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(71) Applicant *(for all designated States except US)*: **REGENTS OF THE UNIVERSITY OF MINNESOTA** [US/US]; 450 McNamara Alumni Center, 200 Oak Street Se, Minneapolis, Minnesota 55455-2070 (US).

(71) Applicants and
(72) Inventors: **SLATON, Joel, W.** [US/US]; 3806 W. 32nd St., Minneapolis, Minnesota 55416 (US). **AHMED, Khalil** [US/US]; 2011 James Ave. S., Minneapolis, Minnesota 55405 (US). **UNGER, Gretchen, M.** [US/US]; 3180 Highpoint Dr., Chaska, Minnesota 55318 (US). **DAVIS, Alan** [US/US]; 450 McNamara Alumni Center, 200 Oak Street Se, Minneapolis, Minnesota 55455 (US). **SLOPER, Dan** [US/US]; 450 McNamara Alumni Center, 200 Oak Street Se, Minneapolis, Minnesota 55455 (US).

(74) Agents: **PARSONS, Angela, M., D.** et al.; Fish & Richardson P.C., P.A., P.o. Box 1022, Minneapolis, Minnesota 55440-1022 (US).

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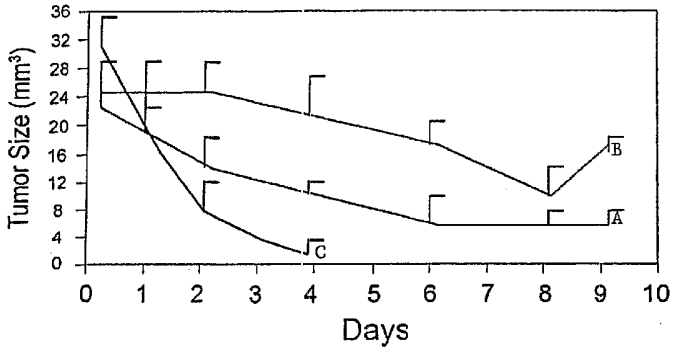
(54) **Title:** CASEIN KINASE 2 ANTISENSE THERAPY



A – 50 µg/ml
B – 100 µg/ml
C – 200 µg/ml

(57) **Abstract:** The invention provides for antisense oligonucleotides that hybridize to casein kinase 2 nucleic acid sequences and methods of using such antisense oligonucleotides to inhibit expression of casein kinase 2 and reduce the size of solid tumors.

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CASEIN KINASE 2 ANTISENSE THERAPY

TECHNICAL FIELD

This invention relates to cancer therapy, and more particularly to cancer therapy using casein kinase 2 antisense molecules.

BACKGROUND

Casein protein kinase 2 (CK2) is a ubiquitous protein serine/threonine kinase that has been implicated in multiple functions in the cell including the regulation of cell growth and proliferation as well as apoptosis (see, for example, Tawfic et al., 2001, *Histol. Histopathol.*, 16:573-82). CK2 is a highly conserved enzyme, and has been suggested to be essential for cell survival. The kinase is a heterotetramer consisting of two catalytic subunits (α and/or α') complexed with two β subunits. One means by which CK2 regulates cell growth is through signaling in the nucleus where nuclear matrix and chromatin appear to be its preferential targets.

CK2 has been strongly implicated in the neoplastic process because of its elevated levels in various tumors. Studies involving transgenic expression of CK2 have provided evidence to suggest that modest overexpression of CK2-α imparts an oncogenic potential to the cells, and further that the incidence of neoplasia is greatly enhanced in the presence of a second oncogenic signal.

SUMMARY

The invention provides for antisense oligonucleotides that hybridize to CK2 nucleic acid sequences and methods of using such antisense oligonucleotides to inhibit expression of CK2 and reduce the size of solid tumors.

This disclosure reports the effects of CK2α antisense delivered by different routes into prostate or bladder tumor xenografts. Prostate cancer xenograft in nude mice was generated using PC3-LN4 cells injected into the subcutis of mice. Intratumoral injection of the CK2α antisense oligonucleotide resulted in eradication of prostate cancer tumors *in vivo* in a dose and time dependent manner. An analysis of the tumors treated with CK2α

antisense oligonucleotide demonstrated a downregulation of CK2 message and nuclear-associated activity. Under similar experimental conditions, normal cells and tissue were relatively resistant to the effect of CK2α antisense oligonucleotide with evidence of minimal apoptosis. Intravenous delivery of naked CK2α phosphorothioate antisense oligonucleotide to nude mice carrying the orthotopic prostate tumor can also effectively downregulate CK2, resulting in induction of apoptosis in the tumor. CK2α antisense oligonucleotides in the phosphodiester form encapsulated in sub 50 nm tenascin nanocapsules also were utilized. When delivered via the intravenous route, these nanoencapsulated formulations were found to be significantly more effective then the naked antisense oligonucleotide.

In one aspect, the invention provides a method of inhibiting the expression of casein kinase 2 in a solid tumor. Such a method includes delivering an antisense oligonucleotide to the tumor, wherein the antisense oligonucleotide hybridizes to casein kinase 2 nucleic acid sequences and reduces the expression thereof.

In another aspect, the invention provides a method of reducing the size of a solid tumor in an individual. Such a method includes delivering an antisense oligonucleotide to the tumor, wherein the antisense oligonucleotide hybridizes to casein kinase 2 nucleic acid sequences and reduces the expression thereof. Generally, a reduction in expression of casein kinase 2 results in a reduction in size of the tumor.

Methods of delivering a casein kinase 2 antisense oligonucleotide can include, for example, intratumorally or intravenously. Further, an antisense oligonucleotide can be encapsulated. In an embodiment of the invention, the antisense oligonucleotide is a phosphorothioate antisense oligonucleotide. A representative antisense oligonucleotide has the sequence shown in SEQ ID NO:1 or SEQ ID NO:2. In another embodiment of the invention, the casein kinase 2 is casein kinase 2-α or a casein kinase 2-β. Representative solid tumors that can be treated by methods of the invention include those that originate in a location such as prostate, bladder, breast, liver, kidney, brain, head, or neck.

In yet another aspect, the invention provides a composition that includes an antisense oligonucleotide and a pharmaceutically acceptable carrier, wherein the antisense oligonucleotide hybridizes to casein kinase 2 nucleic acid sequences and reduces the

expression thereof. Such a composition can be provided in an encapsulated form. In one embodiment, the composition can include an antisense oligonucleotide having the sequence shown in SEQ ID NO:1 or SEQ ID NO:2.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the drawings and detailed description, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 is a graph showing prostate tumor size in athymic nude mice following intratumoral administration of a CK2 antisense oligonucleotide. Tumor size was assessed every 2 days. Each point represents 3 tumors.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

The present invention employs antisense compounds, particularly oligonucleotides, to inhibit the expression of target nucleic acid molecules. As used herein, the term "target nucleic acid" refers to both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. The target nucleic acid can be double-stranded or single-stranded (i.e., a sense or an antisense single strand). In some embodiments, the target nucleic acid encodes a casein kinase 2 (CK2) polypeptide. Thus, a "target nucleic acid" encompasses DNA encoding CK2, RNA (including pre-

mRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA. An "antisense" compound is a compound containing nucleic acids or nucleic acid analogs that can specifically hybridize to a target nucleic acid, and inhibition of expression of a target nucleic acid by an antisense oligonucleotide is generally referred to as "antisense technology."

The term "hybridization," as used herein, means hydrogen bonding, which can be Watson-Crick, Hoogsteen, or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. For example, adenine and thymine, and guanine and cytosine, respectively, are complementary nucleobases (often referred to in the art simply as "bases") that pair through the formation of hydrogen bonds. "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an oligonucleotide is capable of hydrogen bonding with a nucleotide in a target nucleic acid molecule, then the oligonucleotide and the target nucleic acid are considered to be complementary to each other at that position. The oligonucleotide and the target nucleic acid are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides that can hydrogen bond with each other. Thus, "specifically hybridizable" is used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the target nucleic acid.

It is understood in the art that the sequence of an antisense oligonucleotide need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense oligonucleotide is specifically hybridizable when (a) binding of the oligonucleotide to the target nucleic acid interferes with the normal function of the target nucleic acid, and (b) there is sufficient complementarity to avoid non-specific binding of the antisense oligonucleotide to non-target sequences under conditions in which specific binding is desired, i.e., under conditions in which *in vitro* assays are performed or under physiological conditions for *in vivo* assays or therapeutic uses.

Stringency conditions *in vitro* are dependent on temperature, time, and salt concentration (see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY (1989)). Typically, conditions of high to

moderate stringency are used for specific hybridization *in vitro*, such that hybridization occurs between substantially similar nucleic acids, but not between dissimilar nucleic acids. Specific hybridization conditions are hybridization in 5X SSC (0.75 M sodium chloride/0.075 M sodium citrate) for 1 hour at 40°C, followed by washing 10 times in 1X SSC at 40°C and 5 times in 1X SSC at room temperature.

In vivo hybridization conditions consist of intracellular conditions (e.g., physiological pH and intracellular ionic conditions) that govern the hybridization of antisense oligonucleotides with target sequences. *In vivo* conditions can be mimicked *in vitro* by relatively low stringency conditions. For example, hybridization can be carried out *in vitro* in 2X SSC (0.3 M sodium chloride/0.03 M sodium citrate), 0.1% SDS at 37°C. A wash solution containing 4X SSC, 0.1% SDS can be used at 37°C, with a final wash in 1X SSC at 45°C.

The specific hybridization of an antisense molecule with its target nucleic acid can interfere with the normal function of the target nucleic acid. For a target DNA nucleic acid, antisense technology can disrupt replication and transcription. For a target RNA nucleic acid, antisense technology can disrupt, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity of the RNA. The overall effect of such interference with target nucleic acid function is, in the case of a nucleic acid encoding CK2, inhibition of the expression of CK2. In the context of the present invention, "inhibiting expression of CK2" means to disrupt the transcription and/or translation of CK2 nucleic acid sequences resulting in a reduction in the level of CK2 polypeptide or a complete absence of CK2 polypeptide.

Identification of Target Sequences for CK2 Antisense Oligonucleotides

Antisense oligonucleotides are preferably directed at specific targets within a CK2 nucleic acid molecule. A representative CK2-α sequence from mouse can be found in GenBank Accession No. NM 009974; representative CK2-α sequences from human can be found in GenBank Accession Nos. NM 001892, S72393, and X70251; and representative CK2-β sequences from human can be found in GenBank Accession No.

NM 001320. Representative CK2 antisense oligonucleotides are disclosed herein as well as in U.S. Publication No. 2002/0147163.

The targeting process includes the identification of a site or sites within the CK2 nucleic acid molecule where an antisense interaction can occur such that a desired effect, e.g., inhibition of CK2 expression, will result. Traditionally, preferred target sites for antisense oligonucleotides have included the regions encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene. In addition, the ORF has been targeted effectively in antisense technology, as have the 5' and 3' untranslated regions. Furthermore, antisense oligonucleotides have been successfully directed at intron regions and intron-exon junction regions.

Simple knowledge of the sequence and domain structure (e.g., the location of translation initiation codons, exons, or introns) of a target nucleic acid, however, is generally not sufficient to ensure that an antisense oligonucleotide directed to a specific region will effectively bind to and inhibit transcription and/or translation of the target nucleic acid. In its native state, an mRNA molecule is folded into complex secondary and tertiary structures, and sequences that are on the interior of such structures are inaccessible to antisense oligonucleotides. For maximal effectiveness, antisense oligonucleotides can be directed to regions of a target mRNA that are most accessible, i.e., regions at or near the surface of a folded mRNA molecule. Accessible regions of an mRNA molecule can be identified by methods known in the art, including the use of RiboTAG™, or mRNA Accessible Site Tagging (MAST), technology. RiboTAG™ technology is disclosed in PCT Application Number SE01/02054.

CK2 Antisense Oligonucleotides

Once one or more target sites have been identified, antisense oligonucleotides can be synthesized that are sufficiently complementary to the target (i.e., that hybridize with sufficient strength and specificity to give the desired effect). In the context of the present invention, the desired effect is inhibiting the expression of CK2 such that cellular levels of CK2 are reduced. The effectiveness of an antisense oligonucleotide to inhibit expression of a target nucleic acid can be evaluated by measuring levels of CK2 mRNA or protein using, for example, Northern blotting, RT-PCR, Western blotting, ELISA, or

immunohistochemical staining. A description of anti-CK2 antibodies can be found, for example, in Faust et al. (1999, *Int. J. Biochem. Cell. Biol.*, 31:941-9), Nastaincyzyk et al. (1995, *Hybridoma*, 14:335-9) and references therein.

In some embodiments, it may be useful to target multiple accessible regions of a target nucleic acid. In such embodiments, multiple antisense oligonucleotides can be used that each specifically hybridize to a different accessible region. Multiple antisense oligonucleotides can be used together or sequentially.

The antisense oligonucleotides in accordance with this invention can be from about 10 to about 50 nucleotides in length (e.g., 12 to 40, 14 to 30, or 15 to 25 nucleotides in length). Antisense oligonucleotides that are 15 to 23 nucleotides in length are particularly useful. However, an antisense oligonucleotide containing even fewer than 10 nucleotides (for example, a portion of one of the preferred antisense oligonucleotides) is understood to be included within the present invention so long as it demonstrates the desired activity of inhibiting expression of CK2.

An "antisense oligonucleotide" can be an oligonucleotide as described herein. The term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or analogs thereof. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent internucleoside (backbone) linkages, as well as oligonucleotides having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for a nucleic acid target, and increased stability in the presence of nucleases.

It should be noted that an antisense oligonucleotide may consist essentially of a nucleotide sequence that specifically hybridizes with an accessible region in the target nucleic acid. Such antisense oligonucleotides, however, may contain additional flanking sequences of 5 to 10 nucleotides at either end. Flanking sequences can include, for example, additional sequences of the target nucleic acid, sequences complementary to an amplification primer, or sequences corresponding to a restriction enzyme site.

For maximal effectiveness, further criteria can be applied to the design of antisense oligonucleotides. Such criteria are well known in the art, and are widely used,

for example, in the design of oligonucleotide primers. These criteria include the lack of predicted secondary structure of a potential antisense oligonucleotide, an appropriate G and C nucleotide content (e.g., approximately 50%), and the absence of sequence motifs such as single nucleotide repeats (e.g., GGGG runs).

While antisense oligonucleotides are a preferred form of antisense compounds, the present invention includes other oligomeric antisense compounds, including but not limited to, oligonucleotide analogs such as those described below. As is known in the art, a nucleoside is a base-sugar combination, wherein the base portion is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric molecule. The respective ends of this linear polymeric molecule can be further joined to form a circular molecule, although linear molecules are generally preferred. Within the oligonucleotide molecule, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

CK2 antisense oligonucleotides that are useful in the present invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined herein, oligonucleotides having modified backbones include those that have a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone also can be considered to be oligonucleotides.

Modified oligonucleotide backbones can include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotri-esters, methyl and other alkyl phosphonates (e.g., 3'-alkylene phosphonates and chiral phosphonates), phosphinates, phosphoramidates (e.g., 3'-amino phosphoramidate and aminoalkylphosphoramidates), thionophosphoramidates, thionoalkylphosphonates,

thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, as well as 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. References that teach the preparation of such modified backbone oligonucleotides are provided, for example, in U.S. Patent Nos. 4,469,863 and 5,750,666.

CK2 antisense molecules with modified oligonucleotide backbones that do not include a phosphorus atom therein can have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts. References that teach the preparation of such modified backbone oligonucleotides are provided, for example, in U.S. Patent Nos. 5,235,033 and 5,596,086.

In another embodiment, a CK2 antisense compound can be an oligonucleotide analog, in which both the sugar and the internucleoside linkage (i.e., the backbone) of the nucleotide units are replaced with novel groups, while the base units are maintained for hybridization with an appropriate nucleic acid target. One such oligonucleotide analog that has been shown to have excellent hybridization properties is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone (e.g., an aminoethylglycine backbone). The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. References that teach the preparation of such modified backbone oligonucleotides are provided, for example, in Nielsen et al., *Science* 254:1497-1500 (1991), and in U.S. Patent No. 5,539,082.

Other useful CK2 antisense oligonucleotides can have phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular

CH_2NHOCH_2, $CH_2N(CH_3)OCH_2$, $CH_2ON(CH_3)CH_2$, $CH_2N(CH_3)N(CH_3)CH_2$, and $ON(CH_3)CH_2CH_2$ (wherein the native phosphodiester backbone is represented as $OPOCH_2$) as taught in U.S. Patent No. 5,489,677, and the amide backbones disclosed in U.S. Patent No. 5,602,240.

Substituted sugar moieties also can be included in modified oligonucleotides. CK2 antisense oligonucleotides of the invention can comprise one or more of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S-, or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Useful modifications also can include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(C_2)_nCH_3)]_2$, where n and m are from 1 to about 10. In addition, oligonucleotides can comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, groups for improving the pharmacokinetic or pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Other useful modifications include an alkoxyalkoxy group, e.g., 2'-methoxyethoxy (2'-$OCH_2CH_2OCH_3$), a dimethylaminooxyethoxy group (2'-$O(CH_2)_2ON(CH_3)_2$), or a dimethylamino-ethoxyethoxy group (2'-$OCH_2OCH_2N(CH_2)_2$). Other modifications can include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$), or 2'-fluoro (2'-F). Similar modifications also can be made at other positions within the oligonucleotide, such as the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides, and the 5' position of the 5' terminal nucleotide. Oligonucleotides also can have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl group. References that teach the preparation of such substituted sugar moieties include U.S. Patent Nos. 4,981,957 and 5,359,044.

Useful CK2 antisense oligonucleotides also can include nucleobase modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases can include other synthetic and natural

nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Other useful nucleobases include those disclosed, for example, in U.S. Patent No. 3,687,808.

Certain nucleobase substitutions can be particularly useful for increasing the binding affinity of the antisense oligonucleotides of the invention. For example, 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6 to 1.2°C (Sanghvi et al., eds., *Antisense Research and Applications*, pp. 276-278, CRC Press, Boca Raton, FL (1993)). Other useful nucleobase substitutions include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines such as 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

Antisense oligonucleotides of the invention also can be modified by chemical linkage to one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties (e.g., a cholesterol moiety); cholic acid; a thioether moiety (e.g., hexyl-S-tritylthiol); a thiocholesterol moiety; an aliphatic chain (e.g., dodecandiol or undecyl residues); a phospholipid moiety (e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate); a polyamine or a polyethylene glycol chain; adamantane acetic acid; a palmityl moiety; or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety. The preparation of such oligonucleotide conjugates is disclosed in, for example, U.S. Patent Nos. 5,218,105 and 5,214,136.

It is not necessary for all nucleobase positions in a given antisense oligonucleotide to be uniformly modified. More than one of the aforementioned modifications can be incorporated into a single oligonucleotide or even at a single nucleoside within an

oligonucleotide. The present invention also includes antisense oligonucleotides that are chimeric oligonucleotides. "Chimeric" antisense oligonucleotides can contain two or more chemically distinct regions, each made up of at least one monomer unit (e.g., a nucleotide in the case of an oligonucleotide). Chimeric oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer, for example, increased resistance to nuclease degradation, increased cellular uptake, and/or increased affinity for the target nucleic acid. For example, a region of a chimeric oligonucleotide can serve as a substrate for an enzyme such as RNase H, which is capable of cleaving the RNA strand of an RNA:DNA duplex such as that formed between a target mRNA and an antisense oligonucleotide. Cleavage of such a duplex by RNase H, therefore, can greatly enhance the effectiveness of an antisense oligonucleotide.

The CK2 antisense oligonucleotides of the invention are synthesized *in vitro* and do not include antisense compositions of biological origin, except for oligonucleotides that comprise the subject antisense oligonucleotides and that have been purified from or isolated from biological material. Antisense oligonucleotides used in accordance with this invention can be conveniently produced through the well-known technique of solid phase synthesis. Equipment for such synthesis is commercially available from several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art additionally or alternatively can be employed. Similar techniques also can be used to prepare modified oligonucleotides such as phosphorothioates or alkylated derivatives.

Methods for Using CK2 Antisense Oligonucleotides

The antisense oligonucleotides of the invention are useful for research and diagnostics, and for therapeutic use. For example, assays based on hybridization of antisense oligonucleotides to nucleic acids encoding CK2 can be used to evaluate levels of CK2 in a tissue sample. Hybridization of the antisense oligonucleotides of the invention with a nucleic acid encoding CK2 can be detected by means known in the art. Such means can include conjugation of an enzyme to the antisense oligonucleotide, radiolabeling of the antisense oligonucleotide, or any other suitable means of detection.

Those of skill in the art can harness the specificity and sensitivity of antisense technology for therapeutic use. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals, including humans. For therapeutic methods, the cells or tissues are typically within a vertebrate (e.g., a mammal such as a human).

The invention provides therapeutic methods for inhibiting the expression of CK2 in a solid tumor and reducing the size of the solid tumor. By these methods, antisense oligonucleotides in accordance with the invention are introduced intratumorally into a solid tumor to inhibit expression of CK2. The methods and compositions of the present invention can be used to kill cells, inhibit cell growth, inhibit metastasis, inhibit angiogenesis, or otherwise reverse or reduce the malignant phenotype of tumor cells. Significantly, the size of a solid tumor can be reduced by inhibiting the expression of CK2. Solid tumors that can be treated using methods of the invention include those tumors that originate in a location or body part such as prostate, breast, liver, kidney, brain, head, and neck.

The pharmaceutical compositions of the present invention are generally administered intratumorally. Intratumoral administration can be rapid (e.g., by direct injection) or can occur over a period of time (e.g., by slow infusion). The pharmaceutical forms suitable for injection or infusion use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that the form is readily injectable via syringe. It must be stable under the conditions of manufacture and storage, and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

The ability of a CK2 antisense oligonucleotide to inhibit expression of CK2 can be assessed, for example, by measuring levels of CK2 mRNA or protein in a subject before and after treatment. Methods for measuring mRNA and protein levels in tissues or biological samples are well known in the art and include Northern blotting, Western blotting, *in situ* hybridization, and immunohistochemical staining.

Methods for formulating and subsequently administering therapeutic compositions are well known to those skilled in the art. See, for example, Remington, *The Science and Practice of Pharmacy*, 20th Ed., Gennaro & Gennaro, eds., Lippincott, Williams & Wilkins (2000). It is expected that the dosage for intratumoral injection will be determined more by the mass and density of the tumor than by the size of the patient. Dosing also can be dependent upon the severity and responsiveness of the tumor to be treated, with the course of treatment consisting of a single treatment to several days or several months of treatment, or until a cure is effected or a reduction in size of the tumor is achieved. Typically, the antisense oligonucleotide is administered in an inhibitory amount (i.e., in an amount that is effective for inhibiting the production of CK2 in the cells or tissues contacted by the antisense oligonucleotides). Thus, a single intratumoral injection of from about 2.5 μg to about 10 μg or greater of a CK2 antisense oligonucleotide can significantly reduce the size of a 3-5 mm tumor. Other amounts can be used for tumors of various tissue densities, sizes, and intercellular permeabilities. Persons of ordinary skill in the art routinely determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages can vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50} found to be effective in *in vitro* and *in vivo* models. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent subsequent enlargement of the tumor.

The present invention provides pharmaceutical compositions and formulations that include the CK2 antisense oligonucleotides of the invention. A "pharmaceutically acceptable carrier" (also referred to herein as an "excipient") is a pharmaceutically acceptable solvent, suspending agent, or any other pharmacologically inert vehicle for delivering one or more therapeutic compounds (e.g., CK2 antisense oligonucleotides) to a subject. Pharmaceutically acceptable carriers can be selected with intratumoral administration in mind so as to provide for the desired bulk, consistency, and other pertinent transport and chemical properties, when combined with one or more of therapeutic compounds and any other components of a given pharmaceutical composition. Typical pharmaceutically acceptable carriers that do not deleteriously react with nucleic acids include, by way of example and not limitation: water; saline solution;

binding agents (e.g., polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose and other sugars, gelatin, or calcium sulfate); lubricants (e.g., starch, polyethylene glycol, or sodium acetate); disintegrates (e.g., starch or sodium starch glycolate); and wetting agents (e.g., sodium lauryl sulfate).

The CK2 antisense oligonucleotides of the invention further encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound 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 invention provides pharmaceutically acceptable salts of CK2 antisense oligonucleotides. The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the oligonucleotides of the invention (i.e., salts that retain the desired biological activity of the parent oligonucleotide without imparting undesired toxicological effects). Examples of pharmaceutically acceptable salts of oligonucleotides include, but are not limited to, salts formed with cations (e.g., sodium, potassium, calcium, or polyamines such as spermine); acid addition salts formed with inorganic acids (e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, or nitric acid); salts formed with organic acids (e.g., acetic acid, citric acid, oxalic acid, palmitic acid, or fumaric acid); and salts formed from elemental anions (e.g., chlorine, bromine, and iodine).

Certain embodiments of the invention provide pharmaceutical compositions containing (a) one or more antisense oligonucleotides and (b) one or more other agents that function by a non-antisense mechanism. For example, anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, can be included in compositions of the invention. Other non-antisense agents (e.g., chemotherapeutic agents) are also within the scope of this invention. Such combined compounds can be used together or sequentially to treat a solid tumor.

The antisense compositions of the present invention additionally can contain other adjunct components conventionally found in pharmaceutical compositions. Thus, the compositions also can include compatible, pharmaceutically active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or

additional materials useful in maintaining shelf life of the composition or maintaining integrity of the composition during storing or shipping, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents, wetting agents, salts for influencing osmotic pressure, buffers, and stabilizers. When added, however, such materials should not unduly interfere with the biological activities of the antisense components within the compositions of the present invention. The formulations can be sterilized, if desired, provided sterilization does not deleteriously affect the nucleic acid(s) of the formulation.

Nucleic Acid Constructs

Nucleic acid constructs (e.g., a plasmid vector) are capable of transporting a nucleic acid into a host cell. Suitable host cells include prokaryotic or eukaryotic cells (e.g., bacterial cells such as *E. coli*, insect cells, yeast cells, and mammalian cells). Some constructs are capable of autonomously replicating in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell and are replicated with the host genome.

Nucleic acid constructs can be, for example, plasmid vectors or viral vectors (e.g., replication defective retroviruses, adenoviruses, and adeno-associated viruses). Nucleic acid constructs include one or more regulatory sequences operably linked to the nucleic acid of interest (e.g., a nucleic acid encoding a transcript that specifically hybridizes to a CK2 mRNA in its native form). With respect to regulatory elements, "operably linked" means that the regulatory sequence and the nucleic acid of interest are positioned such that nucleotide sequence is transcribed (e.g., when the vector is introduced into the host cell).

Regulatory sequences include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). (See, e.g., Goeddel, *Gene Expression Technology: Methods in Enzymology*, 185, Academic Press, San Diego, CA (1990)). Regulatory sequences include those that direct expression of a nucleotide sequence in

many types of host cells and that direct expression of the nucleotide sequence only in certain host cells (e.g., cell type- or tissue-specific regulatory sequences).

Articles of Manufacture

Antisense oligonucleotides of the invention can be combined with packaging material and sold as kits for inhibiting the expression of CK2. Components and methods for producing articles of manufacture are well known. The articles of manufacture may combine one or more of the antisense oligonucleotides set out in the above sections. In addition, the article of manufacture further may include buffers, hybridization reagents, or other control reagents for reducing and/or monitoring expression of CK2. Instructions describing how the antisense oligonucleotides can be used to inhibit expression of CK2 can be included in such kits.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1—Intratumoral administration of a CK2 antisense oligonucleotide

The effects of interfering with the CK2 signal in various cancer cell lines (ALVA-41, PC-3, LNCaP, and Ca9-22) was examined by employing antisense oligonucleotides against the α and β subunits of CK2. Results demonstrated that antisense CK2α and antisense CK2β elicit a potent apoptotic response in cells when cells were exposed to low concentrations of the antisense oligonucleotide. Results suggested that only a modest down regulation of CK2α is necessary to achieve a potent apoptotic response in cancer cells.

The ability of CK2α antisense oligonucleotides to ablate prostate tumors *in vivo* was determined. A metastatic human prostate cancer cell line, PC3-LN4 ($2x10^6$ cells in 200 μl), was injected into the subcutis of athymic nude mice. The tumors were allowed to grow for 3 weeks until they reached 3-5 mm in size. Mice were randomized into 4 groups with 4 mice each. An antisense CK2α oligonucleotide [5'-cct gct tgg cac ggg tcc

cga cat-3' (SEQ ID NO:1) (High Purity Salt Free, 1.0 umol scale, prepared by MWG Biotech, Highpoint, NC) phosphorothioate; 50 μl of a solution at a concentration of 50 or 200 μg/ml] was injected directly into the tumor on day 0 and day 3, and the tumors were then observed for 7-10 days. A DNA oligonucleotide and saline were used as control treatments.

Treatment with the lower dose (2.5 μg) resulted in a 30-40% reduction in tumor size within 7 days after the second injection. Treatment with the higher dose (10 μg) resulted in a 95% reduction in tumor size by day 7, suggesting that relatively low doses of the CK2α antisense oligonucleotide were effective in inducing apoptosis in this model (Table 1). The experiment to assess tumor size was repeated every two days, and demonstrated a dose-dependent response to intratumoral therapy with a CK2 antisense oligonucleotide (Figure 1).

To assess the effects of antisense CK2α, paraffin embedded tumor sections were stained with hematoxylin and eosin for histology analysis and for TUNEL for apoptosis analysis. Following staining, individual cancer cells were interspersed throughout necrotic and apoptotic stroma. Therefore, intratumoral injection of the CK2α antisense oligonucleotide induced apoptosis in the prostate cancer xenograft model.

Table 1

Therapy	Final Tumor Weight
Saline	225 mg (200-530 mg)
Control oligonucleotides	180 mg (220-460 mg)
CK2 antisense (50 μg/ml)	131 mg (125-290 mg)
CK2 antisense (200 μg/ml)	30 mg (<10-55 mg)

Example 2—Systemic administration of naked antisense CK2α in an orthotopic model of prostate cancer

Because intravenous administration is required to treat tumors which cannot be directly injected or distant metastases, the ability of CK2α antisense oligoncleotides to

ablate prostate and bladder tumors *in vivo* following intravenous administration was determined. First, a metastatic human prostate cancer cell line, PC3-LN4 ($2x10^6$ cells in 200 µl), was injected intraprostatically into male athymic nude mice. The tumors were allowed to grow for 3 weeks until they reached 6-8 mm in size. Mice were randomized into 3 groups with 3-4 mice each. An antisense CK2α oligonucleotide (5'-cct gct tgg cac ggg tcc cga cat-3' (SEQ ID NO:1) phosphorothioate; High purity Salt Free, 1.0 µmol scale, prepared by MWG Biotech, Highpoint, NC; 300 µg in 200 µl of saline or ~ 10 mg/kg) was injected by tail vein on day 0 and day 3, and the tumors were then observed for 7 days. A random sequence DNA oligonucleotide or saline were used in the control treatments. Tumors were collected 10 days after starting treatment, measured, weighed and scored for necrosis. Intravenous treatment with phosphorothioate-CK2α antisense oligonucleotide resulted in a 65% reduction in tumor volume 7 days after a second treatment, suggesting that CK2α was effective at reducing tumor size after only limited intravenous dosing (Table 2).

Table 2. Intravenous antisense CK2 therapy in an orthotopic prostate tumor model

Therapy	Final Tumor Volume (mm^3)	Final Tumor Weight (gm)	Necrosis Score
Saline	1227 (320-2240)	1.25 (0.2-2.3)	1.33 (0-4)
Control oligonucleotides	594 (407-800)	0.93 (0.3-1.5)	1.33 (0-2)
CK2 antisense naked	425 (292-665)	0.53 (0.3-0.9)	0.33 (0-2)

Necrosis scale: 0 = none, 1 = less than 5% of tumor area, 2 = <25% without central necrosis, 3 = <25% including central necrosis, 4 = >25% of tumor area including central necrosis.

Example 3—Systemic administration of nanoencapsulated antisense CK2α in an orthotopic model of prostate cancer

While delivery of antisense oligonucleotides can be given systemically, their efficacy has been limited by the inability to target tumor specifically. A delivery system has been developed by GeneSegues, Inc. (Chaska, MN) that involves encapsulation of molecular sized cargo into nanocapsules (sub 50 nm) coated with a ligand specific for receptors on neoplasms. PC3-LN4 ($2x10^6$ cells in 200 µl) were injected intraprostatically into male athymic nude mice. The tumors were allowed to grow for 3 weeks until they

reached 6-8 mm in size. An CK2α phosphorothioate antisense oligonucleotide (SEQ ID NO:1; 300 μg in 200 μl of saline or ~ 10 mg/kg) was packaged as cargo into nanocapsules (GeneSegues, Inc; See, for example, U.S. Patent No. 6,632,671). This nanoencapsulated antisense CK2α was injected by tail venom on day 0 and day 3, and the tumors were then observed for 7 days. A DNA oligonucleotide and saline were used as control treatments. Tumors were collected 10 days after starting treatment and assessed for tumor reduction and for necrosis. Intravenous treatment with nanoencapsulated CK2a resulted in complete liquification of the prostate tumors after 7 days with resulting cystic lesion with only minimal viable tumor remaining.

Example 4—Induction of Apoptosis, Inhibition of Proliferation in Orthotopic Prostate Cancer Xenograft

To assess the molecular effects of antisense CK2α, paraffin embedded tumor sections were analyzed by immunohistofluorescence for the presence of CK2α, histone deacetylase 1 (HDAC1), and activated Caspase 3 (aC3). Respectively, these proteins characterize the effect of antisense CK2α on its molecular target, the process of cellular differentiation, and the process of apoptosis. HDAC1, a direct target of CK2, is an enzyme that acts upon chromatin to change the availability of chromosome binding sites to transcription factors. High HDAC1 activity corresponds to proliferative activity in cells and low activity is consistent with a program of terminal differentiation and cessation of growth. CK2 immunosignal was bright, nuclear and very uniform throughout the untreated tumor. Concomitantly, there was no evidence of apoptosis or differentiation/HDAC1 inhibition as evidenced respectively by i) no elevation in activated caspase 3 or ii) a bright, uniform HDAC1 immunosignal. In remaining viable tumor regions, antisense CK2 effects a partial reduction in CK2 nuclear immunosignal concomitantly with an abrogation in HDAC1 immunosignal, consistent with induction of tissue differentiation. These data indicate antisense CK2 is able to effect desirable molecular changes in tumor tissue after limited intravenous dosing.

While no evidence of apoptosis was observed following intravenous administration of antisense CK2α, administration of antisense CK2α formulated into a colloidal dosage form for enhanced delivery resulted in i) uniform reduction of CK2

nuclear immunosignal, ii) induction of apoptosis as indicated by uniform bright aC3 immunosignal, and iii) maintenance of HDAC1 inhibition. This induction of apoptosis following delivery enhancement was consistent with the appearance of significant central necrosis and fluid retention observed in nanocapsule-treated tumors at necropsy. Such results in an orthotopic model of prostate cancer are consistent with the apoptosis induction. Therefore, CK2α antisense oligonucleotide induced apoptosis in the prostate cancer xenograft model following intravenous administration.

Example 5—Inhibition of mutant p53 by intravenous administration of antisense CK2α

More than half of human tumors present with a mutation in the p53 tumor suppressor protein that renders it overly stable (Mutp53). By acting as a dominant mutant protein to activate multi-drug transporters (e.g. MDR-1) and multiple growth/survival cascades, the Mutp53 oncoprotein is believed to play a broad role in the phenomenon of drug resistance and the progression of advanced disease. Via direct protein binding, mutant p53 also inactivates wild-type p53, disabling the normal activation of apoptosis following significant cellular damage. Enhanced genomic instability in tumor cells is also highly correlated with Mutp53 and may be mediated by its direct binding to Topoisomerase I and concomitant downregulation of the mismatch DNA repair enzyme, msh. Thus, the consequences of enhanced stability in this central protein to patients are resistance to chemotherapy, radiotherapy and poor prognosis.

An aggressive, human bladder cancer cell line, 253J-BV ($2x10^6$ cells in 200 μl), was injected into the bladder of male athymic nude mice. The tumors were allowed to grow for 3 weeks until they reached 3-4 mm in size. Mice were randomized into 2 groups with 3-4 mice in each group. An CK2 phosphorothioate antisense oligonucleotide (5'-gtc ccg aca tgt cag aca gg-3' (SEQ ID NO:2); Oligos, Etc., Wilsonville, OR; 300 μg in 200 μl of saline or ~ 10 mg/kg) was nanoencapsulated (see, for example, U.S. Patent No. 6,632,671; GeneSegues, Inc.; Chaska, MN) and injected intraperitoneally (i.p) on day 0 and day 3. The tumors were then observed for 7 days. A DNA nonsense oligonucleotide, similarly encapsulated, was used as a comparison. Tumors were collected 10 days after starting treatment and measured and weighed. Intravenous treatment with nanoencapsulated CK2α antisense oligonucleotide resulted in a significant 22% reduction

in tumor volume 7 days after a second treatment. These experiments illustrate that CK2α is effective at reducing the size of solid tumors in organs other than the prostate (Table 3).

Table 3. Intravenous antisense CK2 therapy in an orthotopic, bladder tumor model

Therapy, formulated via targeted, colloidal delivery	Final Tumor Volume (mm^3)	Final Tumor Weight (gm)
Nonsense oligonucleotides	55 ± 2, SE (51-58)	0.17 (0.1-1.2)
CK2 antisense oligonucleotides	43 ± 2, SE (31-53)	0.12 (0.1-1.0)

CK2 is a prime regulator of p53 via direct protein binding in the C-terminus of p53. Protein binding is a mechanism of CK2 stabilization of cellular proteins. It has been shown *in vitro* that the isolated C-terminal region of p53 increases CK2 enzyme activity following binding direct binding to the oncoprotein. Using a pantropic, monoclonal antibody specific for both mutant and normal p53 (clone DO-1), paraffin-embedded sections were examined for p53 using immunohistofluorescence. Only mutant 53, but not wildtype p53, was detectable by immunohistofluorescence. A uniform abrogation of both CK2α nuclear immunosignal and p53 in tumors treated with antisense CK2α was observed relative to nonsense CK2α. Nuclear counterstaining for tissue treated with antisense CK2α was performed to confirm the presence of live tissue in antisense-treated tissue. The reduction in both CK2α and p53 bladder xenograft tissue was confirmed by Western blotting. Tumors were pooled to create a single protein lysate for each treatment. These lysates were electrophoresed on a 4-12% acrylamide gel, transferred to nitrocellulose and CK2 was detected using a polyclonal chicken antibody that detects all 3 CK2 isoforms. A commercial rat cerebellum lysate was used as a positive control reference lane. Equivalent protein loading was confirmed using the Coomassie-stained gel. The Western blot showed that CK2α was reduced below the level of detection for this method for antisense-treated pooled lysate relative to the random oligonucleotide and saline-treated lysates. A reduction in both CK2α and CK2β were observed, consistent with CK2α's role in stabilizing these structures.

p53 was detected using the pantropic, moloclonal DO-1. A pooled lysate of saline-treated bladder tumors was included as a comparison with the other pooled lysates.

A recombinant, commercially-available p53 protein was used as a positive control. The protein contained a 7 kD fusion tag for a total molecular weight of 60 kD. Equivalent protein loading was confirmed using the Coomassie-stained gel. The Western blot showed that p53 was reduced by >70% relative to the nonsense-treated pooled lysate. p53 was markedly elevated in sense-treated lysates relative to the historical control. This result is consistent with both p53's and CK2α's role in mediating response to stress. Based on these results, intravenous administration of antisense CK2α reduces levels of mutant p53 in tumors.

OTHER EMBODIMENTS

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

WHAT IS CLAIMED IS:

1. A method of inhibiting the expression of casein kinase 2 in a solid tumor, comprising:

delivering an antisense oligonucleotide to said tumor, wherein said antisense oligonucleotide hybridizes to casein kinase 2 nucleic acid sequences and reduces the expression thereof.

2. The method of claim 1, wherein said delivery is intratumorally or intravenously.

3. The method of claim 1, wherein said antisense oligonucleotide is an encapsulated antisense oligonucleotide.

4. The method of claim 1, wherein said antisense oligonucleotide is a phosphorothioate antisense oligonucleotide.

5. The method of claim 1, wherein said antisense oligonucleotide has the sequence shown in SEQ ID NO:1 or SEQ ID NO:2.

6. The method of claim 1, wherein said casein kinase 2 is casein kinase 2-α.

7. The method of claim 1, wherein said casein kinase 2 is casein kinase 2-β.

8. The method of claim 1, wherein said solid tumor originates in a location selected from the group consisting of prostate, bladder, breast, liver, kidney, brain, head, and neck.

9. A method of reducing the size of a solid tumor in an individual, comprising

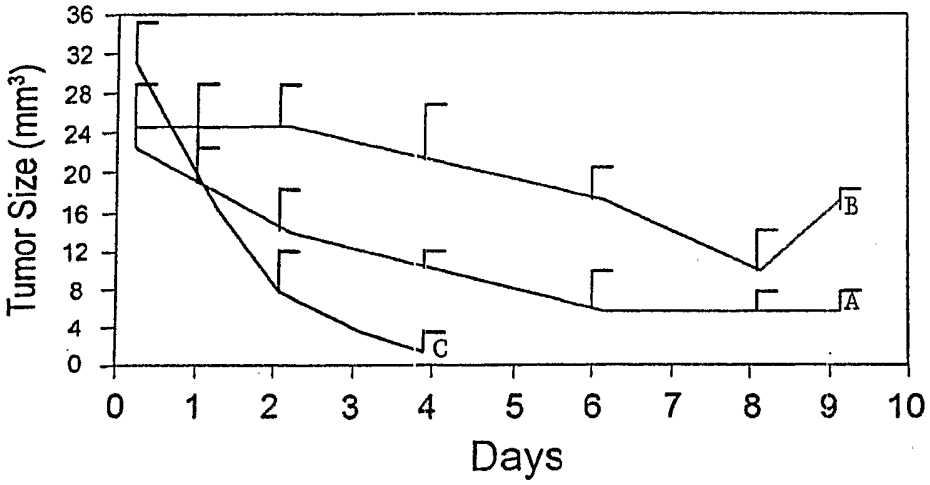
delivering an antisense oligonucleotide to said tumor, wherein said antisense oligonucleotide hybridizes to casein kinase 2 nucleic acid sequences and reduces the expression thereof,

wherein said reduction in expression of casein kinase 2 results in a reduction in size of said tumor.

10. A composition comprising an antisense oligonucleotide and a pharmaceutically acceptable carrier, wherein said antisense oligonucleotide hybridizes to casein kinase 2 nucleic acid sequences and reduces the expression thereof.

11. The composition of claim 10, wherein said composition is encapsulated.

12. The composition of claim 10, wherein said antisense oligonucleotide has the sequence shown in SEQ ID NO:1 or SEQ ID NO:2.



A – 50 μg/ml
B – 100 μg/ml
C – 200 μg/ml

Figure 1