44073	44088	TAATGTCACAAGCAGA	52	636
695977	1687	1702	44076	44091	TTTTAATGTCACAAGC	52	637
695978	1688	1703	44077	44092	CTTTTAATGTCACAAG	9	638

695979	1689	1704	44078	44093	TCTTTTAATGTCACAA	46	639
695980	1690	1705	44079	44094	ATCTTTTAATGTCACA	58	640
695981	1691	1706	44080	44095	AATCTTTTAATGTCAC	44	641
695982	1692	1707	44081	44096	TAATCTTTTAATGTCA	7	642
695995	1778	1793	44167	44182	TCTCTATGAAAGCTCA	50	655
695996	1780	1795	44169	44184	ACTCTCTATGAAAGCT	29	656
695998	1790	1805	44179	44194	ATGCTGTGAAACTCTC	58	658
695999	1792	1807	44181	44196	CCATGCTGTGAAACTC	26	659
696011	1875	1890	44264	44279	GTTGAGCTATCCAAAC	2	671
696012	1878	1893	44267	44282	CTTGTTGAGCTATCCA	63	672
696013	1882	1897	44271	44286	GTATCTTGTTGAGCTA	56	673
696014	1884	1899	44273	44288	TTGTATCTTGTTGAGC	50	674
696016	1914	1929	44303	44318	ATTTGTCAGCAGGACC	35	676
696017	1917	1932	44306	44321	TTGATTTGTCAGCAGG	65	677
696018	1920	1935	44309	44324	CTCTTGATTTGTCAGC	53	678
696025	2025	2040	44414	44429	AATTAATGTCTTGGCA	12	685
696026	2026	2041	44415	44430	AAATTAATGTCTTGGC	47	686
696816	1519	1534	43908	43923	CATGTAAATATAGCCC	5	805
716613	1489	1504	43878	43893	TAATAATCCCCATTTC	9	806
716614	1490	1505	43879	43894	ATAATAATCCCCATTT	4	807
716615	1491	1506	43880	43895	TATAATAATCCCCATT	7	808
716616	1517	1532	43906	43921	TGTAAATATAGCCCCA	34	809
716617	1777	1792	44166	44181	CTCTATGAAAGCTCAA	39	810
716618	1779	1794	44168	44183	CTCTCTATGAAAGCTC	29	811
716619	1786	1801	44175	44190	TGTGAAACTCTCTATG	4	812
716620	1787	1802	44176	44191	CTGTGAAACTCTCTAT	29	813
716621	1788	1803	44177	44192	GCTGTGAAACTCTCTA	42	814
716622	1791	1806	44180	44195	CATGCTGTGAAACTCT	8	815
716623	1876	1891	44265	44280	TGTTGAGCTATCCAAA	37	816
716624	1877	1892	44266	44281	TTGTTGAGCTATCCAA	3	817
716625	1879	1894	44268	44283	TCTTGTTGAGCTATCC	56	818
716626	1881	1896	44270	44285	TATCTTGTTGAGCTAT	33	819
716627	1883	1898	44272	44287	TGTATCTTGTTGAGCT	31	820
716628	1916	1931	44305	44320	TGATTTGTCAGCAGGA	59	821
716629	2027	2042	44416	44431	AAAATTAATGTCTTGG	12	822
716630	2028	2043	44417	44432	AAAAATTAATGTCTTG	20	823
716631	2029	2044	44418	44433	AAAAAATTAATGTCTT	9	824
716632	2030	2045	44419	44434	AAAAAAATTAATGTCT	7	825
716633	2031	2046	44420	44435	AAAAAAAATTAATGTC	14	826

716634	2032	2047	44421	44436	AAAAAAAAATTAATGT	16	827
716635	2033	2048	44422	44437	AAAAAAAAAATTAATG	6	828
716636	2034	2049	44423	44438	TAAAAAAAAAATTAAT	1	829
716637	2035	2050	44424	44439	TTAAAAAAAAAATTAA	0	830
716638	2036	2051	44425	44440	TTTAAAAAAAAAATTA	3	831
716639	2037	2052	44426	44441	GTTTAAAAAAAAAATT	2	832
716640	2038	2053	44427	44442	TGTTTAAAAAAAAAAT	2	833
716641	2039	2054	44428	44443	TTGTTTAAAAAAAAAA	0	834
716642	2040	2055	44429	44444	ATTGTTTAAAAAAAAA	0	835
716643	2041	2056	44430	44445	CATTGTTTAAAAAAAA	0	836
716644	2042	2057	44431	44446	TCATTGTTTAAAAAAA	0	837
716645	2043	2058	44432	44447	TTCATTGTTTAAAAAA	6	838
716646	2044	2059	44433	44448	CTTCATTGTTTAAAAA	11	839
716647	2045	2060	44434	44449	ACTTCATTGTTTAAAA	0	840
716648	2046	2061	44435	44450	CACTTCATTGTTTAAA	12	841

Example 6: Antisense inhibition of human K-Ras in A431 cells

Antisense oligonucleotides were designed targeting a K-Ras nucleic acid and were tested for their effects on K-Ras mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured A431cells at a density of 5,000 cells per well were treated with 2,000 nM antisense oligonucleotide by free uptake. After a treatment period of approximately 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3496_MGB was used to measure mRNA levels. K-Ras mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers or deoxy, MOE, and (S)-cEt gapmers. The 3-10-3 cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. The deoxy, MOE and (S)-cEt oligonucleotides are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; 'd' indicates deoxyribose; the number after 'd' indicates the number of deoxynucleosides; and 'e' indicates a MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages.

All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either SEQ ID NO: 1 or SEQ ID NO: 2. 'N/A' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 14

Inhibition of K-Ras mRNA by gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	Chemistry	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	kkk-d10-kkk	62	122
651600	2095	2110	44484	44499	TAACCAGTGTTAAGAG	kkk-d10-kkk	40	691
651603	2120	2135	44509	44524	GAAAAGTGTTTATGCA	kkk-d10-kkk	60	692
651633	2620	2635	45009	45024	GGGAATTACAAGTATT	kkk-d10-kkk	28	842
651634	2634	2649	45023	45038	AATCTTATGGTTAGGG	kkk-d10-kkk	45	316
651770	4332	4347	46721	46736	TGTGCAATGGTGACAA	kkk-d10-kkk	28	843
652019	1918	1933	44307	44322	CTTGATTTGTCAGCAG	kkk-d10-kkk	51	304
652028	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	kkk-d10-kkk	60	334
652029	2118	2133	44507	44522	AAAGTGTTTATGCAAT	kkk-d10-kkk	29	335
695867	1130	1145	43519	43534	TCCATTTATGTGACTA	kkk-d10-kkk	70	506
696039	2092	2107	44481	44496	CCAGTGTTAAGAGAAC	kkk-d10-kkk	33	710
696042	2107	2122	44496	44511	GCAATGTTAATTTAAC	kkk-d10-kkk	17	713
696043	2113	2128	44502	44517	GTTTATGCAATGTTAA	kkk-d10-kkk	55	714
696044	2115	2130	44504	44519	GTGTTTATGCAATGTT	kkk-d10-kkk	79	715
696045	2127	2142	44516	44531	CAGACTTGAAAAGTGT	kkk-d10-kkk	24	716
696121	2635	2650	45024	45039	AAATCTTATGGTTAGG	kkk-d10-kkk	34	844
696357	4331	4346	46720	46735	GTGCAATGGTGACAAC	kkk-d10-kkk	38	845
696358	4334	4349	46723	46738	ATTGTGCAATGGTGAC	kkk-d10-kkk	57	846
696359	4336	4351	46725	46740	AAATTGTGCAATGGTG	kkk-d10-kkk	48	847
716649	2094	2109	44483	44498	AACCAGTGTTAAGAGA	kkk-d10-kkk	35	848
716650	2108	2123	44497	44512	TGCAATGTTAATTTAA	kkk-d10-kkk	13	849
716651	2109	2124	44498	44513	ATGCAATGTTAATTTA	kkk-d10-kkk	9	850
716652	2110	2125	44499	44514	TATGCAATGTTAATTT	kkk-d10-kkk	8	851
716653	2111	2126	44500	44515	TTATGCAATGTTAATT	kkk-d10-kkk	3	852
716654	2112	2127	44501	44516	TTTATGCAATGTTAAT	kkk-d10-kkk	34	853
716655	2114	2129	44503	44518	TGTTTATGCAATGTTA	kkk-d10-kkk	58	854
716656	2116	2131	44505	44520	AGTGTTTATGCAATGT	kkk-d10-kkk	69	855
716657	2117	2132	44506	44521	AAGTGTTTATGCAATG	kkk-d10-kkk	45	856
716658	2119	2134	44508	44523	AAAAGTGTTTATGCAA	kkk-d10-kkk	32	857

716659	2121	2136	44510	44525	TGAAAAGTGTTTATGC	kkk-d10-kkk	40	858
716660	2122	2137	44511	44526	TTGAAAAGTGTTTATG	kkk-d10-kkk	26	859
716661	2123	2138	44512	44527	CTTGAAAAGTGTTTAT	kkk-d10-kkk	0	860
716662	2124	2139	44513	44528	ACTTGAAAAGTGTTTA	kkk-d10-kkk	16	861
716663	2125	2140	44514	44529	GACTTGAAAAGTGTTT	kkk-d10-kkk	14	862
716664	2126	2141	44515	44530	AGACTTGAAAAGTGTT	kkk-d10-kkk	28	863
716665	2624	2639	45013	45028	TTAGGGGAATTACAAG	kkk-d10-kkk	23	864
716666	2626	2641	45015	45030	GGTTAGGGGAATTACA	kkk-d10-kkk	26	865
716667	2628	2643	45017	45032	ATGGTTAGGGGAATTA	kkk-d10-kkk	33	866
716668	2630	2645	45019	45034	TTATGGTTAGGGGAAT	kkk-d10-kkk	21	867
716669	2632	2647	45021	45036	TCTTATGGTTAGGGGA	kkk-d10-kkk	8	868
716670	2633	2648	45022	45037	ATCTTATGGTTAGGGG	kkk-d10-kkk	20	869
716671	4333	4348	46722	46737	TTGTGCAATGGTGACA	kkk-d10-kkk	29	870
716672	4335	4350	46724	46739	AATTGTGCAATGGTGA	kkk-d10-kkk	46	871
716719	1918	1933	44307	44322	CTTGATTTGTCAGCAG	kkk-d9-kkke	46	872
716720	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	kkk-d9-kkke	44	873
716724	1918	1933	44307	44322	CTTGATTTGTCAGCAG	kkk-d8-kekek	41	874
716725	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	kkk-d8-kekek	51	875
716729	1918	1933	44307	44322	CTTGATTTGTCAGCAG	kkk-d9-keke	46	876
716730	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	kkk-d9-keke	40	877
716734	1918	1933	44307	44322	CTTGATTTGTCAGCAG	kk-d10-keke	54	878
716735	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	kk-d10-keke	42	879
716739	1918	1933	44307	44322	CTTGATTTGTCAGCAG	kk-d9-kekek	49	880
716740	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	kk-d9-kekek	49	881
716744	1918	1933	44307	44322	CTTGATTTGTCAGCAG	k-d10-kekek	45	882
716745	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	k-d10-kekek	44	883
716749	1918	1933	44307	44322	CTTGATTTGTCAGCAG	k-d9-kekeke	44	884
716750	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	k-d9-kekeke	50	885
716754	1918	1933	44307	44322	CTTGATTTGTCAGCAG	kk-d8-kekekk	33	886
716755	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	kk-d8-kekekk	52	887
716759	1918	1933	44307	44322	CTTGATTTGTCAGCAG	kkk-d8-kdkdk	33	888
716760	2093	2108	44482	44497	ACCAGTGTTAAGAGAA	kkk-d8-kdkdk	50	889
716764	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kkk-d9-kkke	55	890
716765	2092	2107	44481	44496	CCAGTGTTAAGAGAAC	kkk-d9-kkke	55	891
716769	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kk-d10-keke	66	892
716770	2092	2107	44481	44496	CCAGTGTTAAGAGAAC	kk-d10-keke	42	893
716774	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kk-d9-kekek	23	894
716775	2092	2107	44481	44496	CCAGTGTTAAGAGAAC	kk-d9-kekek	43	895
716779	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kk-d8-kekekk	29	896

716780	2092	2107	44481	44496	CCAGTGTTAAGAGAAC	kk-d8-kekekk	53	897
716784	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kk-d9-kdkdk	53	898
716785	2092	2107	44481	44496	CCAGTGTTAAGAGAAC	kk-d9-kdkdk	38	899
716789	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kkk-d8-kekek	37	900
716790	2092	2107	44481	44496	CCAGTGTTAAGAGAAC	kkk-d8-kekek	37	901
716794	1916	1931	44305	44320	TGATTTGTCAGCAGGA	k-d10-kekek	47	902
716795	2091	2106	44480	44495	CAGTGTTAAGAGAACT	k-d10-kekek	23	903
716799	1916	1931	44305	44320	TGATTTGTCAGCAGGA	k-d9-kekeke	32	904
716800	2091	2106	44480	44495	CAGTGTTAAGAGAACT	k-d9-kekeke	40	905
716804	1916	1931	44305	44320	TGATTTGTCAGCAGGA	kk-d8-kekekk	32	906
716805	2091	2106	44480	44495	CAGTGTTAAGAGAACT	kk-d8-kekekk	35	907

Table 15

Inhibition of K-Ras mRNA by gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	Chemistry	% Inhibition	SEQ ID NO
540787	2460	2475	44849	44864	GTGTAACATAGGTTAA	kkk-d10-kkk	30	39
540797	2694	2709	45083	45098	TAGGGCATTTCTGATG	kkk-d10-kkk	20	44
540802	2848	2863	45237	45252	CTATTCATACCAGGTT	kkk-d10-kkk	33	120
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	kkk-d10-kkk	60	122
651609	2247	2262	44636	44651	TCCAGTGATACTTTCA	kkk-d10-kkk	51	908
651623	2466	2481	44855	44870	AAGATGGTGTAACATA	kkk-d10-kkk	30	312
651626	2527	2542	44916	44931	GAGAATGGATATTCAA	kkk-d10-kkk	8	909
651635	2654	2669	45043	45058	AGATATCCACAGCAGC	kkk-d10-kkk	58	910
651642	2686	2701	45075	45090	TTCTGATGTGACTCAG	kkk-d10-kkk	26	320
651653	2762	2777	45151	45166	ATTAGTGATTAGGTCA	kkk-d10-kkk	55	911
651666	2855	2870	45244	45259	GTTCTGTCTATTCATA	kkk-d10-kkk	31	912
652034	2248	2263	44637	44652	GTCCAGTGATACTTTC	kkk-d10-kkk	44	340
652050	2434	2449	44823	44838	ATTGTGCTGAGCTTGA	kkk-d10-kkk	51	356
652055	2531	2546	44920	44935	AAACGAGAATGGATAT	kkk-d10-kkk	12	361
652061	2652	2667	45041	45056	ATATCCACAGCAGCAG	kkk-d10-kkk	39	367
652067	2764	2779	45153	45168	AAATTAGTGATTAGGT	kkk-d10-kkk	5	373
663560	2437	2452	44826	44841	CAGATTGTGCTGAGCT	kkk-d10-kkk	29	913
695867	1130	1145	43519	43534	TCCATTTATGTGACTA	kkk-d10-kkk	57	506
695912	1312	1327	43701	43716	GACTAATAGCAGTGGA	kkk-d10-kkk	52	408
696044	2115	2130	44504	44519	GTGTTTATGCAATGTT	kkk-d10-kkk	72	715
696054	2246	2261	44635	44650	CCAGTGATACTTTCAC	kkk-d10-kkk	15	725
696055	2249	2264	44638	44653	AGTCCAGTGATACTTT	kkk-d10-kkk	8	726
696090	2435	2450	44824	44839	GATTGTGCTGAGCTTG	kkk-d10-kkk	63	761

696091	2436	2451	44825	44840	AGATTGTGCTGAGCTT	kkk-d10-kkk	39	762
696092	2438	2453	44827	44842	ACAGATTGTGCTGAGC	kkk-d10-kkk	46	763
696096	2463	2478	44852	44867	ATGGTGTAACATAGGT	kkk-d10-kkk	59	914
696108	2529	2544	44918	44933	ACGAGAATGGATATTC	kkk-d10-kkk	29	915
696116	2563	2578	44952	44967	CAAGATGACACTAATA	kkk-d10-kkk	18	916
696117	2565	2580	44954	44969	GGCAAGATGACACTAA	kkk-d10-kkk	30	917
696118	2567	2582	44956	44971	GAGGCAAGATGACACT	kkk-d10-kkk	0	918
696132	2656	2671	45045	45060	GGAGATATCCACAGCA	kkk-d10-kkk	0	919
696137	2688	2703	45077	45092	ATTTCTGATGTGACTC	kkk-d10-kkk	49	920
696138	2692	2707	45081	45096	GGGCATTTCTGATGTG	kkk-d10-kkk	7	921
696139	2697	2712	45086	45101	ATGTAGGGCATTTCTG	kkk-d10-kkk	9	922
696151	2759	2774	45148	45163	AGTGATTAGGTCAAAT	kkk-d10-kkk	23	923
696152	2761	2776	45150	45165	TTAGTGATTAGGTCAA	kkk-d10-kkk	59	924
696167	2850	2865	45239	45254	GTCTATTCATACCAGG	kkk-d10-kkk	60	925
716673	2461	2476	44850	44865	GGTGTAACATAGGTTA	kkk-d10-kkk	55	926
716674	2462	2477	44851	44866	TGGTGTAACATAGGTT	kkk-d10-kkk	58	927
716675	2464	2479	44853	44868	GATGGTGTAACATAGG	kkk-d10-kkk	60	928
716676	2465	2480	44854	44869	AGATGGTGTAACATAG	kkk-d10-kkk	39	929
716677	2528	2543	44917	44932	CGAGAATGGATATTCA	kkk-d10-kkk	14	930
716678	2530	2545	44919	44934	AACGAGAATGGATATT	kkk-d10-kkk	4	931
716679	2564	2579	44953	44968	GCAAGATGACACTAAT	kkk-d10-kkk	30	932
716680	2566	2581	44955	44970	AGGCAAGATGACACTA	kkk-d10-kkk	21	933
716681	2653	2668	45042	45057	GATATCCACAGCAGCA	kkk-d10-kkk	10	934
716682	2655	2670	45044	45059	GAGATATCCACAGCAG	kkk-d10-kkk	49	935
716683	2687	2702	45076	45091	TTTCTGATGTGACTCA	kkk-d10-kkk	57	936
716684	2689	2704	45078	45093	CATTTCTGATGTGACT	kkk-d10-kkk	32	937
716685	2690	2705	45079	45094	GCATTTCTGATGTGAC	kkk-d10-kkk	24	938
716686	2691	2706	45080	45095	GGCATTTCTGATGTGA	kkk-d10-kkk	8	939
716687	2695	2710	45084	45099	GTAGGGCATTTCTGAT	kkk-d10-kkk	14	940
716688	2696	2711	45085	45100	TGTAGGGCATTTCTGA	kkk-d10-kkk	0	941
716689	2698	2713	45087	45102	GATGTAGGGCATTTCT	kkk-d10-kkk	0	942
716690	2760	2775	45149	45164	TAGTGATTAGGTCAAA	kkk-d10-kkk	21	943
716691	2763	2778	45152	45167	AATTAGTGATTAGGTC	kkk-d10-kkk	32	944
716692	2849	2864	45238	45253	TCTATTCATACCAGGT	kkk-d10-kkk	26	945
716693	2851	2866	45240	45255	TGTCTATTCATACCAG	kkk-d10-kkk	16	946
716694	2852	2867	45241	45256	CTGTCTATTCATACCA	kkk-d10-kkk	34	947
716695	2853	2868	45242	45257	TCTGTCTATTCATACC	kkk-d10-kkk	12	948
716696	2854	2869	45243	45258	TTCTGTCTATTCATAC	kkk-d10-kkk	10	949
716721	2248	2263	44637	44652	GTCCAGTGATACTTTC	kkk-d9-kkke	48	950

716726	2248	2263	44637	44652	GTCCAGTGATACTTTC	kkk-d8-kekek	21	951
716731	2248	2263	44637	44652	GTCCAGTGATACTTTC	kkk-d9-keke	33	952
716736	2248	2263	44637	44652	GTCCAGTGATACTTTC	kk-d10-keke	39	953
716741	2248	2263	44637	44652	GTCCAGTGATACTTTC	kk-d9-kekek	43	954
716746	2248	2263	44637	44652	GTCCAGTGATACTTTC	k-d10-kekek	36	955
716751	2248	2263	44637	44652	GTCCAGTGATACTTTC	k-d9-kekeke	20	956
716756	2248	2263	44637	44652	GTCCAGTGATACTTTC	kk-d8-kekekk	22	957
716761	2248	2263	44637	44652	GTCCAGTGATACTTTC	kkk-d8-kdkdk	30	958
716766	2247	2262	44636	44651	TCCAGTGATACTTTCA	kkk-d9-kkke	47	959
716771	2247	2262	44636	44651	TCCAGTGATACTTTCA	kk-d10-keke	39	960
716776	2247	2262	44636	44651	TCCAGTGATACTTTCA	kk-d9-kekek	37	961
716781	2247	2262	44636	44651	TCCAGTGATACTTTCA	kk-d8-kekekk	24	962
716786	2247	2262	44636	44651	TCCAGTGATACTTTCA	kk-d9-kdkdk	35	963
716791	2247	2262	44636	44651	TCCAGTGATACTTTCA	kkk-d8-kekek	48	964
716796	2246	2261	44635	44650	CCAGTGATACTTTCAC	k-d10-kekek	0	965
716801	2246	2261	44635	44650	CCAGTGATACTTTCAC	k-d9-kekeke	16	966
716806	2246	2261	44635	44650	CCAGTGATACTTTCAC	kk-d8-kekekk	17	967

Table 16

Inhibition of K-Ras mRNA by gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	Chemistry	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	kkk-d10-kkk	66	122
540831	4276	4291	46665	46680	TGCAGTGTGACTCAGT	kkk-d10-kkk	55	61
651720	3715	3730	46104	46119	CTTATGCAGAGAAAAC	kkk-d10-kkk	8	968
651721	3721	3736	46110	46125	TAATTACTTATGCAGA	kkk-d10-kkk	17	969
651759	4273	4288	46662	46677	AGTGTGACTCAGTTAA	kkk-d10-kkk	53	970
651760	4274	4289	46663	46678	CAGTGTGACTCAGTTA	kkk-d10-kkk	53	971
651761	4275	4290	46664	46679	GCAGTGTGACTCAGTT	kkk-d10-kkk	48	972
651795	4620	4635	47009	47024	CCAGTATTAACACAGA	kkk-d10-kkk	37	973
652112	3722	3737	46111	46126	TTAATTACTTATGCAG	kkk-d10-kkk	32	974
652132	4036	4051	46425	46440	ACCATTCAAAGTTCAC	kkk-d10-kkk	34	975
652157	4524	4539	46913	46928	CTTTTTGACAAATGGA	kkk-d10-kkk	22	976
695867	1130	1145	43519	43534	TCCATTTATGTGACTA	kkk-d10-kkk	63	506
696271	3719	3734	46108	46123	ATTACTTATGCAGAGA	kkk-d10-kkk	60	977
696316	4029	4044	46418	46433	AAAGTTCACATAAAGG	kkk-d10-kkk	38	978
696317	4035	4050	46424	46439	CCATTCAAAGTTCACA	kkk-d10-kkk	42	979
696318	4037	4052	46426	46441	AACCATTCAAAGTTCA	kkk-d10-kkk	35	980
696377	4525	4540	46914	46929	ACTTTTTGACAAATGG	kkk-d10-kkk	46	981

696378	4530	4545	46919	46934	TCATTACTTTTTGACA	kkk-d10-kkk	4	982
696385	4617	4632	47006	47021	GTATTAACACAGAAGT	kkk-d10-kkk	0	983
696386	4622	4637	47011	47026	ATCCAGTATTAACACA	kkk-d10-kkk	0	984
696556	N/A	N/A	10213	10228	CTGAATTAGTCTCCAT	kkk-d10-kkk	48	985
716697	3716	3731	46105	46120	ACTTATGCAGAGAAAA	kkk-d10-kkk	37	986
716698	3717	3732	46106	46121	TACTTATGCAGAGAAA	kkk-d10-kkk	0	987
716699	3718	3733	46107	46122	TTACTTATGCAGAGAA	kkk-d10-kkk	46	988
716700	3720	3735	46109	46124	AATTACTTATGCAGAG	kkk-d10-kkk	44	989
716701	4030	4045	46419	46434	CAAAGTTCACATAAAG	kkk-d10-kkk	21	990
716702	4031	4046	46420	46435	TCAAAGTTCACATAAA	kkk-d10-kkk	19	991
716703	4032	4047	46421	46436	TTCAAAGTTCACATAA	kkk-d10-kkk	7	992
716704	4033	4048	46422	46437	ATTCAAAGTTCACATA	kkk-d10-kkk	0	993
716705	4034	4049	46423	46438	CATTCAAAGTTCACAT	kkk-d10-kkk	2	994
716706	4526	4541	46915	46930	TACTTTTTGACAAATG	kkk-d10-kkk	37	995
716707	4527	4542	46916	46931	TTACTTTTTGACAAAT	kkk-d10-kkk	0	996
716708	4528	4543	46917	46932	ATTACTTTTTGACAAA	kkk-d10-kkk	0	997
716709	4529	4544	46918	46933	CATTACTTTTTGACAA	kkk-d10-kkk	8	998
716710	4618	4633	47007	47022	AGTATTAACACAGAAG	kkk-d10-kkk	27	999
716711	4619	4634	47008	47023	CAGTATTAACACAGAA	kkk-d10-kkk	7	1000
716712	4621	4636	47010	47025	TCCAGTATTAACACAG	kkk-d10-kkk	34	1001
716713	N/A	N/A	10204	10219	TCTCCATTAGTAAATA	kkk-d10-kkk	13	1002
716714	N/A	N/A	10209	10224	ATTAGTCTCCATTAGT	kkk-d10-kkk	29	1003
716715	N/A	N/A	10212	10227	TGAATTAGTCTCCATT	kkk-d10-kkk	17	1004
716716	N/A	N/A	10214	10229	TCTGAATTAGTCTCCA	kkk-d10-kkk	62	1005
716717	N/A	N/A	10217	10232	AAATCTGAATTAGTCT	kkk-d10-kkk	25	1006
716718	N/A	N/A	10222	10237	CTTACAAATCTGAATT	kkk-d10-kkk	7	1007
716722	4036	4051	46425	46440	ACCATTCAAAGTTCAC	kkk-d9-kkke	42	1008
716723	4274	4289	46663	46678	CAGTGTGACTCAGTTA	kkk-d9-kkke	42	1009
716727	4036	4051	46425	46440	ACCATTCAAAGTTCAC	kkk-d8-kekek	26	1010
716728	4274	4289	46663	46678	CAGTGTGACTCAGTTA	kkk-d8-kekek	63	1011
716732	4036	4051	46425	46440	ACCATTCAAAGTTCAC	kkk-d9-keke	30	1012
716733	4274	4289	46663	46678	CAGTGTGACTCAGTTA	kkk-d9-keke	55	1013
716737	4036	4051	46425	46440	ACCATTCAAAGTTCAC	kk-d10-keke	41	1014
716738	4274	4289	46663	46678	CAGTGTGACTCAGTTA	kk-d10-keke	51	1015
716742	4036	4051	46425	46440	ACCATTCAAAGTTCAC	kk-d9-kekek	36	1016
716743	4274	4289	46663	46678	CAGTGTGACTCAGTTA	kk-d9-kekek	56	1017
716747	4036	4051	46425	46440	ACCATTCAAAGTTCAC	k-d10-kekek	18	1018
716748	4274	4289	46663	46678	CAGTGTGACTCAGTTA	k-d10-kekek	32	1019
716752	4036	4051	46425	46440	ACCATTCAAAGTTCAC	k-d9-kekeke	15	1020

716753	4274	4289	46663	46678	CAGTGTGACTCAGTTA	k-d9-kekeke	54	1021
716757	4036	4051	46425	46440	ACCATTCAAAGTTCAC	kk-d8-kekekk	7	1022
716758	4274	4289	46663	46678	CAGTGTGACTCAGTTA	kk-d8-kekekk	63	1023
716762	4036	4051	46425	46440	ACCATTCAAAGTTCAC	kkk-d8-kdkdk	12	1024
716763	4274	4289	46663	46678	CAGTGTGACTCAGTTA	kkk-d8-kdkdk	60	1025
716767	4035	4050	46424	46439	CCATTCAAAGTTCACA	kkk-d9-kkke	41	1026
716768	4273	4288	46662	46677	AGTGTGACTCAGTTAA	kkk-d9-kkke	15	1027
716772	4035	4050	46424	46439	CCATTCAAAGTTCACA	kk-d10-keke	63	1028
716773	4273	4288	46662	46677	AGTGTGACTCAGTTAA	kk-d10-keke	41	1029
716777	4035	4050	46424	46439	CCATTCAAAGTTCACA	kk-d9-kekek	36	1030
716778	4273	4288	46662	46677	AGTGTGACTCAGTTAA	kk-d9-kekek	48	1031
716782	4035	4050	46424	46439	CCATTCAAAGTTCACA	kk-d8-kekekk	57	1032
716783	4273	4288	46662	46677	AGTGTGACTCAGTTAA	kk-d8-kekekk	47	1033
716787	4035	4050	46424	46439	CCATTCAAAGTTCACA	kk-d9-kdkdk	31	1034
716788	4273	4288	46662	46677	AGTGTGACTCAGTTAA	kk-d9-kdkdk	38	1035
716792	4035	4050	46424	46439	CCATTCAAAGTTCACA	kkk-d8-kekek	39	1036
716793	4273	4288	46662	46677	AGTGTGACTCAGTTAA	kkk-d8-kekek	29	1037
716797	4034	4049	46423	46438	CATTCAAAGTTCACAT	k-d10-kekek	43	1038
716798	4272	4287	46661	46676	GTGTGACTCAGTTAAA	k-d10-kekek	30	1039
716802	4034	4049	46423	46438	CATTCAAAGTTCACAT	k-d9-kekeke	24	1040
716803	4272	4287	46661	46676	GTGTGACTCAGTTAAA	k-d9-kekeke	25	1041
716807	4034	4049	46423	46438	CATTCAAAGTTCACAT	kk-d8-kekekk	96	1042
716808	4272	4287	46661	46676	GTGTGACTCAGTTAAA	kk-d8-kekekk	38	1043

Example 7: Antisense inhibition of human K-Ras in A431 cells by cEt gapmers

Antisense oligonucleotides were designed targeting a K-Ras nucleic acid and were tested for their effects on K-Ras mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured A431cells at a density of 5,000 cells per well were ttreated with 1,000 nM antisense oligonucleotide by free uptake. After a treatment period of approximately 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3496_MGB was used to measure mRNA levels. K-Ras mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises

of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either SEQ ID NO: 1 or SEQ ID NO: 2. 'N/A' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity. In case the sequence alignment for a target gene in a particular table is not shown, it is understood that none of the oligonucleotides presented in that table align with 100% complementarity with that target gene.

Table 17

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	73	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	67	122
651626	2527	2542	44916	44931	GAGAATGGATATTCAA	26	909
651627	2533	2548	44922	44937	TAAAACGAGAATGGAT	11	1044
651633	2620	2635	45009	45024	GGGAATTACAAGTATT	38	842
651634	2634	2649	45023	45038	AATCTTATGGTTAGGG	63	316
651635	2654	2669	45043	45058	AGATATCCACAGCAGC	68	910
651636	2660	2675	45049	45064	TCATGGAGATATCCAC	35	1045
651644	2713	2728	45102	45117	GCCCTGAGGAAATAAG	11	1046
651651	2750	2765	45139	45154	GTCAAATCCCTTTATG	24	1047
651658	2813	2828	45202	45217	TAATGGATTGGGCAGC	41	1048
651663	2823	2838	45212	45227	CTACTGTCGCTAATGG	38	1049
696095	2458	2473	44847	44862	GTAACATAGGTTAAAA	18	1050
696096	2463	2478	44852	44867	ATGGTGTAACATAGGT	67	914
696097	2468	2483	44857	44872	TGAAGATGGTGTAACA	44	1051
696098	2470	2485	44859	44874	ACTGAAGATGGTGTAA	15	1052
696099	2474	2489	44863	44878	TGGCACTGAAGATGGT	46	1053
696100	2476	2491	44865	44880	ACTGGCACTGAAGATG	39	1054
696101	2480	2495	44869	44884	CAAGACTGGCACTGAA	32	1055
696102	2482	2497	44871	44886	CCCAAGACTGGCACTG	27	1056
696103	2488	2503	44877	44892	ATTTTGCCCAAGACTG	33	1057
696104	2492	2507	44881	44896	CACAATTTTGCCCAAG	36	1058

696105	2499	2514	44888	44903	CCTCTTGCACAATTTT	59	1059
696106	2504	2519	44893	44908	CTTCACCTCTTGCACA	28	1060
696107	2510	2525	44899	44914	TATAAACTTCACCTCT	17	1061
696108	2529	2544	44918	44933	ACGAGAATGGATATTC	58	915
696109	2535	2550	44924	44939	CCTAAAACGAGAATGG	10	1062
696110	2537	2552	44926	44941	GTCCTAAAACGAGAAT	22	1063
696111	2539	2554	44928	44943	GAGTCCTAAAACGAGA	39	1064
696112	2545	2560	44934	44949	GAAGAAGAGTCCTAAA	15	1065
696113	2549	2564	44938	44953	TATGGAAGAAGAGTCC	34	1066
696114	2553	2568	44942	44957	CTAATATGGAAGAAGA	10	1067
696115	2558	2573	44947	44962	TGACACTAATATGGAA	17	1068
696116	2563	2578	44952	44967	CAAGATGACACTAATA	18	916
696117	2565	2580	44954	44969	GGCAAGATGACACTAA	51	917
696118	2567	2582	44956	44971	GAGGCAAGATGACACT	18	918
696119	2585	2600	44974	44989	GGGCATGTGGAAGGTA	20	1069
696120	2609	2624	44998	45013	GTATTAAAACTGCATC	34	1070
696121	2635	2650	45024	45039	AAATCTTATGGTTAGG	40	844
696122	2636	2651	45025	45040	TAAATCTTATGGTTAG	13	1071
696123	2637	2652	45026	45041	GTAAATCTTATGGTTA	26	1072
696124	2638	2653	45027	45042	AGTAAATCTTATGGTT	28	1073
696125	2639	2654	45028	45043	CAGTAAATCTTATGGT	25	1074
696126	2640	2655	45029	45044	GCAGTAAATCTTATGG	50	1075
696127	2641	2656	45030	45045	AGCAGTAAATCTTATG	46	1076
696128	2642	2657	45031	45046	CAGCAGTAAATCTTAT	25	1077
696129	2645	2660	45034	45049	CAGCAGCAGTAAATCT	37	1078
696130	2647	2662	45036	45051	CACAGCAGCAGTAAAT	11	1079
696131	2649	2664	45038	45053	TCCACAGCAGCAGTAA	37	1080
696132	2656	2671	45045	45060	GGAGATATCCACAGCA	23	919
696133	2658	2673	45047	45062	ATGGAGATATCCACAG	17	1081
696134	2664	2679	45053	45068	AACTTCATGGAGATAT	38	1082
696135	2668	2683	45057	45072	GGAAAACTTCATGGAG	37	1083
696136	2671	2686	45060	45075	GTGGGAAAACTTCATG	12	1084
696137	2688	2703	45077	45092	ATTTCTGATGTGACTC	73	920
696138	2692	2707	45081	45096	GGGCATTTCTGATGTG	7	921
696139	2697	2712	45086	45101	ATGTAGGGCATTTCTG	24	922
696140	2701	2716	45090	45105	TAAGATGTAGGGCATT	30	1085
696141	2703	2718	45092	45107	AATAAGATGTAGGGCA	15	1086
696142	2705	2720	45094	45109	GAAATAAGATGTAGGG	26	1087
696143	2715	2730	45104	45119	GAGCCCTGAGGAAATA	19	1088

696144	2718	2733	45107	45122	CTTGAGCCCTGAGGAA	21	1089
696145	2727	2742	45116	45131	TCAGATTCTCTTGAGC	22	1090
696146	2728	2743	45117	45132	GTCAGATTCTCTTGAG	37	1091
696147	2729	2744	45118	45133	TGTCAGATTCTCTTGA	33	1092
696148	2732	2747	45121	45136	ATCTGTCAGATTCTCT	48	1093
696149	2745	2760	45134	45149	ATCCCTTTATGGTATC	14	1094
696150	2748	2763	45137	45152	CAAATCCCTTTATGGT	18	1095
696151	2759	2774	45148	45163	AGTGATTAGGTCAAAT	37	923
696152	2761	2776	45150	45165	TTAGTGATTAGGTCAA	74	924
696153	2766	2781	45155	45170	GAAAATTAGTGATTAG	34	1096
696154	2770	2785	45159	45174	ACCTGAAAATTAGTGA	24	1097
696155	2777	2792	45166	45181	AGCCACCACCTGAAAA	18	1098
696156	2787	2802	45176	45191	TCAAAGCATCAGCCAC	15	1099
696157	2801	2816	45190	45205	CAGCAAAGAGATGTTC	21	1100
696158	2808	2823	45197	45212	GATTGGGCAGCAAAGA	19	1101
696159	2811	2826	45200	45215	ATGGATTGGGCAGCAA	25	1102
696160	2825	2840	45214	45229	TCCTACTGTCGCTAAT	53	1103
696161	2827	2842	45216	45231	AATCCTACTGTCGCTA	37	1104

Table 18

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 2

ISIS NO	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	45370	45385	GCATGAAGATTTCTGG	50	122
540806	45370	45385	GCATGAAGATTTCTGG	51	122
663688	9544	9559	CCAGAGTCAAGTCTTC	31	1105
696498	4399	4414	ATTAGAGTTTGTGTAT	11	1106
696499	4885	4900	TTATAACAAGGTCTCA	0	1107
696500	4949	4964	CGGTAAATATTAATAA	0	1108
696501	4958	4973	TTTCCAATACGGTAAA	9	1109
696502	4960	4975	AATTTCCAATACGGTA	28	1110
696503	5169	5184	TAATAGATTGAATGCA	5	1111
696504	5280	5295	ACTAAGACTTTTCTGG	5	1112
696505	5482	5497	AAGTTAACAACCACTA	5	1113
696506	5562	5577	AATTGTATCACACACG	28	1114
696507	5572	5587	AGGCTAGAAAAATTGT	5	1115
696508	5694	5709	ATTGTAGATAAACACT	13	1116
696509	5734	5749	ATTATAGGATGAGTAG	1	1117
696510	5754	5769	GAGTAAATGCACAACT	0	1118

696511	5904	5919	GATTGTATAACAAACA	5	1119
696512	5994	6009	AGTGAATATATCTCAG	20	1120
696513	6002	6017	AAGATGCAAGTGAATA	4	1121
696514	6729	6744	AGAGAACTCCGAATTA	0	1122
696515	6988	7003	ATCAGATGAGAGTTGA	2	1123
696516	7188	7203	ACTCATGTAGAGACTT	6	1124
696517	7200	7215	TATCATGACTTCACTC	16	1125
696518	7250	7265	TAAATAGCCAGACTGC	12	1126
696519	7292	7307	TACATAGACAGTTCTT	24	1127
696520	7301	7316	CATAAATGCTACATAG	7	1128
696521	7351	7366	ATGAACTGTACTTCAT	0	1129
696522	7471	7486	ACTATCAAATACTCCA	26	1130
696523	7503	7518	ATATTAGAACATGTCA	0	1131
696524	7545	7560	ATTTTCAGCAGGCCTT	23	1132
696525	7666	7681	AACAAGATTTACCTCT	0	1133
696526	7790	7805	CAAGGTACATTTCAGA	38	1134
696527	7935	7950	CAGATAGGATACAAAT	3	1135
696528	7960	7975	CAATAAAAAGATTGTC	0	1136
696529	8011	8026	ACCTTTACATATGATG	4	1137
696530	8034	8049	TTTCACTAGTACAATT	6	1138
696531	8179	8194	AATAGTACCTTTATAT	0	1139
696532	8412	8427	CATCTGCTTGGGATGG	11	1140
696533	8415	8430	CCTCATCTGCTTGGGA	16	1141
696534	8610	8625	CCTAAGAAACAATCTA	16	1142
696535	8751	8766	ACTTTGACCTGTTCTA	2	1143
696536	8789	8804	TCTTCAAGACACTACA	7	1144
696537	8800	8815	CGCAAAGTGTCTCTTC	10	1145
696538	8815	8830	CAGAACTTGCCTCAGC	26	1146
696539	8885	8900	GATTAGTTATCTAATC	0	1147
696540	8996	9011	CTTAAAATTGGAAGCC	30	1148
696541	9060	9075	ATACAGAGACTATTGC	34	1149
696542	9091	9106	TACACATTGAATTAAC	3	1150
696543	9140	9155	ATTAAAATGGGTGCAC	8	1151
696544	9325	9340	CTGATTGGAAACAAAG	13	1152
696545	9542	9557	AGAGTCAAGTCTTCTG	0	1153
696546	9555	9570	ACCAATGCTTCCCAGA	30	1154
696547	9627	9642	AGATAATCTCAGATAC	11	1155
696548	9736	9751	CAACTATTTAACTACT	0	1156
696549	9813	9828	CAATGGCAGTGAAATC	21	1157

696550	9824	9839	ATCAAAACCTGCAATG	3	1158
696551	9876	9891	AGTCATATCCTTTCTA	15	1159
696552	9889	9904	TTCCAAAATGTGCAGT	34	1160
696553	10022	10037	AACAATAGCCACCCTC	20	1161
696554	10141	10156	CAAGAGTACAGTGCAA	46	1162
696555	10179	10194	TTTGAAAGATAGCTAA	0	1163
696556	10213	10228	CTGAATTAGTCTCCAT	61	985
696557	10504	10519	GATCTCTGAACTATAA	0	1164
696558	10734	10749	CCACAATAAAAGCATG	15	1165
696559	10761	10776	CATAATACTTGAACTG	29	1166
696560	10791	10806	TGAATAGGAAACTGTT	0	1167
696561	10823	10838	ACCAATCCAATGATTA	29	1168
696562	10841	10856	ACACTAAAGATGAAAC	6	1169
696563	10867	10882	GGTAAATAAATACTCT	26	1170
696564	11016	11031	TTACATAAGGCTTTTC	16	1171
696565	11079	11094	CAACCATCCCTCATTG	8	1172
696566	11082	11097	ACCCAACCATCCCTCA	3	1173
696567	11694	11709	ATACGAAATCAATCAT	12	1174
696568	11982	11997	AATTTGCCACTTCTGA	19	1175
696569	12000	12015	CAAAATGTGCACTTTC	0	1176
696570	12091	12106	TTCTTAATTTGACCTA	20	1177
696571	12131	12146	GACCAGTAAAGTTTTA	27	1178
696572	12288	12303	CTAGGATTAAGGAATT	9	1179
696573	12369	12384	AATCTGGTCTGTTTTG	32	1180

Table 19

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 2

ISIS NO	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	45370	45385	GCATGAAGATTTCTGG	69	122
540806	45370	45385	GCATGAAGATTTCTGG	73	122
696581	12559	12574	GATTCAACCAATTATG	29	1181
696582	12636	12651	TAATTAGCATGATTGC	14	1182
696583	12753	12768	TATTAAGATCCCAATA	0	1183
696584	12838	12853	TAGTACAGCGAATAGC	0	1184
696585	13223	13238	AAGCAGTGACACTGCT	0	1185
696586	13501	13516	CTCAAGGGTGAAAAAT	3	1186
696587	13593	13608	ATTTTTATGCAGCCAG	37	1187
696588	13672	13687	TAAGATAGCTTCCTGT	12	1188

696589	13684	13699	CTAATTCATATATAAG	0	1189
696590	13800	13815	AGTAAGTGTCTTTTTA	23	1190
696591	13944	13959	CCATAAAGTCTGAGGG	0	1191
696592	13993	14008	GTCAAAGGACATGTAG	13	1192
696593	14208	14223	AGTATATCTAAATCTA	0	1193
696594	14266	14281	ACTCAACACAAGGTGC	4	1194
696595	14514	14529	TACCTCTCATATTATT	4	1195
696596	14611	14626	CCACAAGTGATCACTT	0	1196
696597	14853	14868	GACATTCACTAAAAGT	0	1197
696598	15129	15144	TTTATATACTACACGC	37	1198
696599	15226	15241	TAAAACTGCATACAGG	4	1199
696600	15350	15365	TAGACTTGGGAGTCTT	0	1200
696601	15393	15408	TACATACATGTCTGGT	34	1201
696602	15716	15731	AATTAGCAGTTTTTAG	6	1202
696603	15840	15855	GCAAAAACATAGACGA	9	1203
696604	16077	16092	CAAAGACAGAGCTACC	0	1204
696605	16109	16124	TACCAAAACCACTTGG	0	1205
696606	16313	16328	GTTAAAAATGGGTGGA	11	1206
696607	16473	16488	CAGCATTCCCTGAATC	0	1207
696608	16495	16510	TCTGATAAACCCCAAA	9	1208
696609	16621	16636	CAAAATGTTTTGGCCC	0	1209
696610	16671	16686	GGGAGATCAGATTCAT	17	1210
696611	16679	16694	AATAGGAAGGGAGATC	14	1211
696612	16738	16753	GTATATTAAGTAAGGA	15	1212
696613	16784	16799	GTGAAACTGGACAATC	0	1213
696614	17106	17121	ATTTTTCCAAGGACCG	51	1214
696615	17204	17219	CATGTTGAGGACACAG	16	1215
696616	17383	17398	GTGGAAGAGACATGAA	15	1216
696617	17446	17461	AATCTAGGTGTCACAT	1	1217
696618	17600	17615	CACTTTCCGTTTATAA	1	1218
696619	17640	17655	CGAAAGGTTATTTAAA	4	1219
696620	17704	17719	CACCTGTAGGAAAAGA	5	1220
696621	17715	17730	CTGCTTAATAACACCT	20	1221
696622	17900	17915	ACTGTCATAAGCATAT	7	1222
696623	17902	17917	ATACTGTCATAAGCAT	23	1223
696624	17922	17937	AGCCCTTACTTATATG	0	1224
696625	18046	18061	TACATTCCAAGTATAG	0	1225
696626	18198	18213	ACCAGAACATCAAGTT	9	1226
696627	18203	18218	CATTAACCAGAACATC	7	1227

696628	18220	18235	TCAAGATAAGATAACC	7	1228
696629	18312	18327	CTTCTTTTACACTGAC	29	1229
696630	18523	18538	TACTACTATTCTATAA	0	1230
696631	18658	18673	TAAGACTAGGGAAAAG	30	1231
696632	19173	19188	CTACCTAACAGTCTTG	6	1232
696633	19192	19207	ACTCACCACTACACAC	0	1233
696634	19421	19436	ATGAACGAAGGTAGGT	24	1234
696635	19713	19728	CACAATATAGTCTCCA	38	1235
696636	19761	19776	GGCAATCTGCAGCAAT	9	1236
696637	19828	19843	CTACATCCAACCACCT	0	1237
696638	19926	19941	TTAACATGGCATCCTA	13	1238
696639	19934	19949	AGAGATTCTTAACATG	25	1239
696640	20250	20265	TAACTTAAACTAACTC	4	1240
696641	20285	20300	AATTTGTAGCCTTAGG	42	1241
696642	20289	20304	TACTAATTTGTAGCCT	9	1242
696643	20719	20734	CTAAATAAGGTTTCAG	7	1243
696644	20951	20966	TATACACACGGCATTG	0	1244
696645	21144	21159	CTTAGAAGTGCAATTA	18	1245
696646	21254	21269	GTTTCAAAGTAATCTA	0	1246
696647	21284	21299	TATCGATAGCAAAGTT	7	1247
696648	21398	21413	TCATAAGATGCTTCCA	10	1248
696649	21400	21415	TTTCATAAGATGCTTC	31	1249
696650	21437	21452	AACCTGTAATGTGGGA	31	1250
696651	21442	21457	TAGTCAACCTGTAATG	9	1251
696652	22061	22076	ATTTGAGCATTCAGTT	18	1252
696653	22728	22743	AAAGATGTCTAAGTGC	25	1253
696654	22748	22763	TTACAGTATAAGGAGA	36	1254
696655	22797	22812	GAGAAAGAATGGTCAT	0	1255
696656	23248	23263	AAGAAGCAGGGCTAAC	10	1256
696657	23428	23443	ACTATAAGATTAAGTA	13	1257

Table 20

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 2

ISIS NO	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	45370	45385	GCATGAAGATTTCTGG	63	122
540806	45370	45385	GCATGAAGATTTCTGG	74	122
663670	37371	37386	CTCTCTGCATTGTAAA	31	1258
663729	37497	37512	ACTTACCAGATTACAT	3	1259

696733	34491	34506	GAATTGGAAGCCAATA	26	1260
696734	34493	34508	GAGAATTGGAAGCCAA	29	1261
696735	34496	34511	AGAGAGAATTGGAAGC	30	1262
696736	34502	34517	CAATGCAGAGAGAATT	1	1263
696737	34508	34523	TTCCAGCAATGCAGAG	1	1264
696738	34758	34773	CCAGGTAAAAGCTCAT	31	1265
696739	35416	35431	ATTCTAAGAGCAGTCT	16	1266
696740	35716	35731	TAATTTTTGCATGCAG	28	1267
696741	35718	35733	CTTAATTTTTGCATGC	22	1268
696742	35990	36005	TAAAGCTGGTATATTT	0	1269
696743	36111	36126	AGAAAAGCATACCATC	34	1270
696744	36181	36196	TCCAATCTAGAAAATT	9	1271
696745	36225	36240	ACAATCATATATTGGC	12	1272
696746	36329	36344	TTAGAACAGTGTTCAA	14	1273
696747	36668	36683	ATCCTTACTACAAGTT	15	1274
696748	36798	36813	TACAAGTGAAGCTGAG	30	1275
696749	37039	37054	TTAAAGCCTAAACTGA	2	1276
696750	37045	37060	CTGGAATTAAAGCCTA	0	1277
696751	37258	37273	TTTAAACAGACATCAG	8	1278
696752	37364	37379	CATTGTAAAACACAAC	1	1279
696753	37367	37382	CTGCATTGTAAAACAC	30	1280
696754	37373	37388	CACTCTCTGCATTGTA	12	1281
696755	37493	37508	ACCAGATTACATTATA	28	1282
696756	37495	37510	TTACCAGATTACATTA	8	1283
696757	37499	37514	AAACTTACCAGATTAC	6	1284
696758	37566	37581	AAAGTGGTTGCCACCT	14	1285
696759	37594	37609	AGTTAGAATACTACAC	7	1286
696760	37596	37611	CAAGTTAGAATACTAC	3	1287
696761	37715	37730	ATGCCAAATATAGATT	17	1288
696762	37880	37895	ATATTACTGCTGTCTA	26	1289
696763	37881	37896	AATATTACTGCTGTCT	11	1290
696764	38059	38074	GAAAAGAGGGCGGTAG	17	1291
696765	38181	38196	TGGTAAACCAAATAGG	35	1292
696766	38556	38571	CTATAGCTAAAATGAC	23	1293
696767	38587	38602	CTGCAACACATGTGGA	6	1294
696768	38623	38638	AAAGAGCTGGAGTGGT	15	1295
696769	38886	38901	AAGCATATAATAGTTA	6	1296
696770	38961	38976	ATTCTGGCTAAGATTT	0	1297
696771	39067	39082	GTTTAGAAACGAAAAT	10	1298

696772	39157	39172	ACAAACAATATGCATC	1	1299
696773	39196	39211	CTGTAATTTTATTGCC	18	1300
696774	39341	39356	AGTAGATTAGTACACC	32	1301
696775	39586	39601	ACAATAGGAGGAGAAA	16	1302
696776	39726	39741	AGTCACTGTATAAAAC	16	1303
696777	39750	39765	CAAACAATTGTGACAT	3	1304
696778	39820	39835	AATTACCAAGTATACT	21	1305
696779	40231	40246	CTTTTTCAGGACTAAG	8	1306
696780	40306	40321	CAACCTACACAGAGCA	9	1307
696781	40553	40568	AAAGATTCTAGGCTTA	11	1308
696782	40571	40586	ACTTCCTAAGATTCTG	5	1309
696783	40786	40801	GTGATAGAATCTTAAA	23	1310
696784	40945	40960	AAGGTTTGATTACATA	11	1311
696785	41190	41205	TTTAAGAGAGGTAAAC	0	1312
696786	41301	41316	TACTAAGATTAACGAT	23	1313
696787	41302	41317	CTACTAAGATTAACGA	7	1314
696788	41784	41799	CCACTTTAGGAACAAT	53	1315
696789	41810	41825	CAACACATTAAGTTGT	0	1316
696790	41812	41827	CCCAACACATTAAGTT	21	1317
696791	41844	41859	CTAGACAGCAGAGGGA	7	1318
696792	41994	42009	GTCAATTCTTGTCATG	34	1319
696793	42010	42025	CAAGATCCATACACAA	17	1320
696794	42086	42101	ACCTAATAATCTACAG	12	1321
696795	42094	42109	ACTTTTCAACCTAATA	0	1322
696796	42175	42190	TAGTCATTGTGACCAC	22	1323
696797	42312	42327	TAGCTAAATCATTTGA	20	1324
696798	42339	42354	ACTAATACCTCAGATT	30	1325
696799	42437	42452	TGATATATATTAAGGG	4	1326
696800	42662	42677	ACTTAATTGTCCTTAT	7	1327
696801	42664	42679	ACACTTAATTGTCCTT	28	1328
696802	42736	42751	CTTAATTTGCTACTAT	10	1329
696803	42764	42779	ATAAGGTAACGACTTT	18	1330
696804	42795	42810	GACAAGGATAACCAAT	18	1331
696805	42880	42895	TATATTAGGACTTTTA	5	1332
696806	42986	43001	ATATATACGATGGCTT	0	1333
696807	43412	43427	CTGCATGCACCAAAAG	15	1334

Example 7: Antisense inhibition of human K-Ras in Hep3B cells by cEt gapmers

Antisense oligonucleotides were designed targeting a K-Ras nucleic acid and were tested for their effects on K-Ras mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured Hep3B cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3496_MGB was used to measure mRNA levels. K-Ras mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either SEQ ID NO: 1 or SEQ ID NO: 2. Certain oligonucleotides are targeted to SEQ ID NO: 3. 'N/A' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity. In case the sequence alignment for a target gene in a particular table is not shown, it is understood that none of the oligonucleotides presented in that table align with 100% complementarity with that target gene.

Table 21

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	76	122
651672	2941	2956	45330	45345	CTGTTACCAGGAGTAG	75	1335
651675	2949	2964	45338	45353	ATGTATTACTGTTACC	72	1336
651677	2976	2991	45365	45380	AAGATTTCTGGTTACT	57	1337
651678	2978	2993	45367	45382	TGAAGATTTCTGGTTA	52	1338
651679	2980	2995	45369	45384	CATGAAGATTTCTGGT	51	1339
651681	2984	2999	45373	45388	ATTGCATGAAGATTTC	60	1340
651691	3379	3394	45768	45783	ATTATGTCTCTTGTTT	39	1341

651696	3481	3496	45870	45885	CTTCTTTGCAAAACTA	62	1342
651707	3622	3637	46011	46026	ACAGTTATGCCAAATA	67	1343
651708	3624	3639	46013	46028	TCACAGTTATGCCAAA	58	1344
651709	3626	3641	46015	46030	AATCACAGTTATGCCA	44	1345
651711	3630	3645	46019	46034	AAAGAATCACAGTTAT	17	1346
651712	3632	3647	46021	46036	TAAAAGAATCACAGTT	24	1347
651713	3642	3657	46031	46046	GTAATTGTCCTAAAAG	19	1348
651724	3786	3801	46175	46190	TGTGAACTAGTTCAGG	59	1349
651726	3800	3815	46189	46204	GAAGTTTCCTTGTCTG	68	1350
651732	3891	3906	46280	46295	TACTGTGTAAGTCTTA	60	1351
651733	3893	3908	46282	46297	GGTACTGTGTAAGTCT	76	1352
651734	3895	3910	46284	46299	GAGGTACTGTGTAAGT	58	1353
651736	3899	3914	46288	46303	AAACGAGGTACTGTGT	36	1354
651739	3939	3954	46328	46343	CTGCAGTTCCTGAAGT	17	1355
651741	3943	3958	46332	46347	AGCACTGCAGTTCCTG	72	1356
651742	3945	3960	46334	46349	TAAGCACTGCAGTTCC	65	1357
651743	3947	3962	46336	46351	CATAAGCACTGCAGTT	27	1358
652079	2925	2940	45314	45329	TCCTAGTTATAGATTA	44	1359
652080	2934	2949	45323	45338	CAGGAGTAGTCCTAGT	39	1360
652081	2962	2977	45351	45366	CTAAAACAATGGAATG	12	1361
652082	3004	3019	45393	45408	CATGAATTAAAGTATT	0	1362
652083	3013	3028	45402	45417	AGTAAGCTTCATGAAT	19	1363
652084	3038	3053	45427	45442	CGAGACTCTGACACCA	35	1364
652085	3271	3286	45660	45675	GTTTATGAGGCCAAGG	50	1365
652086	3280	3295	45669	45684	GCAAAACAGGTTTATG	59	1366
652087	3289	3304	45678	45693	ATGAGTTCTGCAAAAC	63	1367
652088	3325	3340	45714	45729	CATCTGGTAGGCACTC	62	1368
652089	3351	3366	45740	45755	TACCCAGTGCCTTGTG	15	1369
652090	3360	3375	45749	45764	GATACCATATACCCAG	64	1370
652091	3393	3408	45782	45797	ACCTAAGGACCGGGAT	30	1371
652092	3403	3418	45792	45807	CACTAGCACTACCTAA	8	1372
652093	3421	3436	45810	45825	GTAAGATATTACAGAC	56	1373
652094	3434	3449	45823	45838	ACCAAAGGCCTTAGTA	29	1374
652095	3469	3484	45858	45873	ACTAAAATACGCATCG	66	1375
652096	3490	3505	45879	45894	ACCAAACCCCTTCTTT	7	1376
652097	3500	3515	45889	45904	TGGCACAGAGACCAAA	8	1377
652098	3509	3524	45898	45913	TTATAGAGCTGGCACA	23	1378
652099	3518	3533	45907	45922	GCAAAACAATTATAGA	15	1379
652100	3538	3553	45927	45942	AGAGTTTCAGTGGAAT	74	1380

652101	3547	3562	45936	45951	CTTGATCGAAGAGTTT	73	1381
652102	3556	3571	45945	45960	ATAAAGTAGCTTGATC	31	1382
652103	3566	3581	45955	45970	AGTGATTTACATAAAG	38	1383
652104	3591	3606	45980	45995	CAAGTTTATTCCTTTA	57	1384
652105	3600	3615	45989	46004	CAATATAATCAAGTTT	0	1385
652106	3651	3666	46040	46055	ATGTGTACAGTAATTG	40	1386
652107	3661	3676	46050	46065	ATACACCTTAATGTGT	0	1387
652108	3678	3693	46067	46082	CAATATGAATATCTGA	17	1388
652109	3694	3709	46083	46098	TATTACACATTTGGGT	35	1389
652110	3703	3718	46092	46107	AAACTGGAATATTACA	16	1390
652111	3713	3728	46102	46117	TATGCAGAGAAAACTG	37	1391
652112	3722	3737	46111	46126	TTAATTACTTATGCAG	44	974
652113	3750	3765	46139	46154	GATAAAACTATTAATT	0	1392
652114	3759	3774	46148	46163	TTGTACCCAGATAAAA	21	1393
652115	3770	3785	46159	46174	CACCTGTTTATTTGTA	36	1394
652116	3809	3824	46198	46213	TTTTACATAGAAGTTT	24	1395
652117	3818	3833	46207	46222	CATAGTGATTTTTACA	43	1396
652118	3827	3842	46216	46231	TTCAGAAATCATAGTG	60	1397
652119	3839	3854	46228	46243	TTCACATAGCAATTCA	63	1398
652120	3848	3863	46237	46252	ATCTGTAGTTTCACAT	51	1399
652121	3857	3872	46246	46261	GTTCCAAAGATCTGTA	65	1400
652122	3876	3891	46265	46280	AACACCCTACCTAAAC	10	1401
652123	3931	3946	46320	46335	CCTGAAGTATGGCCAT	64	1402
652124	3959	3974	46348	46363	TAAATATCCCCTCATA	10	1403
652125	3968	3983	46357	46372	CAAGAGGCCTAAATAT	12	1404
652126	3977	3992	46366	46381	TCAAAAATTCAAGAGG	36	1405
652127	3986	4001	46375	46390	CCATCTACATCAAAAA	26	1406
652128	3995	4010	46384	46399	AAAAAATGCCCATCTA	21	1407
652129	4006	4021	46395	46410	CCACTACCTTAAAAAA	5	1408
652130	4018	4033	46407	46422	AAAGGTAATTAACCAC	0	1409
652131	4027	4042	46416	46431	AGTTCACATAAAGGTA	69	1410

Table 22

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	77	122
540832	4277	4292	46666	46681	ATGCAGTGTGACTCAG	56	135
540834	4279	4294	46668	46683	CTATGCAGTGTGACTC	50	136

540836	4338	4353	46727	46742	CAAAATTGTGCAATGG	52	137
540839	4343	4358	46732	46747	TAGGACAAAATTGTGC	42	64
540846	4579	4594	46968	46983	AAGGTAACTGCTGGGT	69	67
651757	4267	4282	46656	46671	ACTCAGTTAAATAGAG	2	1411
651759	4273	4288	46662	46677	AGTGTGACTCAGTTAA	64	970
651760	4274	4289	46663	46678	CAGTGTGACTCAGTTA	72	971
651762	4280	4295	46669	46684	CCTATGCAGTGTGACT	51	1412
651763	4281	4296	46670	46685	TCCTATGCAGTGTGAC	48	1413
651764	4283	4298	46672	46687	ATTCCTATGCAGTGTG	55	1414
651765	4287	4302	46676	46691	CTAAATTCCTATGCAG	30	1415
651768	4308	4323	46697	46712	ATAACCTATAAAAGTT	8	1416
651771	4340	4355	46729	46744	GACAAAATTGTGCAAT	27	1417
651772	4342	4357	46731	46746	AGGACAAAATTGTGCA	60	1418
651773	4344	4359	46733	46748	TTAGGACAAAATTGTG	28	1419
651774	4346	4361	46735	46750	TATTAGGACAAAATTG	0	1420
651775	4348	4363	46737	46752	TATATTAGGACAAAAT	0	1421
651780	4472	4487	46861	46876	CCCTAAAAAAAGTTAT	3	1422
651786	4574	4589	46963	46978	AACTGCTGGGTTCTAA	34	1423
651787	4576	4591	46965	46980	GTAACTGCTGGGTTCT	49	1424
651790	4582	4597	46971	46986	TTTAAGGTAACTGCTG	41	1425
651796	4626	4641	47015	47030	TGCTATCCAGTATTAA	46	1426
651800	4731	4746	47120	47135	TCTTAATCTAGTTATG	3	1427
651801	4761	4776	47150	47165	GCACTTCAAACTATTA	70	1428
651808	4893	4908	47282	47297	ACTTTCGGATAAAACA	19	1429
652132	4036	4051	46425	46440	ACCATTCAAAGTTCAC	73	975
652133	4046	4061	46435	46450	CTTTTGTTAAACCATT	42	1430
652134	4071	4086	46460	46475	CTTTAAAATCTCTACA	0	1431
652135	4080	4095	46469	46484	ATTCTCCCCCTTTAAA	15	1432
652136	4112	4127	46501	46516	GCTGTAATAATTAGGT	52	1433
652137	4121	4136	46510	46525	GTCTTTAAGGCTGTAA	41	1434
652138	4134	4149	46523	46538	AACAAGGATTTTTGTC	0	1435
652139	4143	4158	46532	46547	AAAAACTTCAACAAGG	34	1436
652140	4172	4187	46561	46576	CTAAGTCTATGTAATT	0	1437
652141	4181	4196	46570	46585	TGTTAATGCCTAAGTC	21	1438
652142	4190	4205	46579	46594	CCACAAACATGTTAAT	12	1439
652143	4200	4215	46589	46604	CTATATTCTTCCACAA	43	1440
652144	4225	4240	46614	46629	ACTCAAATGATACAAT	0	1441
652145	4249	4264	46638	46653	TAGAATGCCTACTTGG	30	1442
652146	4298	4313	46687	46702	AAAGTTAGGTTCTAAA	4	1443

652147	4317	4332	46706	46721	ACAGTTTTGATAACCT	57	1444
652148	4327	4342	46716	46731	AATGGTGACAACAGTT	58	1445
652149	4374	4389	46763	46778	AACATGCCCCACAAAG	20	1446
652150	4383	4398	46772	46787	CTGTAACTTAACATGC	28	1447
652151	4403	4418	46792	46807	ATGAGATGAACTTGTG	60	1448
652152	4412	4427	46801	46816	GGAATACAAATGAGAT	43	1449
652153	4461	4476	46850	46865	GTTATATACTGTTTGA	59	1450
652154	4496	4511	46885	46900	GTTTTTGCTGTCTAAA	53	1451
652155	4505	4520	46894	46909	CTTCAGATAGTTTTTG	39	1452
652156	4514	4529	46903	46918	AATGGAAATCTTCAGA	49	1453
652157	4524	4539	46913	46928	CTTTTTGACAAATGGA	71	976
652158	4537	4552	46926	46941	CAAGAAATCATTACTT	0	1454
652159	4551	4566	46940	46955	TACTACACAATTATCA	20	1455
652160	4561	4576	46950	46965	TAAAAAACATTACTAC	0	1456
652161	4606	4621	46995	47010	GAAGTTACTAAATATA	0	1457
652162	4615	4630	47004	47019	ATTAACACAGAAGTTA	22	1458
652163	4635	4650	47024	47039	CAGAATTCATGCTATC	64	1459
652164	4647	4662	47036	47051	AGTTTCTCAATGCAGA	32	1460
652165	4660	4675	47049	47064	ATGACAGCTATTCAGT	47	1461
652166	4670	4685	47059	47074	GTTTCATTTTATGACA	36	1462
652167	4681	4696	47070	47085	TTAGAAAGAAAGTTTC	0	1463
652168	4693	4708	47082	47097	GAGTATCTTTCTTTAG	28	1464
652169	4702	4717	47091	47106	AACTCATGTGAGTATC	54	1465
652170	4711	4726	47100	47115	TTCTTCAAGAACTCAT	44	1466
652171	4722	4737	47111	47126	AGTTATGACTATTCTT	31	1467
652172	4740	4755	47129	47144	AAACACAGATCTTAAT	0	1468
652173	4752	4767	47141	47156	ACTATTAAACTAAAAC	8	1469
652174	4770	4785	47159	47174	CCCAAACAGGCACTTC	62	1470
652175	4779	4794	47168	47183	TATCATTATCCCAAAC	4	1471
652176	4788	4803	47177	47192	TAAATTACCTATCATT	0	1472
652177	4800	4815	47189	47204	CCTAAATTCATCTAAA	0	1473
652178	4821	4836	47210	47225	CTGCAGATAACTTTTT	12	1474
652179	4831	4846	47220	47235	CTCAACATATCTGCAG	9	1475
652180	4877	4892	47266	47281	CTGTAACCCAGTTAGC	43	1476
652181	4902	4917	47291	47306	GAATTGGAAACTTTCG	29	1477
652182	4915	4930	47304	47319	ACACAAGACAGTGGAA	36	1478

Table 23

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	78	122
651824	5227	5242	47616	47631	CAAATTTTAGATCACT	5	1479
651827	N/A	N/A	37388	37403	ATAAAAAGCATCCTCC	27	1480
651831	N/A	N/A	37463	37478	TTTCACACAGCCAGGA	34	1481
652183	4971	4986	47360	47375	TACTAGTAAGAAATTG	9	1482
652184	4980	4995	47369	47384	AAGAAATAGTACTAGT	26	1483
652185	5013	5028	47402	47417	GTTAAAATACATTCCA	42	1484
652186	5028	5043	47417	47432	CACTATACAAAAATAG	4	1485
652187	5037	5052	47426	47441	TTCAGTTTACACTATA	45	1486
652188	5048	5063	47437	47452	AATGTGCATGTTTCAG	46	1487
652189	5061	5076	47450	47465	GCACAATGTACAAAAT	11	1488
652190	5076	5091	47465	47480	GTCCCACAAAAGAAAG	18	1489
652191	5097	5112	47486	47501	CAACTGGATCACACTG	27	1490
652192	5108	5123	47497	47512	ATGATGGAAAACAACT	19	1491
652193	5118	5133	47507	47522	GCGCAACCAAATGATG	12	1492
652194	5137	5152	47526	47541	GACCAACATTCCTAGG	24	1493
652195	5146	5161	47535	47550	GTTTGATATGACCAAC	33	1494
652196	5159	5174	47548	47563	GGTCATTTTTAATGTT	30	1495
652197	5168	5183	47557	47572	TAAAAGAGTGGTCATT	0	1496
652198	5200	5215	47589	47604	ACTCCTATAAACATTT	22	1497
652199	5209	5224	47598	47613	ACAGCACATACTCCTA	49	1498
652200	5218	5233	47607	47622	GATCACTTCACAGCAC	41	1499
652201	5249	5264	47638	47653	ACAGTTCATGACAAAA	40	1500
652202	5259	5274	47648	47663	TAGGAGTAGTACAGTT	17	1501
652203	5268	5283	47657	47672	TACAATAATTAGGAGT	4	1502
652206	N/A	N/A	37417	37432	CTGTATTGTCGGATCT	62	1503
652207	N/A	N/A	37430	37445	GATTTTTTTCAATCTG	36	1504
652208	N/A	N/A	37486	37501	TACATTATAATGCATT	21	1505
652209	N/A	N/A	2653	2668	ATGCAGCAGGGAAGGC	6	1506
652210	N/A	N/A	4249	4264	TCCAAAGGAGTCTTAC	47	1507
652211	N/A	N/A	7796	7811	GAAACCCAAGGTACAT	67	1508
652212	N/A	N/A	8388	8403	CTCCATGACCTTCAAG	49	1509
652213	N/A	N/A	9042	9057	AGGCAGTCTACTTCAA	44	1510
652214	N/A	N/A	9464	9479	CCAAATAAAGGCTTAA	0	1511
652215	N/A	N/A	10358	10373	TACAAGTAAAGGTGAT	27	1512
652216	N/A	N/A	10751	10766	GAACTGAATTATAAGT	31	1513

652217	N/A	N/A	11315	11330	TATCAAGGTTTGGATC	37	1514
652218	N/A	N/A	11502	11517	TAAAATTGCTGTGTGT	37	1515
652219	N/A	N/A	11687	11702	ATCAATCATATAAGAC	45	1516
652220	N/A	N/A	12032	12047	TCACAACTATTCTACA	15	1517
652221	N/A	N/A	12408	12423	CTAGAGATACCTAAAA	11	1518
652222	N/A	N/A	13439	13454	AATCTATGTTACTTAG	19	1519
652223	N/A	N/A	13991	14006	CAAAGGACATGTAGTT	35	1520
652224	N/A	N/A	14347	14362	AGCCCAATGGTATAAG	20	1521
652225	N/A	N/A	14965	14980	ATCACAGGGAAGGATA	6	1522
652226	N/A	N/A	15751	15766	AATAATCAGAGTGGAC	20	1523
652227	N/A	N/A	16942	16957	ACAGGAGCTAAGGCAA	13	1524
652228	N/A	N/A	17144	17159	AACTTTTCCGGCATCA	23	1525
652229	N/A	N/A	17450	17465	TGAAAATCTAGGTGTC	31	1526
652230	N/A	N/A	17739	17754	AGTATTGTAAGGACTT	41	1527
652231	N/A	N/A	17984	17999	TAACTTTTACTAAAGG	4	1528
652232	N/A	N/A	18104	18119	ACTCAGGCAGTGACTC	38	1529
652233	N/A	N/A	18935	18950	ATGTAACAGTGTGCAA	41	1530
652234	N/A	N/A	18964	18979	GAATGTTCACGACAAA	58	1531
652235	N/A	N/A	19258	19273	AATTGTTTAAGTCTAT	1	1532
652236	N/A	N/A	19785	19800	GTCCATGATAACTATT	37	1533
652237	N/A	N/A	21645	21660	GTACAGATTGGCCAGG	32	1534
652238	N/A	N/A	25871	25886	ACTCCACTGCTCTAAT	8	1535
652239	N/A	N/A	26391	26406	ACTAGACTATACAGTA	7	1536
652240	N/A	N/A	26720	26735	CTAGAAAGATTTTGAT	0	1537
652241	N/A	N/A	31136	31151	AAGTTAGGGCATAAAA	17	1538
652242	N/A	N/A	31818	31833	TATTAAAGTTAGCCTG	34	1539
652243	N/A	N/A	33116	33131	GTTCAAAATATTGATC	18	1540
652244	N/A	N/A	33201	33216	AAAAACCACTACTTGG	22	1541
652245	N/A	N/A	34689	34704	AAGTTATAATGTCAAT	6	1542
652246	N/A	N/A	35767	35782	ACAGAGAATTGGCAAC	48	1543
652247	N/A	N/A	35770	35785	CACACAGAGAATTGGC	71	1544
652248	N/A	N/A	35803	35818	CACCAGTACCATTTGC	36	1545
652249	N/A	N/A	36056	36071	ATATATAGTGCAAATT	15	1546
652250	N/A	N/A	36325	36340	AACAGTGTTCAATCAT	57	1547
652251	N/A	N/A	36875	36890	TCTCAAAGGTGAGTCA	41	1548
652252	N/A	N/A	37321	37336	AGTAATTTACTGGGAA	35	1549
652253	N/A	N/A	38872	38887	TAAGAATAGTATTCTG	2	1550
652254	N/A	N/A	41315	41330	CTCCTTTACTGTACTA	23	1551
652255	N/A	N/A	42293	42308	GTCTTATAGTTTACCA	55	1552

652256	N/A	N/A	42551	42566	GTAAAATCCATTGGAT	15	1553

Table 24

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID 2: Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	74	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	85	122
651666	2855	2870	45244	45259	GTTCTGTCTATTCATA	43	912
651673	2943	2958	45332	45347	TACTGTTACCAGGAGT	44	1554
651674	2945	2960	45334	45349	ATTACTGTTACCAGGA	69	1555
651676	2965	2980	45354	45369	TTACTAAAACAATGGA	14	1556
651683	3008	3023	45397	45412	GCTTCATGAATTAAAG	23	1557
651692	3381	3396	45770	45785	GGATTATGTCTCTTGT	68	1558
651693	3400	3415	45789	45804	TAGCACTACCTAAGGA	2	1559
696162	2829	2844	45218	45233	AAAATCCTACTGTCGC	50	1560
696163	2835	2850	45224	45239	GTTTGAAAAATCCTAC	12	1561
696164	2838	2853	45227	45242	CAGGTTTGAAAAATCC	57	1562
696165	2843	2858	45232	45247	CATACCAGGTTTGAAA	37	1563
696166	2845	2860	45234	45249	TTCATACCAGGTTTGA	50	1564
696167	2850	2865	45239	45254	GTCTATTCATACCAGG	79	925
696168	2860	2875	45249	45264	ATAGGGTTCTGTCTAT	5	1565
696169	2866	2881	45255	45270	CACTGGATAGGGTTCT	51	1566
696170	2869	2884	45258	45273	TTCCACTGGATAGGGT	31	1567
696171	2871	2886	45260	45275	CCTTCCACTGGATAGG	3	1568
696172	2876	2891	45265	45280	ATTCTCCTTCCACTGG	42	1569
696173	2892	2907	45281	45296	GCACTATCTTTATTAA	19	1570
696174	2898	2913	45287	45302	CTTTCAGCACTATCTT	48	1571
696175	2900	2915	45289	45304	TTCTTTCAGCACTATC	51	1572
696176	2903	2918	45292	45307	GAATTCTTTCAGCACT	73	1573
696177	2909	2924	45298	45313	CCTAAGGAATTCTTTC	42	1574
696178	2911	2926	45300	45315	TACCTAAGGAATTCTT	33	1575
696179	2913	2928	45302	45317	ATTACCTAAGGAATTC	21	1576
696180	2917	2932	45306	45321	ATAGATTACCTAAGGA	37	1577
696181	2919	2934	45308	45323	TTATAGATTACCTAAG	14	1578
696182	2921	2936	45310	45325	AGTTATAGATTACCTA	10	1579
696183	2923	2938	45312	45327	CTAGTTATAGATTACC	34	1580
696184	2927	2942	45316	45331	AGTCCTAGTTATAGAT	35	1581
696185	2930	2945	45319	45334	AGTAGTCCTAGTTATA	44	1582

696186	2935	2950	45324	45339	CCAGGAGTAGTCCTAG	42	1583
696187	2936	2951	45325	45340	ACCAGGAGTAGTCCTA	62	1584
696188	2937	2952	45326	45341	TACCAGGAGTAGTCCT	45	1585
696189	2938	2953	45327	45342	TTACCAGGAGTAGTCC	46	1586
696190	2940	2955	45329	45344	TGTTACCAGGAGTAGT	35	1587
696191	2942	2957	45331	45346	ACTGTTACCAGGAGTA	29	1588
696192	2946	2961	45335	45350	TATTACTGTTACCAGG	68	1589
696193	2951	2966	45340	45355	GAATGTATTACTGTTA	56	1590
696194	2954	2969	45343	45358	ATGGAATGTATTACTG	52	1591
696195	2967	2982	45356	45371	GGTTACTAAAACAATG	27	1592
696196	2969	2984	45358	45373	CTGGTTACTAAAACAA	42	1593
696197	2973	2988	45362	45377	ATTTCTGGTTACTAAA	18	1594
696198	2983	2998	45372	45387	TTGCATGAAGATTTCT	59	1595
696199	2987	3002	45376	45391	TTCATTGCATGAAGAT	40	1596
696200	2989	3004	45378	45393	TTTTCATTGCATGAAG	39	1597
696201	3011	3026	45400	45415	TAAGCTTCATGAATTA	19	1598
696202	3273	3288	45662	45677	AGGTTTATGAGGCCAA	36	1599
696203	3276	3291	45665	45680	AACAGGTTTATGAGGC	50	1600
696204	3285	3300	45674	45689	GTTCTGCAAAACAGGT	64	1601
696205	3287	3302	45676	45691	GAGTTCTGCAAAACAG	57	1602
696206	3342	3357	N/A	N/A	CCTTGTGCGGTGACTG	11	1603
696207	3354	3369	45743	45758	ATATACCCAGTGCCTT	14	1604
696208	3356	3371	45745	45760	CCATATACCCAGTGCC	55	1605
696209	3358	3373	45747	45762	TACCATATACCCAGTG	47	1606
696210	3383	3398	45772	45787	CGGGATTATGTCTCTT	69	1607
696211	3390	3405	45779	45794	TAAGGACCGGGATTAT	19	1608
696212	3395	3410	45784	45799	CTACCTAAGGACCGGG	51	1609
696213	3397	3412	45786	45801	CACTACCTAAGGACCG	32	1610
696214	3405	3420	45794	45809	CACACTAGCACTACCT	41	1611
696215	3407	3422	45796	45811	ACCACACTAGCACTAC	48	1612
696216	3409	3424	45798	45813	AGACCACACTAGCACT	48	1613
696217	3411	3426	45800	45815	ACAGACCACACTAGCA	43	1614
696218	3413	3428	45802	45817	TTACAGACCACACTAG	16	1615
696219	3418	3433	45807	45822	AGATATTACAGACCAC	76	1616
696220	3423	3438	45812	45827	TAGTAAGATATTACAG	24	1617
696221	3425	3440	45814	45829	CTTAGTAAGATATTAC	5	1618
696222	3430	3445	45819	45834	AAGGCCTTAGTAAGAT	25	1619
696223	3432	3447	45821	45836	CAAAGGCCTTAGTAAG	18	1620
696224	3436	3451	45825	45840	ATACCAAAGGCCTTAG	48	1621

696225	3438	3453	45827	45842	GTATACCAAAGGCCTT	49	1622
696226	3471	3486	45860	45875	AAACTAAAATACGCAT	2	1623
696227	3473	3488	45862	45877	CAAAACTAAAATACGC	34	1624
696228	3486	3501	45875	45890	AACCCCTTCTTTGCAA	34	1625
696229	3492	3507	45881	45896	AGACCAAACCCCTTCT	45	1626
696230	3494	3509	45883	45898	AGAGACCAAACCCCTT	35	1627
696231	3496	3511	45885	45900	ACAGAGACCAAACCCC	28	1628

Table 25

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID 2: Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	44	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	58	122
651703	3548	3563	45937	45952	GCTTGATCGAAGAGTT	62	1629
651704	3554	3569	45943	45958	AAAGTAGCTTGATCGA	69	1630
651705	3568	3583	45957	45972	GAAGTGATTTACATAA	40	1631
651706	3595	3610	45984	45999	TAATCAAGTTTATTCC	40	1632
651719	3700	3715	46089	46104	CTGGAATATTACACAT	48	1633
651720	3715	3730	46104	46119	CTTATGCAGAGAAAAC	26	968
651727	3806	3821	46195	46210	TACATAGAAGTTTCCT	38	1634
651728	3812	3827	46201	46216	GATTTTTACATAGAAG	18	1635
651730	3859	3874	46248	46263	GTGTTCCAAAGATCTG	59	1636
696232	3498	3513	45887	45902	GCACAGAGACCAAACC	31	1637
696233	3507	3522	45896	45911	ATAGAGCTGGCACAGA	33	1638
696234	3511	3526	45900	45915	AATTATAGAGCTGGCA	29	1639
696235	3513	3528	45902	45917	ACAATTATAGAGCTGG	53	1640
696236	3515	3530	45904	45919	AAACAATTATAGAGCT	8	1641
696237	3536	3551	45925	45940	AGTTTCAGTGGAATCG	66	1642
696238	3537	3552	45926	45941	GAGTTTCAGTGGAATC	56	1643
696239	3543	3558	45932	45947	ATCGAAGAGTTTCAGT	49	1644
696240	3544	3559	45933	45948	GATCGAAGAGTTTCAG	51	1645
696241	3549	3564	45938	45953	AGCTTGATCGAAGAGT	71	1646
696242	3550	3565	45939	45954	TAGCTTGATCGAAGAG	62	1647
696243	3551	3566	45940	45955	GTAGCTTGATCGAAGA	53	1648
696244	3552	3567	45941	45956	AGTAGCTTGATCGAAG	56	1649
696245	3553	3568	45942	45957	AAGTAGCTTGATCGAA	50	1650
696246	3558	3573	45947	45962	ACATAAAGTAGCTTGA	21	1651
696247	3560	3575	45949	45964	TTACATAAAGTAGCTT	37	1652

696248	3563	3578	45952	45967	GATTTACATAAAGTAG	11	1653
696249	3572	3587	45961	45976	CAATGAAGTGATTTAC	34	1654
696250	3593	3608	45982	45997	ATCAAGTTTATTCCTT	57	1655
696251	3594	3609	45983	45998	AATCAAGTTTATTCCT	46	1656
696252	3596	3611	45985	46000	ATAATCAAGTTTATTC	23	1657
696253	3636	3651	46025	46040	GTCCTAAAAGAATCAC	46	1658
696254	3639	3654	46028	46043	ATTGTCCTAAAAGAAT	41	1659
696255	3644	3659	46033	46048	CAGTAATTGTCCTAAA	35	1660
696256	3646	3661	46035	46050	TACAGTAATTGTCCTA	48	1661
696257	3649	3664	46038	46053	GTGTACAGTAATTGTC	43	1662
696258	3653	3668	46042	46057	TAATGTGTACAGTAAT	0	1663
696259	3655	3670	46044	46059	CTTAATGTGTACAGTA	46	1664
696260	3657	3672	46046	46061	ACCTTAATGTGTACAG	39	1665
696261	3659	3674	46048	46063	ACACCTTAATGTGTAC	15	1666
696262	3663	3678	46052	46067	ACATACACCTTAATGT	0	1667
696263	3665	3680	46054	46069	TGACATACACCTTAAT	37	1668
696264	3667	3682	46056	46071	TCTGACATACACCTTA	33	1669
696265	3669	3684	46058	46073	TATCTGACATACACCT	50	1670
696266	3671	3686	46060	46075	AATATCTGACATACAC	39	1671
696267	3680	3695	46069	46084	GTCAATATGAATATCT	53	1672
696268	3686	3701	46075	46090	ATTTGGGTCAATATGA	31	1673
696269	3690	3705	46079	46094	ACACATTTGGGTCAAT	37	1674
696270	3692	3707	46081	46096	TTACACATTTGGGTCA	47	1675
696271	3719	3734	46108	46123	ATTACTTATGCAGAGA	73	977
696272	3755	3770	46144	46159	ACCCAGATAAAACTAT	16	1676
696273	3757	3772	46146	46161	GTACCCAGATAAAACT	12	1677
696274	3761	3776	46150	46165	ATTTGTACCCAGATAA	29	1678
696275	3763	3778	46152	46167	TTATTTGTACCCAGAT	38	1679
696276	3765	3780	46154	46169	GTTTATTTGTACCCAG	67	1680
696277	3773	3788	46162	46177	AGGCACCTGTTTATTT	46	1681
696278	3777	3792	46166	46181	GTTCAGGCACCTGTTT	30	1682
696279	3782	3797	46171	46186	AACTAGTTCAGGCACC	39	1683
696280	3791	3806	46180	46195	TTGTCTGTGAACTAGT	54	1684
696281	3793	3808	46182	46197	CCTTGTCTGTGAACTA	63	1685
696282	3802	3817	46191	46206	TAGAAGTTTCCTTGTC	50	1686
696283	3804	3819	46193	46208	CATAGAAGTTTCCTTG	51	1687
696284	3825	3840	46214	46229	CAGAAATCATAGTGAT	37	1688
696285	3837	3852	46226	46241	CACATAGCAATTCAGA	50	1689
696286	3841	3856	46230	46245	GTTTCACATAGCAATT	47	769

696287	3844	3859	46233	46248	GTAGTTTCACATAGCA	79	770
696288	3846	3861	46235	46250	CTGTAGTTTCACATAG	51	771
696289	3850	3865	46239	46254	AGATCTGTAGTTTCAC	74	1690
696290	3852	3867	46241	46256	AAAGATCTGTAGTTTC	47	1691
696291	3854	3869	46243	46258	CCAAAGATCTGTAGTT	43	1692
696292	3861	3876	46250	46265	CAGTGTTCCAAAGATC	56	1693
696293	3873	3888	46262	46277	ACCCTACCTAAACAGT	22	1694
696294	3878	3893	46267	46282	TTAACACCCTACCTAA	18	1695
696295	3880	3895	46269	46284	TCTTAACACCCTACCT	20	1696
696296	3887	3902	46276	46291	GTGTAAGTCTTAACAC	19	1697
696297	3892	3907	46281	46296	GTACTGTGTAAGTCTT	59	1698
696298	3902	3917	46291	46306	TAGAAACGAGGTACTG	41	1699
696299	3905	3920	46294	46309	GTGTAGAAACGAGGTA	73	1700

Table 26

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID 2: Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	37	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	60	122
651745	3972	3987	46361	46376	AATTCAAGAGGCCTAA	1	1701
651749	4087	4102	46476	46491	TTCTAGAATTCTCCCC	50	1702
651750	4140	4155	46529	46544	AACTTCAACAAGGATT	30	1703
651755	4233	4248	46622	46637	GAACATTCACTCAAAT	8	1704
651756	4252	4267	46641	46656	GCCTAGAATGCCTACT	38	1705
651758	4271	4286	46660	46675	TGTGACTCAGTTAAAT	33	1706
651766	4296	4311	46685	46700	AGTTAGGTTCTAAATT	0	1707
651767	4302	4317	46691	46706	TATAAAAGTTAGGTTC	0	1708
663588	4185	4200	46574	46589	AACATGTTAATGCCTA	46	1709
696300	3910	3925	46299	46314	TCTCTGTGTAGAAACG	41	1710
696301	3935	3950	46324	46339	AGTTCCTGAAGTATGG	59	1711
696302	3944	3959	46333	46348	AAGCACTGCAGTTCCT	52	1712
696303	3949	3964	46338	46353	CTCATAAGCACTGCAG	54	1713
696304	3951	3966	46340	46355	CCCTCATAAGCACTGC	57	1714
696305	3956	3971	46345	46360	ATATCCCCTCATAAGC	21	1715
696306	3961	3976	46350	46365	CCTAAATATCCCCTCA	20	1716
696307	3963	3978	46352	46367	GGCCTAAATATCCCCT	29	1717
696308	3970	3985	46359	46374	TTCAAGAGGCCTAAAT	31	1718
696309	3988	4003	46377	46392	GCCCATCTACATCAAA	51	1719

696310	3992	4007	46381	46396	AAATGCCCATCTACAT	16	1720
696311	4009	4024	46398	46413	TAACCACTACCTTAAA	21	1721
696312	4011	4026	46400	46415	ATTAACCACTACCTTA	0	1722
696313	4015	4030	46404	46419	GGTAATTAACCACTAC	20	1723
696314	4020	4035	46409	46424	ATAAAGGTAATTAACC	0	1724
696315	4028	4043	46417	46432	AAGTTCACATAAAGGT	43	1725
696316	4029	4044	46418	46433	AAAGTTCACATAAAGG	37	978
696317	4035	4050	46424	46439	CCATTCAAAGTTCACA	71	979
696318	4037	4052	46426	46441	AACCATTCAAAGTTCA	67	980
696319	4038	4053	46427	46442	AAACCATTCAAAGTTC	26	1726
696320	4043	4058	46432	46447	TTGTTAAACCATTCAA	21	1727
696321	4075	4090	46464	46479	CCCCCTTTAAAATCTC	10	1728
696322	4084	4099	46473	46488	TAGAATTCTCCCCCTT	45	1729
696323	4109	4124	46498	46513	GTAATAATTAGGTAAC	18	1730
696324	4114	4129	46503	46518	AGGCTGTAATAATTAG	39	1731
696325	4116	4131	46505	46520	TAAGGCTGTAATAATT	6	1732
696326	4119	4134	46508	46523	CTTTAAGGCTGTAATA	15	1733
696327	4136	4151	46525	46540	TCAACAAGGATTTTTG	3	1734
696328	4138	4153	46527	46542	CTTCAACAAGGATTTT	40	1735
696329	4169	4184	46558	46573	AGTCTATGTAATTTAG	21	1736
696330	4174	4189	46563	46578	GCCTAAGTCTATGTAA	28	1737
696331	4176	4191	46565	46580	ATGCCTAAGTCTATGT	30	1738
696332	4178	4193	46567	46582	TAATGCCTAAGTCTAT	24	1739
696333	4183	4198	46572	46587	CATGTTAATGCCTAAG	52	1740
696334	4187	4202	46576	46591	CAAACATGTTAATGCC	42	1741
696335	4202	4217	46591	46606	TGCTATATTCTTCCAC	53	1742
696336	4227	4242	46616	46631	TCACTCAAATGATACA	58	1743
696337	4230	4245	46619	46634	CATTCACTCAAATGAT	27	1744
696338	4235	4250	46624	46639	GGGAACATTCACTCAA	47	1745
696339	4242	4257	46631	46646	CCTACTTGGGAACATT	34	1746
696340	4244	4259	46633	46648	TGCCTACTTGGGAACA	22	1747
696341	4254	4269	46643	46658	GAGCCTAGAATGCCTA	33	1748
696342	4257	4272	46646	46661	ATAGAGCCTAGAATGC	0	1749
696343	4259	4274	46648	46663	AAATAGAGCCTAGAAT	0	1750
696344	4263	4278	46652	46667	AGTTAAATAGAGCCTA	13	1751
696345	4268	4283	46657	46672	GACTCAGTTAAATAGA	33	1752
696346	4269	4284	46658	46673	TGACTCAGTTAAATAG	23	1753
696347	4272	4287	46661	46676	GTGTGACTCAGTTAAA	51	1754
696348	4289	4304	46678	46693	TTCTAAATTCCTATGC	4	1755

696349	4292	4307	46681	46696	AGGTTCTAAATTCCTA	3	1756
696350	4300	4315	46689	46704	TAAAAGTTAGGTTCTA	8	1757
696351	4310	4325	46699	46714	TGATAACCTATAAAAG	20	1758
696352	4314	4329	46703	46718	GTTTTGATAACCTATA	32	1759
696353	4319	4334	46708	46723	CAACAGTTTTGATAAC	36	1760
696354	4321	4336	46710	46725	GACAACAGTTTTGATA	38	1761
696355	4323	4338	46712	46727	GTGACAACAGTTTTGA	60	1762
696356	4329	4344	46718	46733	GCAATGGTGACAACAG	69	1763
696357	4331	4346	46720	46735	GTGCAATGGTGACAAC	22	845
696358	4334	4349	46723	46738	ATTGTGCAATGGTGAC	63	846
696359	4336	4351	46725	46740	AAATTGTGCAATGGTG	39	847
696360	4353	4368	46742	46757	ATGTATATATTAGGAC	20	1764
696361	4355	4370	46744	46759	CTATGTATATATTAGG	22	1765
696362	4367	4382	46756	46771	CCCACAAAGTTTCTAT	22	1766
696363	4369	4384	46758	46773	GCCCCACAAAGTTTCT	23	1767
696364	4376	4391	46765	46780	TTAACATGCCCCACAA	20	1768
696365	4378	4393	46767	46782	ACTTAACATGCCCCAC	47	1769
696366	4380	4395	46769	46784	TAACTTAACATGCCCC	45	1770
696367	4385	4400	46774	46789	AACTGTAACTTAACAT	0	1771

Table 27

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	72	122
540806	2981	2996	45370	45385	GCATGAAGATTTCTGG	77	122
651778	4389	4404	46778	46793	TGCAAACTGTAACTTA	43	1772
651781	4502	4517	46891	46906	CAGATAGTTTTTGCTG	13	1773
651782	4508	4523	46897	46912	AATCTTCAGATAGTTT	48	1774
651784	4566	4581	46955	46970	GGTTCTAAAAAACATT	21	1775
651791	4584	4599	46973	46988	GCTTTAAGGTAACTGC	45	1776
651792	4591	4606	46980	46995	AAATTCAGCTTTAAGG	31	1777
651795	4620	4635	47009	47024	CCAGTATTAACACAGA	70	973
651798	4705	4720	47094	47109	AAGAACTCATGTGAGT	40	1778
651799	4719	4734	47108	47123	TATGACTATTCTTCAA	46	1779
651804	4792	4807	47181	47196	CATCTAAATTACCTAT	0	1780
651809	4899	4914	47288	47303	TTGGAAACTTTCGGAT	32	1781
663600	4637	4652	47026	47041	TGCAGAATTCATGCTA	15	1782
696368	4387	4402	46776	46791	CAAACTGTAACTTAAC	31	1783

696369	4406	4421	46795	46810	CAAATGAGATGAACTT	29	1784
696370	4408	4423	46797	46812	TACAAATGAGATGAAC	13	1785
696371	4458	4473	46847	46862	ATATACTGTTTGAAGA	14	1786
696372	4475	4490	46864	46879	ATCCCCTAAAAAAAGT	14	1787
696373	4498	4513	46887	46902	TAGTTTTTGCTGTCTA	29	1788
696374	4500	4515	46889	46904	GATAGTTTTTGCTGTC	37	1789
696375	4510	4525	46899	46914	GAAATCTTCAGATAGT	43	1790
696376	4512	4527	46901	46916	TGGAAATCTTCAGATA	32	1791
696377	4525	4540	46914	46929	ACTTTTTGACAAATGG	76	981
696378	4530	4545	46919	46934	TCATTACTTTTTGACA	0	982
696379	4544	4559	46933	46948	CAATTATCAAGAAATC	2	1792
696380	4549	4564	46938	46953	CTACACAATTATCAAG	8	1793
696381	4554	4569	46943	46958	CATTACTACACAATTA	3	1794
696382	4556	4571	46945	46960	AACATTACTACACAAT	16	1795
696383	4586	4601	46975	46990	CAGCTTTAAGGTAACT	51	1796
696384	4589	4604	46978	46993	ATTCAGCTTTAAGGTA	57	1797
696385	4617	4632	47006	47021	GTATTAACACAGAAGT	46	983
696386	4622	4637	47011	47026	ATCCAGTATTAACACA	43	984
696387	4628	4643	47017	47032	CATGCTATCCAGTATT	45	1798
696388	4631	4646	47020	47035	ATTCATGCTATCCAGT	52	1799
696389	4633	4648	47022	47037	GAATTCATGCTATCCA	57	1800
696390	4639	4654	47028	47043	AATGCAGAATTCATGC	43	1801
696391	4662	4677	47051	47066	TTATGACAGCTATTCA	48	1802
696392	4697	4712	47086	47101	ATGTGAGTATCTTTCT	46	1803
696393	4700	4715	47089	47104	CTCATGTGAGTATCTT	62	1804
696394	4707	4722	47096	47111	TCAAGAACTCATGTGA	33	1805
696395	4709	4724	47098	47113	CTTCAAGAACTCATGT	7	1806
696396	4713	4728	47102	47117	TATTCTTCAAGAACTC	50	1807
696397	4715	4730	47104	47119	ACTATTCTTCAAGAAC	39	1808
696398	4717	4732	47106	47121	TGACTATTCTTCAAGA	39	1809
696399	4724	4739	47113	47128	CTAGTTATGACTATTC	56	1810
696400	4726	4741	47115	47130	ATCTAGTTATGACTAT	19	1811
696401	4728	4743	47117	47132	TAATCTAGTTATGACT	9	1812
696402	4733	4748	47122	47137	GATCTTAATCTAGTTA	25	1813
696403	4735	4750	47124	47139	CAGATCTTAATCTAGT	51	1814
696404	4737	4752	47126	47141	CACAGATCTTAATCTA	39	1815
696405	4763	4778	47152	47167	AGGCACTTCAAACTAT	39	1816
696406	4766	4781	47155	47170	AACAGGCACTTCAAAC	20	1817
696407	4768	4783	47157	47172	CAAACAGGCACTTCAA	33	1818

696408	4772	4787	47161	47176	ATCCCAAACAGGCACT	45	1819
696409	4775	4790	47164	47179	ATTATCCCAAACAGGC	53	1820
696410	4782	4797	47171	47186	ACCTATCATTATCCCA	48	1821
696411	4785	4800	47174	47189	ATTACCTATCATTATC	24	1822
696412	4790	4805	47179	47194	TCTAAATTACCTATCA	16	1823
696413	4795	4810	47184	47199	ATTCATCTAAATTACC	4	1824
696414	4802	4817	47191	47206	CCCCTAAATTCATCTA	18	1825
696415	4824	4839	47213	47228	TATCTGCAGATAACTT	3	1826
696416	4826	4841	47215	47230	CATATCTGCAGATAAC	3	1827
696417	4828	4843	47217	47232	AACATATCTGCAGATA	0	1828
696418	4833	4848	47222	47237	CCCTCAACATATCTGC	20	1829
696419	4869	4884	47258	47273	CAGTTAGCTCTGTGGG	48	1830
696420	4879	4894	47268	47283	CACTGTAACCCAGTTA	37	1831
696421	4881	4896	47270	47285	AACACTGTAACCCAGT	58	1832
696422	4883	4898	47272	47287	AAAACACTGTAACCCA	44	1833
696423	4889	4904	47278	47293	TCGGATAAAACACTGT	46	1834
696424	4891	4906	47280	47295	TTTCGGATAAAACACT	14	1835
696425	4895	4910	47284	47299	AAACTTTCGGATAAAA	0	1836
696426	4897	4912	47286	47301	GGAAACTTTCGGATAA	56	1837
696427	4904	4919	47293	47308	TGGAATTGGAAACTTT	48	1838
696428	4906	4921	47295	47310	AGTGGAATTGGAAACT	14	1839
696429	4913	4928	47302	47317	ACAAGACAGTGGAATT	31	1840
696430	4917	4932	47306	47321	AAACACAAGACAGTGG	17	1841
696431	4959	4974	47348	47363	ATTGGCACTCAAAGGA	35	1842
696432	4961	4976	47350	47365	AAATTGGCACTCAAAG	30	1843

Table 28

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1, 2 and 3

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO: 3 Start Site	SEQ ID NO: 3 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	2981	2996	45370	45385	3105	3120	GCATGAAGATTTCTGG	77	122
540806	2981	2996	45370	45385	3105	3120	GCATGAAGATTTCTGG	81	122
651813	5033	5048	47422	47437	5157	5172	GTTTACACTATACAAA	18	1844
651814	5039	5054	47428	47443	5163	5178	GTTTCAGTTTACACTA	43	1845
651815	5054	5069	47443	47458	5178	5193	GTACAAAATGTGCATG	26	1846
651816	5103	5118	47492	47507	5227	5242	GGAAAACAACTGGATC	40	1847
651818	5144	5159	47533	47548	5268	5283	TTGATATGACCAACAT	0	1848
651830	N/A	N/A	37421	37436	681	696	CAATCTGTATTGTCGG	67	1849
651837	N/A	N/A	3302	3317	N/A	N/A	AGTCTATTTCAGGCGG	76	1850

663623	5065	5080	47454	47469	5189	5204	GAAAGCACAATGTACA	24	1851
663626	5086	5101	47475	47490	5210	5225	CACTGCATATGTCCCA	54	1852
663627	5094	5109	47483	47498	5218	5233	CTGGATCACACTGCAT	49	1853
663630	5123	5138	47512	47527	5247	5262	GGTCAGCGCAACCAAA	57	1854
663635	5150	5165	47539	47554	5274	5289	TAATGTTTGATATGAC	0	1855
696433	4968	4983	47357	47372	5092	5107	TAGTAAGAAATTGGCA	32	1856
696434	4973	4988	47362	47377	5097	5112	AGTACTAGTAAGAAAT	10	1857
696435	4975	4990	47364	47379	5099	5114	ATAGTACTAGTAAGAA	19	1858
696436	4977	4992	47366	47381	5101	5116	AAATAGTACTAGTAAG	9	1859
696437	4984	4999	47373	47388	5108	5123	CATTAAGAAATAGTAC	12	1860
696438	5000	5015	47389	47404	5124	5139	CCAGGTAAACATGTTA	59	1861
696439	5002	5017	47391	47406	5126	5141	TTCCAGGTAAACATGT	38	1862
696440	5004	5019	47393	47408	5128	5143	CATTCCAGGTAAACAT	47	1863
696441	5035	5050	47424	47439	5159	5174	CAGTTTACACTATACA	46	1864
696442	5041	5056	47430	47445	5165	5180	ATGTTTCAGTTTACAC	34	1865
696443	5045	5060	47434	47449	5169	5184	GTGCATGTTTCAGTTT	44	1866
696444	5079	5094	47468	47483	5203	5218	TATGTCCCACAAAAGA	38	1867
696445	5082	5097	47471	47486	5206	5221	GCATATGTCCCACAAA	51	1868
696446	5084	5099	47473	47488	5208	5223	CTGCATATGTCCCACA	62	1869
696447	5099	5114	47488	47503	5223	5238	AACAACTGGATCACAC	27	1870
696448	5101	5116	47490	47505	5225	5240	AAAACAACTGGATCAC	23	1871
696449	5115	5130	47504	47519	5239	5254	CAACCAAATGATGGAA	54	1872
696450	5128	5143	47517	47532	5252	5267	TCCTAGGTCAGCGCAA	33	1873
696451	5130	5145	47519	47534	5254	5269	ATTCCTAGGTCAGCGC	71	1874
696452	5134	5149	47523	47538	5258	5273	CAACATTCCTAGGTCA	25	1875
696453	5140	5155	47529	47544	5264	5279	TATGACCAACATTCCT	29	1876
696454	5142	5157	47531	47546	5266	5281	GATATGACCAACATTC	27	1877
696455	5148	5163	47537	47552	5272	5287	ATGTTTGATATGACCA	35	1878
696456	5170	5185	47559	47574	5294	5309	ATTAAAAGAGTGGTCA	25	1879
696457	5172	5187	47561	47576	5296	5311	CAATTAAAAGAGTGGT	13	1880
696458	5206	5221	47595	47610	5330	5345	GCACATACTCCTATAA	29	1881
696459	5213	5228	47602	47617	5337	5352	CTTCACAGCACATACT	17	1882
696460	5215	5230	47604	47619	5339	5354	CACTTCACAGCACATA	43	1883
696461	5221	5236	47610	47625	5345	5360	TTAGATCACTTCACAG	32	1884
696462	5223	5238	47612	47627	5347	5362	TTTTAGATCACTTCAC	25	1885
696463	5251	5266	47640	47655	5375	5390	GTACAGTTCATGACAA	35	1886
696464	5254	5269	47643	47658	5378	5393	GTAGTACAGTTCATGA	33	1887
696465	5256	5271	47645	47660	5380	5395	GAGTAGTACAGTTCAT	34	1888
696466	5261	5276	47650	47665	5385	5400	ATTAGGAGTAGTACAG	12	1889

696467	5263	5278	47652	47667	5387	5402	TAATTAGGAGTAGTAC	12	1890
696468	5265	5280	47654	47669	5389	5404	AATAATTAGGAGTAGT	16	1891
696469	5271	5286	47660	47675	5395	5410	CATTACAATAATTAGG	0	1892
696470	5293	5308	47682	47697	5417	5432	GTCACTGTAACTATTT	7	1893
696473	N/A	N/A	37399	37414	659	674	CTCACCAATGTATAAA	17	1894
696474	N/A	N/A	37406	37421	666	681	GATCTCCCTCACCAAT	4	1895
696475	N/A	N/A	37413	37428	673	688	ATTGTCGGATCTCCCT	39	1896
696476	N/A	N/A	37419	37434	679	694	ATCTGTATTGTCGGAT	25	1897
696477	N/A	N/A	37423	37438	683	698	TTCAATCTGTATTGTC	48	1898
696478	N/A	N/A	37453	37468	713	728	CCAGGAGTCTTTTCTT	35	1899
696479	N/A	N/A	37458	37473	718	733	CACAGCCAGGAGTCTT	43	1900
696480	N/A	N/A	37465	37480	725	740	ATTTTCACACAGCCAG	58	1901
696481	N/A	N/A	37467	37482	727	742	TAATTTTCACACAGCC	52	1902
696482	N/A	N/A	37489	37504	749	764	GATTACATTATAATGC	15	1903
696483	N/A	N/A	37492	37507	752	767	CCAGATTACATTATAA	28	1904
696484	N/A	N/A	N/A	N/A	756	771	ACACCCAGATTACATT	12	1905
696485	N/A	N/A	N/A	N/A	761	776	CATCAACACCCAGATT	0	1906
696486	N/A	N/A	N/A	N/A	763	778	ATCATCAACACCCAGA	37	1907
696487	N/A	N/A	2233	2248	N/A	N/A	CGGCAAAGAGGGTCGG	0	1908
696488	N/A	N/A	2550	2565	N/A	N/A	AACCTCCACCGCACCC	2	1909
696489	N/A	N/A	2715	2730	N/A	N/A	ACCACTATCCGTCCAG	45	1910
696490	N/A	N/A	2817	2832	N/A	N/A	CCAAACACAATAACCT	32	1911
696491	N/A	N/A	3068	3083	N/A	N/A	CAACTAGCAAGGAAAA	17	1912
696492	N/A	N/A	3175	3190	N/A	N/A	AGTATAAAAGAGACGA	25	1913
696493	N/A	N/A	3951	3966	N/A	N/A	GTTAATTCTGAGCTGA	53	1914
696494	N/A	N/A	3991	4006	N/A	N/A	CATTTTGGACCTCAGT	33	1915
696495	N/A	N/A	3993	4008	N/A	N/A	AGCATTTTGGACCTCA	71	1916
696496	N/A	N/A	4065	4080	N/A	N/A	ATGGCTACAGTCTCAA	35	1917
696497	N/A	N/A	4079	4094	N/A	N/A	CAAATATACTGTGGAT	26	1918

Table 29

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	% Inhibition	SEQ ID NO
540806	45370	45385	GCATGAAGATTTCTGG	69	122
540806	45370	45385	GCATGAAGATTTCTGG	77	122
663720	27165	27180	TAATTTGTTCTCTGGG	22	1919
696658	23436	23451	GAACTGCAACTATAAG	18	1920
696659	23439	23454	AGAGAACTGCAACTAT	22	1921

696660	23507	23522	ATCTCTAAAGAGCAAT	24	1922
696661	23579	23594	CAATACTCAAGATTCT	18	1923
696662	23688	23703	CAACTCTATTATTCAA	14	1924
696663	24168	24183	CTTAAAATTAACTACC	0	1925
696664	24292	24307	CAGGTACAGAATTCTA	31	1926
696665	24486	24501	AACCTGTATATACATG	20	1927
696666	24583	24598	GAACCAGTTAAGTATC	28	1928
696667	24605	24620	GGATTTTTGGACGAGG	45	1929
696668	24889	24904	ATAGGTTGAGCATTAA	34	1930
696669	24895	24910	TTTCATATAGGTTGAG	28	1931
696670	25198	25213	AAATCTTTGTGCATTG	16	1932
696671	25489	25504	TTATTACAGTGCACCT	5	1933
696672	25494	25509	CTGGATTATTACAGTG	1	1934
696673	25499	25514	ACAGTCTGGATTATTA	5	1935
696674	25501	25516	ACACAGTCTGGATTAT	0	1936
696675	25503	25518	AAACACAGTCTGGATT	12	1937
696676	25696	25711	ACCTATAATGGTGAAT	1	1938
696677	25698	25713	CCACCTATAATGGTGA	0	1939
696678	25701	25716	AACCCACCTATAATGG	8	1940
696679	25704	25719	TTAAACCCACCTATAA	10	1941
696680	25706	25721	ATTTAAACCCACCTAT	0	1942
696681	25855	25870	CCCCCAAGAACTTCAT	3	1943
696682	26058	26073	GTTAAAGTGACACCAT	40	1944
696683	26101	26116	ATCCAAGCAATTCTAT	5	1945
696684	26252	26267	CCCTCAAAGAAATAGA	11	1946
696685	26395	26410	TATTACTAGACTATAC	0	1947
696686	26396	26411	CTATTACTAGACTATA	0	1948
696687	26489	26504	CCATTAGCTGGGTAAA	31	1949
696688	26520	26535	CAGAATTGGCTCAAAT	13	1950
696689	26910	26925	TTAATATGCAGGTAGA	43	1951
696690	26921	26936	AACCTAATAGGTTAAT	4	1952
696691	26939	26954	GAAGTATAGTAAAACT	23	1953
696692	27497	27512	AGCCAAAAGCAGTACC	64	1954
696693	28073	28088	TAGAAAGTATCCCTGT	21	1955
696694	28150	28165	GGTTATACTACCAAGG	46	1956
696695	28205	28220	ACAGGTTTGTATCCCT	48	1957
696696	28230	28245	AGTCATTAGTACAGTT	44	1958
696697	28284	28299	CCAAGTGTAGGTTTAG	58	1959
696698	28347	28362	AGTAAAGTAAGGTTAA	24	1960

696699	28799	28814	GTATAATGGTATAGCA	50	1961
696700	28874	28889	TAACACTGTAGTACGA	10	1962
696701	29016	29031	TATAGATGGATCAATT	28	1963
696702	29038	29053	AGCCCTAAACAAATTG	36	1964
696703	29443	29458	GTAAAGTGATATATGA	22	1965
696704	30003	30018	CTCTTTTTATGTCCTC	56	1966
696705	30110	30125	ATTAGTACTTCTGAGG	41	1967
696706	30205	30220	CCTAAAAATCTCTTAT	0	1968
696707	30356	30371	AAGTATTCTTTCATAC	5	1969
696708	30418	30433	TACATAATAACATCAG	24	1970
696709	30590	30605	CTTTAAAGTCTTCCAG	43	1971
696710	30673	30688	ATTTTCACCAGTAACT	19	1972
696711	30706	30721	TAACAAAATACTCTGC	0	1973
696712	31090	31105	GCACACTAATTTTGTT	41	1974
696713	31124	31139	AAAACAACTTGCCGAT	52	1975
696714	31150	31165	GATCAAGACCCCAAAA	26	1976
696715	31361	31376	AACGATTTTTGCATTT	52	1977
696716	31759	31774	ACTAAAGTTACCCAGA	38	1978
696717	31816	31831	TTAAAGTTAGCCTGTA	29	1979
696718	32195	32210	AAATACTAGAGACCAG	0	1980
696719	32583	32598	TATGTAACGCATTATA	36	1981
696720	32735	32750	GTCCAAAGGGACCAGG	31	1982
696721	32833	32848	AACCCTCCCACTTTTG	17	1983
696722	33039	33054	AAAGCATTCTTTAACG	26	1984
696723	33293	33308	ACAAGATGTATTCTAA	24	1985
696724	33365	33380	CAACACATCAAATACC	22	1986
696725	33478	33493	CCAAAGTATCATTCTA	41	1987
696726	33514	33529	GAAACAAAGCACTCCA	44	1988
696727	33551	33566	CTCAACTATTATCTGA	23	1989
696728	33642	33657	CTTTAAGAACAACTGA	35	1990
696729	34076	34091	TAGCACACAATAATTT	14	1991
696730	34367	34382	ATAAGAAACTTAGGTT	8	1992
696731	34412	34427	TAATTAACAGCACAGG	64	1993
696732	34488	34503	TTGGAAGCCAATAATT	19	1994

Example 8: Antisense inhibition of human K-Ras in HepG2 cells by cEt gapmers

Antisense oligonucleotides were designed targeting a K-Ras nucleic acid and were tested for their effects on K-Ras mRNA in vitro. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 4,000 nM antisense oligonucleotide. After a treatment period of

approximately 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS132 was used to measure mRNA levels. K-Ras mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt sugar modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either SEQ ID NO: 1 or SEQ ID NO: 2. Certain antisense oligonucleotides target the target sequence with one mismatch. These antisense oligonucleotides are presented in the Table below with bold underlining on the mismatched nucleoside.

Table 30

Inhibition of K-Ras mRNA by 3-10-3 cEt gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Mismatches	Sequence	% Inhibition	SEQ ID NO
540737	219	234	7585	7600	0	TACGCCACCAGCTCCA	68	1995
540738	220	235	7586	7601	0	CTACGCCACCAGCTCC	64	1996
540739	221	236	7587	7602	0	CCTACGCCACCAGCTC	86	1997
540740	222	237	7588	7603	0	GCCTACGCCACCAGCT	67	1998
540801	2818	2833	45207	45222	0	GTCGCTAATGGATTGG	85	46
540806	2981	2996	45370	45385	0	GCATGAAGATTTCTGG	52	122
540806	2981	2996	45370	45385	0	GCATGAAGATTTCTGG	85	122
540808	3376	3391	45765	45780	0	ATGTCTCTTGTTTGGG	85	123
651530	1313	1328	43702	43717	0	TGACTAATAGCAGTGG	90	239
651634	2634	2649	45023	45038	0	AATCTTATGGTTAGGG	85	316
651645	2733	2748	45122	45137	0	TATCTGTCAGATTCTC	87	321
651733	3893	3908	46282	46297	0	GGTACTGTGTAAGTCT	90	1352
651972	1213	1228	43602	43617	0	TCATCAGGAAGCCCAT	79	226
651987	1447	1462	43836	43851	0	GCTATTAGGAGTCTTT	89	272
651990	1493	1508	43882	43897	0	GCTATAATAATCCCCA	88	275
652004	1685	1700	44074	44089	0	TTAATGTCACAAGCAG	92	289

652019	1918	1933	44307	44322	0	CTTGATTTGTCAGCAG	92	304
652132	4036	4051	46425	46440	0	ACCATTCAAAGTTCAC	88	975
663454	225	240	7591	7606	0	CTTGCCTACGCCACCA	66	1999
667541	211	226	7577	7592	0	CAGCTCCAACTACCAC	87	2000
667542	212	227	7578	7593	0	CCAGCTCCAACTACCA	81	2001
667543	213	228	7579	7594	0	ACCAGCTCCAACTACC	76	2002
667544	214	229	7580	7595	0	CACCAGCTCCAACTAC	64	2003
667545	215	230	7581	7596	0	CCACCAGCTCCAACTA	70	2004
667546	216	231	7582	7597	0	GCCACCAGCTCCAACT	70	2005
667547	217	232	7583	7598	0	CGCCACCAGCTCCAAC	78	2006
667548	218	233	7584	7599	0	ACGCCACCAGCTCCAA	89	2007
667549	223	238	7589	7604	0	TGCCTACGCCACCAGC	78	2008
667550	224	239	7590	7605	0	TTGCCTACGCCACCAG	82	550
667551	226	241	7592	7607	0	TCTTGCCTACGCCACC	67	2009
667552	227	242	7593	7608	0	CTCTTGCCTACGCCAC	74	2010
667553	211	226	7577	7592	1	**A**AGCTCCAACTACCAC	81	2011
667554	212	227	7578	7593	1	C**A**AGCTCCAACTACCA	82	2012
667555	213	228	7579	7594	1	AC**A**AGCTCCAACTACC	41	2013
667556	214	229	7580	7595	1	CAC**A**AGCTCCAACTAC	34	2014
667557	215	230	7581	7596	1	CCAC**A**AGCTCCAACTA	49	2015
667558	216	231	7582	7597	1	GCCAC**A**AGCTCCAACT	47	2016
667559	217	232	7583	7598	1	CGCCAC**A**AGCTCCAAC	55	2017
667560	218	233	7584	7599	1	ACGCCAC**A**AGCTCCAA	64	2018
667561	219	234	7585	7600	1	TACGCCAC**A**AGCTCCA	72	2019
667562	220	235	7586	7601	1	CTACGCCAC**A**AGCTCC	60	2020
667563	221	236	7587	7602	1	CCTACGCCAC**A**AGCTC	56	2021
667564	222	237	7588	7603	1	GCCTACGCCAC**A**AGCT	47	2022
667565	223	238	7589	7604	1	TGCCTACGCCAC**A**AGC	47	2023
667566	224	239	7590	7605	1	TTGCCTACGCCAC**A**AG	62	2024
667567	225	240	7591	7606	1	CTTGCCTACGCCAC**A**A	62	2025
667568	226	241	7592	7607	1	TCTTGCCTACGCCAC**A**	73	2026
667569	212	227	7578	7593	1	**T**CAGCTCCAACTACCA	79	2027
667570	213	228	7579	7594	1	A**T**CAGCTCCAACTACC	61	2028
667571	214	229	7580	7595	1	CA**T**CAGCTCCAACTAC	28	2029
667572	215	230	7581	7596	1	CCA**T**CAGCTCCAACTA	36	2030
667573	216	231	7582	7597	1	GCCA**T**CAGCTCCAACT	6	2031
667574	217	232	7583	7598	1	CGCCA**T**CAGCTCCAAC	16	2032
667575	218	233	7584	7599	1	ACGCCA**T**CAGCTCCAA	57	2033
667576	219	234	7585	7600	1	TACGCCA**T**CAGCTCCA	57	2034

667577	220	235	7586	7601	1	CTACGCCA**T**CAGCTCC	58	2035
667578	221	236	7587	7602	1	CCTACGCCA**T**CAGCTC	58	2036
667579	222	237	7588	7603	1	GCCTACGCCA**T**CAGCT	0	2037
667580	223	238	7589	7604	1	TGCCTACGCCA**T**CAGC	40	2038
667581	224	239	7590	7605	1	TTGCCTACGCCA**T**CAG	58	2039
667582	225	240	7591	7606	1	CTTGCCTACGCCA**T**CA	53	2040
667583	226	241	7592	7607	1	TCTTGCCTACGCCA**T**C	58	2041
667584	227	242	7593	7608	1	CTCTTGCCTACGCCA**T**	73	2042
667585	212	227	7578	7593	1	**A**CAGCTCCAACTACCA	83	2043
667586	213	228	7579	7594	1	A**A**CAGCTCCAACTACC	62	2044
667587	214	229	7580	7595	1	CA**A**CAGCTCCAACTAC	28	2045
667588	215	230	7581	7596	1	CCA**A**CAGCTCCAACTA	35	2046
667589	216	231	7582	7597	1	GCCA**A**CAGCTCCAACT	26	2047
667590	217	232	7583	7598	1	CGCCA**A**CAGCTCCAAC	37	2048
667591	218	233	7584	7599	1	ACGCCA**A**CAGCTCCAA	83	2049
667592	219	234	7585	7600	1	TACGCCA**A**CAGCTCCA	77	2050
667593	220	235	7586	7601	1	CTACGCCA**A**CAGCTCC	70	2051
667594	221	236	7587	7602	1	CCTACGCCA**A**CAGCTC	64	2052
667595	222	237	7588	7603	1	GCCTACGCCA**A**CAGCT	29	2053
667596	223	238	7589	7604	1	TGCCTACGCCA**A**CAGC	24	2054
667597	224	239	7590	7605	1	TTGCCTACGCCA**A**CAG	51	2055
667598	225	240	7591	7606	1	CTTGCCTACGCCA**A**CA	45	2056
667599	226	241	7592	7607	1	TCTTGCCTACGCCA**A**C	63	2057
667600	227	242	7593	7608	1	CTCTTGCCTACGCCA**A**	72	2058

Example 9: Antisense inhibition of human K-Ras in A431 cells

Antisense oligonucleotides were designed targeting a K-Ras nucleic acid and were tested for their effects on K-Ras mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured A431cells at a density of 5,000 cells per well were treated with 2,000 nM antisense oligonucleotide by free uptake. After a treatment period of approximately 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3496_MGB was used to measure mRNA levels. K-Ras mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 3-10-3 cEt gapmers or deoxy, MOE, and (S)-cEt gapmers. The 3-10-3 cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three nucleosides each. The deoxy, MOE and (S)-cEt oligonucleotides are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; 'd' indicates deoxyribose; the number after 'd' indicates the number of deoxynucleosides; and 'e' indicates a MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either SEQ ID NO: 1 or SEQ ID NO: 2. 'N/A' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 31

Inhibition of K-Ras mRNA by gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	Chemistry	% Inhibition	SEQ ID NO
651987	1447	1462	43836	43851	GCTATTAGGAGTCTTT	kkk-d10-kkk	49	272
651987	1447	1462	43836	43851	GCTATTAGGAGTCTTT	kkk-d10-kkk	62	272
695867	1130	1145	43519	43534	TCCATTTATGTGACTA	kkk-d10-kkk	46	506
695924	1441	1456	43830	43845	AGGAGTCTTTATAGTA	kkk-d10-kkk	65	420
695998	1790	1805	44179	44194	ATGCTGTGAAACTCTC	kkk-d10-kkk	52	658
696017	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kkk-d10-kkk	66	677
696044	2115	2130	44504	44519	GTGTTTATGCAATGTT	kkk-d10-kkk	74	715
696091	2436	2451	44825	44840	AGATTGTGCTGAGCTT	kkk-d10-kkk	29	762
696096	2463	2478	44852	44867	ATGGTGTAACATAGGT	kkk-d10-kkk	47	914
696152	2761	2776	45150	45165	TTAGTGATTAGGTCAA	kkk-d10-kkk	51	924
716764	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kkk-d9-kkke	30	890
716769	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kk-d10-keke	45	892
716774	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kk-d9-kekek	22	894
716779	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kk-d8-kekekk	0	896
716789	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kkk-d8-kekek	12	900
716804	1916	1931	44305	44320	TGATTTGTCAGCAGGA	kk-d8-kekekk	17	906
740162	1128	1143	43517	43532	CATTTATGTGACTAGA	k-d10-kekek	24	2059
740163	1439	1454	43828	43843	GAGTCTTTATAGTAAT	k-d10-kekek	11	2060
740164	1915	1930	44304	44319	GATTTGTCAGCAGGAC	k-d10-kekek	57	2061

740168	1130	1145	43519	43534	TCCATTTATGTGACTA	k-d10-kekek	38	2062
740169	1441	1456	43830	43845	AGGAGTCTTTATAGTA	k-d10-kekek	31	2063
740170	1917	1932	44306	44321	TTGATTTGTCAGCAGG	k-d10-kekek	17	2064
740174	1128	1143	43517	43532	CATTTATGTGACTAGA	k-d9-kekeke	24	2065
740175	1439	1454	43828	43843	GAGTCTTTATAGTAAT	k-d9-kekeke	21	2066
740176	1915	1930	44304	44319	GATTTGTCAGCAGGAC	k-d9-kekeke	32	2067
740180	1130	1145	43519	43534	TCCATTTATGTGACTA	k-d9-kekeke	15	2068
740181	1441	1456	43830	43845	AGGAGTCTTTATAGTA	k-d9-kekeke	24	2069
740182	1917	1932	44306	44321	TTGATTTGTCAGCAGG	k-d9-kekeke	22	2070
740186	1129	1144	43518	43533	CCATTTATGTGACTAG	kk-d10-keke	46	2071
740187	1440	1455	43829	43844	GGAGTCTTTATAGTAA	kk-d10-keke	55	2072
740188	1916	1931	44305	44320	TGATTTGTCAGCAGGA	kk-d10-keke	55	2073
740192	1130	1145	43519	43534	TCCATTTATGTGACTA	kk-d10-keke	22	2074
740193	1441	1456	43830	43845	AGGAGTCTTTATAGTA	kk-d10-keke	40	2075
740197	1129	1144	43518	43533	CCATTTATGTGACTAG	kk-d8-kekekk	0	2076
740198	1440	1455	43829	43844	GGAGTCTTTATAGTAA	kk-d8-kekekk	25	2077
740202	1130	1145	43519	43534	TCCATTTATGTGACTA	kk-d8-kekekk	29	2078
740203	1441	1456	43830	43845	AGGAGTCTTTATAGTA	kk-d8-kekekk	21	2079
740207	1129	1144	43518	43533	CCATTTATGTGACTAG	kk-d9-kdkdk	43	2080
740208	1440	1455	43829	43844	GGAGTCTTTATAGTAA	kk-d9-kdkdk	29	2081
740209	1916	1931	44305	44320	TGATTTGTCAGCAGGA	kk-d9-kdkdk	15	2082
740213	1129	1144	43518	43533	CCATTTATGTGACTAG	kk-d9-kekek	25	2083
740214	1440	1455	43829	43844	GGAGTCTTTATAGTAA	kk-d9-kekek	21	2084
740215	1916	1931	44305	44320	TGATTTGTCAGCAGGA	kk-d9-kekek	45	2085
740219	1130	1145	43519	43534	TCCATTTATGTGACTA	kk-d9-kekek	32	2086
740220	1441	1456	43830	43845	AGGAGTCTTTATAGTA	kk-d9-kekek	31	2087
740224	1128	1143	43517	43532	CATTTATGTGACTAGA	kk-d8-kekekk	20	2088
740225	1439	1454	43828	43843	GAGTCTTTATAGTAAT	kk-d8-kekekk	0	2089
740226	1915	1930	44304	44319	GATTTGTCAGCAGGAC	kk-d8-kekekk	0	2090
740230	1130	1145	43519	43534	TCCATTTATGTGACTA	kkk-d8-kdkdk	16	2091
740231	1441	1456	43830	43845	AGGAGTCTTTATAGTA	kkk-d8-kdkdk	30	2092
740232	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kkk-d8-kdkdk	19	2093
740236	1130	1145	43519	43534	TCCATTTATGTGACTA	kkk-d8-kekek	0	2094
740237	1441	1456	43830	43845	AGGAGTCTTTATAGTA	kkk-d8-kekek	22	2095
740241	1129	1144	43518	43533	CCATTTATGTGACTAG	kkk-d8-kekek	36	2096
740242	1440	1455	43829	43844	GGAGTCTTTATAGTAA	kkk-d8-kekek	0	2097
740243	1916	1931	44305	44320	TGATTTGTCAGCAGGA	kkk-d8-kekek	41	2098
740247	1130	1145	43519	43534	TCCATTTATGTGACTA	kkk-d9-keke	8	2099
740248	1441	1456	43830	43845	AGGAGTCTTTATAGTA	kkk-d9-keke	31	2100

740249	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kkk-d9-keke	40	2101
740253	1129	1144	43518	43533	CCATTTATGTGACTAG	kkk-d9-kkke	49	2102
740254	1440	1455	43829	43844	GGAGTCTTTATAGTAA	kkk-d9-kkke	41	2103
740255	1916	1931	44305	44320	TGATTTGTCAGCAGGA	kkk-d9-kkke	63	2104
740259	1130	1145	43519	43534	TCCATTTATGTGACTA	kkk-d9-kkke	23	2105
740260	1441	1456	43830	43845	AGGAGTCTTTATAGTA	kkk-d9-kkke	6	2106
740273	1788	1803	44177	44192	GCTGTGAAACTCTCTA	k-d10-kekek	41	2107
740275	1790	1805	44179	44194	ATGCTGTGAAACTCTC	k-d10-kekek	18	2108
740277	1788	1803	44177	44192	GCTGTGAAACTCTCTA	k-d9-kekeke	2	2109
740279	1790	1805	44179	44194	ATGCTGTGAAACTCTC	k-d9-kekeke	24	2110
740281	1789	1804	44178	44193	TGCTGTGAAACTCTCT	kk-d10-keke	30	2111
740283	1790	1805	44179	44194	ATGCTGTGAAACTCTC	kk-d10-keke	35	2112
740285	1788	1803	44177	44192	GCTGTGAAACTCTCTA	kk-d8-kekekk	16	2113
740287	1789	1804	44178	44193	TGCTGTGAAACTCTCT	kk-d8-kekekk	5	2114
740289	1790	1805	44179	44194	ATGCTGTGAAACTCTC	kk-d8-kekekk	25	2115
740291	1789	1804	44178	44193	TGCTGTGAAACTCTCT	kk-d9-kdkdk	26	2116
740293	1789	1804	44178	44193	TGCTGTGAAACTCTCT	kk-d9-kekek	0	2117
740295	1790	1805	44179	44194	ATGCTGTGAAACTCTC	kk-d9-kekek	16	2118
740297	1790	1805	44179	44194	ATGCTGTGAAACTCTC	kkk-d9-keke	2	2119
740299	1789	1804	44178	44193	TGCTGTGAAACTCTCT	kkk-d9-kkke	23	2120
740301	1790	1805	44179	44194	ATGCTGTGAAACTCTC	kkk-d9-kkke	37	2121

Table 32

Inhibition of K-Ras mRNA by gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence	Chemistry	% Inhibition	SEQ ID NO
651987	1447	1462	43836	43851	GCTATTAGGAGTCTTT	kkk-d10-kkk	55	272
651987	1447	1462	43836	43851	GCTATTAGGAGTCTTT	kkk-d10-kkk	65	272
695867	1130	1145	43519	43534	TCCATTTATGTGACTA	kkk-d10-kkk	52	506
695924	1441	1456	43830	43845	AGGAGTCTTTATAGTA	kkk-d10-kkk	57	420
695998	1790	1805	44179	44194	ATGCTGTGAAACTCTC	kkk-d10-kkk	39	658
696017	1917	1932	44306	44321	TTGATTTGTCAGCAGG	kkk-d10-kkk	74	677
696044	2115	2130	44504	44519	GTGTTTATGCAATGTT	kkk-d10-kkk	60	715
696091	2436	2451	44825	44840	AGATTGTGCTGAGCTT	kkk-d10-kkk	11	762
696096	2463	2478	44852	44867	ATGGTGTAACATAGGT	kkk-d10-kkk	52	914
696152	2761	2776	45150	45165	TTAGTGATTAGGTCAA	kkk-d10-kkk	52	924
740165	2113	2128	44502	44517	GTTTATGCAATGTTAA	k-d10-kekek	37	2122
740166	2434	2449	44823	44838	ATTGTGCTGAGCTTGA	k-d10-kekek	34	2123
740167	2461	2476	44850	44865	GGTGTAACATAGGTTA	k-d10-kekek	64	2124

740171	2115	2130	44504	44519	GTGTTTATGCAATGTT	k-d10-kekek	53	2125
740172	2436	2451	44825	44840	AGATTGTGCTGAGCTT	k-d10-kekek	7	2126
740173	2463	2478	44852	44867	ATGGTGTAACATAGGT	k-d10-kekek	47	2127
740177	2113	2128	44502	44517	GTTTATGCAATGTTAA	k-d9-kekeke	31	2128
740178	2434	2449	44823	44838	ATTGTGCTGAGCTTGA	k-d9-kekeke	18	2129
740179	2461	2476	44850	44865	GGTGTAACATAGGTTA	k-d9-kekeke	57	2130
740183	2115	2130	44504	44519	GTGTTTATGCAATGTT	k-d9-kekeke	41	2131
740184	2436	2451	44825	44840	AGATTGTGCTGAGCTT	k-d9-kekeke	0	2132
740185	2463	2478	44852	44867	ATGGTGTAACATAGGT	k-d9-kekeke	39	2133
740189	2114	2129	44503	44518	TGTTTATGCAATGTTA	kk-d10-keke	23	2134
740190	2435	2450	44824	44839	GATTGTGCTGAGCTTG	kk-d10-keke	19	2135
740191	2462	2477	44851	44866	TGGTGTAACATAGGTT	kk-d10-keke	67	2136
740194	2115	2130	44504	44519	GTGTTTATGCAATGTT	kk-d10-keke	73	2137
740195	2436	2451	44825	44840	AGATTGTGCTGAGCTT	kk-d10-keke	40	2138
740196	2463	2478	44852	44867	ATGGTGTAACATAGGT	kk-d10-keke	65	2139
740199	2114	2129	44503	44518	TGTTTATGCAATGTTA	kk-d8-kekekk	56	2140
740200	2435	2450	44824	44839	GATTGTGCTGAGCTTG	kk-d8-kekekk	14	2141
740201	2462	2477	44851	44866	TGGTGTAACATAGGTT	kk-d8-kekekk	55	2142
740204	2115	2130	44504	44519	GTGTTTATGCAATGTT	kk-d8-kekekk	9	2143
740205	2436	2451	44825	44840	AGATTGTGCTGAGCTT	kk-d8-kekekk	0	2144
740206	2463	2478	44852	44867	ATGGTGTAACATAGGT	kk-d8-kekekk	40	2145
740210	2114	2129	44503	44518	TGTTTATGCAATGTTA	kk-d9-kdkdk	46	2146
740211	2435	2450	44824	44839	GATTGTGCTGAGCTTG	kk-d9-kdkdk	57	2147
740212	2462	2477	44851	44866	TGGTGTAACATAGGTT	kk-d9-kdkdk	57	2148
740216	2114	2129	44503	44518	TGTTTATGCAATGTTA	kk-d9-kekek	56	2149
740217	2435	2450	44824	44839	GATTGTGCTGAGCTTG	kk-d9-kekek	45	2150
740218	2462	2477	44851	44866	TGGTGTAACATAGGTT	kk-d9-kekek	62	2151
740221	2115	2130	44504	44519	GTGTTTATGCAATGTT	kk-d9-kekek	65	2152
740222	2436	2451	44825	44840	AGATTGTGCTGAGCTT	kk-d9-kekek	38	2153
740223	2463	2478	44852	44867	ATGGTGTAACATAGGT	kk-d9-kekek	53	2154
740227	2113	2128	44502	44517	GTTTATGCAATGTTAA	kk-d8-kekekk	57	2155
740228	2434	2449	44823	44838	ATTGTGCTGAGCTTGA	kk-d8-kekekk	31	2156
740229	2461	2476	44850	44865	GGTGTAACATAGGTTA	kk-d8-kekekk	54	2157
740233	2115	2130	44504	44519	GTGTTTATGCAATGTT	kkk-d8-kdkdk	65	2158
740234	2436	2451	44825	44840	AGATTGTGCTGAGCTT	kkk-d8-kdkdk	42	2159
740235	2463	2478	44852	44867	ATGGTGTAACATAGGT	kkk-d8-kdkdk	36	2160
740238	2115	2130	44504	44519	GTGTTTATGCAATGTT	kkk-d8-kekek	66	2161
740239	2436	2451	44825	44840	AGATTGTGCTGAGCTT	kkk-d8-kekek	26	2162
740240	2463	2478	44852	44867	ATGGTGTAACATAGGT	kkk-d8-kekek	43	2163

740244	2114	2129	44503	44518	TGTTTATGCAATGTTA	kkk-d8-kekek	52	2164
740245	2435	2450	44824	44839	GATTGTGCTGAGCTTG	kkk-d8-kekek	37	2165
740246	2462	2477	44851	44866	TGGTGTAACATAGGTT	kkk-d8-kekek	67	2166
740250	2115	2130	44504	44519	GTGTTTATGCAATGTT	kkk-d9-keke	66	2167
740251	2436	2451	44825	44840	AGATTGTGCTGAGCTT	kkk-d9-keke	43	2168
740252	2463	2478	44852	44867	ATGGTGTAACATAGGT	kkk-d9-keke	43	2169
740256	2114	2129	44503	44518	TGTTTATGCAATGTTA	kkk-d9-kkke	72	2170
740257	2435	2450	44824	44839	GATTGTGCTGAGCTTG	kkk-d9-kkke	30	2171
740258	2462	2477	44851	44866	TGGTGTAACATAGGTT	kkk-d9-kkke	56	2172
740261	2115	2130	44504	44519	GTGTTTATGCAATGTT	kkk-d9-kkke	59	2173
740262	2436	2451	44825	44840	AGATTGTGCTGAGCTT	kkk-d9-kkke	42	2174
740263	2463	2478	44852	44867	ATGGTGTAACATAGGT	kkk-d9-kkke	47	2175
740274	2759	2774	45148	45163	AGTGATTAGGTCAAAT	k-d10-kekek	21	2176
740276	2761	2776	45150	45165	TTAGTGATTAGGTCAA	k-d10-kekek	12	2177
740278	2759	2774	45148	45163	AGTGATTAGGTCAAAT	k-d9-kekeke	12	2178
740280	2761	2776	45150	45165	TTAGTGATTAGGTCAA	k-d9-kekeke	0	2179
740282	2760	2775	45149	45164	TAGTGATTAGGTCAAA	kk-d10-keke	34	2180
740284	2761	2776	45150	45165	TTAGTGATTAGGTCAA	kk-d10-keke	22	2181
740286	2759	2774	45148	45163	AGTGATTAGGTCAAAT	kk-d8-kekekk	46	2182
740288	2760	2775	45149	45164	TAGTGATTAGGTCAAA	kk-d8-kekekk	42	2183
740290	2761	2776	45150	45165	TTAGTGATTAGGTCAA	kk-d8-kekekk	33	2184
740292	2760	2775	45149	45164	TAGTGATTAGGTCAAA	kk-d9-kdkdk	25	2185
740294	2760	2775	45149	45164	TAGTGATTAGGTCAAA	kk-d9-kekek	51	2186
740296	2761	2776	45150	45165	TTAGTGATTAGGTCAA	kk-d9-kekek	48	2187
740298	2761	2776	45150	45165	TTAGTGATTAGGTCAA	kkk-d9-keke	32	2188
740300	2760	2775	45149	45164	TAGTGATTAGGTCAAA	kkk-d9-kkke	43	2189
740302	2761	2776	45150	45165	TTAGTGATTAGGTCAA	kkk-d9-kkke	53	2190

Example 10: Dose-dependent inhibition of human K-Ras mRNA expression in A431 cells

Antisense oligonucleotides described in the studies above were tested at various doses in A431 cells. Isis No. 549148 (3-10-3 cEt gapmer, GGCTACTACGCCGTCA, designated herein as SEQ ID NO: 2191) or ISIS 141923 (5-10-5 MOE gapmer, CCTTCCCTGAAGGTTCCTCC, designated herein as SEQ ID NO: 2192), control oligonucleotides that do not target K-Ras, were included in each experiment as negative controls.

Study 1

Cells were plated at a density of 5,000 cells per well. Cells were incubated with concentrations of antisense oligonucleotide specified in the tables below. Each table represents a separate experiment. After approximately 72 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human K-Ras primer probe set RTS3496_MGB, described above, was used to measure mRNA levels. K-Ras mRNA levels were normalized to beta-actin mRNA levels or RIBOGREEN©. Results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

For some antisense oligonucleotides, the half maximal inhibitory concentration (IC_{50}) is also presented. As illustrated in the tables below, oligonucleotides were successfully taken up by the cells and K-Ras mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 33

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

ISIS No.	Inhibition (%)					IC_{50} (nM)
	12.3 nM	37.0 nM	111 nM	333 nM	1000 nM	
549148	10	10	8	9	18	> 1000
651530	24	64	84	92	93	24
651555	23	58	73	86	88	30
651587	31	68	84	89	91	22
651987	51	79	86	90	93	12
695785	25	58	75	86	88	30
695823	24	46	68	82	89	74
695980	44	74	86	92	95	18
695995	29	61	81	91	93	24

Table 34

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

ISIS No.	Inhibition (%)				
	49.4 nM	148.1 nM	444.4 nM	1333 nM	4000 nM
540806	0	1	54	73	80
651530	5	27	62	82	90
651634	0	11	59	78	86
651645	0	11	26	65	79
651733	0	0	25	44	56
651760	0	10	61	79	87
651972	1	6	38	60	80
651987	2	25	71	86	89

651990	0	18	46	65	79
652004	0	9	55	82	90
652019	0	14	53	75	85
652028	0	0	31	73	81
652034	0	0	35	61	78
652100	0	18	47	69	80
652132	0	23	61	78	88

Table 35

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

Isis No.	Inhibition (%)				
	62 nM	185 nM	556 nM	1667 nM	5000 nM
141923	0	0	4	0	22
651588	8	13	22	60	75
651987	10	33	50	77	83
651990	4	1	24	55	68
652028	17	5	39	59	72
716583	9	34	46	45	70
716587	18	18	45	61	74
716588	7	24	46	67	77
716600	22	30	56	79	83
716608	22	36	53	64	75
716612	20	28	45	66	75
716625	5	28	38	61	82
716628	2	19	58	72	81
716655	13	21	40	53	79
716656	11	27	46	65	83
716769	8	25	48	62	83

Table 36

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

Isis No.	Inhibition (%)				IC_{50} (μM)
	148 nM	444 nM	1333 nM	4000 nM	
540806	15	14	55	62	1.7
651587	6	32	63	76	1.0
651990	5	23	34	59	2.7
652004	3	29	62	73	1.1
663455	0	23	38	47	3.7
695815	28	26	40	58	2.7
695909	0	18	32	50	4.0
695940	0	11	32	55	3.2
695958	0	8	33	36	8.8
695976	3	26	57	73	1.2

695977	14	19	44	62	2.1
695980	17	40	60	78	0.8
695981	1	28	51	65	1.6
695995	13	22	52	64	1.6
696105	7	22	45	58	2.3
696108	3	40	47	65	1.5
696117	2	17	41	59	2.4
696160	10	7	15	33	>4
696176	2	0	0	10	>4
696289	3	0	0	2	>4

Table 37

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

ISIS No.	Inhibition (%)				
	19.5 nM	78.1 nM	312.5 nM	1250 nM	5000 nM
141923	10	15	12	0	14
651530	59	86	93	95	96
651555	49	83	93	96	96
651587	53	85	93	95	96
651987	73	91	94	95	95
695785	40	82	94	96	97
695980	52	85	95	96	97
695995	53	82	93	95	96

Table 38

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

ISIS No.	Inhibition (%)					IC_{50} (μM)
	19.5 nM	78.1 nM	312.5 nM	1250 nM	5000 nM	
141923	0	7	3	0	2	>5
651530	24	55	82	90	93	0.07
651555	13	62	80	91	93	0.09
651587	17	55	76	88	93	0.10
651987	39	72	87	91	93	0.02
695785	20	40	71	84	90	0.13
695980	31	67	86	93	96	0.04
695995	18	54	78	8	92	0.09

Table 39

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

ISIS No.	Inhibition (%)					IC_{50} (nM)
	12.3 nM	37.0 nM	111 nM	333 nM	1000 nM	
549148	0	0	0	0	0	> 1000
651987	19	41	60	80	92	60

696018	23	66	73	80	83	30
716744	39	62	76	83	88	20
716749	28	51	68	75	81	35
716754	27	55	60	70	76	40
746273	23	52	67	77	81	35
746274	29	49	65	70	74	35
746275	62	84	91	95	95	9
746276	49	70	81	87	88	12
746277	7	26	38	46	57	450
746278	22	36	46	50	64	200
746279	37	57	74	77	89	25
746280	47	61	74	84	88	15
746281	0	36	50	69	73	100
746282	20	35	50	70	70	100
746283	6	24	30	54	60	300
746284	21	47	60	72	78	45
746285	37	60	72	79	81	25
746286	48	72	84	90	92	12
746287	32	62	71	80	82	25

Table 40

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

Isis No.	Inhibition (%)				IC_{50} (μM)
	148 nM	444 nM	1333 nM	4000 nM	
141923	0	0	0	0	NA
651499	22	49	63	73	0.7
651530	44	63	78	89	0.2
651541	31	61	71	80	0.4
651555	30	59	75	85	0.4
651603	36	59	70	80	0.3
651634	22	54	69	80	0.5
651635	31	52	65	74	0.5
651704	26	35	43	56	2.3
651795	22	47	59	74	0.7
651837	3	15	27	38	5
651987	42	70	85	88	0.2
695785	35	63	75	79	0.3
695809	32	52	71	80	0.4
695823	19	41	63	73	0.8
695847	25	55	77	87	0.4
695852	20	33	49	65	1.4

695867	59	79	86	92	0.04
695883	17	18	43	57	2.7
695885	21	55	69	84	0.5
695912	45	69	72	82	0.1
695917	32	55	73	76	0.4
695924	44	65	73	83	0.2
695930	31	54	75	82	0.4
695998	37	54	74	80	0.3
696012	31	69	77	89	0.3
696013	33	64	73	83	0.3
696017	55	65	85	90	0.1
696018	37	58	73	80	0.3
696026	34	68	61	76	0.3
696043	28	43	69	81	0.6
696044	55	76	85	90	0.1
696090	43	64	78	83	0.2
696091	24	38	44	66	1.4
696096	23	62	74	82	0.4
696137	42	66	74	83	0.2
696152	27	61	73	81	0.4
696167	0	55	67	77	0.8
696176	0	0	11	21	>4
696219	7	37	51	63	1.5
696241	14	18	15	32	5
696271	34	53	69	76	0.4
696276	26	39	52	63	1.2
696287	29	40	57	72	0.8
696289	2	13	7	7	>4
696299	29	30	50	62	1.5
696317	15	49	66	75	0.7
696318	14	38	47	60	1.7
696355	16	38	53	65	1.2
696356	29	33	55	64	1.2
696358	28	40	60	69	0.8
696377	15	42	59	75	0.9
696495	18	48	52	71	0.9
696554	3	28	47	60	1.9
696556	25	46	62	73	0.7

Table 41

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

Isis No.	Inhibition (%)				IC_{50} (μM)
	185 nM	556 nM	1667 nM	5000 nM	
141923	0	0	0	0	> 10,000
651987	16	47	67	77	1.3
695867	29	59	65	78	N/A
695924	14	42	58	77	1.5
695998	26	42	47	72	N/A
696017	41	60	77	87	NA
696044	31	52	77	89	0.9
696096	17	36	59	75	N/A
696152	32	56	68	82	N/A
740164	16	46	62	78	N/A
740167	15	39	62	80	1.0
740179	1	35	56	71	1.5
740187	27	45	57	82	N/A
740188	31	48	67	8	N/A
740191	28	62	74	87	N/A
740194	44	71	83	92	N/A
740196	22	39	60	69	1.1
740199	22	47	68	76	0.8
740201	11	33	58	68	1.4
740211	0	17	48	58	2.7
740212	13	33	63	75	1.2
740216	50	46	69	82	0.3
740218	21	45	66	83	0.8
740221	22	54	77	83	0.6
740227	27	46	45	79	1
740229	0	2	40	58	3.5
740233	27	47	73	81	0.7
740238	33	55	69	78	NA
740246	31	53	79	82	NA
740250	30	55	80	85	0.5
740253	28	47	53	66	NA
740255	26	52	70	80	NA
740256	43	57	78	90	NA
740258	21	46	69	80	0.8
740261	19	63	79	88	0.5

Table 42

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

Isis No.	Inhibition (%)					IC_{50} (μM)
	62 nM	185 nM	556 nM	1667 nM	5000 nM	
141923	2	0	0	0	0	>5
651588	12	0	21	47	69	2.2
651634	11	2	2	35	52	>5
651653	3	2	37	47	71	1.4
651987	0	26	44	58	77	1.0
651634	11	2	2	35	52	>5
651653	3	25	37	47	71	1.4
652028	3	3	19	43	60	3.2
716583	0	6	5	31	65	4.2
716587	0	9	17	43	64	2.3
716588	0	13	35	52	71	1.5
716600	0	14	35	53	72	1.4
716608	0	0	27	45	70	2
716612	16	3	34	39	69	2.2
716625	0	3	29	40	64	2.5
716628	0	0	36	58	74	1.3
716655	0	0	27	53	68	2
716656	0	9	21	46	67	2
716673	3	7	29	47	67	2
716674	1	0	35	61	78	1.2
716675	8	8	30	56	68	1.6
716683	19	0	23	43	60	2.7
716716	0	0	26	48	68	2
716728	1	28	24	46	71	1.7
716758	10	0	36	58	67	1.6
716763	0	10	24	45	66	2
716772	0	19	32	50	67	1.7
716782	0	0	19	31	39	>5
716807	3	5	7	9	16	>5

Study 2

A431 cells were plated at a density of 10,000 cells per well. Cells were incubated with concentrations of antisense oligonucleotide specified in the tables below. Each table represents a separate experiment. After approximately 48 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. Human K-Ras primer probe set RTS3496_MGB, described above, was used to measure mRNA levels. K-Ras mRNA levels were normalized to RIBOGREEN©. Results are presented as percent inhibition of K-Ras , relative to untreated control cells.

A negative value for percent inhibition indicates that the K-Ras mRNA level was higher than in untreated cells.

For some antisense oligonucleotides, the half maximal inhibitory concentration (IC_{50}) is also presented. As illustrated in the tables below, oligonucleotides were successfully taken up by the cells and K-Ras mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 43

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

Isis No.	Inhibition (%)				
	7.81 nM	31.25 nM	125.0 nM	500.0 nM	2000 nM
141923	0	0	0	0	0
651987	30	39	68	87	93
696018	24	41	64	87	93
696044	29	54	83	94	97
716600	20	45	70	89	94
716655	2	20	49	79	92
740233	29	46	70	86	93
746275	25	47	65	85	91

Table 44

Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

Isis No.	Inhibition (%)					IC_{50} (μM)
	7.81 nM	31.25 nM	125.0 nM	500.0 nM	2000 nM	
141923	2	1	0	3	4	>2
651987	18	30	67	87	93	0.06
696018	9	26	57	80	90	0.11
696044	15	44	83	92	95	0.04
716600	13	36	67	88	94	0.06
716655	10	23	53	82	92	0.11
740233	7	32	58	80	89	0.09
746275	6	24	61	84	92	0.09

Study 3

Hep3B cells were plated at a density of 20,000 cells per well. Cells were transfected using electroporation with increasing concentrations of antisense oligonucleotide, as shown below. After a treatment period of approximately 24 hours, RNA was isolated from the cells and human K-Ras mRNA levels were measured by quantitative real-time PCR. Human K-Ras primer probe set RTS3496_MGB, described above, was used to measure mRNA levels. K-Ras mRNA levels were normalized to Ribogreen. Results are presented as percent inhibition of K-Ras , relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) is also presented. As illustrated in the table below, K-Ras mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 45

Dose-dependent inhibition of human K-Ras mRNA expression by electroporation of ISIS oligonucleotides

ISIS No.	Inhibition (%)					IC_{50} (μM)
	61.7 nM	185.2 nM	555.6 nM	1666.7 nM	5000.0 nM	
540806	14	36	67	75	90	0.4
651530	25	39	66	82	92	0.3
651551	38	65	80	87	93	0.1
651586	28	51	73	86	91	0.2
651595	15	40	65	80	37	0.3
651634	14	36	57	74	90	0.4
651645	9	43	67	83	84	0.4
651646	44	65	79	90	93	0.1
651647	12	40	61	83	89	0.4
651672	2	15	47	69	83	0.8
651733	16	42	66	79	86	0.3
651741	14	34	57	82	88	0.4
651760	8	37	56	74	89	0.5
651923	6	27	54	84	95	0.5
651951	13	30	55	76	89	0.5
651953	19	40	64	83	89	0.3
651959	9	31	66	79	85	0.4
651972	25	33	58	82	86	0.4
651987	35	46	66	82	90	0.2
651990	18	36	64	84	91	0.3
652002	13	20	53	77	85	0.6
652004	18	48	67	85	91	0.3
652019	21	37	68	84	89	0.3
652028	17	41	58	77	89	0.4
652034	11	36	63	79	87	0.4
652050	21	34	63	79	87	0.4
652100	2	32	58	72	88	0.5
652101	16	25	52	72	86	0.6
652132	19	42	72	76	83	0.3
652157	0	22	54	77	88	0.6

Example 11: Dose-dependent inhibition of antisense oligonucleotides targeting K-Ras in cynomolgus monkey primary hepatocytes

At the time this study was undertaken, the cynomolgus monkey genomic sequence was not available in the National Center for Biotechnology Information (NCBI) database; therefore, cross-reactivity with the cynomolgus monkey gene sequence could not be confirmed. Instead, the sequences of the ISIS antisense oligonucleotides used in the cynomolgus monkeys were compared to a rhesus monkey genomic DNA sequence for complementarity. It is expected that ISIS oligonucleotides with complementarity to the rhesus monkey sequence are fully cross-reactive with the cynomolgus monkey sequence as well. The human antisense oligonucleotides tested had at most one mismatch with the rhesus genomic sequence (the complement of GENBANK Accession NC_007868.1 truncated from nucleotide 25479955 to 25525362, designated herein as SEQ ID NO: 2194). In the table below, the number of mismatches of the oligonucleotides with respect to the rhesus genomic sequence is indicated as "# MM."

Table 46

Isis No.	Sequence	Chemistry	# MM	SEQ ID NO.
141923	CCTTCCCTGAAGGTTCCTCC	5-10-5 MOE		2192
651987	GCTATTAGGAGTCTTT	kkk-10-kkk	0	272
696018	CTCTTGATTTGTCAGC	kkk-10-kkk	0	678
696044	GTGTTTATGCAATGTT	kkk-10-kkk	1	715
716600	CCATTTATGTGACTAG	kkk-10-kkk	1	790
716655	TGTTTATGCAATGTTA	kkk-10-kkk	1	854
740233	GTGTTTATGCAATGTT	kkk-8-kdkdk	1	2158
746275	TCTTGATTTGTCAGCA	kk-10-keke	0	804

Antisense oligonucleotides described above were tested at various doses in cynomolgus monkey hepatocytes for ability to reduce K-Ras expression. Cryopreserved cynomolgus monkey primary hepatocytes were plated at a density of 35,000 cells per well and transfected using electroporation with various concentrations of antisense oligonucleotide, as specified in the Tables below. After a treatment period of approximately 24 hours, the cells were washed and lysed, and RNA was isolated. Monkey K-Ras mRNA levels were measured by quantitative real-time PCR, using primer probe set RTS3496_MGB, as described above. K-Ras mRNA target levels were adjusted according to total RNA content, as measured by RIBOGREEN®. In the tables below, results are presented as percent inhibition of K-Ras, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the antisense oligonucleotide did not inhibit mRNA levels.

Table 47

Dose dependent inhibition of monkey K-Ras mRNA expression by electroporation of ISIS oligonucleotides into in primary cynomolgus monkey hepatocytes

ISIS No.	Inhibition (%)					IC_{50} (μM)
	19.5 nM	78.1 nM	312.5 nM	1250 nM	5000 nM	
141923	0	4	0	0	0	>5
651530	37	64	80	83	84	0.03
651555	26	63	68	90	94	0.07
651587	48	69	75	85	78	0.01
651987	50	75	81	88	87	0.01
695785	14	40	72	81	83	0.2
695980	0	0	17	28	50	6
695995	33	46	70	74	90	0.1

Table 48

Dose dependent inhibition of monkey K-Ras mRNA expression by electroporation of ISIS oligonucleotides into in primary cynomolgus monkey hepatocytes

Isis No.	Inhibition (%)					IC_{50} (μM)
	7.81 nM	31.25 nM	125.0 nM	500.0 nM	2000 nM	
141923	2	0	2	0	0	>2
651987	0	20	64	83	91	0.09
696018	11	26	57	83	91	0.10
696044	0	1	0	0	0	>2
716600	2	19	52	79	91	0.14
716655	0	0	0	0	4	>2
740233	4	0	0	0	0	>2
746275	8	22	51	78	88	0.13

Example 12: Tolerability of antisense oligonucleotides targeting human K-Ras mRNA in Lean BALB/c mice

Treatment

Six-to-seven week old male BALB/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously two times a week for four weeks (for a total of 8 treatments) at 100 mg/kg/week with the

antisense oligonucleotides or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration.

Plasma chemistry markers

To evaluate the effect of antisense oligonucleotides on liver and kidney function, plasma levels of ALT transaminase, albumin, blood urea nitrogen (BUN) and total bilirubin were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below. Antisense oligonucleotides causing changes in the levels of any of the liver or kidney function markers outside the expected range for antisense oligonucleotides were excluded from further studies.

Table 49

Plasma chemistry markers in male BALB/c mice

ISIS No.	ALT (U/L)	Albumin (g/dL)	BUN (mg/dL)	T. Bilirubin (mg/dL)
PBS	65	2.8	25	0.17
549148	57	2.8	26	0.27
651530	143	2.7	26	0.22
651551	5927	3.3	26	4.62
651634	56	2.7	29	0.21
651645	4944	3.1	27	0.25
651646	1449	1.8	25	0.27
651647	2061	2.7	23	0.25
651672	4070	2.9	31	0.48
651923	4157	3.1	21	0.36
651972	2105	3.3	29	0.22
651987	71	2.7	29	0.16
651990	32	2.5	30	0.18
652002	106	3.2	29	0.20
652004	145	3.0	31	0.19
652019	5205	3.6	24	0.41
652028	3712	3.8	17	4.43
652034	1167	3.6	27	0.32
652101	48	3.1	23	0.22
652132	1399	3.3	28	0.22
652157	68	2.9	24	0.23

Body and organ weights

Body weights of BALB/c mice were measured at days 1 and 27, and the average body weight for each group is presented in the Table below. Liver, spleen and kidney weights were measured at the end of the study, and are presented in the Table below. Antisense oligonucleotides that caused any changes in

organ weights outside the expected range for antisense oligonucleotides were excluded from further studies.

Table 50

Body and organ weights (in grams)

ISIS No.	body (g)		kidney (g)	liver (g)	spleen (g)
	Day 1	Day 27			
PBS	24	25	0.4	1.7	0.09
549148	24	27	0.5	1.5	0.10
651530	23	27	0.5	1.7	0.13
651551	24	23	0.4	1.7	0.09
651634	24	27	0.3	1.7	0.12
651645	24	27	0.4	1.4	0.14
651646	24	21	0.4	1.4	0.18
651647	24	25	0.4	1.5	0.18
651672	23	20	0.4	1.4	0.07
651733	24	N/A	N/A	N/A	N/A
651760	23	N/A	N/A	N/A	N/A
651923	23	25	0.3	1.9	0.15
651972	24	25	0.4	1.5	0.11
651987	24	27	0.4	1.9	0.11
651990	24	28	0.4	2.2	0.12
652002	24	29	0.4	1.8	0.13
652004	23	27	0.4	1.5	0.10
652019	24	24	0.4	1.8	0.14
652028	23	18	0.3	1.0	0.05
652034	23	25	0.4	1.4	0.11
652100	25	N/A	N/A	N/A	N/A
652101	23	28	0.5	1.4	0.10
652132	25	27	0.4	1.8	0.09
652157	23	28	0.5	1.7	0.13

Example 13: Pharmacodynamics and toxicological profile of antisense oligonucleotides targeting K-Ras in an A431 epidermoid carcinoma xenograft model

Female, 6-8 week old NCr nude mice (Taconic Biosciences, Hudson, NY) were inoculated with human epidermoid carcinoma A431 cells and treated with an antisense oligonucleotide described in the tables above or with PBS. Effects of the oligonucleotides on K-Ras mRNA expression in the tumor and tolerability in the mice were evaluated.

Treatment

The mice each were inoculated with $5x10^6$ A431 cells in 50% Matrigel (BD Bioscience) for tumor development. Antisense oligonucleotide treatment started at day 10-14 after tumor inoculation when the mean tumor size reached approximately 200 mm^3. The mice were subcutaneously injected with 50 mg/kg three times per week (150 mg/kg/week) for three weeks, for a total of nine doses, with an antisense oligonucleotide or PBS. The body weights of the mice were measured once per week. Three weeks after the start of treatment, the mice were sacrificed, K-Ras mRNA levels in the tumor, spleen weights, and body weights were measured.

Study 1

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis and measurement of human K-Ras mRNA levels using primer probe set RTS3496_MGB, described herein above. Results are presented as average percent inhibition of K-Ras for each treatment group, relative to PBS control, normalized to glyceraldehyde-3-phosphate dehydrogenase or beta-actin mRNA levels. As shown in the Tables below, treatment with Isis antisense oligonucleotides resulted in reduction of human K-Ras mRNA in comparison to the PBS control.

Table 51

Antisense mediated inhibition of human K-Ras mRNA expression in A431 xenograft model

ISIS No.	Inhibition (%)
651499	39
651530	55
651541	32
651555	51
651587	51
651603	41
651634	37
651795	46
651987	50
651990	46
652004	47
695815	24
695823	54
695847	50
695867	68
695912	34
695930	45
695976	42

695980	56
695981	35
695995	47
696026	35
696317	40
696816	31

Body weight measurements

Body weights were measured throughout the treatment period. The data is presented in in the Table below as the average for each treatment group at various time points. Spleen weights were measured at the end of the study and are presented in in the Table below.

Table 52

Body and spleen weight measurements in A431 xenograft model

Isis No.	Body weight (g)				Spleen (g)
	Day 1	Day 6	Day 12	Day 19	Day 20
PBS	21.5	23.0	23.4	22.9	0.09
481464	21.4	22.4	22.0	22.4	0.11
549148	21.8	23.0	22.1	23.0	0.10
560131	19.9	20.4	20.2	19.9	0.10
651499	20.8	21.9	22.2	22.5	0.13
651530	21.1	22.5	23.1	22.1	0.10
651541	20.5	21.4	21.4	21.0	0.09
651555	21.5	22.2	22.4	22.1	0.09
651587	21.5	22.6	22.6	20.8	0.11
651603	22.7	24.7	24.8	22.0	0.36
651634	20.6	21.9	21.9	21.9	0.09
651635	20.3	21.7	17.9	N/A	N/A
651795	21.4	22.8	23.3	22.8	0.09
651987	22.8	24.2	24.1	24.1	0.09
651990	22.3	23.3	23.3	23.0	0.12
652004	21.0	22.3	23.3	22.8	0.16
695815	22.7	23.4	23.4	21.4	0.09
695823	21.4	22.6	22.5	21.9	0.09
695847	21.5	22.1	22.1	20.4	0.09
695867	21.1	22.6	22.2	19.6	0.06
695912	21.4	22.3	22.4	22.5	0.09
695930	21.7	22.2	22.8	22.5	0.09
695976	20.5	22.1	22.1	21.6	0.11
695980	21.1	22.5	22.1	21.2	0.07

695981	19.9	21.6	22.1	21.7	0.08
695995	20.7	21.8	22.0	21.6	0.10
696026	21.7	22.8	23.4	22.8	0.09
696317	21.5	23.1	23.0	22.9	0.13
696816	21.6	22.3	22.3	22.1	0.12

Plasma chemistry markers

To evaluate the effect of antisense oligonucleotides on liver and kidney function, plasma levels of transaminases, total bilirubin and blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below.

Table 53
Plasma chemistry markers in A431 xenograft model

ISIS No.	ALT (U/L)	AST (U/L)	Albumin g/dL	T. Bilirubin (mg/dL)	BUN (mg/dL)
PBS	37.0	117.5	3.1	0.17	22
481464	74.0	158.8	2.8	0.13	21
549148	48.3	107.7	3.2	0.16	20
560131	88.3	198.0	3.0	0.13	31
651499	69.8	142.0	2.8	0.17	22
651530	163.0	261.8	3.2	0.15	20
651541	37.3	67.3	2.7	0.14	22
651555	53.3	111.5	3.2	0.17	24
651587	151.8	239.3	3.3	0.13	16
651603	585.3	766.0	2.5	0.18	29
651634	54.3	134.8	3.1	0.15	20
651795	76.0	139.8	3.0	0.13	24
651987	51.3	115.5	3.0	0.13	18
651990	33.8	76.5	2.5	0.13	20
652004	96.3	154.0	3.0	0.12	21
695815	40.3	95.8	2.9	0.13	22
695823	27.7	116.7	2.9	0.15	22
695847	326.3	396.0	2.9	0.16	20
695867	722.8	1200.5	3.1	0.94	19
695912	28.8	80.5	3.1	0.16	23
695930	50.5	131.8	3.0	0.20	22
695976	101.8	281.0	2.7	0.17	20
695980	42.5	104.0	3.1	0.19	25
695981	53.8	132.3	2.8	0.12	21
695995	37.8	122.5	2.9	0.13	22
696026	56.5	101.5	3.1	0.19	23

696317	46.8	133.5	2.7	0.10	23
696816	44.0	83.3	2.7	0.13	24

Study 2

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis and measurement of human K-Ras mRNA levels using primer probe set RTS3496_MGB, described herein above. Results are presented as average percent inhibition of K-Ras for each treatment group, relative to PBS control. As shown in the Table below, treatment with Isis antisense oligonucleotides resulted in reduction of human K-Ras mRNA in comparison to the PBS control.

Table 54

Antisense mediated inhibition of human K-Ras mRNA expression in A431 xenograft model

ISIS No.	Inhibition (%)
481464	33
549148	28
651987	64
695785	54
695809	28
695917	45
695924	87
695977	36
695998	55
696012	24
696013	54
696017	70
696018	71
696043	41
696044	72
696091	79
696096	75
696108	53
696117	40
696137	45
696152	58
696167	53
696271	50
696287	47
696358	46

696377	37
696556	41

Body weight measurements

Body weights were measured throughout the treatment period. The data is presented in in the Table below as the average for each treatment group at various time points. Spleen weights were measured at the end of the study (Day 21) and are presented in in the Table below.

Table 55

Body and spleen weight measurements in A431 xenograft model

Isis No.	Body weight (g)		Spleen (g)
	Day 8	Day 19	Day 21
PBS	23.2	23.5	0.09
481464	20.9	21.2	0.09
549148	22.8	22.0	0.10
651987	20.8	20.5	0.09
695785	21.7	21.5	0.09
695809	22.0	21.5	0.08
695917	21.7	21.6	0.10
695924	22.4	18.8	0.05
695977	22.3	22.9	0.10
695998	22.9	23.2	0.10
696012	23.0	22.5	0.11
696013	21.6	22.7	0.14
696017	24.4	23.1	0.10
696018	23.0	23.1	0.09
696043	22.5	22.2	0.13
696044	21.6	20.5	0.09
696090	22.4	N/A	N/A
696091	22.1	20.8	0.08
696096	21.7	19.8	0.07
696108	22.5	22.8	0.11
696117	22.6	22.6	0.10
696137	23.0	21.9	0.10
696152	22.2	22.3	0.12
696167	22.7	22.5	0.12
696271	21.4	21.5	0.09
696287	24.0	24.4	0.15
696358	22.2	21.2	0.08
696377	23.2	23.6	0.10

696556	23.3	22.9	0.10

Plasma chemistry markers

To evaluate the effect of antisense oligonucleotides on liver and kidney function, plasma levels of transaminases, total bilirubin and blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below.

Table 56
Plasma chemistry markers in A431 xenograft model

ISIS No.	ALT (U/L)	AST (U/L)	Albumin g/dL	T. Bilirubin (mg/dL)	BUN (mg/dL)
PBS	35.5	73.6	3.0	0.13	20
481464	42.3	91.0	2.9	0.10	21
549148	33.3	81.3	2.9	0.13	14
651987	43.5	101.0	2.8	0.12	25
695785	56.5	146.5	2.8	0.17	25
695809	264.8	312.0	2.7	0.18	17
695917	270.0	389.3	2.8	0.16	19
695924	540.0	5421.0	3.4	2.20	48
695977	32.0	70.0	2.9	0.15	22
695998	3197.3	3515.8	3.0	2.96	17
696012	41.0	135.3	2.8	0.15	21
696013	687.0	1511.0	2.4	0.17	20
696017	4189.3	3429.0	2.6	0.84	18
696018	126.8	197.8	3.2	0.18	19
696043	57.0	146.0	2.8	0.15	21
696044	734.5	871.3	3.0	0.24	16
696091	2168.0	6776.0	2.5	7.61	35
696096	4400.3	2436.0	2.1	2.35	18
696108	45.8	105.0	3.0	0.13	19
696117	1699.3	1426.3	3.2	0.22	18
696137	5308.3	6041.3	2.9	0.52	24
696152	1818.3	1798.0	2.9	0.30	22
696167	271.0	312.5	3.0	0.17	17
696271	41.3	84.8	2.9	0.16	20
696287	92.0	183.3	2.8	0.13	17
696358	2284.3	1820.0	3.0	0.27	18
696377	34.8	106.5	3.0	0.12	18
696556	36.0	87.0	3.0	0.15	17

Study 3

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis and measurement of human K-Ras mRNA levels using primer probe set RTS3496_MGB, described herein above. Results are presented as average percent inhibition of K-Ras for each treatment group, relative to PBS control. As shown in the Table below, treatment with Isis antisense oligonucleotides resulted in reduction of human K-Ras mRNA in comparison to the PBS control.

Table 57

Antisense mediated inhibition of human K-Ras mRNA expression in A431 xenograft model

ISIS No.	Inhibition (%)
651588	69
651653	41
651987	51
716587	40
716588	37
716600	55
716608	43
716612	39
716628	48
716655	49
716656	64
716673	54
716683	50
716716	51
716758	74
716769	52
716772	47

Body weight measurements

Body weights were measured throughout the treatment period. The data is presented in in the Table below as the average for each treatment group at various time points. Organ weights were measured at the end of the study (Day 23) and are presented in in the Table below.

Table 58

Body and organ weight measurements in A431 xenograft model

Isis No.	Body weight (g)				Organ weights (g)		
	Day -1	Day 7	Day 14	Day 21	kidney	liver	spleen
PBS	20.5	21.0	21.5	21.9	0.30	1.06	0.09
651588	19.9	20.9	20.3	18.0	0.29	1.41	0.07

651653	20.1	21.1	20.5	20.4	0.32	1.63	0.13
651987	21.2	21.6	21.7	22.2	0.33	1.42	0.12
716587	21.7	23.2	21.4	22.4	0.36	2.38	0.15
716588	20.4	21.7	21.7	22.1	0.33	1.74	0.09
716600	20.3	21.3	22.0	22.7	0.34	N/A	0.13
716608	21.0	19.9	17.3	20.9	0.39	2.07	0.11
716612	21.7	22.1	22.4	23.5	0.34	2.78	0.14
716625	20.1	20.3	17.2	N/A	N/A	N/A	N/A
716628	21.0	21.7	20.8	20.1	0.36	1.55	0.12
716655	19.6	20.6	20.9	21.5	0.33	1.47	0.13
716656	20.5	20.9	19.9	18.5	0.29	1.69	0.07
716673	19.2	20.5	19.0	18.9	0.32	2.47	0.08
716674	20.3	21.0	N/A	N/A	N/A	N/A	N/A
716675	21.4	20.0	N/A	N/A	N/A	N/A	N/A
716683	21.2	21.0	19.2	19.9	0.35	2.58	0.09
716716	19.7	20.5	20.8	21.4	0.34	1.43	0.11
716728	21.7	18.4	N/A	N/A	N/A	N/A	N/A
716758	20.6	20.3	17.6	16.2	0.30	1.29	0.05
716763	21.8	N/A	N/A	N/A	N/A	N/A	N/A
716769	19.6	20.6	20.8	20.7	0.38	1.54	0.12
716772	19.9	20.9	21.3	22.1	0.29	1.16	0.10

Plasma chemistry markers

To evaluate the effect of antisense oligonucleotides on liver and kidney function, plasma levels of transaminases, total bilirubin and blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below.

Table 59
Plasma chemistry markers in A431 xenograft model

ISIS No.	ALT (U/L)	AST (U/L)	T. Bilirubin (mg/dL)	BUN (mg/dL)
PBS	31.4	109.8	0.17	20
651588	4064.3	3039.5	0.21	23
651653	1900.5	1571.3	0.20	21
651987	88.5	150.8	0.11	20
716587	640.0	401.5	0.22	18
716588	1418.8	1067.8	0.17	20
716600	217.3	316.0	0.11	19
716608	950.0	1622.0	0.26	22

716612	281.0	267.5	0.10	19
716628	2662.7	3046.0	0.18	18
716655	125.0	208.8	0.11	23
716656	3306.3	2432.3	2.43	24
716673	3574.3	2518.5	1.01	24
716683	2644.5	3775.0	0.22	28
716716	321.0	586.0	0.20	23
716758	2724.8	1988.3	0.28	27
716769	3563.5	4408.3	0.63	19
716772	45.3	117.5	0.10	22

Study 4

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis and measurement of human K-Ras mRNA levels using primer probe set RTS3496_MGB, described herein above. Results are presented as average percent inhibition of K-Ras for each treatment group, relative to PBS control. As shown in the Tables below, treatment with Isis antisense oligonucleotides resulted in reduction of human K-Ras mRNA in comparison to the PBS control.

Table 60

Antisense mediated inhibition of human K-Ras mRNA expression in A431 xenograft model

ISIS No.	Inhibition (%)
481464	0
651987	45
696816	14
740167	40
740171	31
740173	50
740179	50
740187	39
740188	62
740191	53
740194	56
740196	62
740199	57
740201	51
740212	41
740216	42
740218	48
740221	59

740223	57
740227	42
740233	54
740238	38
740244	22
740246	47
740250	50
740255	47
740256	67
740261	0
740294	30

Body weight measurements

Body weights were measured throughout the treatment period. The data is presented in in the Table below as the average for each treatment group at various time points. Organ weights were measured at the end of the study and are presented in in the Table below.

Table 61
Body weight measurements in A431 xenograft model

Isis No.	Body weight (g)					Organ weights (g)		
	Day 3	Day 8	Day 15	Day 22	Day 24	kidney	liver	spleen
PBS	22.4	23.0	23.3	23.6	23.3	0.33	1.27	0.08
481464	19.9	20.5	20.8	21.9	22.1	0.30	1.32	0.11
651987	22.8	23.4	22.9	23.7	24.1	0.35	1.59	0.13
696816	21.1	21.7	21.6	22.7	23.0	0.33	1.33	0.11
740167	20.3	20.7	20.4	20.9	20.5	0.30	1.26	0.09
740171	22.1	22.4	23.1	24.2	24.5	0.36	1.51	0.37
740173	21.0	21.6	21.5	21.0	20.9	0.31	2.38	0.14
740179	21.4	20.9	22.2	22.8	22.7	0.36	1.41	0.12
740187	23.0	23.2	23.4	24.2	24.4	0.34	1.53	0.11
740188	22.6	21.7	19.6	19.4	19.7	0.35	3.23	0.03
740191	23.9	24.7	25.2	24.6	24.7	0.37	1.51	0.13
740194	23.5	24.3	23.4	21.7	21.8	0.40	1.61	0.16
740196	21.2	21.7	18.0	21.9	23.8	0.37	2.66	0.09
740199	21.5	22.3	21.8	21.5	21.8	0.39	2.40	0.12
740201	22.7	22.9	23.1	23.7	23.5	0.36	1.70	0.13
740212	21.9	22.4	22.2	22.5	22.3	0.33	1.44	0.14
740216	22.1	22.7	22.4	22.3	22.5	0.41	1.89	0.16
740218	20.8	21.4	21.3	21.0	21.7	0.33	2.72	0.13
740221	23.0	23.9	22.1	22.7	23.4	0.41	3.43	0.09

740223	20.6	21.1	21.1	21.2	21.2	0.36	1.28	0.09
740227	20.3	21.2	21.0	21.6	21.4	0.34	2.24	0.14
740233	21.8	22.4	22.5	22.8	22.9	0.36	1.37	0.10
740238	23.8	24.5	24.5	25.0	25.1	0.38	1.61	0.13
740244	21.3	22.8	22.7	22.9	23.2	0.38	1.65	0.12
740246	20.9	21.5	21.5	21.9	21.7	0.37	3.48	0.13
740250	23.1	23.2	19.8	20.5	21.5	0.39	2.69	0.10
740255	21.9	22.6	22.4	22.7	22.7	0.33	2.18	0.09
740256	21.1	21.3	19.5	21.2	21.5	0.37	2.81	0.09
740258	21.8	21.2	17.3	N/A	N/A	N/A	N/A	N/A
740261	21.3	21.9	21.0	22.3	22.8	0.33	1.42	0.12
740294	20.8	21.5	21.9	22.0	21.8	0.36	1.75	0.11
740302	22.8	22.9	N/A	N/A	N/A	N/A	N/A	N/A

Plasma chemistry markers

To evaluate the effect of antisense oligonucleotides on liver and kidney function, plasma levels of transaminases, total bilirubin and blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below.

Table 62
Plasma chemistry markers in A431 xenograft model

ISIS No.	ALT (U/L)	AST (U/L)	Albumin g/dL	T. Bilirubin (mg/dL)	BUN (mg/dL)
PBS	45.0	85.3	3.2	0.14	25
481464	47.0	138.8	2.8	0.14	21
651987	68.3	239.3	2.9	0.18	18
696816	55.5	139.5	2.5	0.14	31
740167	66.0	167.0	2.8	0.16	18
740171	59.5	182.3	3.0	0.18	18
740173	1375.8	964.8	2.8	5.05	19
740179	105.5	262.3	3.1	0.21	17
740187	129.3	164.5	2.9	0.14	22
740188	3098.0	3275.0	2.3	0.24	50
740191	69.8	161.8	2.9	0.14	18
740194	2729.8	3065.5	2.5	0.68	20
740196	2553.0	1607.3	3.2	0.17	24
740199	3552.8	3428.5	3.4	0.28	19
740201	190.5	367.0	2.9	0.19	19
740212	59.8	184.3	3.2	0.19	22
740216	2297.0	2663.5	2.9	0.95	20

740218	3984.8	2519.8	2.1	2.77	21
740221	4076.0	2571.5	2.4	0.33	22
740223	142.3	355.5	3.2	0.19	18
740227	3511.5	3993.0	2.4	0.62	16
740233	67.3	162.5	2.8	0.16	21
740238	165.0	362.0	2.8	0.16	20
740244	118.3	276.0	3.1	0.18	20
740246	3198.8	1579.8	2.2	0.42	22
740250	1710.5	1364.3	2.8	0.16	25
740255	1579.5	827.3	2.8	0.34	21
740256	3725.5	2068.5	2.8	0.21	27
740261	84.3	238.8	3.0	0.20	22
740294	1596.3	1357.0	3.0	0.31	22

Study 5

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis and measurement of human K-Ras mRNA levels using primer probe set RTS3496_MGB, described herein above. Results are presented as average percent inhibition of K-Ras for each treatment group, relative to PBS control, normalized to glyceraldehyde-3-phosphate dehydrogenase or beta-actin mRNA levels. As shown in the Tables below, treatment with Isis antisense oligonucleotides resulted in reduction of human K-Ras mRNA in comparison to the PBS control.

Table 63

Antisense mediated inhibition of human K-Ras mRNA expression in A431 xenograft model

ISIS No.	Inhibition (%)
651555	48
651987	36
695823	26
695980	35
696018	47
716744	25
716749	0
716754	26
746273	9
746275	51
746276	26
746279	31
746280	43
746285	24

746286	51
746287	45

Body weight measurements

Body weights were measured throughout the treatment period. The data is presented in the Table below as the average for each treatment group at various time points. At the end of the study (day 23), organ weights were measured and are presented in the Table below.

Table 64

Body and organ weight measurements in A431 xenograft model

Isis No.	Body weight (g)				Organ weights (g)		
	Day 1	Day 8	Day 15	Day 23	kidney	liver	spleen
PBS	19.2	19.8	20.2	20.6	0.34	1.11	0.10
651555	18.0	17.8	18.4	18.9	0.33	1.26	0.08
651987	19.1	19.4	20.2	21.4	0.30	1.34	0.14
695823	18.8	19.6	19.4	19.8	0.26	1.17	0.09
695980	20.1	20.2	20.7	21.6	0.37	1.32	0.09
696018	19.0	20.3	19.7	19.8	0.31	1.32	0.08
716744	18.3	18.3	18.8	19.8	0.28	1.17	0.09
716749	19.8	20.9	20.6	21.3	0.31	1.27	0.09
716754	19.4	19.2	19.5	20.0	0.28	1.11	0.09
746273	18.8	19.3	19.4	20.1	0.28	1.04	0.08
746275	18.1	18.3	18.7	19.7	0.29	1.12	0.07
746276	19.9	20.9	21.1	21.7	0.33	1.31	0.09
746279	18.7	19.2	19.9	20.6	0.30	1.22	0.11
746280	18.5	19.3	19.5	20.3	0.29	1.19	0.10
746285	20.2	20.5	20.8	21.3	0.33	1.33	0.08
746286	19.8	19.9	19.9	19.7	0.31	1.41	0.06
746287	19.2	19.6	19.1	19.4	0.30	1.51	0.07

Plasma chemistry markers

To evaluate the effect of antisense oligonucleotides on liver and kidney function, plasma levels of transaminases, total bilirubin and blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below.

Table 65

Plasma chemistry markers in A431 xenograft model

ISIS No.	ALT (U/L)	AST (U/L)	T. Bilirubin (mg/dL)	BUN (mg/dL)
PBS	41.8	125.0	0.12	21

651555	65.3	207.3	0.16	23
651987	47.5	185.3	0.17	23
695823	35.0	106.8	0.15	21
695980	36.5	115.3	0.11	18
696018	381.5	634.8	0.16	21
716744	37.3	103.5	.09	22
716749	43.0	141.0	.12	22
716754	82.8	232.3	.12	23
746273	38.8	133.3	.13	22
746275	79.5	275.5	0.18	24
746276	95.8	340.3	.19	18
746279	85.8	276.5	.17	22
746280	84.8	281.0	.18	24
746285	336.0	696.8	.20	21
746286	1345.8	1871.5	.21	20
746287	1454.0	2072.5	.31	22

Table 66
Plasma chemistry markers in A431 xenograft model

ISIS No.	ALT (U/L)	AST (U/L)	T. Bilirubin (mg/dL)	BUN (mg/dL)
PBS	35.5	73.6	0.13	20
651987	43.5	101.0	0.12	25
695785	56.5	146.5	0.17	25
696018	126.8	197.8	0.18	19
696044	734.5	871.3	0.24	16

Example 14: Tolerability of antisense oligonucleotides targeting human K-Ras mRNA in Sprague-Dawley rats

The antisense oligonucleotides described in the studies above were also tested for *in vivo* tolerability in Sprague-Dawley rats.

Groups of four Sprague-Dawley rats were injected subcutaneously once per week for 6 weeks, for a total of 7 treatments, with 50 mg/kg of an antisense oligonucleotide. A control group of rats was injected subcutaneously once per week for 6 weeks with PBS. Two days after the last dose rats were euthanized and organs and plasma were harvested for further analysis. Body weights were measured throughout the study.

To evaluate the effect of the antisense oligonucleotides on hepatic function, plasma concentrations of transaminases (ALT, AST), Albumin (Alb) and total bilirubin (T. Bil.) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY).

To evaluate the effect of the antisense oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine (Cre) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Albumin (Alb) was also measured. Total urine protein (Micro Total Protein (MTP)) and urine creatinine levels as well as the ratio of total urine protein to creatinine (MTP/CREA) were also determined.

Liver, spleen, and kidney weights were measured at the end of the study.

The results are presented in the Tables below and show that many antisense oligonucleotides targeting human K-Ras were well tolerated in Sprague Dawley rats.

Table 67
Body and organ weights

Isis No.	Body weight (g) on indicated study day							Liver (g)	Spleen (g)	Kidney (g)
	1	8	15	22	29	36	43			
PBS	348.8	371.0	407.0	425.8	443.3	463.0	478.0	16.9	0.84	3.6
651530	351.3	377.8	408.5	432.0	449.5	461.5	466.0	17.1	1.26	3.5
651555	369.8	385.5	414.3	424.3	433.3	439.8	443.3	17.7	2.50	3.6
651587	357.0	375.3	412.0	429.3	437.0	442.5	450.5	16.8	1.40	3.7
651987	350.8	375.3	403.0	415.5	425.8	434.5	439.3	19.2	1.74	3.5
695785	351.5	364.3	384.5	389.5	393.0	395.3	409.5	17.8	2.51	3.9
695823	352.8	372.5	398.5	414.3	423.8	434.0	440.8	16.7	1.23	3.4
695980	363.5	379.8	407.8	416.8	419.5	422.8	429.3	17.6	3.06	3.8
695995	332.3	349.5	371.8	372.5	370.0	368.8	374.3	13.4	1.29	2.9

Table 68
Plasma and urine clinical chemistry

	plasma						urine		
Isis No.	ALT (U/L)	AST (U/L)	Alb (g/dL)	BUN (mg/dL)	Cre (mg/dL)	T. Bil. (mg/dL)	Cre (mg/dL)	MTP (mg/dL)	MTP /CREA
PBS	74.3	83.3	3.3	21.6	0.32	0.14	118.5	117.8	1.0
549148	56.5	89.8	3.4	22.5	0.42	0.13	96.8	366.3	3.8
651530	90.8	96.0	3.4	20.6	0.38	0.14	104.8	415.8	4.0
651555	95.3	116.0	3.0	27.8	0.40	0.13	84.5	547.3	6.5

651587	88.8	102.8	3.1	40.6	0.66	0.15	66.5	675.0	10.2
651987	62.8	65.8	2.1	46.5	0.58	0.19	92.8	569.8	6.1
695785	97.0	87.3	3.0	24.8	0.38	0.13	63.0	585.3	9.3
695823	73.5	87.8	3.9	27.1	0.47	0.16	89.3	421.3	4.7
695980	69.0	109.8	3.3	27.1	0.50	0.13	71.0	488.8	6.9
695995	240.3	203.5	4.0	29.3	0.53	0.25	61.0	244.8	4.0

Example 15: Tolerability of antisense oligonucleotides targeting human K-Ras mRNA in Sprague-Dawley rats

The antisense oligonucleotides described in the studies above were also tested for *in vivo* tolerability in Sprague-Dawley rats.

Groups of four Sprague-Dawley rats were injected subcutaneously once per week for 6 weeks, for a total of 7 treatments, with 50 mg/kg of an antisense oligonucleotide. A control group of rats was injected subcutaneously once per week for 6 weeks with PBS. Two days after the last dose rats were euthanized and organs and plasma were harvested for further analysis. Body weights were measured throughout the study.

To evaluate the effect of the antisense oligonucleotides on hepatic function, plasma concentrations of transaminases (ALT, AST), Albumin (Alb) and total bilirubin (T. Bil.) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY).

To evaluate the effect of the antisense oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine (Cre) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Albumin (Alb) was also measured. Total urine protein (Micro Total Protein (MTP)) and urine creatinine levels as well as the ratio of total urine protein to creatinine (MTP/CREA) were also determined.

Liver, spleen, and kidney weights were measured at the end of the study.

The results are presented in the Tables below and show that many antisense oligonucleotides targeting human K-Ras were well tolerated in Sprague Dawley rats.

Table 69
Body and organ weights

Isis No.	Body weight (g) on indicated study day							Liver (g)	Spleen (g)	Kidney (g)
	1	8	15	22	29	36	43			
PBS	252.3	321.5	377.8	417.8	456.8	453.8	492.8	20.5	0.91	3.42

696018	243.3	298.8	329.5	344.5	348.8	340.8	333.3	13.5	2.03	3.38
696044	245.5	299.0	336.5	350.5	345.0	351.5	348.5	15.8	2.57	3.52
716600	240.0	287.5	316.8	333.5	347.0	351.5	360.3	17.2	1.87	3.28
716655	247.8	305.0	341.8	360.3	370.5	374.3	363.5	16.6	3.23	4.86
740233	247.0	305.3	347.3	375.3	393.0	389.0	383.5	16.9	2.13	3.82
746275	242.8	305.5	350.8	372.0	392.5	401.8	405.0	17.5	2.51	3.85

Table 70
Plasma and urine clinical chemistry

Isis No.	plasma						urine		
	ALT (U/L)	AST (U/L)	Alb (g/dL)	BUN (mg/dL)	Cre (mg/dL)	T. Bil. (mg/dL)	Cre (mg/dL)	MTP (mg/dL)	MTP /CREA
PBS	42.0	59.5	3.3	17.1	0.32	0.14	60.8	69.3	1.14
696018	47.5	84.8	2.9	20.5	0.41	0.19	32.0	113.3	3.54
696044	49.5	104.8	2.2	20.7	0.34	0.12	42.0	543.5	12.94
716600	38.3	71.8	2.5	17.2	0.32	0.10	60.3	455.3	7.56
716655	48.3	62.8	1.8	60.9	0.66	0.08	67.5	618.5	9.16
740233	53.3	126.3	2.8	19.3	0.33	0.13	52.5	421.0	8.02
746275	212.8	222.0	2.9	18.6	0.37	0.17	50.5	330.8	6.55

Example 16: Comparative evaluation of potency for Gen1.0 and Gen2.5 human K-RAS antisense oligonucleotides

Antisense oligonucleotides described above and Isis No. 6957 were tested at various doses in A431 cells. Isis No. 6957, described in US Patent No. 6,784,290, consists of 2'-deoxynucleosides linked via phosphorothioate internucleoside linkages, and the sequence is CAGTGCCTGCGCCGCGCTCG (SEQ ID NO: 2193). Isis No. 549148, which does not target K-Ras, was included as a negative control. A431 cells were plated at a density of 10,000 cells per well and incubated with concentrations of antisense oligonucleotide specified in Table 24 below. After 24 hours, RNA was isolated from the cells and K-Ras mRNA levels were measured by quantitative real-time PCR. RTS3496_MGB primer probe set was used to measure K-Ras mRNA levels. K-Ras mRNA levels were normalized to beta-actin mRNA levels. Results are presented as percent inhibition of K-Ras mRNA, relative to untreated control cells.

As illustrated in the Table below, the new antisense oligonucleotides were much more potent than Isis No. 6957, which exhibited minimal inhibition of K-Ras.

Table 71
Dose-dependent inhibition of human K-Ras mRNA expression by free-uptake of ISIS oligonucleotides

ISIS No.	Inhibition (%)					
	41.2 nM	123 nM	370 nM	1111 nM	3333 nM	10000 nM
6957	0	1	2	0	7	17
549148	6	4	1	2	3	1
651530	14	26	54	69	90	92
651555	15	32	57	63	77	84
651587	17	22	69	78	80	87
651987	27	40	70	79	88	91
695785	13	39	53	73	85	87
695823	18	31	43	67	77	81
695980	19	37	65	76	84	88
695995	18	32	45	74	84	90

Example 17: Pharmacodynamics and toxicological profile of human K-Ras antisense oligonucleotides in COLO205 adenocarcinoma xenograft model

Female, 6-8 week old NCr nude mice (Taconic Biosciences, Hudson, NY) were inoculated with human colorectal adenocarcinoma COLO205 cells and treated with antisense oligonucleotides or with PBS. K-Ras expression and tolerability of the oligonucleotides in the mice were evaluated.

Treatment

For tumor development, the mice were each inoculated in the right lateral fat pad with 3×10^6 COLO205 cells in 50% Matrigel (BD Bioscience). Antisense oligonucleotide treatment started around day 10 after tumor inoculation when the mean tumor size reached approximately 200 mm^3. The mice were subcutaneously injected with 30 or 50 mg/kg/week three times per week for three weeks, for a total of nine doses at 150 or 250 mg/kg/week, with antisense oligonucleotide or PBS. RNA was extracted from tumor tissue for real-time PCR analysis. The mice were euthanized 24 hours after the last dose, and organs and plasma were harvested for further analysis. Body weights were measured throughout the study. Liver, spleen, and kidney weights were measured at the end of the study. The results are presented in the Tables below, demonstrating that many antisense oligonucleotides targeting human K-Rasresulted in reduction of K-Ras mRNA levels, and were well tolerated.

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis and measurement of human K-Ras mRNA levels using primer probe set RTS3496_MGB, described herein above. Results are presented

as average percent inhibition of K-Ras for each treatment group, relative to PBS control, normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA levels. As shown in the tables below, treatment with Isis antisense oligonucleotides resulted in reduction of human K-Ras mRNA in comparison to the PBS control.

Table 72

Inhibition of human K-Ras mRNA expression in COLO205 xenograft model

ISIS No.	mg/kg/week	Inhibition (%)
PBS	N/A	0
651555	250	47
	150	23
651987	250	36
	150	30
695980	250	41
	150	28
696044	250	43
	150	45
716600	250	49
	150	43
716655	250	36
	150	36
716772	250	33
	150	27
740179	250	24
	150	28
740256	150	46

Body weight measurements

Body weights were measured throughout the treatment period. The data is presented in the tables below as the average for each treatment group at various time points. At the end of the study, organ weights were measured and are presented in the table below.

Table 73

Body and organ weight measurements

ISIS No.	mg/kg/ week	Body weight (g)				Organ weight (g)		
		day 1	day 9	day 16	day 23	kidney	liver	spleen
PBS	N/A	21.9	21.5	19.7	21.5	0.3	1.1	0.10
651555	250	20.4	19.0	18.2	17.6	0.3	1.0	0.05
	150	18.5	21.9	19.9	21.3	0.2	1.2	0.09
651987	250	20.7	20.3	18.0	16.7	0.3	1.1	0.05

	150	20.6	18.1	16.0	16.4	0.2	1.0	0.06
695980	250	20.6	19.7	17.9	19.4	0.4	1.2	0.14
	150	19.5	19.8	20.3	20.9	0.3	1.1	0.06
696044	250	21.1	20.7	17.0	18.0	0.3	1.7	0.14
	150	21.1	20.7	19.3	19.1	0.3	1.6	0.09
716600	250	19.9	19.7	18.5	18.2	0.3	1.5	0.11
	150	20.3	20.2	19.7	19.2	0.3	1.3	0.07
716655	250	22.8	19.1	18.3	16.7	0.3	1.4	0.05
	150	23.8	19.1	17.5	17.1	0.3	1.4	0.03
716772	250	21.5	20.0	17.6	19.7	0.3	1.3	0.09
	150	20.7	18.5	17.3	17.1	0.2	1.1	0.06
740179	250	21.0	22.3	20.4	21.6	0.3	1.8	0.18
	150	20.9	17.4	17.9	18.0	0.3	1.2	0.09
740256	150	21.2	21.2	18.9	19.9	0.3	1.4	0.09

Plasma chemistry markers

Using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY), plasma concentrations of transaminases (ALT, AST) and total bilirubin (T. Bil.) were measured to evaluate the effect of the antisense oligonucleotides on hepatic function, and plasma concentrations of blood urea nitrogen (BUN) were measured to evaluate the effect of the antisense oligonucleotides on kidney function. Albumin (Alb) was also measured. The results are presented in the Tables below and show that many antisense oligonucleotides targeting human K-Ras mRNA were well tolerated in the COLO205 adenocarcinoma xenograft model.

Table 74

Plasma clinical chemistry

ISIS No.	mg/kg/ week	ALT (U/L)	AST (U/L)	Alb (g/dL)	BUN (mg/dL)	T. bil (mg/dL)
PBS	N/A	29.5	127.0	3.7	16.7	0.14
651555	250	218.3	548.0	3.7	23.6	0.16
	150	47.8	145.3	3.6	18.5	0.15
651987	250	371.8	620.8	3.8	23.1	0.19
	150	232.8	493.0	3.9	20.9	0.20
695980	250	64.3	248.3	3.4	22.4	0.11
	150	37.0	146.3	3.9	20.1	0.15
696044	250	1176.5	1267.0	3.7	20.2	0.85
	150	96.8	298.8	4.4	20.8	0.24
716600	250	647.3	1128.0	3.5	22.9	0.38
	150	49.3	294.0	4.1	19.7	0.18
716655	250	367.5	923.0	3.9	29.2	0.94

	150	86.5	398.0	4.4	27.8	0.17
716772	250	65.0	285.0	4.0	29.3	0.13
	150	50.8	301.5	4.0	28.7	0.13
740179	250	396.5	400.0	3.6	20.5	0.15
	150	95.7	255.7	4.3	20.6	0.16
740256	150	225.3	488.5	3.6	22.0	0.32

Example 18: Effect of human K-Ras antisense oligonucleotides on proliferation of H460 cells (3D assays)

An *in vitro* three-dimensional (3D) model was used to assess the effects of human K-Ras antisense oligonucleotides on mutant K-Ras cancer tumor cell growth. Human mutant K-Ras non-small-cell lung cancer cells (NCI-H460) were grown as spheroids on Thermo Scientific™ Nunclon™ Sphera™ ultra-low attachment microwell plates. Cancer spheroids simulate the 3D structures of tumor growth, allowing the study of tumor progression and efficacy of antisense oligonucleotides *in vitro*.

Treatment

NCI-H460 cells were plated at a density of 1000 cells per well and incubated with various doses of antisense oligonucleotide or with PBS for a period of eight days. K-Ras mRNA expression and effects of the oligonucleotides on spheroid volume were evaluated and are presented in the tables below.

RNA analysis

At day six, RNA was isolated from the cells for real-time PCR analysis and human K-Ras mRNA levels were measured using primer probe set RTS3496_MGB, described herein above. Results are presented as average percent inhibition of K-Ras for each treatment group, relative to PBS control, normalized to beta-actin mRNA levels. Treatment with Isis antisense oligonucleotides resulted in reduction of human K-Ras mRNA in comparison to the PBS control. The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented.

Table 75

Dose-dependent inhibition of human K-Ras mRNA expression by antisense oligonucleotides

ISIS No.	Inhibition (%)					IC_{50} (μM)
	12.3 nM	37.0 nM	111 nM	333 nM	1000 nM	
651530	21.9	53.3	71.8	81.6	93.1	0.33
651555	19.9	44.2	75.9	84.3	94.5	0.43
651587	36.2	60.1	79.1	86.4	93.7	0.23
651987	32.9	69.9	81.8	85.7	93.3	0.2
695785	3.6	33.3	63.1	75.9	77.6	0.65

695823	20.4	37.3	56.2	79.3	84.3	0.75
695980	29.0	65.3	82.9	83.4	94.0	0.23
695995	20.8	49.7	69.0	81.4	86.5	0.35
696018	19.4	55.4	76.6	81.5	91.4	0.3
696044	43.7	76.8	86.8	94.2	97.6	0.15
716600	20.4	52.4	79.9	87.5	95.6	0.33
716655	10.8	41.0	73.4	82.7	92.5	0.7
716772	20.1	54.7	74.6	79.0	87.1	0.3
740179	17.2	52.2	79.0	84.7	93.6	0.33
740191	33.0	64.3	80.6	90.0	95.0	0.23
740223	12.9	52.7	75.3	83.4	92.7	0.38
740256	24.9	65.6	80.1	88.2	94.6	0.3
746275	16.5	67.6	79.6	87.8	94.5	0.3

Spheroid volume analysis

At day eight, H460 spheroids were photographed and their relative volume was measurement using ImageJ. Results are presented as average percent reduction in spheroid volume for each treatment group, relative to PBS control. The half maximal growth inhibitory concentration (GI_{50}) of each oligonucleotide is also presented.

Table 76
Relative spheroid volume at 8 days relative to untreated NCI-H460 cells

ISIS No.	GI_{50} (μM)
651530	1.7
651555	2.0
651587	1.1
651987	1.1
695785	5.0
695823	6.0
695980	0.7
695995	2.5
696018	0.8
696044	0.8
716600	1.3
716655	2.2
716772	4.0
740179	1.1
740191	1.2
740223	1.5
740256	0.8

746275	0.9

Example 19: H358 xenograft study of tumor volume

A K-Ras mutant mouse xenograft model for non-small cell lung cancer (NSCLC) was generated and used to study the efficacy of lead antisense oligonucleotides ISIS Nos. 651987 and 746275, as compared to untreated mice and to mice treated with ISIS No. 549148 as a negative control. The mice each were inoculated with NCI-H358 human NSCLC cells for tumor development.

Treatment

Thirty-two female, athymic nude mice (CrTac:NCr-*Foxn1*^{nu}; Taconic Biosciences, Inc., Hudson, NY), 6-8 weeks old with starting weights of 19-21 g, were divided into four groups, eight subjects per treatment group with exception of the control group treated with ISIS No. 549148, which contained five subjects. The mice were inoculated with $5x10^6$ NCI-H358 cells in 50% Matrigel (BD Bioscience) into the mammary fat pad. Antisense oligonucleotide treatment started at day 10-14 after tumor inoculation when the mean tumor size reached approximately 200 mm^3. The mice were subcutaneously injected with antisense oligonucleotide at 50 mg/kg, five times per week (250 mg/kg/week) for 4.5 weeks (for a total of 22 doses), or with PBS as untreated control. Effects of KRAS antisesnse oligonucleotides on tumor K-Ras mRNA expression and tumor growth as well as tolerability of KRAS oligonucleotides in mice were evaluated. The body weights of the mice were measured once per week. At the end of the study (day 33), the mice were sacrificed, organs and tumor harvested, and K-Ras mRNA levels in the tumor were measured.

RNA analysis

RNA was extracted from tumor tissue for real-time PCR analysis and measurement of human K-Ras mRNA levels using primer probe set RTS3496_MGB, described herein above. Results are presented the Table below as average percent inhibition of K-Ras for each treatment group, relative to PBS control, normalized to beta-actin mRNA levels.

Table 77

Percent inhibition of human K-Ras mRNA expression relative to control in H358 xenograft model

ISIS No.	Inhibition (%)
549148	0
651987	36
746275	56

Body weight measurements

Body weights were measured throughout the treatment period. At the end of the study (day 33), organs were weighed and the data is presented in the Table below as the average for each treatment group at various time points.

Table 78
Body and organ weight measurements in H358 xenograft model

Isis No.	Body weight (g)					Organ weights (g)		
	Day 6	Day 15	Day 20	Day 26	Day 32	kidney	liver	spleen
PBS	22.6	23.2	23.4	23.0	23.2	0.32	1.29	0.14
549148	20.6	21.3	21.8	22.0	22.6	0.34	1.83	0.18
651987	23.1	22.5	21.7	21.2	21.0	0.34	1.54	0.10
746275	22.0	22.3	22.6	22.6	22.9	0.34	1.62	0.15

Plasma chemistry markers

To evaluate the effect of antisense oligonucleotides on liver and kidney function, plasma levels of transaminases, total bilirubin and blood urea nitrogen (BUN) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below.

Table 79
Plasma chemistry markers in H358 xenograft model

ISIS No.	ALT (U/L)	AST (U/L)	Albumin g/dL	BUN (mg/dL)	T. Bilirubin (mg/dL)
PBS	27.5	74.5	2.7	19.1	0.14
549148	68.6	102.2	2.8	19.0	0.11
651987	235.6	347.3	2.5	21.6	0.14
746275	258.6	403.8	2.4	23.5	0.12

Tumor volume

To evaluate the effect of antisense oligonucleotides on tumor volume, tumor sites were measured at various time points. The results are presented in the Table below.

Table 80
Relative tumor volume, % from day one in H358 xenograft model

Isis No.	Day 1	Day 5	Day 14	Day 19	Day 25	Day 31
PBS	100	186	337	378	1138	1908
549148	100	170	322	353	968	1109

651987	100	141	184	154	381	387
746275	100	142	162	148	265	250

Two lead antisense oligonucleotides, ISIS 651987 and ISIS 746275, inhibited tumor growth over the course of the study.

Example 20: Effect of a KRAS ASO on the proliferation of KRAS mutant and KRAS wild type tumour cells *in vitro* (3D)

The effect of 651987 on KRAS mRNA levels and proliferation in 3D was assessed *in vitro* in a panel of lung, colon and pancreatic cancer cell lines expressing mutant or wild type KRAS. The correlation between down regulation of KRAS mRNA (IC_{50}) and inhibition of growth in soft agar (IC_{50}) is shown in the Table below. The observations from this study show that 651987 down-regulates mutant and wild type *KRAS* isoforms and has selective phenotypic effects on KRAS mutant cells *in vitro*.

RNA analysis

For analysis of effect on KRAS mRNA expression cells were plated into 96-well plates and treated with dose responses of KRAS ASO for a minimum of 48 hours. For analysis of mRNA expression cell lysates prepared using FastLane Cell Probe kit (Qiagen) were used in real-time one-step RT-PCR reactions performed on a ABI 7900HT instrument (Applied Biosystems, Thermo Fisher Scientific) or a Lightcycler 480 instrument (Roche). Gene expression values were calculated using the using the comparative Ct (-ΔΔCt) method as previously described in User Bulletin #2 ABI PRISM 7700 Sequence Detection System 10/2001, using GAPDH or 18S rRNA CT values for normalisation. ABI FAM MGB Assay Probes for human KRAS (Hs00364284_g1), human GAPDH (4333764F), eukaryotic 18S rRNA (4333760F) were from Thermo Fisher Scientific.

3D colony assays

Colony assays were performed in 96 well plates. Cells (500 - 2000 cells per well) were seeded in 75µl of 0.3% agar onto a 50µl 1% agar layer in 10% RPMI-1640 growth media. The agar layers were then covered with 50µl of media containing treatment taking into account the entire volume of agar and media. Colonies were grown for 7 to 24 days depending upon the cell line and colony formation assessed by scanning on a GelCount scanner (Oxford Optronix, Abingdon, UK) and counting colonies of a

specified diameter. PC9 cells were obtained from Akiko Hiraide, Preclinical Sciences R&D, AZ, Japan. All other cells were obtained from ATCC.

Table 81

Details of the cell lines used in this study including results of STR finger print testing, *KRAS* and other key mutations. Correlation between IC_{50} (μM) of KRAS mRNA down-regulation and inhibition of colony formation by 651987 in KRAS wild type and mutant cell lines.

Cell Line	Tissue Type	STR finger print tested	KRAS Mutation	Other mutations/amplifications/ deletions	KRAS mRNA knockdown (IC50 μM)	Inhibition of colony formation (IC50 μM)
A549	Lung	Pass	G12S	CDKN2A, STK11	1.1810	1.0065
NCI-H358	Lung	Pass	G12C	KRAS amp, PIK3CA, STK11	0.5520	0.3313
NCI-H460	Lung	Pass	Q61H	CDKN2A, TP53, STK11	0.3240	0.3936
NCI-H2122	Lung	Pass	G12C	CDKN2A, TP53, STK11	0.3740	0.4580
SW900	Lung	Pass	G12V	KRAS amp, TP53, NF1	0.7470	0.3248
SW480	Colon	Pass	G12V	KRAS amp, TP53	0.4530	1.5303
PANC1	Pancreas	Pass	G12D	CDKN2A, TP53	0.2200	0.2573
PC9	Lung	Pass	WT	CDKN2A, EGFR	0.0710	5.9597
NCI-H1437	Lung	Pass	WT	TP53, MEK	0.1010	6.7957
NCI-H1299	Lung	Pass	WT	NRAS	0.7130	>10.0
NCI-H1793	Lung	Pass	WT	CDKN2A, TP53	0.0410	>10.0
COLO201	Colon	Pass	WT	BRAF, TP53	0.2270	>10.0

Example 21: Tolerability of antisense oligonucleotides targeting K-Ras in cynomolgus monkeys

Eight antisense oligonucleotides were compared for their relative efficacy, tolerability, pharmacokinetic and pharmacodynamic profiles in a repeated-dose study of male cynomolgus monkeys following six weeks of treatment by subcutaneous administration. These antisense oligonucleotides used in the study are described in the table below.

Table 82

Isis No.	Sequence	Chemistry	SEQ ID NO.
651530	TGACTAATAGCAGTGG	kkk-10-kkk	239
651555	TTTAATGTCACAAGCA	kkk-10-kkk	615
651587	GATTTGTCAGCAGGAC	kkk-10-kkk	621
651987	GCTATTAGGAGTCTTT	kkk-10-kkk	272
695785	AATGGTGAATATCTTC	kkk-10-kkk	569
695823	AGGTAAAAGCTAACAG	kkk-10-kkk	607
695980	ATCTTTTAATGTCACA	kkk-10-kkk	640
695995	TCTCTATGAAAGCTCA	kkk-10-kkk	655

Treatment

Prior to the study, the monkeys were kept in quarantine during which the animals were observed daily for general health. The monkeys were two to three years old and weighed two to three kg. Observations were recorded for all animals once daily during the acclimation and pre-treatment period, twice daily (before and after dosing on the day of dosing, in the morning and afternoon on non-dosing day) during the treatment period, and prior to the necropsy.

All study animals were weighed once prior to group assignment during the acclimation period and once weekly during the treatment period. Body weights were taken prior to the necropsy on the day of scheduled sacrifice. Blood samples were collected from the cephalic or femoral vein for evaluation of hematology, coagulation, and clinical chemistry. Fresh urine samples were collected from all available animals for urinalysis/urine chemistry parameters. Animals were fasted overnight prior to blood collection for clinical chemistry and urine collection.

At the end of the study, the monkeys were sacrificed, necropsied and organs removed. The protocols described in the Example were approved by the Institutional Animal Care and Use Committee (IACUC).

Thirty-six male cynomolgus monkeys were divided into nine groups of four monkeys each, with one group treated with 0.9% saline as a negative control. The eight antisense oligonucleotides were subcutaneously administered 40 mg/kg every other day for the first week (Days 1, 3, 5 and 7) for a total

of four loading doses, and once a week thereafter (days 14, 21, 28, 35, and 42 or 43) for 6 weeks. Several clinical endpoints were measured over the course of the study. Tail bleeds were conducted at 1 week prior to the first subcutaneous administration, then again at days 9, 16, 30, 44, 58, 72, and 86.

Body and organ weights

Body weight was assessed weekly. Body weights at some of these time points and organ weights (at day 44) are presented in the Table below. No remarkable effects of the antisense oligonucleotides on body weight were observed.

Table 83

Body and organ weights of cynomolgus monkeys treated with antisense oligonucleotides

Isis No.	Body (g)				Organ (g)							
	day 1	day 14	day 28	day 44	heart	kidney	liver	mandibular lymph node	mesenteric lymph node	spleen	testes	thymus
Saline	2632	2653	2737	2788	11.1	12.5	54.4	0.5	1.5	3.2	1.1	4.1
651530	2503	2563	2646	2695	10.2	14.0	59.3	0.6	1.5	3.0	1.3	2.3
651555	2572	2628	2718	2735	10.6	15.0	65.0	0.5	2.3	4.8	1.5	3.6
651587	2326	2354	2409	2393	9.3	13.7	53.4	0.6	1.4	3.3	1.2	1.7
651987	2579	2615	2715	2833	10.5	15.4	70.5	0.7	2.0	5.1	1.1	2.5
695785	2652	2730	2756	2753	11.1	16.4	68.5	0.6	2.5	3.5	0.9	2.5
695823	2741	2819	2883	2930	10.0	13.5	62.3	0.5	1.7	3.3	1.5	3.0
695980	2856	2947	2990	3008	11.0	14.5	72.4	0.6	1.9	4.7	1.3	3.2
695995	2874	2991	3074	3283	11.8	13.9	66.4	0.6	2.3	3.4	2.2	2.5

RNA analysis

At the end of the study, RNA was extracted from monkey livers and kidneys for real-time PCR analysis of measurement of mRNA expression of K-Ras. As above, primer probe set RTS3496_MGB was used, and the results for each group were averaged and presented as percent inhibition of mRNA, relative to the PBS control, normalized with rhesus cyclophilin A. The results of two trials were averaged and are presented in the Table below.

Table 84

Percent inhibition of K-Ras mRNA in the cynomolgus monkey liver relative to the PBS control

ISIS No.	% inhibition	SEQ ID NO
651530	73	239
651555	81	615
651587	78	621
651987	84	272

695785	88	569
695823	71	607
695980	45	640
695995	71	655

Table 85

Inhibition of K-Ras mRNA relative to the PBS control in various monkey tissues after treatment with ISIS 651987

Oligo ID	651987
Tissue	% Inhibition
Liver	84
Kidney	69
Lung	23
Duodenum	53
Pancreas	21
Heart	32

Hematology

To evaluate any effect of ISIS oligonucleotides in cynomolgus monkeys on hematologic parameters, blood samples of approximately 1.3 mL of blood were collected on day 44 from each of the study animals in tubes containing K_2-EDTA. Samples were analyzed for red blood cell (RBC) count, white blood cells (WBC) count, basophil count (BAS), as well as for platelet count (PLT) and mean platelet volume (MPV) using an ADVIA120 hematology analyzer (Bayer, USA). The data is presented in the Table below.

Table 86

Hematology

ISIS No.					
	BAS 10^3/uL	PLT 10^3/uL	MPV fL	RBC 10^6/uL	WBC 10^3/uL
Saline	0.02	346	8.4	5	12
651530	0.02	385	8.3	5	11
651555	0.03	340	8.8	6	12
651587	0.03	450	7.3	6	12
651987	0.02	362	8.1	6	9
695785	0.03	339	8.5	6	11
695823	0.03	305	8.3	6	9

695980	0.02	301	8.4	5	7
695995	0.03	288	11.2	5	12

The data indicate the oligonucleotides did not cause any significant changes in hematologic parameters outside the expected range for antisense oligonucleotides at this dose. These antisense oligonucleotides were well tolerated in terms of hematologic parameters in the monkeys.

Liver and kidney function

To evaluate the effect of these antisense oligonucleotides on liver and kidney function, samples of blood, plasma, serum and urine were collected from all study groups on day 44. The blood samples were collected via femoral venipuncture, 48 hrs post-dosing. The monkeys were fasted overnight prior to blood collection. Approximately 1.5 mL of blood was collected from each animal into tubes without anticoagulant for serum separation. Levels of the various markers were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Total urine protein and urine creatinine levels were measured, and the ratio of total urine protein to creatinine (P/C Ratio) was determined.

To evaluate the effect of the antisense oligonucleotides on hepatic function, plasma concentrations of transaminases (ALT, AST), Albumin (Alb) and total bilirubin ("T. Bil") were measured. To evaluate the effect of the antisense oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine (Cre) were measured. Urine levels of albumin (Alb), creatinine (Cre) and total urine protein (Micro Total Protein (MTP)) were measured, and the ratio of total urine protein to creatinine (P/C ratio) was determined.

To evaluate any inflammatory effect of the ISIS oligonucleotides in cynomolgus monkeys, C-reactive protein (CRP), which is synthesized in the liver and which serves as a marker of inflammation, was measured on day 42. For this, blood samples were taken from fasted monkeys, the tubes were kept at room temperature for a minimum of 90 min., and centrifuged at 3,000 rpm for 10 min at room temperature to obtain serum. The results are presented in the Tables below and indicate that most of the antisense oligonucleotides targeting human K-Ras were well tolerated in cynomolgus monkeys.

Table 87

Serum and urine clinical chemistry

ISIS No.	Serum (day 44)								Urine (day 44)			
	C3 mg/dL	ALT U/L	AST U/L	Alb g/dL	BUN mg/dL	CRP mg/L	Cre mg/dL	T.bil mg/dL	Alb g/dL	Cre mg/dL	MTP mg/dL	P/C ratio
Saline	103.3	43.3	60.0	4.0	25.1	0.27	0.78	0.24	1.0	21.5	0.00	0.00
651530	78.9	42.9	75.6	4.1	25.1	0.14	0.79	0.25	2.1	32.5	0.23	0.01
651555	101.8	83.1	92.7	4.0	24.6	0.21	0.73	0.19	11.8	34.0	1.75	0.06

651587	93.9	93.7	94.3	4.0	22.4	0.13	0.81	0.22	8.4	76.2	5.10	0.07
651987	83.1	67.0	77.9	3.9	27.6	0.23	0.81	0.21	4.8	59.3	0.96	0.02
695785	100.4	72.2	107.1	4.1	24.6	0.11	0.81	0.20	6.6	42.9	0.51	0.01
695823	97.1	68.5	67.9	4.2	23.9	0.14	0.85	0.23	4.2	33.6	0.12	0.00
695980	85.9	60.2	63.8	4.0	27.2	0.19	0.84	0.26	4.4	37.4	0.17	0.01
695995	93.4	47.8	82.5	4.1	28.2	0.28	0.90	0.25	3.5	44.2	0.09	0.00

Complement C3 levels

C3 levels were measured on several days during the study period prior to dosing and on Day 42 (pre- and post-dosing). When compared on Day 42 pre-dose to concurrent control (saline) and baseline (Day -14 pre-dose), a decreasing trend was noted in all antisense oligonucleotide-treated groups except animals treated with ISIS No. 651555. The lowest C3 level (82 % of Day 42 pre-dose baseline value) was shown in in animals treated with ISIS No. 651987 on Day 42 compared to pre-dose. The results of the complement C3 analysis are shown in the Table below.

Table 88

C3 Analysis on Day 42 as compared to baseline and control groups (mg/dL)

ISIS No.	Day -14 (pre-dose)	Day 42 (pre-dose)	% decrease compared to baseline (pre-dose on Day -14)	% decrease compared to control group (pre-dose on Day 42)
Saline	110	103	-7	0
651530	94	81	-14	-21
651555	108	110	+2	+7
651587	105	93	-12	-9
651987	109	89	-18	-13
695785	112	101	-10	-2
695823	105	98	-7	-4
695980	91	86	-6	-16
695995	108	95	-12	-7

Decreased C3 levels (approximately decreased by 6 to 18 % compared to baseline control) were observed in all oligonucleotide-treated groups except animals treated with ISIS No. 651555. The lowest C3 level was shown in animals treated with ISIS No. 651987.

Table 89

Concentrations of ISIS antisense oligonucleotide in liver and lung in cynomolgus monkeys

ISIS No.	Study day	Concentration		Liver /lung ratio
		Liver (μg/g)	Lung (μg/g)	
695785	44	658	69	9.6
695823	45	339	29	11.7
695995	45	556	38	14.5

Table 90

K-Ras concentrations in liver and kidney cortex in cynomolgus monkeys following 6 weeks of dosing

ISIS No.	Liver	Kidney	Kidney /liver ratio
651530	416	1012	2.4
651555	467	1127	2.4
651587	318	1070	3.4
651987	310	832	2.7
695785	587	1719	2.9
695823	519	2748	5.3
695980	601	2807	4.7
695995	464	1325	2.9

In conclusion, subcutaneous injection of eight antisense oligonucleotides targeting K-Ras mRNA for 6 weeks was well tolerated with no overt toxicity. No treatment-related changes in mortality, body weight, coagulation and urinalysis/urine chemistry were observed in this study.

Example 22: Tolerability of antisense oligonucleotides targeting K-Ras in cynomolgus monkeys

Six antisense oligonucleotides were compared for their relative efficacy, tolerability, pharmacokinetic and pharmacodynamic profiles in a repeated-dose study of male cynomolgus monkeys following six weeks of treatment by subcutaneous administration. These antisense oligonucleotides used in the study are described in the table below.

Table 91

Isis No.	Sequence	Chemistry	SEQ ID NO.
696018	CTCTTGATTTGTCAGC	kkk-10-kkk	678
696044	GTGTTTATGCAATGTT	kkk-10-kkk	715
716600	CCATTTATGTGACTAG	kkk-10-kkk	790
716655	TGTTTATGCAATGTTA	kkk-10-kkk	854
740233	GTGTTTATGCAATGTT	kkk-8-kdkdk	2158
746275	TCTTGATTTGTCAGCA	kk-10-keke	804

Treatment

Prior to the study, the monkeys were kept in quarantine during which the animals were observed daily for general health. The monkeys were two to three years old and weighed two to three kg. Observations were recorded for all animals once daily during the acclimation and pre-treatment period, twice daily (before and after dosing on the day of dosing, in the morning and afternoon on non-dosing day) during the treatment period, and prior to the necropsy.

All study animals were weighed once prior to group assignment during the acclimation period and once weekly during the treatment period. Body weights were taken prior to the necropsy on the day of scheduled sacrifice. Blood samples were collected from the cephalic or femoral vein for evaluation of hematology, coagulation, and clinical chemistry. Fresh urine samples were collected from all available animals for urinalysis/urine chemistry parameters. Animals were fasted overnight prior to blood collection for clinical chemistry and urine collection.

At the end of the study, the monkeys were sacrificed, necropsied and organs removed. The protocols described in the Example were approved by the Institutional Animal Care and Use Committee (IACUC).

Twenty-eight male cynomolgus monkeys were divided into seven groups of four monkeys each, with one group treated with 0.9% saline as a negative control. The six antisense oligonucleotides were subcutaneously administered 40 mg/kg every other day for the first week (Days 1, 3, 5 and 7) for a total of four loading doses, and once a week thereafter (days 14, 21, 28, 35, and 4) for 6 weeks. Several clinical endpoints were measured over the course of the study. Tail bleeds were conducted at 2 weeks and 1 week prior to the first subcutaneous administration, then again at days 16, 30, and 44. Serum was tested at 2 weeks prior to the first subcutaneous administration and at day 42 and urine was collected at 1 week prior to study start and at day 44.

Body and organ weights

Body weight was assessed weekly. Body weights at some of these time points and organ weights (at day 44) are presented in the Table below. No remarkable effects of the antisense oligonucleotides on body weight were observed.

Table 92

Body weights of cynomolgus monkeys treated with antisense oligonucleotides

Isis No.	Body (g)							
	day 1	day 7	day 14	day 21	day 28	day 35	day 42	day 44
PBS	2512.5	2630.8	2536.0	2542.5	2606.3	2565.8	2557.8	2,614.5
696018	2478.0	2596.8	2537.3	2527.5	2586.0	2539.3	2534.0	2,596.8

696044	2507.0	2624.5	2574.5	2607.0	2656.0	2610.3	2589.5	2,630.0
716600	2458.5	2537.5	2496.0	2503.8	2560.8	2526.5	2528.5	2,543.8
716655	2454.3	2506.8	2470.8	2508.8	2569.5	2520.8	2551.8	2,599.8
740233	2522.5	2554.3	2501.0	2545.5	2591.5	2540.5	2541.8	2,601.0
746275	2365.5	2411.0	2374.5	2408.5	2469.3	2419.5	2410.8	2,421.8

Table 93

Organ weights of cynomolgus monkeys treated with antisense oligonucleotides

Isis No.	Organ (g)							
	heart	kidney	liver	mandibular lymph node	mesenteric lymph node	spleen	testes	thymus
PBS	8.89	14.10	58.39	0.29	1.03	2.62	0.80	2.20
696018	10.06	13.72	67.02	0.51	2.67	4.03	1.20	0.68
696044	9.25	17.02	77.61	0.57	2.82	6.23	1.46	1.24
716600	8.96	13.34	69.54	0.43	2.43	3.20	1.29	2.38
716655	8.67	14.85	69.76	0.48	2.05	3.45	1.32	1.04
740233	10.05	15.86	79.39	0.79	3.13	6.23	1.07	1.33
746275	8.40	12.96	65.13	0.87	4.10	5.14	0.94	1.12

Hematology

To evaluate any effect of ISIS oligonucleotides in cynomolgus monkeys on hematologic parameters, blood samples of approximately 1.3 mL of blood were collected on day 44 from each of the study animals in tubes containing K_2-EDTA. Samples were analyzed for red blood cell (RBC) count, white blood cells (WBC) count, basophil count (BAS), as well as for platelet count (PLT) and mean platelet volume (MPV) using an ADVIA120 hematology analyzer (Bayer, USA). The data is presented in the Table below.

Table 94

Hematology

ISIS No.	BAS 10^3/uL	PLT 10^3/uL	MPV fL	RBC 10^6/uL	WBC 10^3/uL
PBS	.043	379.3	8.00	5.51	14.04
696018	.055	352.0	8.78	6.16	13.12
696044	.048	293.0	8.75	5.23	10.23
716600	.038	454.0	7.73	5.94	14.73
716655	.030	408.0	6.88	6.04	11.47
740233	.035	352.3	7.40	5.59	7.99
746275	.043	352.8	7.13	5.53	9.24

The data indicate the oligonucleotides did not cause any significant changes in hematologic parameters outside the expected range for antisense oligonucleotides at this dose. These antisense oligonucleotides were well tolerated in terms of hematologic parameters in the monkeys.

Liver and kidney function

To evaluate the effect of these antisense oligonucleotides on liver and kidney function, samples of blood, plasma, serum and urine were collected from all study groups on day 44. The blood samples were collected via femoral venipuncture, 48 hrs post-dosing. The monkeys were fasted overnight prior to blood collection. Approximately 1.5 mL of blood was collected from each animal into tubes without anticoagulant for serum separation. Levels of the various markers were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Total urine protein and urine creatinine levels were measured, and the ratio of total urine protein to creatinine (P/C Ratio) was determined.

To evaluate the effect of the antisense oligonucleotides on hepatic function, plasma concentrations of transaminases (ALT, AST), Albumin (Alb) and total bilirubin ("T. Bil") were measured. To evaluate the effect of the antisense oligonucleotides on kidney function, plasma concentrations of blood urea nitrogen (BUN) and creatinine (Cre) were measured. Urine levels of albumin (Alb), creatinine (Cre) and total urine protein (Micro Total Protein (MTP)) were measured, and the ratio of total urine protein to creatinine (P/C ratio) was determined.

To evaluate any inflammatory effect of the ISIS oligonucleotides in cynomolgus monkeys, C-reactive protein (CRP), which is synthesized in the liver and which serves as a marker of inflammation, was measured on day 42. For this, blood samples were taken from fasted monkeys, the tubes were kept at room temperature for a minimum of 90 min., and centrifuged at 3,000 rpm for 10 min at room temperature to obtain serum. The results are presented in the Tables below and indicate that most of the antisense oligonucleotides targeting human K-Ras were well tolerated in cynomolgus monkeys.

Table 95
Serum and urine clinical chemistry

ISIS No.	Serum (day 44)								Urine (day 42)		
	C3 mg/dL	ALT U/L	AST U/L	Alb g/dL	BUN mg/dL	CRP mg/L	Cre mg/dL	T.bil mg/dL	Alb g/dL	Cre mg/dL	P/C ratio
PBS	125.5	45.5	74.0	4.3	27.1	.158	.718	.206	.150	35.31	.000
696018	114.5	79.2	95.1	3.9	34.1	.278	.988	.157	3.000	42.10	.175
696044	105.1	39.2	96.9	3.7	29.1	.435	.795	.235	1.075	36.83	.120
716600	92.0	72.0	130.7	3.9	33.9	.190	.903	.192	.450	39.47	.018
716655	89.5	63.5	76.5	3.7	27.0	.153	.753	.189	.225	36.03	.003
740233	90.6	58.9	101.8	3.7	24.2	.283	.668	.190	.125	34.68	.000

746275	85.4	46.2	82.9	3.5	25.3	.428	.795	.184	1.600	46.52	.073

RNA analysis

At the end of the study, RNA was extracted from various monkey tissues for real-time PCR analysis of measurement of mRNA expression of K-Ras for the animals treated with ISIS 746275. As above, primer probe set RTS3496_MGB was used, and the results for each group were averaged and presented as percent inhibition of mRNA, relative to the PBS control, normalized with rhesus cyclophilin A.

Table 96

Inhibition of K-Ras mRNA relative to the PBS control in various monkey tissues after treatment with ISIS 746275

Tissue	% Inhibition
Liver	69
Kidney	51
Lung	27
Duodenum	39
Pancreas	0
Heart	28

WHAT IS CLAIMED:

1. A compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, or 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 13-2190.

2. A compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 13-2190.

3. A compound comprising a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 13-2190.

4. A compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides complementary within nucleobases 463-478, 877-892, 1129-1144, 1313-1328, 1447-1462, 1686-1701, 1690-1705, 1778-1793, 1915-1930, 1919-1934, 1920-1935, 2114-2129, 2115-2130, 2461-2476, 2462-2477, 2463-2478, 4035-4050 of SEQ ID NO: 1, wherein said modified oligonucleotide is at least 85%, 90%, 95%, or 100% complementary to SEQ ID NO: 1.

5. A compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides having a nucleobase sequence comprising at least 8, 9, 10, 11, or 12 contiguous nucleobases of any of the nucleobase sequences of any one of SEQ ID NOs: 272, 804, 239, 569, 607, 615, 621, 640, 655, 678, 715, 790, 854, 1028, 2130, 2136, 2142, 2154, and 2158.

6. A compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising the nucleobase sequences of any one of SEQ ID NOs: 272, 804, 239, 569, 607, 615, 621, 640, 655, 678, 715, 790, 854, 1028, 2130, 2136, 2142, 2154, and 2158.

7. A compound comprising a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of any one of SEQ ID NOs: 272, 804, 239, 569, 607, 615, 621, 640, 655, 678, 715, 790, 854, 1028, 2130, 2136, 2142, 2154, and 2158.

8. The compound of any one of claims1-7, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

9. A compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 272, 239, 569, 607, 615, 621, 640, 655, 678, 715, 790, and 854, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a constrained ethyl (cEt) nucleoside; wherein each internucleoside linkage is a phosphorothioate linkage and wherein each cytosine is a 5-methylcytosine.

10. A compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 2130, wherein the modified oligonucleotide comprises:

a gap segment consisting of nine linked deoxynucleosides;

a 5' wing segment consisting of one linked nucleoside; and

a 3' wing segment consisting of six linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, and 2'-O-methoxyethyl nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

11. A compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 804, 1028, and 2136, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, and a 2'-O-methoxyethyl nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

12. A compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 2142, wherein the modified oligonucleotide comprises:

a gap segment consisting of eight linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of six linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

13. A compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 2154, wherein the modified oligonucleotide comprises:

a gap segment consisting of nine linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, a cEt nucleoside, a 2'-O-methoxyethyl nucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

14. A compound comprising a modified oligonucleotide consisting of 16 to 80 linked

nucleosides having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 2158, wherein the modified oligonucleotide comprises:

a gap segment consisting of eight linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a cEt nucleoside, a cEt nucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt nucleoside, a deoxynucleoside, a cEt nucleoside, a deoxynucleoside, and a cEt nucleoside in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

15. The compound of any one of claims 1-14, wherein the oligonucleotide is at least 80%, 85%, 90%, 95% or 100% complementary to SEQ ID NO: 1 or 2.

16. The compound of any one of claims 1-15, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar, or at least one modified nucleobase.

17. The compound of claim 16, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

18. The compound of claim 16 or 17, wherein the modified sugar is a bicyclic sugar.

19. The compound of claim 18, wherein the bicyclic sugar is selected from the group consisting of: 4'-(CH_2)-O-2' (LNA); 4'-$(CH_2)_2$-O-2' (ENA); and 4'-$CH(CH_3)$-O-2' (cEt).

20. The compound of any one of claims 16-19, wherein the modified sugar is 2'-O-methoxyethyl.

21. The compound of any one of claims 16-20, wherein the modified nucleobase is a 5-methylcytosine.

22. The compound of any one of claims 1-21, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

23. The compound of any one of claims 1-22, wherein the compound is single-stranded.

24. The compound of any one of claims 1-23, wherein the compound is double-stranded.

25. The compound of any one of claims 1-24, wherein the compound comprises ribonucleotides.

26. The compound of claim 25, wherein the compound comprises a double-stranded RNA oligonucleotide, wherein one strand of the double-stranded RNA oligonucleotide is the modified oligonucleotide.

27. The compound of any one of claims 1-24, wherein the compound comprises deoxyribonucleotides.

. 28. The compound of any one of claims 1-27, wherein the modified oligonucleotide consists of 10 to 30, 12 to 30, 15 to 30, 16 to 30, or 16 linked nucleosides.

29. The compound of any one of claims 1-28, wherein the compound comprises a conjugate and the modified oligonucleotide.

30. The compound of any one of claims 1-28, wherein the compound consists of a conjugate and the modified oligonucleotide.

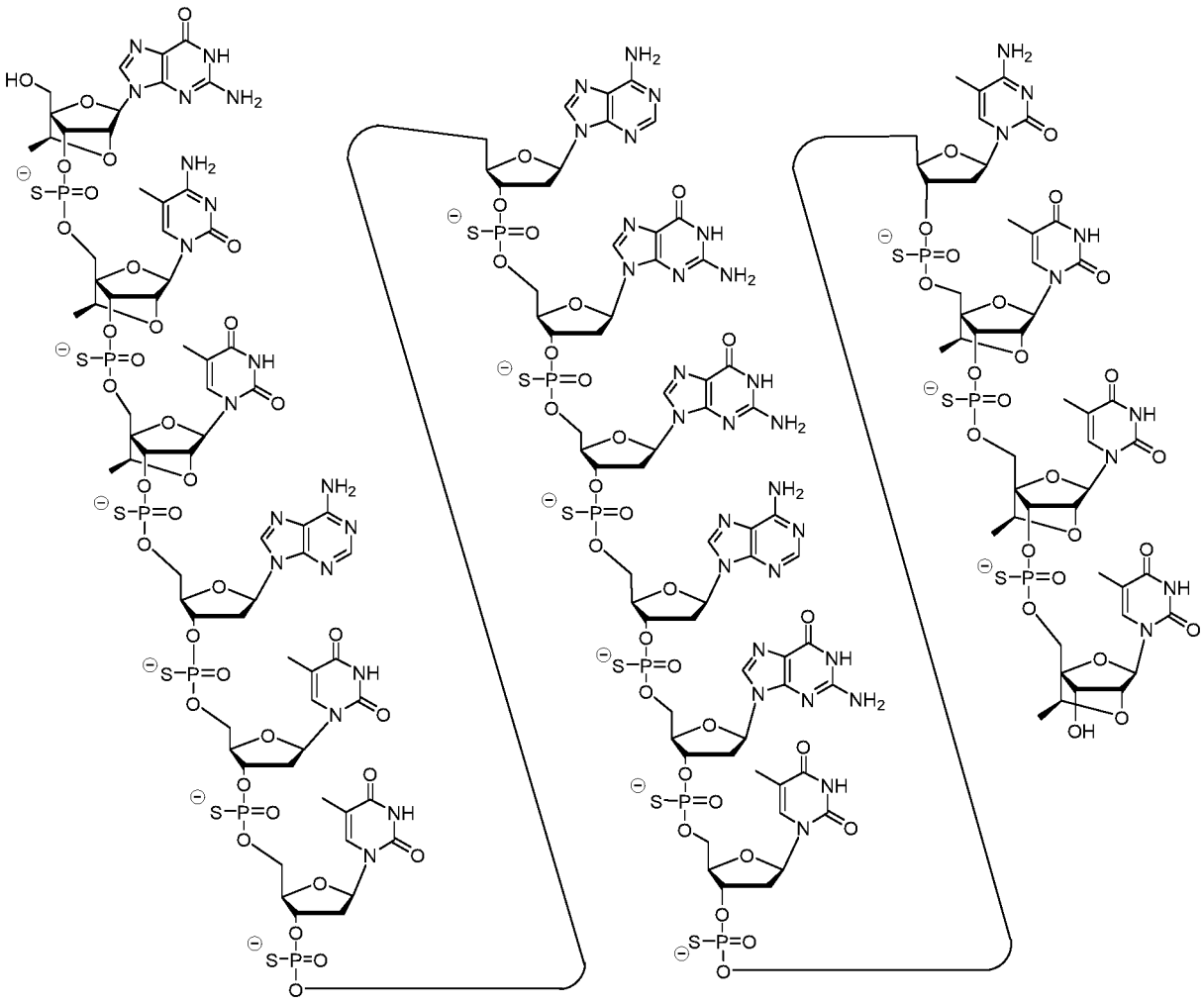
31. The compound of any one of claims 1-28, wherein the compound consists of the modified oligonucleotide.

32. A compound consisting of a pharmaceutically acceptable salt of any of the compounds of claims 1-31.

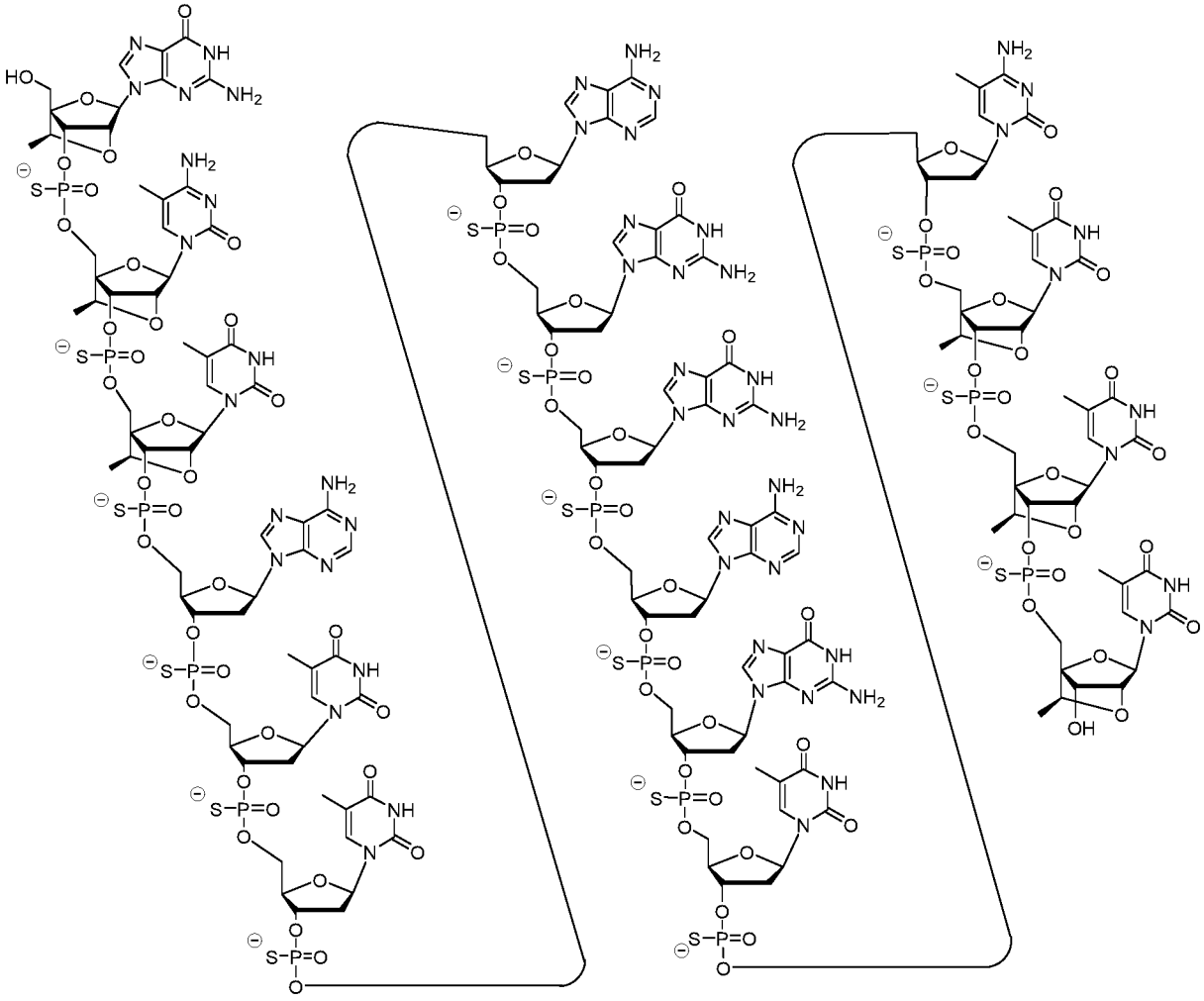
33. The compound of claim 32, wherein the pharmaceutically acceptable salt is a sodium salt.

34. The compound of claim 32, wherein the pharmaceutically acceptable salt is a potassium salt.

35. A compound comprising ISIS 651987, or a pharmaceutically acceptable salt thereof, having the formula:



36. A compound consisting of ISIS 651987, or a pharmaceutically acceptable salt thereof, having the formula:



37. The compound of claim 35 or 36, wherein the pharmaceutically acceptable salt is a sodium salt.

38. A composition comprising the compound of any one of claims 1-37 and a pharmaceutically acceptable carrier.

39. A method of treating, preventing, or ameliorating cancer in an individual comprising administering to the individual the compound of any one of claims 1-37 or composition of claim 38, thereby treating, preventing, or ameliorating cancer in the individual.

40. The method of claim 39, wherein the cancer is lung cancer, non-small cell lung carcinoma (NSCLC), small-cell lung carcinoma (SCLC)), gastrointestinal cancer, large intestinal cancer, small intestinal cancer, colon cancer, colorectal cancer, bladder cancer, liver cancer, stomach cancer, esophageal cancer, pancreatic cancer, biliary tract cancer, breast cancer, ovarian cancer, endometrial cancer, cervical cancer, prostate cancer, hematopoetic cancer, brain cancer, glioblastoma, malignant

peripheral nerve sheath tumor (MPNST), neurofibromatosis type 1 (NF1) mutant MPNST, neurofibroma, leukemia, myeloid leukemia, or lymphoma.

41. The method of claim 39 or 40, wherein administering the compound reduces the number of cancer cells in the individual, reduces the size of a tumor in the individual, reduces or inhibits growth or proliferation of a tumor in the individual, prevents metastasis or reduces the extent of metastasis in the individual, or extends survival of the individual.

42. A method of inhibiting expression of KRAS in a cell comprising contacting the cell with the compound of any one of claims 1-37 or composition of claim 38, thereby inhibiting expression of KRAS in the cell.

43. Use of the compound of any one of claims 1-37 or composition of claim 38 for treating, preventing, or ameliorating cancer in an individual.

44. Use of the compound of any one of claims 1-37 or composition of claim 38 for the manufacture of a medicament for treating cancer.

45. The use of claim 43 or 44, wherein the cancer is lung cancer, non-small cell lung carcinoma (NSCLC), small-cell lung carcinoma (SCLC)), gastrointestinal cancer, large intestinal cancer, small intestinal cancer, colon cancer, colorectal cancer, bladder cancer, liver cancer, stomach cancer, esophageal cancer, pancreatic cancer, biliary tract cancer, breast cancer, ovarian cancer, endometrial cancer, cervical cancer, prostate cancer, hematopoetic cancer, brain cancer, glioblastoma, malignant peripheral nerve sheath tumor (MPNST), neurofibromatosis type 1 (NF1) mutant MPNST, neurofibroma, leukemia, myeloid leukemia, or lymphoma.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/53334

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68, C07H 21/04, A61K 31/7088, C12N 15/113 (2017.01)
CPC - C07H 21/02, C12N 2310/14, C12N 2310/344, C12N 2310/3521, C12N 15/1135, C12N 2310/11
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)
IPC(8) - C12Q 1/68, C07H 21/04, A61K 31/7088, C12N 15/113 (2017.01)
CPC - C07H 21/02, C12N 2310/14, C12N 2310/344, C12N 2310/3521, C12N 15/1135, C12N 2310/11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12N 2310/315, C12N 2310/321, C12N 2310/322, C12N 2310/3231, C12N 2310/3233, C12N 2310/3519, C12N 2310/3525, C12N 2310/3533, C12N 2320/33; USPC- 514/44A, 536/24.5

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
GenCore 6.4.1; SEQ ID NO: 13

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2010/115202 A2 (DICERNA PHARMACEUTICALS INC., et al.) 07 October 2010 (07.10.2010) claims 74, 80, 83; SEQ ID NO:990, nucleotides 22-7, 100% identity to SEQ ID NO:13	1-3 --------- 8/(1-3)
Y	Wang, et al. Antisense knockdown of Kras inhibits fibrosis in a rat model of unilateral ureteric obstruction. Am J Pathol. 2012, 180(1):82-90; Abstract, pg 83, col 1; pg 88, Fig 6 and its legend	8/(1-3)

☐ Further documents are listed in the continuation of Box C. ☐

* 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
09 January 2017	23 FEB 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/53334

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 15-34, 38-45
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

********** See Supplemental Sheet to continue **********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 8/(1-3), restricted to SEQ ID NO:13

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/53334

Continuation of Box III. Observations where unity of invention is lacking:

Group I+: claims 1-14, 35-37, directed to an oligonucleotide of a specific nucleic acid sequence. The compound will be searched to the extent that the nucleic acid sequence encompasses SEQ ID NO:13. It is believed that claims 1-3, 8/(1-3) encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a modified oligonucleotide of SEQ ID NO:13. [NOTE: Claim 4 is excluded because SEQ ID NO:13 is not at least 85% complementary to SEQ ID NO:1, please see instant application, pg 72, Table 1.] Additional nucleic acid sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected nucleic acid sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a compound comprising a modified oligonucleotide of SEQ ID NO:272 (ISIS 651987), i.e., claims 1-9, 35-37.

The inventions listed as Group I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features
The special technical feature of each invention of Group I+ is a specific nucleic acid sequence recited therein, because no significant structural similarities can readily be ascertained among the specific nucleic acid sequences, and further because SEQ ID NO:13 was known in the art at the time of the invention (please see WO 2010/115206 A2 to DICERNA PHARMACEUTICALS INC. (07 October 2010) (hereinafter "Dicerna"); SEQ ID 947, nucleotides 20-5, 100% identity to SEQ ID NO:13).

Common Technical Features
The inventions of Group I+ share the technical feature of a compound comprising a modified oligonucleotide consisting of 8 to 80 linked nucleosides. Some inventions of Group I+ share the technical feature of a compound comprising a modified oligonucleotide comprising a gap segment consisting of linked deoxynucleosides; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides; wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar. However, these shared technical features do not represent a contribution over prior art as being anticipated by a paper titled "Antisense knockdown of Kras inhibits fibrosis in a rat model of unilateral ureteric obstruction" by Wang, et al. (Am J Pathol. 2012, 180(1):82-90) (hereinafter "Wang").

Wand discloses a compound comprising a modified oligonucleotide comprising a gap segment consisting of linked deoxynucleosides; a 5' wing segment consisting of linked nucleosides; and a 3' wing segment consisting of linked nucleosides; wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar (pg 83, col 1, "ASOs... are 20-nucleotide, fully phosphorothioate "gapmers." The five sugar residues on each end are 2=-O-methoxy-ethyl modified. The central 10 sugars are deoxyribonucleotides. All pyrimidine bases are 5-methyl substituted (T and me5-C). The oligonucleotide sequences were as follows: ASO 1, 5'-ATTCACATGACTATACACCT-3'; ASO 2, 5'-CACACTTATTCCCTACTAGG-3'..."). As said technical features were known in the art at the time of the invention, these cannot be considered special technical feature that would otherwise unify the groups.

Some inventions of Group I+ share the technical feature of a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides comprising: a gap segment consisting of ten linked deoxynucleosides; a 5' wing segment consisting of three linked nucleosides; and a 3' wing segment consisting of three linked nucleosides; wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a constrained ethyl (cEt) nucleoside; wherein each internucleoside linkage is a phosphorothioate linkage and wherein each cytosine is a 5-methylcytosine. However, these shared technical features do not represent a contribution over prior art as being obvious over Wang, as above, in view of a paper titled "Oligonucleotide analogues as modulators of the expression and function of noncoding RNAs (ncRNAs): emerging therapeutics applications" by Avitabile, et al. (J Med Chem. 2014, 57(24):10220-40) (hereinafter "Avitabile") as follows:

Wang discloses a compound comprising a modified oligonucleotide consisting of 16 to 80 linked nucleosides comprising: a gap segment consisting of ten linked deoxynucleosides; a 5' wing segment consisting of three linked nucleosides; and a 3' wing segment consisting of three linked nucleosides; wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each internucleoside linkage is a phosphorothioate linkage and wherein each cytosine is a 5-methylcytosine (pg 83, col 1, "ASOs... are 20-nucleotide, fully phosphorothioate "gapmers." The five sugar residues on each end are 2'-O-methoxy-ethyl modified. The central 10 sugars are deoxyribonucleotides. All pyrimidine bases are 5-methyl substituted (T and me5-C). The oligonucleotide sequences were as follows: ASO 1, 5'-ATTCACATGACTATACACCT-3'; ASO 2, 5'-CACACTTATTCCCTACTAGG-3'..."), but does not disclose that each nucleoside of each wing segment comprises a constrained ethyl (cEt) nucleoside.
Avitabile discloses cEt, 2',4'-constrained 2'-O-ethyl modified RNA (pg 10228, col 1 to col 2, "Between the analogues with a constrained backbone, we also report the 2,4'- constrained 2'-O-ethyl modified cEt... the sugar adopts a C3'-endo pucker; thus, cEt-modified monomers confer an improved thermal stability to duplexes in a way which is comparable to LNAs. Also the stability to nucleases is higher than that found for LNAs and 2'-MOEs. Experiments in which cEt modified oligonucleotides were tested as antisense in animals demonstrate that they possess an enhanced potency and therapeutic profile"). It would have been 0obvious to one of ordinary skill in the art to combine, in the course of routine experimentation and with a reasonable expectation of success, Wang and Avitabile by incorporating into the ASO1 and ASO2 of Wang constrained ethyl (cEt) nucleosides disclosed by Avitabile, to improve their potency and therapeutic profile, as disclosed by Avitabile. As said technical feature would have been obvious to one of ordinary skill in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

The inventions of Group I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note re item 4: Claims 15-34, 38-45 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.

摘要

本发明实施例提供了用于抑制 KRAS 表达的方法、化合物、和组合物，这些方法、化合物、和组合物可以用于治疗、预防、或缓解与 KRAS 相关的疾病。