COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF Eg5 GENE

Related Applications

This application claims the benefit of U.S. Provisional Application No. 60/787,762, filed March 31, 2006, and U.S. Provisional Application No. 60/870,259, filed December 15, 2006. ~~Both prior applications are incorporated herein by reference in their entirety.~~

Field of the Invention

This invention relates to double-stranded ribonucleic acid (dsRNA), and its use in mediating RNA interference to inhibit the expression of the Eg5 gene and the use of the dsRNA to treat pathological processes mediated by Eg5 expression, such as cancer, alone or in combination with a dsRNA targeting vacular endothelian growth factor (VEGF).

Background of the Invention

The maintenance of cell populations within an organism is governed by the cellular processes of cell division and programmed cell death. Within normal cells, the cellular events associated with the initiation and completion of each process is highly regulated. In proliferative disease such as cancer, one or both of these processes may be perturbed. For example, a cancer cell may have lost its regulation (checkpoint control) of the cell division cycle through either the overexpression of a positive regulator or the loss of a negative regulator, perhaps by mutation.

Alternatively, a cancer cell may have lost the ability to undergo programmed cell death through the overexpression of a negative regulator. Hence, there is a need to

interference (RNAi). WO 99/32619 (Fire et al.) discloses the use of a dsRNA of at least 25 nucleotides in length to inhibit the expression of genes in *C. elegans*. dsRNA has also been shown to degrade target RNA in other organisms, including plants (see, e.g., WO 99/53050, Waterhouse et al.; and WO 99/61631, Heifetz et al.), *Drosophila* (see, e.g., Yang, D., et al., *Curr. Biol.* (2000) 10:1191-1200), and mammals (see WO 00/44895, Limmer; and DE 101 00 586.5, Kreutzer et al.). This natural mechanism has now become the focus for the development of a new class of pharmaceutical agents for treating disorders that are caused by the aberrant or unwanted regulation of a gene.

Despite significant advances in the field of RNAi and advances in the treatment of pathological processes mediated by Eg5 expression, there remains a need for an agent that can selectively and efficiently silence the Eg5 gene using the cell's own RNAi machinery that has both high biological activity and in vivo stability, and that can effectively inhibit expression of a target Eg5 gene for use in treating pathological processes mediated by Eg5 expression.

Summary of the Invention

The invention is defined in the claims.

The invention ~~provides~~ relates to double-stranded ribonucleic acid (dsRNA), as well as compositions and in vitro methods for inhibiting the expression of the Eg5 gene in a cell or mammal using such dsRNA, alone or in combination with a dsRNA targeting VEGF. The invention also provides compositions and methods for treating pathological conditions and diseases caused by the expression of the Eg5 gene, such as in cancer. The dsRNA of the invention comprises an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is ~~substantially~~ fully complementary to at least part of an mRNA transcript of the Eg5 gene, said part being as defined in the claims.

In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of the Eg5 gene. The dsRNA

comprises at least two sequences that are complementary to each other. The dsRNA comprises a sense strand comprising a first sequence and an antisense strand comprising a second sequence. The antisense strand comprises a nucleotide sequence which is ~~substantially~~ fully complementary to at least part of an mRNA encoding Eg5, and the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length. The dsRNA, upon contacting with a cell expressing the Eg5, inhibits the expression of the Eg5 gene by at least 40%. Said part of an mRNA ecoding Eg5 is defined in the claims.

~~For example, the~~Described are dsRNA molecules ~~of the invention~~ which can be comprised of a first sequence of the dsRNA that is selected from the group consisting of the sense sequences of Tables 1-3 and the second sequence is selected from the group consisting of the antisense sequences of Tables 1-3.

The dsRNA molecules of the invention can be comprised of naturally occurring nucleotides or can be comprised of at least one modified nucleotide, such as a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative. Alternatively, the modified nucleotide may be chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide. ~~Generally, s~~Such modified sequence ~~will be~~ is based on a first sequence of said dsRNA ~~selected from the group consisting of the sense sequences of Tables 1-3~~ and a second sequence ~~selected from the group consisting of the antisense sequences of Tables 1-3~~ as defined in the claims.

In another embodiment, the invention provides a cell comprising one of the dsRNAs of the invention. The cell is generally a mammalian cell, such as a human cell.

In another embodiment, the invention provides a pharmaceutical composition for inhibiting the expression of the Eg5 gene in an organism, generally a human

subject, comprising one or more of the dsRNA of the invention and a pharmaceutically acceptable carrier or delivery vehicle.

In another embodiment, the invention provides ~~a~~ an in vitro method for inhibiting the expression of the Eg5 gene in a cell, comprising the following steps:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNA), wherein the dsRNA comprises at least two sequences that are complementary to each other. The dsRNA comprises a sense strand comprising a first sequence and an antisense strand comprising a second sequence. The antisense strand comprises a region of complementarity which is ~~substantially~~ fully complementary to at least a part of a mRNA encoding Eg5, ~~and~~ wherein the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and wherein the dsRNA, upon contact with a cell expressing the Eg5, inhibits expression of the Eg5 gene by at least 40%; and wherein said part of an mRNA encoding Eg5 is defined in the claims; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the Eg5 gene, thereby inhibiting expression of the Eg5 gene in the cell.

In another embodiment, the invention provides ~~methods~~ the dsRNA defined above for use in treating, preventing or managing pathological processes mediated by Eg5 expression, e.g. cancer~~,~~. Described is the ~~comprising~~ administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of one or more of the dsRNAs of the invention.

In another embodiment, the invention provides vectors for inhibiting the expression of the Eg5 gene in a cell, comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention.

In another embodiment, the invention provides a cell comprising a vector for inhibiting the expression of the Eg5 gene in a cell. The vector comprises a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention.

In a further embodiment, the invention provides the Eg5 dsRNA and the uses thereof as described above in combination with a second dsRNA targeting the VEGF mRNA. A combination of a dsRNA targeting Eg5 and a second dsRNA targeting VEGF provides complementary and synergiatic activity for treating hyperproliferative discords, particulary hepatic carcinoma.

Brief Description of the Figures

No Figures are presented

Detailed Description of the Invention

The invention provides double-stranded ribonucleic acid (dsRNA) as defined in the claims, as well as compositions and in vitro methods for inhibiting the expression of the Eg5 gene in a cell or mammal using the dsRNA. The invention also provides compositions ~~and methods~~ for treating pathological conditions and diseases in a mammal caused by the expression of the Eg5 gene using dsRNA. dsRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The invention further provides this dsRNA in combination with a second dsRNA that inhibits the expression of the VEGF gene.

The dsRNAs of the invention comprises an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is ~~substantially~~ fully complementary to at least part of an mRNA transcript of the Eg5 gene, said part being defined above. The use of these dsRNAs enables the targeted degradation of mRNAs of genes that are implicated in replication and or maintenance of cancer cells in mammals. Using cell-based and animal assays, the present inventors have demonstrated that very low dosages of these

dsRNA can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of the Eg5 gene. Thus, the ~~methods and~~ compositions of the invention comprising these dsRNAs are useful for treating pathological processes mediated by Eg5 expression, e.g. cancer, by targeting a gene involved in mitotic division.

The following detailed description discloses how to make and use the dsRNA and compositions containing dsRNA to inhibit the expression of the Eg5 gene, as well as compositions ~~and methods~~ for treating diseases and disorders caused by the expression of Eg5, such as cancer, alone or in combination with a second dsRNA targeting the VEGF gene. The pharmaceutical compositions of the invention comprise a dsRNA having an antisense strand comprising a region of complementarity which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is substantially complementary to at least part of an RNA transcript of the Eg5 gene, together with a pharmaceutically acceptable carrier. As discussed above, such compositions can further include a second dsRNA targeting VEGF.

Accordingly, certain aspects of the invention provide pharmaceutical compositions comprising the dsRNA of the invention together with a pharmaceutically acceptable carrier, in vitro methods of using the compositions to inhibit expression of the Eg5 gene, and ~~methods of using the~~ pharmaceutical compositions ~~to~~ for use in treating diseases caused by expression of the Eg5 gene. The invention further provides the above pharmaceutical compositions further containing a second dsRNA designed to inhibit the expression of VEGF.

I. Definitions

For convenience, the meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in other parts of this specification and its definition provided in this section, the definition in this section shall prevail.

"G," "C," "A" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil may be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base may base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of the invention by a nucleotide containing, for example, inosine. Sequences comprising such replacement moieties are embodiments of the invention.

As used herein, "Eg5" refers to the human kinesin family member 11, which is also known as KIF11, Eg5, HKSP, KNSL1 or TRIP5. Eg5 sequence can be found as NCBI GeneID:3832, HGNC ID: HGNC:6388 and RefSeq ID number:NM_004523.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of the Eg5 gene, including mRNA that is a product of RNA processing of a primary transcription product. The target sequence according to the invention is defined in the claims.

organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for in vivo delivery, dsRNA can be injected into a tissue site or administered systemically. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection.

The terms "silence" and "inhibit the expression of", in as far as they refer to the Eg5 gene, herein refer to the at least partial suppression of the expression of the Eg5 gene, as manifested by a reduction of the amount of mRNA transcribed from the Eg5 gene which may be isolated from a first cell or group of cells in which the Eg5 gene is transcribed and which has or have been treated such that the expression of the Eg5 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition is usually expressed in terms of

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

Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to Eg5 gene transcription, e.g. the amount of protein encoded by the Eg5 gene which is secreted by a cell, or the number of cells displaying a certain phenotype, e.g apoptosis. In principle, Eg5 gene silencing may be determined in any cell expressing the target, either constitutively or by genomic engineering, and by any appropriate assay. However, when a reference is needed in order to determine whether a given dsRNA inhibits the expression of the Eg5 gene by a certain degree and therefore is encompassed by the instant invention, the assay provided in the Examples below shall serve as such reference.

For example, in certain instances, expression of the Eg5 gene (or VEGF gene) is suppressed by at least about 20%, 25%, 35%, or 50% by administration of the double-stranded oligonucleotide of the invention. ~~In some embodiment, t~~The Eg5 gene may

~~is~~ be suppressed by at least about 60%, 70%, or 80% by administration of the double-stranded oligonucleotide of the invention. In some embodiments, the Eg5 gene is suppressed by at least about 85%, 90%, or 95% by administration of the double-stranded oligonucleotide of the invention. Tables 1-3 provides values for inhibition of expression using various Eg5 dsRNA molecules at various concentrations.

As used herein in the context of Eg5 expression, the terms "treat", "treatment", and the like, refer to relief from or alleviation of pathological processes mediated by Eg5 expression. In the context of the present invention insofar as it relates to any of the other conditions recited herein below (other than pathological processes mediated by Eg5 expression), the terms "treat", "treatment", and the like mean to relieve or alleviate at least one symptom associated with such condition, or to slow or reverse the progression of such condition, such as the slowing and progression of hepatic carcinoma.

As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of pathological processes mediated by Eg5 expression or an overt symptom of pathological processes mediated by Eg5 expression (alone or in combination with VEGF expression). The specific amount that is therapeutically effective can be readily determined by ordinary medical practitioner, and may vary depending on factors known in the art, such as, e.g. the type of pathological processes mediated by Eg5 expression, the patient's history and age, the stage of pathological processes mediated by Eg5 expression, and the administration of other anti-pathological processes mediated by Eg5 expression agents.

As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of a dsRNA and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," "therapeutically

effective amount" or simply "effective amount" refers to that amount of an RNA effective to produce the intended pharmacological, therapeutic or preventive result. For example, if a given clinical treatment is considered effective when there is at least a 25% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least a 25% reduction in that parameter.

The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

As used herein, a "transformed cell" is a cell into which a vector has been introduced from which a dsRNA molecule may be expressed.

II. Double-stranded ribonucleic acid (dsRNA)

In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules as defined in the claims for inhibiting the expression of the Eg5 gene (alone or incombinaton with a second dsRNA for inhibiting the expression of VEGF) in a cell or mammal, wherein the dsRNA comprises an antisense strand comprising a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of the Eg5 gene, and wherein the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in

length, and wherein said dsRNA, upon contact with a cell expressing said Eg5 gene, inhibits the expression of said Eg5 gene by at least 40%. The dsRNA comprises two RNA strands that are sufficiently complementary to hybridize to form a duplex structure. One strand of the dsRNA (the antisense strand) comprises a region of complementarity that is ~~substantially complementary, and generally~~ fully complementary, to ~~a~~ the above defined target sequence, derived from the sequence of an mRNA formed during the expression of the Eg5 gene, the other strand (the sense strand) comprises a region which is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. Generally, the duplex structure is between 15 and 30, more generally between 18 and 25, yet more generally between 19 and 24, and most generally between 19 and 21 base pairs in length. Similarly, the region of complementarity to the target sequence is between 15 and 30, more generally between 18 and 25, yet more generally between 19 and 24, and most generally between 19 and 21 nucleotides in length. The dsRNA of the invention may further comprise one or more single-stranded nucleotide overhang(s). The dsRNA can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc. In a preferred embodiment, the Eg5 gene is the human Eg5 gene. In specific embodiments, the ~~antisense strand of the dsRNA comprises~~ first sequence is the ~~sense~~ sequences of ~~Tables 1-3~~ SEQ ID NO: 135 and the second sequence is ~~selected from the group consisting of~~ the ~~antisense~~ sequences of ~~Tables 1-3~~ SEQ ID NO: 136. Alternative antisense agents that target elsewhere in the target sequence provided in Tables 1-3 can readily be determined using the target sequence and the flanking Eg5 sequence. In embodiments using a second dsRNA targeting VEGF, such agents are exemplified in the Examples and in co-pending US Serial Nos: 11/078,073 and 11/340,080~~, herein incorporated by reference~~.

~~The~~ Described is also a dsRNA ~~will comprise~~ comprising at least two nucleotide sequence selected from the groups of sequences provided in Tables 1-3. One of the two sequences is complementary to the other of the two sequences, with

one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of the Eg5 gene. As such, the dsRNA will comprises two oligonucleotides, wherein one oligonucleotide is described as the sense strand in Tables 1-3 and the second oligonucleotide is described as the antisense strand in Tables 1-3

The skilled person is well aware that dsRNAs comprising a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir et al., EMBO 2001, 20:6877-6888). However, others have found that shorter or longer dsRNAs can be effective as well. In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Tables 1-3, the dsRNAs of the invention can comprise at least one strand of a length of minimally 21 nt. It can be reasonably expected that shorter dsRNAs comprising one of the sequences of Tables 1-3 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs comprising a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Tables 1-3, and differing in their ability to inhibit the expression of the Eg5 gene in a FACS assay as described herein below by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention. Further dsRNAs that cleave within the target sequence provided in Tables 1-3 can readily be made using the Eg5 sequence and the target sequence provided.

In addition, the RNAi agents provided in Tables 1-3 identify a site in the Eg5 mRNA that is susceptible to RNAi based cleavage. ~~As such the present invention~~Described are further ~~includes~~ RNAi agents that target within the sequence targeted by one of the agents of the present invention. As used herein a second RNAi agent is said to target within the sequence of a first RNAi agent if the second RNAi agent cleaves the message anywhere within the mRNA that is complementary to the antisense strand of the first RNAi agent. Such a second agent will generally consist of at least 15 contiguous nucleotides from one of the sequences provided in Tables 1-3 coupled to additional nucleotide sequences taken from the region contiguous to the

selected sequence in the Eg5 gene. For example, the last 15 nucleotides of SEQ ID NO:1 combined with the next 6 nucleotides from the target Eg5 gene produces a single strand agent of 21 nucleotides that is based on one of the sequences provided in Tables 1-3.

~~The dsRNA of the invention can~~Described is a dsRNA which contains one or more mismatches to the target sequence. ~~In a preferred embodiment, the~~Such dsRNA of the invention may contain~~s~~ no more than 3 mismatches. If the antisense strand of the dsRNA contains mismatches to a target sequence, it is preferable that the area of mismatch not be located in the center of the region of complementarity. If the antisense strand of the dsRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to 5 nucleotides from either end, for example 5, 4, 3, 2, or 1 nucleotide from either the 5' or 3' end of the region of complementarity. For example, for a 23 nucleotide dsRNA strand which is complementary to a region of the Eg5 gene, the dsRNA generally does not contain any mismatch within the central 13 nucleotides. The methods described within the invention can be used to determine whether a dsRNA containing a mismatch to a target sequence is effective in inhibiting the expression of the Eg5 gene. Consideration of the efficacy of dsRNAs with mismatches in inhibiting expression of the Eg5 gene is important, especially if the particular region of complementarity in the Eg5 gene is known to have polymorphic sequence variation within the population.

In one embodiment, at least one end of the dsRNA has a single-stranded nucleotide overhang of 1 to 4, generally 1 or 2 nucleotides. dsRNAs having at least one nucleotide overhang have unexpectedly superior inhibitory properties than their blunt-ended counterparts. Moreover, the present inventors have discovered that the presence of only one nucleotide overhang strengthens the interference activity of the

including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those) having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, ~~each of which is herein incorporated by reference~~.

Preferred modified dsRNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or ore 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 CH2 component parts.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938;

5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, each of which is herein incorporated by reference.

In other preferred dsRNA mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an dsRNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an dsRNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., Science, 1991, 254, 1497-1500.

Most preferred embodiments of the invention are dsRNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH.sub.2--NH--CH.sub.2--, --CH.sub.2--N(CH.sub.3)--O--CH.sub.2-- [known as a methylene (methylimino) or MMI backbone], --CH.sub.2--O--N(CH.sub.3)--CH.sub.2--, --CH.sub.2--N(CH.sub.3)--N(CH.sub.3)--CH.sub.2-- and --N(CH.sub.3)--CH.sub.2--CH.sub.2-- [wherein the native phosphodiester backbone is represented as --O--P--O--CH.sub.2--] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. Also preferred are dsRNAs having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

Most preferred embodiments of the invention are dsRNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--CH_2--, --CH_2--N(CH_3)--O--CH_2--[known as a methylene (methylimino) or MMI backbone], --CH_2--O--N(CH_3)--CH_2--, --CH_2--N(CH_3)--N(CH_3)--CH_2-- and --N(CH_3)--CH_2--CH_2--[wherein the native phosphodiester backbone is represented as --O--P--O--CH_2--] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. Also preferred are dsRNAs having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

Modified dsRNAs may also contain one or more substituted sugar moieties. Preferred dsRNAs comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C.sub.1 to C.sub.10 alkyl or C.sub.2 to C.sub.10 alkenyl and alkynyl. Particularly preferred are O[(CH.sub.2).sub.nO].sub.mCH.sub.3, O(CH.sub.2).sub.nOCH.sub.3, O(CH.sub.2).sub.nNH.sub.2, O(CH.sub.2).sub.nCH.sub.3, O(CH.sub.2).sub.nONH.sub.2, and O(CH.sub.2).sub.nON[(CH.sub.2).sub.nCH.sub.3)].sub.2, where n and m are from 1 to about 10. Other preferred dsRNAs comprise one of the following at the 2' position: C.sub.1 to C.sub.10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH.sub.3, OCN, Cl, Br, CN, CF.sub.3, OCF.sub.3, SOCH.sub.3, SO.sub.2CH.sub.3, ONO.sub.2, NO.sub.2, N.sub.3, NH.sub.2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an dsRNA, or a group for improving the pharmacodynamic properties of an dsRNA, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O--CH.sub.2CH.sub.2OCH.sub.3, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chim. Acta, 1995, 78, 486-504) i.e., an alkoxy-alkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a O(CH.sub.2).sub.2ON(CH.sub.3).sub.2 group, also known as 2'-DMAOE, as described in examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O--CH.sub.2--O--CH.sub.2--N(CH.sub.2).sub.2, also described in examples hereinbelow.

Other preferred modifications include 2'-methoxy (2'-OCH.sub.3), 2'-aminopropoxy (2'-OCH.sub.2CH.sub.2CH.sub.2NH.sub.2) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the dsRNA, particularly

Modified dsRNAs may also contain one or more substituted sugar moieties. Preferred dsRNAs comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred dsRNAs 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, a group for improving the pharmacokinetic properties of an dsRNA, or a group for improving the pharmacodynamic properties of an dsRNA, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chim. Acta, 1995, 78, 486-504) i.e., an alkoxy-alkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O--CH_2--O--CH_2--$N(CH_2)_2$, also described in examples hereinbelow.

Other preferred modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the dsRNA, particularly

the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. DsRNAs may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920~~, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety~~.

DsRNAs may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, DsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993.

Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2.degree. C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., DsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; ~~and~~ 5,681,941, ~~each of which is herein incorporated by reference,~~ and U.S. Pat. No. 5,750,692~~, also herein incorporated by reference~~.

Another modification of the dsRNAs of the invention involves chemically linking to the dsRNA one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the dsRNA. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acid. Sci. USA, 199, 86, 6553-6556), cholic acid (Manoharan et al., Biorg. Med. Chem. Let., 1994 4 1053-1060), a thioether, e.g., beryl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Biorg. Med. Chem. Let., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J, 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-

hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-Hphosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937).

Representative U.S. patents that teach the preparation of such dsRNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, ~~each of which is herein incorporated by reference~~.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an dsRNA. The present invention also includes dsRNA compounds which are chimeric compounds. "Chimeric" dsRNA compounds or "chimeras," in the context of this invention, are dsRNA compounds, particularly dsRNAs, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an dsRNA compound. These dsRNAs typically contain at

comprising, for example, either the U6 or H1 RNA promoters, or the cytomegalovirus (CMV) promoter.

A suitable AV vector for expressing the dsRNA of the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H et al. (2002), Nat. Biotech. 20: 1006-1010.

Suitable AAV vectors for expressing the dsRNA of the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R et al. (1987), J. Virol. 61: 3096-3101; Fisher K J et al. (1996), J. Virol, 70: 520-532; Samulski R et al. (1989), J. Virol. 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

III. Pharmaceutical compositions comprising dsRNA

In one embodiment, the invention provides pharmaceutical compositions comprising a dsRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical composition comprising the dsRNA is useful for treating a disease or disorder associated with the expression or activity of the Eg5 gene, such as pathological processes mediated by Eg5 expression. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery.

In another embodiment, such compositions will further comprise a second dsRNA that inhibits VEGF expression. dsRNA directed to VEGF are described in the Examples and in co-pending US Serial Nos: 11/078,073 and 11/340,080.

The pharmaceutical compositions of the invention are to be administered in dosages sufficient to inhibit expression of the Eg5 gene (and VEGF expression when a second

acceptable salt thereof. Topical formulations are described in detail in U.S. patent application Ser. No. 09/315,298 filed on May 20~~, 1999 which is incorporated herein by reference in its entirety~~.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which dsRNAs of the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Preferred fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (e.g. sodium). Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs of the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and

starches. Particularly preferred complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (e.g. p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. application. Ser. No. 08/886,829 (filed Jul. 1, 1997), Ser. No. 09/108,673 (filed Jul. 1, 1998), Ser. No. 09/256,515 (filed Feb. 23, 1999), Ser. No. 09/082,624 (filed May 21, 1998) and Ser. No. 09/315,298 (filed May 20, 1999), ~~each of which is incorporated herein by reference in their entirety~~.

Compositions and formulations for parenteral, intrathecal, intraventricular or intrahepatic administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly perfered are formulations that target the liver when treating hepatic disorders such as hepatic carcinoma.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques

~~In one embodiment of the present invention, t~~The compositions of dsRNAs and nucleic acids ~~are~~ may be formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Methods for treating diseases caused by expression of the Eg5 gene

~~The invention relates in particular to~~ Described is the use of a dsRNA or a pharmaceutical composition prepared therefrom for the treatment of cancer, e.g., for inhibiting tumor growth and tumor metastasis. For example, the dsRNA or a pharmaceutical composition prepared therefrom may be used for the treatment of solid tumors, like breast cancer, lung cancer, head and neck cancer, brain cancer, abdominal cancer, colon cancer, colorectal cancer, esophagus cancer, gastrointestinal cancer, glioma, liver cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, retinoblastoma, Wilm's tumor, multiple myeloma and for the treatment of skin cancer, like melanoma, for the treatment of lymphomas and blood cancer. ~~The invention~~Described is further ~~relates to~~ the use of an dsRNA according to the invention or a pharmaceutical composition prepared therefrom for inhibiting eg5 expression and/or for inhibiting accumulation of ascites fluid and pleural effusion in different types of cancer, e.g., breast cancer, lung cancer, head cancer, neck cancer, brain cancer, abdominal cancer, colon cancer, colorectal cancer, esophagus cancer, gastrointestinal cancer, glioma, liver cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, retinoblastoma, Wilm's tumor, multiple myeloma, skin cancer, melanoma, lymphomas and blood cancer. Owing to the inhibitory effect on eg5 expression, an dsRNA according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life.

~~The invention furthermore relates to~~Also described is the use of an dsRNA or a pharmaceutical composition thereof, e.g., for treating cancer or for preventing tumor metastasis, in combination with other pharmaceuticals and/or other therapeutic methods, e.g., with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating cancer and/or for preventing tumor metastasis. Preference is given to a combination with radiation therapy and chemotherapeutic agents, such as cisplatin, cyclophosphamide, 5-

fluorouracil, adriamycin, daunorubicin or tamoxifen. Other emobiments include the use of a second dsRNA used to inhibit the expression of VEGF.

~~The invention can also be practiced by including with~~Described is also a specific RNAi agent, in combination with another anti-cancer chemotherapeutic agent, such as any conventional chemotherapeutic agent, or another dsRNA used to inhibt the expression of VEGF. The combination of a specific binding agent with such other agents can potentiate the chemotherapeutic protocol. Numerous chemotherapeutic protocols will present themselves in the mind of the skilled practitioner as being capable of incorporation into the method of the invention. Any chemotherapeutic agent can be used, including alkylating agents, antimetabolites, hormones and antagonists, radioisotopes, as well as natural products. For example, the compound of the invention can be administered with antibiotics such as doxorubicin and other anthracycline analogs, nitrogen mustards such as cyclophosphamide, pyrimidine analogs such as 5-fluorouracil, cisplatin, hydroxyurea, taxol and its natural and synthetic derivatives, and the like. As another example, in the case of mixed tumors, such as adenocarcinoma of the breast, where the tumors include gonadotropin-dependent and gonadotropin-independent cells, the compound can be administered in conjunction with leuprolide or goserelin (synthetic peptide analogs of LH-RH). Other antineoplastic protocols include the use of a tetracycline compound with another treatment modality, e.g., surgery, radiation, etc., also referred to herein as "adjunct antineoplastic modalities." Thus, the method of the invention can be employed with such conventional regimens with the benefit of reducing side effects and enhancing efficacy.

Methods for inhibiting expression of the Eg5 gene

~~In yet another aspect, the invention provides~~Described is furthermore a method for inhibiting the expression of the Eg5 gene in a mammal. The method comprises administering a composition of the invention to the mammal such that expression of the target Eg5 gene is silenced. Because of their high specificity, the dsRNAs of the invention specifically target RNAs (primary or processed) of the target Eg5 gene.

Compositions and methods for inhibiting the expression of these Eg5 genes using dsRNAs can be performed as described elsewhere herein.

~~In one embodiment, the~~Such method may comprise~~s~~ administering a composition comprising a dsRNA, wherein the dsRNA comprises a nucleotide sequence which is complementary to at least a part of an RNA transcript of the Eg5 gene of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. ~~In preferred embodiments, t~~The compositions ~~are~~ may be administered by intravenous infusion or injection.

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

Silencing of liver Eg5/KSP in juvenile rats following single-bolus administration of LNP01 formulated siRNA

From birth until approximately 23 days of age, Eg5/KSP expression can be detected in the growing rat liver. Target silencing with a formulated Eg5/KSP siRNA was evaluated in juvenile rats.

KSP Duplex Tested

Duplex ID	Target	Sense	Antisense
AD6248	~~VEGF~~KSP	AccGAAGuGuuGuuuGuccTsT (SEQ ID NO:1238)	GGAcAAAcAAcACUUCGGUTsT (SEQ ID NO:1239)

Methods

Dosing of animals. Male, juvenile Sprague-Dawley rats (19 days old) were administered single doses of lipidoid ("LNP01") formulated siRNA via tail vein injection. Groups of ten animals received doses of 10 milligrams per kilogram (mg/kg) bodyweight of either AD6248 or an unspecific siRNA. Dose level refers to the amount of siRNA duplex administered in the formulation. A third group received phosphate-buffered saline. Animals were sacrificed two days after siRNA administration. Livers were dissected, flash frozen in liquid Nitrogen and pulverized into powders.

mRNA measurements. Levels of Eg5/KSP mRNA were measured in livers from all treatment groups. Samples of each liver powder (approximately ten milligrams) were homogenized in tissue lysis buffer containing proteinase K. Levels of Eg5/KSP and GAPDH mRNA were measured in triplicate for each sample using the Quantigene branched DNA assay (GenoSpectra). Mean values for Eg5/KSP were normalized to mean GAPDH values for each sample. Group means were determined and normalized to the PBS group for each experiment.

siRNA duplexes administered

Duplex ID	Target	Sense	Antisense
AD12115	Eg5/KSP	ucGAGAAucuAAAcuAAcuTsT (SEQ ID NO:1240)	AGUuAGUUuAGAUUCUCGATsT (SEQ ID NO: 1241)
AD3133	VEGF	GcAcAuAGGAGAGAuGAGCUsU (SEQ ID NO:1242)	AAGCUcAUCUCUCCuAuGuGCusG (SEQ ID NO:1243)

Key: A,G,C,U-ribonucleotides; c,u-2'-O-Me ribonucleotides; ~~s-phorphorothioate~~ s-phosphorothioate.

Methods

Dosing of animals. Adult, female Sprague-Dawley rats were administered lipidoid ("LNP01") formulated siRNA by a two-hour infusion into the femoral vein. Groups of four animals received doses of 5, 10 and 15 milligrams per kilogram (mg/kg) bodyweight of formulated siRNA. Dose level refers to the total amount of siRNA duplex administered in the formulation. A fourth group received phosphate-buffered saline. Animals were sacrificed 72 hours after the end of the siRNA infusion. Livers were dissected, flash frozen in liquid Nitrogen and pulverized into powders.

Formulation Procedure

The lipidoid ND98·4HCl (MW 1487) (Formula 1), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) were used to prepare lipid-siRNA nanoparticles. Stock solutions of each in ethanol were prepared: ND98, 133 mg/mL; Cholesterol, 25 mg/mL, PEG-Ceramide C16, 100 mg/mL. ND98, Cholesterol, and PEG-Ceramide C16 stock solutions were then combined in a 42:48:10 molar ratio. Combined lipid solution was mixed rapidly with aqueous siRNA (in sodium acetate pH 5) such that the final ethanol concentration was 35-45% and the final sodium acetate concentration was 100-300 mM. Lipid-siRNA nanoparticles formed spontaneously upon mixing. Depending on the desired particle

5. An isolated cell comprising the dsRNA of claim 1.

6. A pharmaceutical composition for inhibiting the expression of the Eg5 gene comprising the dsRNA of claim 1 or 2.

7. The pharmaceutical composition of claim 6, further comprising a dsRNA that inhibits the expression of the VEGF gene.

8. An *in vitro* method for inhibiting the expression of the Eg5 gene in a cell, the method comprising:

   (a) introducing into the cell the dsRNA of claim 2; and

   (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the Eg5 gene,

   thereby inhibiting expression of the Eg5 gene in the cell.

9. The method of claim 8 wherein a second dsRNA that inhibits the expression of VEGF is introduced into said cell.

10. A dsRNA of claim 1 or 2 for treating, preventing or managing pathological processes mediated by Eg5 expression.

11. The dsRNA of claim 10, wherein a second dsRNA that inhibits the expression of VEGF is to be administered.

12. A vector for inhibiting the expression of the Eg5 gene in a cell, said vector comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a dsRNA, wherein one of the strands of said dsRNA is fully complementary to at least a part of a mRNA encoding Eg5, wherein said dsRNA is less than 30 base pairs in length, wherein said part of a mRNA encoding Eg5 consists of the sequence UCGAGAAUCUAAACUAACU, and wherein said dsRNA, upon contact with a cell expressing said Eg5, inhibits the expression of said Eg5 gene by at least 40%.

13. An isolated cell comprising the vector of claim 12.

14. The pharmaceutical composition of claim 7, the method of claim 9, or the dsRNA of claim 11, to the extent said claims refer back to claim 2, wherein the dsRNA that inhibits expression of VEGF consists of a sense and an antisense strand, said sense strand consisting of the sequence GcAcAuAGGAGAGAuGAGCUsU, said antisense strand consisting of the sequence AAGCUcAUCUCUCCuAuGuGCusG, wherein A, G, C and U represent ribonucleotidesl c and u represent 2'-O-Me ribonucleotides; and s represents phosphorothioate.

15. Use of the dsRNA of claim 1 or 2 for the preparation of a medicament for treatment, prevention or management of pathological processes mediated by Eg5 expression.