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(54) **Title:** COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF A GENE FROM THE JC VIRUS

(57) **Abstract:** The invention relates to a double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a gene from the JC Virus (JC virus genome), comprising an antisense strand having a nucleotide sequence which is less that 30 nucleotides in length, generally 19-25 nucleotides in length, and which is substantially complementary to at least a part of a gene from the JC Virus. The invention also relates to a pharmaceutical composition comprising the dsRNA together with a pharmaceutically acceptable carrier; methods for treating diseases caused by JC virus expression and the expression of a gene from the JC Virus using the pharmaceutical composition; and methods for inhibiting the expression of a gene from the JC Virus in a cell.

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COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF A GENE FROM THE JC VIRUS

Related Applications

This application claims the benefit of U.S. Provisional Application No. 60/795,765, filed April 28, 2006, which is incorporated herein by reference in its 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 one of the genes of the JC virus and the use of the dsRNA to treat pathological processes mediated by JC virus infection, such as PML.

Background of the Invention

Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease of the central nervous system which results from reactivation of the latent polyomavirus JC virus (JCV) and its productive replication in glial cells of the human brain (Berger, J. R. (1995) J. Neurovirol. 1:5-18). Once a rare disease primarily seen in patients with impaired immune systems due to lymphoproliferative and myeloproliferative disorders, PML has become one of the major neurologic problems among patients with AIDS (Cinque, P., (2003). J. Neurovirol. 9(Suppl. 1):88-92).

It has been reported that between 4 and 8% of AIDS patients exhibit signs of PML, and JCV has been detected in the cerebrospinal fluid of affected patients, suggesting that there is active replication of the virus in the brain (Berger, J. R. (1995) J. Neurovirol. 1:5-18, Clifford, D. B., (2001) J. Neurovirol. 4:279). In addition, PML has recently been seen in patients undergoing experimental treatment with Tysbari, an anti VLA4 antobody, in combination with interferon. The histological hallmarks of PML include multifocal demyelinated lesions with enlarged eosinophilic nuclei in oligodendrocytes and enlarged bizarre astrocytes with lobulated hyperchromatic nuclei within white matter tracts of the brain (Cinque, P., (2003). J. Neurovirol. 9(Suppl. 1):88-92), although in some instances atypical features that include a unifocal pattern of

demyelination and involvement of the gray matter have been reported (Sweeney, B. J., (1994). J. Neurol. Neurosurg. Psychiatry 57:994-997). Earlier observations from in vitro cell culture studies and an in vivo evaluation of JCV in clinical samples led to early assumptions that oligodendrocytes and astrocytes are the only cells which support productive viral infections (Gordon, J. (1998) Int. J. Mol. Med. 1:647-655). Accordingly, molecular studies have provided evidence for cell-type-specific transcription of the viral early genome in cells derived from the central nervous system (Raj, G. V., (1995) Virology 10:283-291). However, subsequent studies have shown low, but detectable, levels of JCV gene expression in nonneural cells, including B cells, and noticeably high levels of production of the viral early protein in several neural and nonneural tumor cells in humans (Gordon, J. (1998) Int. J. Mol. Med. 1:647-655, Khalili, K., 2003. Oncogene 22:5181-5191).

Like the other polyomaviruses, JCV is a small DNA virus whose genome can be divided into three regions that encompass the transcription control region; the genes responsible for the expression of the viral early protein, T antigen; and the genes encoding the viral late proteins, VP1, VP2, and VP3. In addition, the late genome is also responsible for production of an auxiliary viral protein, agnoprotein. T-antigen expression is pivotal for initiation of the viral lytic cycle, as this protein stimulates transcription of the late genes and induces the process of viral DNA replication. Recent studies have ascribed an important role for agnoprotein in the transcription and replication of JCV, as inhibition of its production significantly reduced viral gene expression and replication (M. Safak et al., unpublished observations). Furthermore, the agnoprotein dysregulates the cell cycle by altering the expression of several cyclins and their associated kinases (Darbinyan, A., (2002) Oncogene 21:5574-5581).

Thus far, there are no effective therapies for the suppression of JCV replication and the treatment of PML. Cytosine arabinoside (AraC) has been tested for the treatment of PML patients, and the outcome in some instances revealed a remission of JCV-associated demyelination (Aksamit, A. (2001) J. Neurovirol. 7:386-390). Reports from the AIDS Clinical Trial Group Organized Trial 243, however, have suggested that there is no difference in the survival of human immunodeficiency virus type 1 (HIV-1)-infected patients with PML and that of the control population, although in other reports it has been suggested that the failure of AraC

in the AIDS Clinical Trial Group trial may have been due to insufficient delivery of the AraC via the intravenous and intrathecal routes (Levy, R. M.,(2001) J. Neurovirol. 7:382-385). Based on in vitro studies showing the ability of inhibitors of topoisomerase to suppress JCV DNA replication, the topoisomerase inhibitor topotecan was used for the treatment of AIDS-PML patients, and the results suggested that topotecan treatment may be associated with a decreased lesion size and prolonged survival (Royal, W., III, (2003) J. Neurovirol. 9:411-419).

Double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA 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.

Recent reports have indicated that in vitro, RNAi may show some promise in reducing JC virus replication (Radhakrishnan, S. (2004) J. Vir. 78:7264-7269, Orba, Y. (2004) J. Vir. 78:7270-7273). However, the RNAi agents examined were not designed against all know JC Virus strains and were not selected for stability and other properties need for in vivo therapeutic RNAi agents. Accordingly, despite significant advances in the field of RNAi, there remains a need for an agent that can selectively and efficiently silence a gene in the JC virus using the cell's own RNAi machinery that has both high biological activity and in vivo stability, and that can effectively inhibit replication of the JC virus for use in treating pathological processes mediated by JC virus infection.

Summary of the Invention

The invention provides double-stranded ribonucleic acid (dsRNA), as well as compositions and methods for inhibiting the expression of the JC virus in a cell or mammal using such dsRNA. The invention also provides compositions and methods for treating pathological

conditions and diseases caused by JC viral infection, such as PML. 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 complementary to at least part of an mRNA transcript of a gene from the JC Virus.

In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression one of the genes of the JC virus and viral replication. 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 complementary to at least part of an mRNA encoded by a gene from the JC Virus, 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 infected with the JC virus, inhibits the expression of a gene from the JC Virus by at least 40%.

For example, the dsRNA molecules of the invention can be comprised of a first sequence of the dsRNA that is selected from the group consisting of the sense sequences of Tables 1a and b and the second sequence is selected from the group consisting of the antisense sequences of Tables 1a and b. 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, such modified sequence will be based on a first sequence of said dsRNA selected from the group consisting of the sense sequences of Tables 1a and b and a second sequence selected from the group consisting of the antisense sequences of Tables 1a and b.

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 replication of the JC virus 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 method for inhibiting the expression of a gene in the JC Virus 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 complementary to at least a part of a mRNA encoded by the JC virus, 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 infected with the JC virus, inhibits expression of a gene from the JC Virus by at least 40%; and

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

In another embodiment, the invention provides methods for treating, preventing or managing pathological processes mediated by JC virus infection, e.g. such as PML, 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 a gene of the JC virus 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 a gene of the JC virus 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.

Brief Description of the Figures

No Figures are presented.

Detailed Description of the Invention

The invention provides double-stranded ribonucleic acid (dsRNA), as well as compositions and methods for inhibiting the expression of a gene from the JC Virus 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 JC virus infection using dsRNA. dsRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi).

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 complementary to at least part of an mRNA transcript of a gene from the JC Virus. The use of these dsRNAs enables the targeted degradation of mRNAs of genes that are implicated in replication and or maintenance of JC virus infection and the occurance of PML in a subject infected with the JC virus. 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 a gene from the JC Virus. Thus, the methods and compositions of the invention comprising these dsRNAs are useful for treating pathological processes mediated by JC viral infection, e.g. cancer, by targeting a gene involved in JC virus relication and/or maintainance in a cell.

The following detailed description discloses how to make and use the dsRNA and compositions containing dsRNA to inhibit the expression of a gene from the JC virus, as well as compositions and methods for treating diseases and disorders caused by the infection with the JC virus, such as PML. 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 a gene from the JC Virus, together with a pharmaceutically acceptable carrier.

Accordingly, certain aspects of the invention provide pharmaceutical compositions comprising the dsRNA of the invention together with a pharmaceutically acceptable carrier, methods of using the compositions to inhibit expression of a gene in a gene from the JC Virus, and methods of using the pharmaceutical compositions to treat diseases caused by infection with the JC virus.

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, "JC virus" refers to the latent polyomavirus JC Virus that has a reference sequence NC_001699. In addition, further accession numbers of various JCVirus sequences are

AB038249.1-AB038255.1, AB048545.1 - AB048582.1, AB074575.1 - AB074591.1, AB077855.1 - AB077879.1, AB081005.1 - AB081030.1, AB081600.1 - AB081618.1, AB081654.1, AB092578.1 - AB092587.1, AB103387.1, AB103402.1 - AB103423.1, AB104487.1, AB113118.1 - AB113145.1, AB118651.1 - AB118659.1, AB126981.1 - AB127027.1, AB127342.1, AB127344.1, AB127346.1 - AB127349.1, AB127352.1 - AB127353.1, AB198940.1 - AB198954.1, AB220939.1 - AB220943.1, AF004349.1 - AF004350.1, AF015526.1 - AF015537.1, AF015684.1, AF030085.1, AF281599.1 - AF281626.1, AF295731.1 - AF295739.1, AF300945.1 - AF300967.1, AF363830.1 - AF363834.1, AF396422.1 - AF396435.1, AY121907.1 - AY121915.1, NC_001699.1, U61771.1, U73500.1 - U73502.1.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a gene from the JC Virus, including mRNA that is a product of RNA processing of a primary transcription product.

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

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

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

"Complementary" sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled.

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

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

The term "double-stranded RNA" or "dsRNA", as used herein, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary, as defined above, nucleic acid strands,. The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop". Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker". The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, a dsRNA may comprise one or more nucleotide overhangs.

As used herein, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that protrude from the duplex structure of a dsRNA when a 3'-end of one strand of the dsRNA extends beyond the 5'-end of the other strand, or vice versa. "Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the dsRNA, i.e., no nucleotide overhang. A "blunt ended" dsRNA is a dsRNA that is double-stranded over its entire length, i.e., no nucleotide overhang at either end of the molecule.

The term "antisense strand" refers to the strand of a dsRNA which includes a region that is substantially complementary to a target sequence. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated in the terminal regions and, if present, are generally in a terminal region or regions, e.g., within 6, 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus.

The term "sense strand," as used herein, refers to the strand of a dsRNA that includes a region that is substantially complementary to a region of the antisense strand.

"Introducing into a cell", when referring to a dsRNA, means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of dsRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells in vitro; a dsRNA may also be "introduced into a cell", wherein the cell is part of a living 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 a gene from the JC Virus, herein refer to the at least partial suppression of the expression of a gene from the JC Virus, as manifested by a reduction of the amount of mRNA transcribed from a gene from the JC Virus which may be isolated from a first cell or group of cells in which a gene from the JC Virus is transcribed and which has or have been treated such that the expression of a gene from the JC Virus 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 JC virus genome transcription, e.g. the amount of protein encoded by a gene from the JC Virus, or the number of cells displaying a certain phenotype, e.g infection with the JC Virus. In principle, JC virus genome 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 a gene from the JC Virus 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 a gene from the JC Virus is suppressed by at least about 20%, 25%, 35%, or 50% by administration of the double-stranded oligonucleotide of the invention. In some embodiment, a gene from the JC Virus is suppressed by at least about 60%, 70%, or 80% by administration of the double-stranded oligonucleotide of the invention. In some embodiments, a gene from the JC Virus is suppressed by at least about 85%, 90%, or 95% by administration of the double-stranded oligonucleotide of the invention.

As used herein in the context of JC virus expression, the terms "treat", "treatment", and the like, refer to relief from or alleviation of pathological processes mediated by JC virus infection. 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 JC virus 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.

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 JC virus infection or an overt symptom of pathological processes mediated by JC virus 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 JC virus infection, the patient's history and age, the stage of pathological processes mediated by JC virus infection, and the administration of other anti-pathological processes mediated by JC virus infection.

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 for inhibiting the expression of a gene from the JC Virus 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 a gene from the JC Virus, 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 the gene from the JC virus, inhibits the expression of the JC virus 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 target sequence, derived from the sequence of an mRNA formed during the expression of a gene from the JC Virus, 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, a gene from the JC Virus is the human JC virus genome. In specific embodiments, the antisense strand of the dsRNA comprises the sense sequences of Tables 1a and b and the second sequence is selected from the group consisting of the antisense sequences of Tables 1a and b. Alternative antisense agents that target elsewhere in the target sequence provided in Tables 1a and b can readily be determined using the target sequence and the flanking JC virus sequence.

In further embodiments, the dsRNA comprises at least one nucleotide sequence selected from the groups of sequences provided in Tables 1a and b. In other embodiments, the dsRNA comprises at least two sequences selected from this group, wherein one of the at least two sequences is complementary to another of the at least two sequences, and one of the at least two sequences is substantially complementary to a sequence of an mRNA generated in the expression of a gene from the JC Virus. Generally, the dsRNA comprises two oligonucleotides, wherein one oligonucleotide is described as the sense strand in Tables 1a and b and the second oligonucleotide is described as the antisense strand in Tables 1a and b

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 1a and b, 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 1a and b 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 1a and b, and differing in their ability to inhibit the expression of a gene from the JC Virus 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 1a and b can readily be made using the JC virus sequence and the target sequence provided.

In addition, the RNAi agents provided in Tables 1a and b identify a site in the JC virus mRNA that is susceptible to RNAi based cleavage. As such the present invention 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 1a and b coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a gene from the JC Virus. For example, the last 15 nucleotides of SEQ ID NO:1 combined with the next 6 nucleotides from the target JC virus genome produces a single strand agent of 21 nucleotides that is based on one of the sequences provided in Tables 1a and b.

The dsRNA of the invention can contain one or more mismatches to the target sequence. In a preferred embodiment, the dsRNA of the invention contains 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 a gene from the JC Virus, 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 a gene from the JC Virus. Consideration of the efficacy of dsRNAs with mismatches in inhibiting expression of a gene from the JC Virus is important, especially if the particular region of complementarity in a gene from the JC Virus 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 dsRNA, without affecting its overall stability. dsRNA having only one overhang has proven particularly stable and effective in vivo, as well as in a variety of cells, cell culture mediums, blood, and serum. Generally, the single-stranded overhang is located at the 3'-terminal end of the antisense strand or, alternatively, at the 3'-terminal end of the sense strand. The dsRNA may also have a blunt end, generally located at the 5'-end of the antisense strand. Such dsRNAs have improved stability and inhibitory activity, thus allowing administration at low dosages, i.e., less than 5 mg/kg body weight of the recipient per day. Generally, the antisense strand of the dsRNA has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In yet another embodiment, the dsRNA is chemically modified to enhance stability. The nucleic acids of the invention may be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry", Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Specific examples of preferred dsRNA compounds useful in this invention include dsRNAs containing modified backbones or no natural internucleoside linkages. As

defined in this specification, dsRNAs having modified backbones include those that retain 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 dsRNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligomicleosides.

Preferred modified dsRNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those) having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, 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.

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.su- b.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 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 least one region wherein the dsRNA is modified so as to confer upon the dsRNA increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the dsRNA may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of dsRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter dsRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxydsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the dsRNA may be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to dsRNAs in order to enhance the activity, cellular distribution or cellular uptake of the dsRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan et al., Bioorg. Med. Chem.

Let., 1993, 3:2765), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10:111; Kabanov et al., FEBS Lett., 1990, 259:327; Svinarchuk et al., Biochimie, 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36:3651; Shea et al., Nucl. Acids Res., 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36:3651), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277:923). Representative United States patents that teach the preparation of such dsRNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of dsRNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction may be performed either with the dsRNA still bound to the solid support or following cleavage of the dsRNA in solution phase. Purification of the dsRNA conjugate by HPLC typically affords the pure conjugate.

Vector encoded RNAi agents

The dsRNA of the invention can also be expressed from recombinant viral vectors intracellularly in vivo. The recombinant viral vectors of the invention comprise sequences encoding the dsRNA of the invention and any suitable promoter for expressing the dsRNA sequences. Suitable promoters include, for example, the U6 or H1 RNA pol III promoter sequences and the cytomegalovirus promoter. Selection of other suitable promoters is within the skill in the art. The recombinant viral vectors of the invention can also comprise inducible or regulatable promoters for expression of the dsRNA in a particular tissue or in a particular intracellular environment. The use of recombinant viral vectors to deliver dsRNA of the invention to cells in vivo is discussed in more detail below.

dsRNA of the invention can be expressed from a recombinant viral vector either as two separate, complementary RNA molecules, or as a single RNA molecule with two complementary regions.

Any viral vector capable of accepting the coding sequences for the dsRNA molecule(s) to be expressed can be used, for example vectors derived from adenovirus (AV); adeno-associated virus (AAV); retroviruses (e.g, lentiviruses (LV), Rhabdoviruses, murine leukemia virus); herpes virus, and the like. The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate.

For example, lentiviral vectors of the invention can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors of the invention can be made to target different cells by engineering the vectors to express different capsid protein serotypes. For example, an AAV vector expressing a serotype 2 capsid on a serotype 2 genome is called AAV 2/2. This serotype 2 capsid gene in the AAV 2/2 vector can be replaced by a serotype 5 capsid gene to produce an AAV 2/5 vector. Techniques for constructing AAV vectors which express different capsid protein serotypes are within the skill in the art; see, e.g., Rabinowitz J E et al. (2002), J Virol 76:791-801, the entire disclosure of which is herein incorporated by reference.

Selection of recombinant viral vectors suitable for use in the invention, methods for inserting nucleic acid sequences for expressing the dsRNA into the vector, and methods of delivering the viral vector to the cells of interest are within the skill in the art. See, for example, Dornburg R (1995), Gene Therap. 2: 301-310; Eglitis M A (1988), Biotechniques 6: 608-614; Miller A D (1990), Hum Gene Therap. 1: 5-14; Anderson W F (1998), Nature 392: 25-30; and Rubinson D A et al., Nat. Genet. 33: 401-406, the entire disclosures of which are herein incorporated by reference.

Preferred viral vectors are those derived from AV and AAV. In a particularly preferred embodiment, the dsRNA of the invention is expressed as two separate, complementary single-

stranded RNA molecules from a recombinant AAV vector 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 a gene from the JC Virus and/or viral infection, such as PML. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery.

The pharmaceutical compositions of the invention are administered in dosages sufficient to inhibit expression of a gene from the JC Virus. The present inventors have found that, because of their improved efficiency, compositions comprising the dsRNA of the invention can be administered at surprisingly low dosages. A maximum dosage of 5 mg dsRNA per kilogram body weight of recipient per day is sufficient to inhibit or completely suppress expression of a gene from the JC Virus.

In general, a suitable dose of dsRNA will be in the range of 0.01 to 5.0 milligrams per kilogram body weight of the recipient per day, generally in the range of 1 microgram to 1 mg per kilogram body weight per day. The pharmaceutical composition may be administered once daily, or the dsRNA may be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the dsRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, e.g., using a conventional sustained release formulation which provides sustained release of the dsRNA over a several day period. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and in vivo half-lives for the individual dsRNAs encompassed by the invention can be made using conventional methodologies or on the basis of in vivo testing using an appropriate animal model, as described elsewhere herein.

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as pathological processes mediated by JC virus expression. Such models are used for in vivo testing of dsRNA, as well as for determining a therapeutically effective dose.

The present invention also includes pharmaceutical compositions and formulations which include the dsRNA compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes

intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration.

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.

Compositions and formulations for parenteral, intrathecal or intraventricular 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.

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 include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Liposomes

There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present

invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages in vivo.

In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

Further advantages of liposomes include; liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes and as the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the

administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al., Biochem. Biophys. Res. Commun., 1987, 147, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al., Journal of Controlled Release, 1992, 19, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of

skin herpes sores while delivery of interferon via other means (e.g. as a solution or as an emulsion) were ineffective (Weiner et al., Journal of Drug Targeting, 1992, 2, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal formulation to the administration of interferon using an aqueous system, and concluded that the liposomal formulation was superior to aqueous administration (du Plessis et al., Antiviral Research, 1992, 18, 259-265).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome.TM. I (glyceryl dilaurate/cholesterol/po- lyoxyethylene-10-stearyl ether) and Novasome.TM. II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu et al. S.T.P.Pharma. Sci., 1994, 4, 6, 466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G.sub.M1, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen et al., FEBS Letters, 1987, 223, 42; Wu et al., Cancer Research, 1993, 53, 3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (Ann. N.Y. Acad. Sci., 1987, 507, 64) reported the ability of monosialoganglioside G.sub.M1, galactocerebroside sulfate and phosphatidylinositol to improve

blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (Proc. Natl. Acad. Sci. U.S.A., 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G.sub.M1 or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphat- idylcholine are disclosed in WO 97/13499 (Lim et al).

Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto et al. (Bull. Chem. Soc. Jpn., 1980, 53, 2778) described liposomes comprising a nonionic detergent, 2C.sub.1215G, that contains a PEG moiety. Illum et al. (FEBS Lett., 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (e.g., PEG) are described by Sears (U.S. Pat. Nos. 4,426,330 and 4,534,899). Klibanov et al. (FEBS Lett., 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume et al. (Biochimica et Biophysica Acta, 1990, 1029, 91) extended such observations to other PEG-derivatized phospholipids, e.g., DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle et al. (U.S. Pat. Nos. 5,013,556 and 5,356,633) and Martin et al. (U.S. Pat. No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Pat. No. 5,225,212 (both to Martin et al.) and in WO 94/20073 (Zalipsky et al.) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi et al). U.S. Pat. No. 5,540,935 (Miyazaki et al.) and U.S. Pat. No. 5,556,948 (Tagawa et al.) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

A limited number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry et al. discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 to Tagawa et al. discloses protein-bonded liposomes and asserts that the contents of such liposomes may include an dsRNA RNA. U.S. Pat. No. 5,665,710 to Rahman et al. describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love et al. discloses liposomes comprising dsRNA dsRNAs targeted to the raf gene.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g. they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and

ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

Agents that enhance uptake of dsRNAs at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al., PCT Application WO 97/30731), are also known to enhance the cellular uptake of dsRNAs.

Other agents may be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity per se) but is recognized as a nucleic acid by in vivo processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao et al., DsRNA Res. Dev., 1995, 5, 115-121; Takakura et al., DsRNA & Nucl. Acid Drug Dev., 1996, 6, 177-183.

Excipients

In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient may be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc).

Pharmaceutically acceptable organic or inorganic excipient suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Other Components

The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Certain embodiments of the invention provide pharmaceutical compositions containing (a) one or more antisense compounds and (b) one or more other chemotherapeutic agents which

function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphor- amide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). See, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed. 1987, pp. 1206-1228, Berkow et al., eds., Rahway, N.J. When used with the compounds of the invention, such chemotherapeutic agents may be used individually (e.g., 5-FU and oligonucleotide), sequentially (e.g., 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (e.g., 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). 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, may also be combined in compositions of the invention. See, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 2499-2506 and 46-49, respectively). Other non-antisense chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulation a range of dosage for use in humans. The dosage of compositions of the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (e.g., achieving a decreased concentration of the polypeptide) that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

In addition to their administration individually or as a plurality, as discussed above, the dsRNAs of the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by JC virus expression. In any event, the administering physician can adjust the amount and timing of dsRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

Methods for treating diseases caused by expression of a gene from the JC Virus

The invention relates in particular to the use of a dsRNA or a pharmaceutical composition prepared therefrom for the treatment or prevention of pathological conditions associated with JC Virus infection, e.g., PML. Owing to the inhibitory effect on JC virus expression, an dsRNA according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life, particularly in a patient being treated with an anti-VLA4 antibody as part of treatment for MS.

The invention furthermore relates to the use of an dsRNA or a pharmaceutical composition thereof for treating PML 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.

The invention can also be practiced by including with a specific RNAi agent, in combination with another anti-cancer chemotherapeutic agent, such as any conventional chemotherapeutic agent. 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 a gene from the JC Virus

In yet another aspect, the invention provides a method for inhibiting the expression of a gene from the JC Virus in a mammal. The method comprises administering a composition of the invention to the mammal such that expression of the target JC virus genome is silenced. Because of their high specificity, the dsRNAs of the invention specifically target RNAs (primary or processed) of the target JC virus gene. Compositions and methods for inhibiting the expression of these JC virus genes using dsRNAs can be performed as described elsewhere herein.

In one embodiment, the method comprises 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 a gene from the JC Virus, to 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, administration. In preferred embodiments, the compositions are 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.

EXAMPLES

Design of JCV siRNAs

Full-length genome sequences to JC virus available on April 10, 2006, were obtained, resulting in a target pool of 388 sequences (accession numbers: AB038249.1 - AB038255.1; AB048545.1 - AB048582.1; AB074575.1 - AB074591.1; ; AB077855.1 - AB077879.1; AB081005.1 - AB081030.1; AB081600.1 - AB081618.1; AB081654.1; AB092578.1 - AB092587.1; AB103387.1; AB103402.1 - AB103423.1; AB104487.1; AB113118.1 - AB113145.1; AB118651.1 - AB118659.1; AB126981.1 - AB127027.1; AB127342.1; AB127344.1; AB127346.1 - AB127349.1; AB127352.1 - AB127353.1; AB198940.1 - AB198954.1; AB220939.1 - AB220943.1; AF004349.1 - AF004350.1; AF015526.1 - AF015537.1; AF015684.1; AF030085.1; AF281599.1 - AF281626.1; AF295731.1 - AF295739.1; AF300945.1 - AF300967.1; AF363830.1 - AF363834.1; AF396422.1 -

AF396435.1; AY121907.1 - AY121915.1; NC_001699.1; U61771.1; U73500.1 - U73502.1). NC_001699 was defined as reference sequence.

The siRNA selection process was run as follows: ClustalW multiple alignment was used to generate a global alignment of all sequences from the target pool. An IUPAC consensus sequence was then generated.

All conserved 19mer target sequences from the IUPAC consensus represented by stretches containing only A, T, C or G bases, which are therefore present in all sequences of the target pool were selected. In order to only select siRNAs that target transcribed sequence parts of the JC virus, candidate target sequences were selected out of the pool of conserved 19mer target sequences. For this, candidate target sequences covering regions between nucleotide 163-2594 and between 2527-5115 relative to reference sequence were extracted for late and early genes, respectively. Further, as sequences for early genes are in reverse complement orientation compared with genomic sequences, candidate target sequences of these genes were transferred to reverse complement sequences and replaced the former pool of candidate target sequences.

In order to rank candidate target sequences and their respective siRNAs and select appropriate ones, their predicted potential for interacting with irrelevant targets (off-target potential) was taken as a ranking parameter. siRNAs with low off-target potential were defined as preferable and assumed to be more specific *in vivo*.

For predicting siRNA-specific off-target potential, the following assumptions were made:

1) positions 2 to 9 (counting 5' to 3') of a strand (seed region) may contribute more to off-target potential than rest of sequence (non-seed and cleavage site region)

2) positions 10 and 11 (counting 5' to 3') of a strand (cleavage site region) may contribute more to off-target potential than non-seed region

3) an off-target score can be calculated for each hit, based on identity to siRNA sequence and position of mismatches

4) assuming potential abortion of sense strand activity by internal modifications introduced, only off-target potential of antisense strand will be relevant

To identify potential off-target genes, 19mer input sequences were subjected to a homology search against publically available human mRNA sequences.

To this purpose, fastA (version 3.4) searches were performed with all 19mer candidate target sequences against a human RefSeq database (downloaded available version from ftp://ftp.ncbi.nih.gov/refseq/ on November 7, 2006). FastA searches were executed with parameters-values-pairs –f 50 -g 50 in order to take into account the homology over the full length of the 19mer without any gaps. In order to ensure the listing of all relevant off-target hits in the fastA output file the parameter –E 30000 was used in addition. A scoring matrix was applied for the run that assessed every nucleotide match with a score of 13 and every mismatch with a score of -7. The search resulted in a list of potential off-targets for each candidate siRNA.

To sort the resulting list of potential off-targets for each siRNA, fastA output files were analyzed to identify the best off-target and its off-target score. The following off-target properties for each 19mer input sequence were extracted for each off-target to calculate the off-target score:

- Number of mismatches in non-seed region
- Number of mismatches in seed region
- Number of mismatches in cleavage site region

The off-target score was calculated for considering assumption 1 to 3 as follows:

Off-target score = number of seed mismatches * 10

+ number of cleavage site mismatches * 1.2

+ number of non-seed mismatches * 1

The most relevant off-target gene for input each 19mer input sequences was defined as the gene with the lowest off-target score. Accordingly, the lowest off-target score was defined as the relevant off-target score for the corresponding siRNA.

In order to generate a ranking for siRNAs, calculated relevant off-target scores were transferred into a result table. All siRNAs were sorted according to the off-target score (descending).

An off-target score of 2.2 was defined as cut-off for siRNA selection (specificity criterion). In addition, all sequences with only one mismatch in the seed region were eliminated from the screening set. The selection procedure resulted in a set of 93 JCV specific siRNAs (Table 1a).

An expanded screening was generated by re-calculating the predicted specificity based on the newly available human RefSeq database (Human mRNA sequences in RefSeq release version 21 (downloaded January 12, 2007)) and selecting only 208 siRNAs that did not contain more than 3 G's in a row and had an off-target score of at least 2 for the antisense strand (Table 1b).

Synthesis of JCV siRNAs

All siRNAs were synthesized in 0.2 µmole synthesis scale on an ABI3900 DNA synthesizer according to standard procedures.

For the initial screening set (93 different siRNA sequences), 4 different strategies of chemical modification were used:

a) exo/endo light (EEL): - sense strand: 2'-O-methyl @ all pyrimidines, PTO between nucleotides 20 and 21 (counting from 5'-end), dTdT at 3'-end (nucleotides 20 and 21)

- antisense strand: 2'-O-methyl at pyrimidines in 5'-UA-3' and 5'-CA-3' motives, PTO between nucleotides 20 and 21 (counting from 5'-end), dTdT at 3'-end (nucleotides 20 and 21)

b) EEL plus 2'-O-methyl in position 2 of antisense strand (only if no 5'-UA-3' and 5'-CA-3' at 5'-end, otherwise already covered by EEL)

c) EEL plus 2'-O-methyl in position 2 of sense strand (only if no pyrimidine in position 2, otherwise already covered by EEL)

d) EEL plus 2'-O-methyl in position 2 of sense and antisense strand (only if not already covered by a, b, and c) (Table 1a)

For the expanded screening set (208 different siRNA sequences), siRNAs were composed of unmodified RNA oligonucleotides with dT/dT overhangs (dTdT at 3'-end (nucleotides 20 and 21) of antisense and sense strands) (Table 1b).

Screening of JCV siRNAs

Construction of reporter-systems encoding JCV transcripts

The sequence of the early JCV transcript (E) was synthesized at GENEART (Regensburg, Germany) and cloned into GENEART standard vectors. The sequence of the late JCV transcript was subdivided in a first approach into two fragments: L1, including the transcript sequence of the VP1 protein, and LA23, including the sequences of VP2, VP3 and the Agnoprotein. Due to cloning problems with fragment LA23, this sequence was subdivided in a second approach into two fragments (LA23 1-700 and LA23 701--1438). All sequences were synthesized at GENEART and cloned into GENEART standard vectors. All fragments (E, L1, LA23 1-700 and LA23 701-1438) were subcloned into psiCheck-2 (Promega, Mannheim, Germany) via XhoI and NotI (both NEB, Frankfurt, Germany), resulting in constructs with the JCV sequences between the stop-codon and the polyA-signal of Renilla luciferase.

L1

CTCGAGACTTTTAGGGTTGTACGGGACTGTAACACCTGCTCTTGAAGCATATGAAGATGGCCCCA
ACAAAAAGAAAAGGAGAAAGGAAGGACCCCGTGCAAGTTCCAAAACTTCTTATAAGAGGAGGAGT
AGAAGTTCTAGAAGTTAAAACTGGGGTTGACTCAATTACAGAGGTAGAATGCTTTTTAACTCCAG
AAATGGGTGACCCAGATGAGCATCTTAGGGGTTTTAGTAAGTCAATTTCTATATCAGATACATTT
GAAAGTGACTCCCCAAATAAGGACATGCTTCCTTGTTACAGTGTGGCCAGAATTCCACTACCCAA
TCTAAATGAGGATCTAACCTGTGGAAATATACTAATGTGGGAGGCTGTGACCTTAAAAACTGAGG
TTCTAGGGGTGACAACTTTGATGAATGTGCACTCTAATGGTCAAGCAACTCATGACAATGGTGCA

GGAAAGCCAGTGCAGGGCACCAGCTTTCATTTTTTTTCTGTTGGCGGGGAGGCTTTAGAATTACA
GGGGGTGGTTTTTAATTACAGAACAAACTACCCAGATGGAACAATTTTTCCAAAGAATGCAACAG
TGCAATCTCAAGTAATGAACACAGAGCACAAGGCGTACCTAGATAAGAACAAAGCATATCCTGTT
GAATGTTGGGTTCCTGATCCCACCAGAAATGAAAACACAAGATATTTTGGGACACTAACAGGAGG
AGAAAATGTTCCTCCAGTTCTTCATATAACAAACACTGCCACAACAGTGCTGCTTGATGAATTTG
GTGTTGGGCCACTTTGCAAAGGTGACAACTTGTATTTGTCAGCTGTTGATGTTTGTGGAATGTTT
ACTAACAGATCTGGTTCCCAGCAGTGGAGAGGACTGTCCAGATATTTAAGGTTCAGCTCAGAAA
AAGGAGGGTTAAAAACCCCTACCCAATTTCTTTCCTTCTTACTGATTTGATTAACAGAAGGACCC
CTAGAGTTGATGGGCAACCTATGTATGGTATGGATGCTCAGGTAGAGGAGGTTAGAGTTTTTGAG
GGGACAGAGGAACTTCCAGGGGACCCAGACATGATGAGATATGTTGACAGATATGGACAGTTGCA
AACAAAGATGCTGTAATCAAAATCCTTTATTGTAATATGCAGTACATTTTAATAAAGTATAACCA
GCTTTACTTTACAGTTGCAGTCAT**GCGGCCGC** (SEQ ID NO:931)

E

CTCGAGCCGCCTCCAAGCTTACTCAGAAGTAGTAAGGGCGTGGAGGCTTTTTAGGAGGCC
AGGGAAATTCCCTTGTTTTTCCCTTTTTTGCAGTAATTTTTTGCTGCAAAAAGCTAAAATGGACA
AAGTGCTGAATAGGGAGGAATCCATGGAGCTTATGGATTTATTAGGCCTTGATAGGTCTGCATGG
GGGAACATTCCTGTCATGAGAAAAGCTTATCTGAAAAAATGCAAAGAACTCCACCCTGATAAAGG
TGGGGACGAAGACAAGATGAAGAGAATGAATTTTTTATATAAAAAAAATGGAACAAGGTGTAAAAG
TTGCTCATCAGCCTGATTTTGGTACATGGAATAGTTCAGAGGTTGGTTGTGATTTTCCTCCTAAT
TCTGATACCCTTTATTGCAAGGAATGGCCTAACTGTGCCACTAATCCTTCAGTGCATTGCCCCTG
TTTAATGTGCATGCTAAAATTAAGGCATAGAAACAGAAAATTTTTAAGAAGCAGCCCACTTGTGT
GGATAGATTGCTATTGCTTTGATTGCTTCAGACAATGGTTTGGGTGTGACTTAACCCAAGAAGCT
CTTCATTGCTGGGAGAAAGTTCTTGGAGACACCCCCTACAGGGATCTAAAGCTTTAAGTGCCAAC
CTATGGAACAGATGAATGGGAATCCTGGTGGAATACATTTAATGAGAAGTGGGATGAAGACCTGT
TTTGCCATGAAGAAATGTTTGCCAGTGATGATGAAAACACAGGATCCCAACACTCTACCCCACCT
AAAAAGAAAAAAAAAGGTAGAAGACCCTAAAGACTTTCCTGTAGATCTGCATGCATTCCTCAGTCA
AGCTGTGTTTAGTAATAGAACTGTTGCTTCTTTTGCTGTGTATACCACTAAAGAAAAAGCTCAAA
TTTTATATAAGAAACTTATGGAAAAATATTCTGTAACTTTTATAAGTAGACATGGTTTTGGGGGT
CATAATATTTTGTTTTTCTTAACACCACATAGACATAGAGTGTCAGCAATTAATAACTACTGTCA
AAAACTATGTACCTTTAGTTTTTTAATTTGTAAAGGTGTGAATAAGGAATACTTGTTTTATAGTG

CCCTGTGTAGACAGCCATATGCAGTAGTGGAAGAAAGTATTCAGGGGGGCCTTAAGGAGCATGAC
TTTAACCCAGAAGAACCAGAAGAAACTAAGCAGGTTTCATGGAAATTAGTTACACAGTATGCCTT
GGAAACCAAGTGTGAGGATGTTTTTTTGCTTATGGGCATGTACTTAGACTTTCAGGAAAACCCAC
AGCAATGCAAAAAATGTGAAAAAAAGGATCAGCCAAATCACTTTAACCATCATGAAAAACACTAT
TATAATGCCCAAATTTTTGCAGATAGCAAAAATCAAAAAAGCATTTGCCAGCAGGCTGTTGATAC
TGTAGCAGCCAAACAAAGGGTTGACAGCATCCACATGACCAGAGAAGAAATGTTAGTTGAAAGGT
TTAATTTCTTGCTTGATAAAATGGACTTAATTTTTGGGGCACATGGCAATGCTGTTTTAGAGCAA
TATATGGCTGGGGTGGCCTGGATTCATTGCTTGCTGCCTCAAATGGACACTGTTATTTATGACTT
TCTAAAATGCATTGTATTAAACATTCCAAAAAAAAGGTACTGGCTATTCAAGGGGCCAATAGACA
GTGGCAAAACTACTTTAGCTGCAGCTTTACTTGATCTCTGTGGGGGAAAGTCATTAAATGTTAAT
ATGCCATTAGAAAGATTAAACTTTGAATTAGGAGTGGGTATAGATCAGTTTATGGTTGTATTTGA
GGATGTAAAAGGCACTGGTGCAGAGTCAAGGGATTTACCTTCAGGGCATGGCATAAGCAACCTTG
ATTGCTTAAGAGATTACTTAGATGGAAGTGTAAAAGTTAATTTAGAGAGAAAACACCAAAACAAA
AGAACACAGGTGTTTCCACCTGGAATTGTAACCATGAATGAATATTCAGTGCCTAGAACTTTACA
GGCCAGATTTGTAAGGCAGATAGATTTTAGACCAAAGGCCTACCTGAGAAAATCACTAAGCTGCT
CTGAGTATTTGCTAGAAAAAAGGATTTTGCAAAGTGGTATGACTTTGCTTTTGCTTTTAATCTGG
TTTAGGCCAGTTGCTGACTTTGCAGCTGCCATTCATGAGAGGATTGTGCAGTGGAAAGAAAGGCT
GGATTTAGAAATAAGCATGTATACATTTTCTACTATGAAAGCTAATGTTGGTATGGGGAGACCCA
TTCTTGACTTTCCTAGAGAGGAAGATTCTGAAGCAGAAGACTCTGGACATGGATCAAGCACTGAA
TCACAATCACAATGCTTTTCCCAGGTCTCAGAAGCCTCTGGTGCAGACACACAGGAAAACTGCAC
TTTTCACATCTGTAAAGGCTTTCAATGTTTCAAAAAACCAAAGACCCCTCCCCCAAAATAACTGC
AACTGT**GCGGCCGC** **(SEQ ID NO:932)**

LA23 1-700

CTCGAGCAGCTAACAGCCAGTAAACAAAGCACAAGGGCAAGTGGAAAGCAGCCAAGGGAACATGT
TTTGCGAGCCAGAGCTGTTTTGGCTTGTCACCAGCTGGCCATGGTTCTTCGCCAGCTGTCACGTA
AGGCTTCTGTGAAAGTTAGTAAAACCTGGAGTGGAACTAAAAAAAGAGCTCAAAGGATTTTAATT
TTTTTGTTAGAATTTTTGCTGGACTTTTGCACAGGTGAAGACAGTGTAGACGGGAAAAAAAGACA
GAGACACAGTGGTTTGACTGAGCAGACATACAGTGCTTTGCCTGAACCAAAAGCTACATAGGTAA
GTAATGTTTTTTTTTGTGTTTTCAGGTTCATGGGTGCCGCACTTGCACTTTTGGGGGACCTAGTT
GCTACTGTTTCTGAGGCTGCTGCTGCCACAGGATTTTCAGTAGCTGAAATTGCTGCTGGAGAGGC

TGCTGCTACTATAGAAGTTGAAATTGCATCCCTTGCTACTGTAGAGGGGATTACAAGTACCTCTG
AGGCTATAGCTGCTATAGGCCTTACTCCTGAAACATATGCTGTAATAACTGGAGCTCCGGGGGCT
GTAGCTGGGTTTGCTGCATTGGTTCAAACTGTAACTGGTGGTAGTGCTATTGCTCAGTTGGGATA
TAGATTTTTTGCTGACTGGGATCATAAAGTTTCAACAGTTGGGCTTTTTC**GCGGCCGC** **(SEQ**
ID NO:933)

LA23 701-1438

CTCGAGAGCAGCCAGCTATGGCTTTACAATTATTTAATCCAGAAGACTACTATGATATTTTATTT
CCTGGAGTGAATGCCTTTGTTAACAATATTCACTATTTAGATCCTAGACATTGGGGCCCGTCCTT
GTTCTCCACAATCTCCCAGGCTTTTTGGAATCTTGTTAGAGATGATTTGCCAGCCTTAACCTCTC
AGGAAATTCAGAGAAGAACCCAAAAACTATTTGTTGAAAGTTTAGCAAGGTTTTTGGAAGAAACT
ACTTGGGCAATAGTTAATTCACCAGCTAACTTATATAATTATATTTCAGACTATTATTCTAGATT
GTCTCCAGTTAGGCCCTCTATGGTAAGGCAAGTTGCCCAAAGGGAGGGAACCTATATTTCTTTTG
GCCACTCATACACCCAAAGTATAGATGATGCAGACAGCATTCAAGAAGTTACCCAAAGGCTAGAT
TTAAAAACCCCAAATGTGCAATCTGGTGAATTTATAGAAAGAAGTATTGCACCAGGAGGTGCAAA
TCAAAGATCTGCTCCTCAATGGATGTTGCCTTTACTTTTAGGGTTGTACGGGACTGTAACACCTG
CTCTTGAAGCATATGAAGATGGCCCCAACAAAAAGAAAAGGAGAAAGGAAGGACCCCGTGCAAGT
TCCAAAACTTCTTATAAGAGGAGGAGTAGAAGTTCTAGAAGTTAAAACTGGGGTTGACTCAATTA
CAGAGGTAGAATGCT**GCGGCCGC** **(SEQ ID NO:934)**

Screen of JCV siRNAs in transfected cells

Cos-7 cells (DSMZ, Braunschweig, Germany, # ACC-60) were seeded at 1.5 x 10^4 cells / well on white 96-well plates with clear bottoms (Greiner Bio-One GmbH, Frickenhausen, Germany) in 75 µl of growth medium. Directly after seeding the cells, 50 ng of the corresponding reporter-plasmid per well was transfected with Lipofectamine™ 2000 (Invitrogen GmbH, Karlsruhe, Germany), with the plasmid diluted in Opti-MEM to a final volume of 12.5 µl per well, prepared as a mastermix for the whole plate.

4 h after plasmid transfection, growth medium was removed from cells and replaced by 100 µl / well of fresh medium. siRNA transfections were performed using Lipofectamine™ 2000 (Invitrogen GmbH, Karlsruhe, Germany) as described by the manufacturer. Cells were incubated for 24 h at 37°C and 5 % CO_2 in a humidified incubator (Heraeus GmbH, Hanau, Germany). For the primary screen, all siRNAs were screened at a final concentration of 30nM. Selected sequences were rescreened at a siRNA concentration of 300pM. Each siRNA was tested in quadruplicate for each concentration.

Cells were lysed by removing growth medium and application of 150 µl of a 1:1 mixture consisting of medium and substrate from the Dual-Glo Luciferase Assay System (Promega, Mannheim, Germany).The luciferase assay was performed according to the manufacturer's protocol for Dual-Glo Luciferase assay and luminescence was measured in a Victor-Light 1420 Luminescence Counter (Perkin Elmer, Rodgau-Jügesheim, Germany). Values obtained with Renilla luciferase were normalized to the respective values obtained with Firefly luciferase in order to correct for transfection efficacy. Renilla/Firefly luciferase activities obtained after transfection with siRNAs directed against a JCV gene were normalized to Renilla/Firefly luciferase activities obtained after transfection of an unrelated control siRNA set to 100%. Tables 1a and b provides the results where the siRNAs, the sequences of which are given in Tables 1a and b, were tested at a single dose of 30nM. The percentage inhibition ± standard deviation, compared to the unrelated control siRNA, is indicated in the column 'Remaining luciferase activity (% of control)'. A number of JCV siRNAs at 30nM were effective at reducing levels of the targeted mRNA by more than 70% in Cos-7 cells (i.e. remaining luciferase activity was less than 30%).

Selected JCV siRNAs from the single dose screen were further characterized by dose response curves. Transfections of JCV siRNAs for generation of dose response curves were performed with the following siRNA concentrations according to the above protocol:

- from 33nM in 3-fold dilutions down to 0.005nM (for fragment L1)

- from 24nM in 4-fold dilutions down to 0.001nM (for fragment E and fragments LA23 1-700 and LA23 701–1438).

IC50 values were determined by parameterized curve fitting using the program XLfit (IDBS, Guildford, Great Britain). Table 2 provides the results from two independent experiments for 32 selected JCV siRNAs. The mean IC50 from these two independent experiments is shown. Several JCV siRNAs (AD-12622, AD-12677, AD-12709, AD-12710, AD-12722, AD-12724, AD-12728, AD-12763, AD-12767, AD-12768, AD-12769, AD-12771, AD-12774, AD-12775, AD-12777, AD-12781, AD-12784, AD-12795, AD-12813, AD-12821, AD-12823, AD-12824, AD-12825, AD-12827, AD-12829, AD-12842) were particularly potent in this experimental paradigm, and exhibited IC50 values between 70pM and 1nM.

Table 2: IC50s

Duplex name	Mean IC50 [nM]
AD-12599	2.37
AD-12622	0.57
AD-12666	3.7
AD-12677	0.49
AD-12709	0.19
AD-12710	0.47
AD-12712	2.33
AD-12722	0.12
AD-12724	0.26
AD-12728	0.8
AD-12761	1.2
AD-12763	0.95
AD-12767	0.09
AD-12768	0.19
AD-12769	0.35
AD-12771	0.35
AD-12774	0.13
AD-12775	0.18
AD-12777	0.17
AD-12778	12.65
AD-12781	0.18
AD-12784	0.44
AD-12795	0.65
AD-12813	0.2
AD-12818	1.88
AD-12821	0.07
AD-12823	0.46
AD-12824	0.25

AD-12825	0.52
AD-12827	0.15
AD-12829	0.14
AD-12842	0.44

Screen of JCV siRNAs against live JC Virus in SVG-A cells

Cells and Virus

SVG-A cells (human fetal glial cells transformed by SV40 T antigen) obtained from Walter Atwood at Brown University were cultured in Eagle's Minimum Essential Media (ATCC, Manassas, Virginia) supplemented to contain 10% fetal bovine serum (FBS) (Omega Scientific, Tarzana, California), Penicillin 100U/ml, Streptomycin 100ug/ml (Invitrogen, Carlsbad California) at 37°C in an atmosphere with 5% CO_2 in a humidified incubator (Heraeus HERAcell, Thermo Electron Corporation, Ashville, North Carolina). The Mad-1-SVEΔ strain of JCV obtained from Walter Atwood at Brown University was used in all experiments; viral stocks were prepared using SVG-A cells according to standard published methods (Liu and Atwood, Propagation and assay of the JC Virus, Methods Mol Biol. 2001; 165:9-17).

Prophylaxis assay

SVG-A cells were seeded on glass coverslips in 6-well dishes 24 hours prior to transfection in the media described above minus antibiotics. Cells were transfected with the indicated concentration of siRNA (10nM, 50nM, or 100nM) using Lipofectamine™ 2000 according to the manufacturer's instructions (Invitrogen, Carlsbad, California). Twenty-four hours post-transfection cells were washed with media containing 2% FBS and then infected with a 1:25 dilution of JCV virus stock (Mad-1-SVEΔ strain) diluted in 2% FBS media. Cells were rocked every 15 minutes by hand several times to get equal virus binding across the entire coverslip for one hour and then additional 10% FBS media was added and the infection was allowed to proceed for 72 hours. Seventy two hours post-infection, cells were fixed in acetone and stained for late viral protein (VP1) by standard immunofluoresence methods using hybridoma supernatant PAB597 recognizing JCV VP1 (obtained from Walter Atwood at Brown University) with goat anti-mouse Alexa Fluor 488 secondary antibody (Invitrogen, Carlsbad,

California). Infected cells were scored by counting VP1-immunoreactive cells using a fluorescence microscope (Zeiss, Imager.Z1, Thornwood, New Jersey) and data were expressed as the percentage of infected cells counted for the control coverslips transfected with Luciferase siRNA. Table 3 shows the results of the prophylaxis assays at different siRNA concentrations (10nM, 50nM or 100nM). The VP1 siRNAs were the most potent as a group, followed by the T antigen siRNAs, with the VP2/3 siRNAs being the least potent. The VP1 siRNAs most effective in reducing virus were consistently AD-12622, AD-12728, AD-12795, and AD-12842. The most potent T antigen siRNA was AD-12813.

Table 3. Prophylaxis Assay

		Remaining Virus (% of Luciferase Control)		
Duplex Number	Targeted JCV Transcript	50nM	10nM	100nM
AD-12599	VP1	79.9	ND	ND
AD-12709	VP1	46.0	ND	ND
AD-12710	VP1	25.9	ND	ND
AD-12784	VP1	30.9	ND	ND
AD-12712	VP1	29.7	ND	ND
AD-12724	VP1	30.5	38.9	25.8
AD-12622	VP1	22.9	28.2	9.1
AD-12728	VP1	21.1	22.2	ND
AD-12795	VP1	13.6	16.9	8.5
AD-12842	VP1	16.0	23.4	12.7
AD-12761	VP1	26.4	52.3	ND

AD-12818	VP1	24.0	50.2	28.0
AD-12666	VP1	54.1	ND	ND
AD-12763	VP1	39.5	ND	ND
AD-12722	T Antigen	43.6	82.1	ND
AD-12813	T Antigen	21.5	48.8	19.4
AD-12767	T Antigen	37.6	52.2	30.9
AD-12821	T Antigen	33.0	51.2	30.8
AD-12774	T Antigen	74.0	89.2	ND
AD-12827	T Antigen	77.0	92.0	ND
AD-12775	T Antigen	81.6	95.4	ND
AD-12777	T Antigen	73.3	93.9	ND
AD-12829	T Antigen	78.6	93.6	ND
AD-12781	T Antigen	38.8	62.6	34.4
AD-12768	VP2/3	73.9	92.4	ND
AD-12771	VP2/3	51.6	83.6	ND
AD-12824	VP2/3	42.1	79.0	43.7
AD-12769	VP2/3	35.2	78.0	39.7
AD-12823	VP2/3	38.1	78.1	42.0
AD-12677	VP2/3	99.1	102.1	ND
AD-12825	VP2/3	100.8	99.1	ND

ND indicates no data.

Post-infection treatment assay

SVG-A cells were seeded on glass coverslips in 6-well dishes 24 hours prior to infection in 10% FBS media. Cells were washed with media containing 2% FBS and then infected with a 1:25 dilution of JCV virus stock diluted in 2% FBS media. Cells were rocked by hand approximately 8-10 times to get equal virus binding across the entire coverslip every 15 minutes for one hour and then additional 10% FBS media was added. Twenty-four and forty-eight hours postinfection, cells were washed with 10% FBS media containing no antibiotics and then transfected with 50nM of the indicated siRNA using Lipofectamine™ 2000 according to the manufacturer's instructions (Invitrogen, Carlsbad, California). Seventy-two hours postinfection, cells were fixed in acetone and stained for late viral protein (VP1) by standard immunofluoresence methods using hybridoma supernatant PAB597 recognizing JCV VP1 (obtained from Walter Atwood at Brown University) with goat anti-mouse Alexa Fluor 488 secondary antibody (Molecular Probes, Eugene, Oregon). Infected cells were scored by counting VP1-immunoreactive cells using a fluorescence microscope (Zeiss, Imager.Z1, Thornwood, New Jersey) and data were expressed as the percentage of infected cells counted for control coverslips transfected with Luciferase siRNA. Table 4 shows the results of the post-infection treatment experiments. All of the siRNAs tested in the treatment assay showed significant antiviral activity against JCV, such that the remaining virus was significantly less than that in the luciferase siRNA control.

Table 4. Treatment Assay

Duplex Number	Targeted JCV Transcript	Remaining Virus (% of Luciferase Control)
AD-12724	VP1	38.9
AD-12622	VP1	28.2
AD-12795	VP1	16.9
AD-12842	VP1	23.4
AD-12818	VP1	ND
AD-12813	T Antigen	48
AD-12767	T Antigen	56.9
AD-12821	T Antigen	75.8
AD-12781	T Antigen	75.8
AD-12824	VP2/3	60.4
AD-12769	VP2/3	70.7
AD-12823	VP2/3	72.4

ND indicates no data.

Prophylaxis administration of JCV siRNAs inhibits the production of active progeny JC virus

SVG-A cells were seeded in 6-well dishes 24 hours prior to transfection in the media described above minus antibiotics. Cells were transfected with 10nM of the indicated siRNA using Lipofectamine™ 2000 according to the manufacturer's instructions (Invitrogen, Carlsbad, California). Twenty-four hours post-transfection cells were washed with media containing 2% FBS and then infected with a 1:25 dilution of JCV virus stock (Mad-1-SVEΔ strain) diluted in 2% FBS media. Cells were rocked every 15 minutes by hand several times to get equal virus

binding across the entire coverslip for one hour and then additional 10% FBS media was added and the infection was allowed to proceed for 6 days. Six days post-infection, progeny virus was collected either by removal of overlay media from infected cells or by scraping cells and performing virus preparations. The virus preparations consisted of scraping cells into the supernatant media, vortexing, freeze-thawing the re-suspended cells 2 times with vortexing in between, then spinning down the cell debris and taking the supernatant. Fresh SVG-A cells seeded on glass coverslips were infected secondarily with virus collected by either method using the same procedure done with the initial infection to determine the amount of infectious virus produced by cells transfected with the various siRNAs. At 72 hours post-infection of coverslips, cells were fixed in acetone and stained for late viral protein (VP1) by standard immunofluoresence methods using hybridoma supernatant PAB597 recognizing JCV VP1 (obtained from Walter Atwood at Brown University) with goat anti-mouse Alexa Fluor 488 secondary antibody (Invitrogen, Carlsbad, California). Infected cells were scored by counting VP1-immunoreactive cells using a fluorescence microscope (Zeiss, Imager.Z1, Thornwood, New Jersey) and data were expressed as the percentage of infected cells counted for the control coverslips transfected with Luciferase siRNA. Table 5 shows the results for selected siRNAs, demonstrating the ability of prophylaxis siRNA treatment to inhibit active progeny virus production by either method of virus collection. Transfection with siRNAs targeting VP1 (AD-12622 and AD-12842) had the greatest effect on inhibiting the production of active progeny virus regardless of whether virus was collected from media or from infected cell preparations. The T antigen siRNA AD-12813 had the next strongest inhibitory effect, whereas the VP2/3 siRNAs AD-12824 and AD-12769 still showed some albeit a lesser ability to inhibit active progeny JCV production.

Table 5. Prophylaxis administration of JCV siRNAs inhibits the production of active progeny JC virus capable of secondary infection

		Remaining Virus (% of Luciferase Control)	
Duplex Name	Targeted Transcript	Media	Virus Preparation
AD-12622	VP1	30.8	24.9
AD-12842	VP1	33.3	26.9
AD-12813	T Antigen	57.8	38.7
AD-12824	VP2/3	83.6	57.6
AD-12769	VP2/3	79.1	52.2

Stability in cerebrospinal fluid (CSF) of selected siRNAs targeting JCV

Eleven selected JCV siRNAs were tested for stability at 5 uM over 48h at 37°C in human CSF, as well as in PBS for comparison. 30µl of human cerebrospinal fluid (CSF) was mixed with 3 µl of 50 µM duplex (siRNA) solution (150pmole/well) in a 96-well plate, sealed to avoid evaporation and incubated for the indicated time at 37°C. Incubation of the siRNA in 30ul PBS for 48h served as a control for non-specific degradation. Reactions were stopped by the addition of 4ul proteinase K (20mg/ml) and 25ul of proteinase K buffer, and an incubation for 20' at 42°C. Samples were then spin filtered through a 0.2 µm 96 well filter plate at 3000 rpm for 20'. Incubation wells were washed with 50ul Millipore water twice and the combined washing solutions were spin filtered also.

Samples were analyzed by ion exchange HPLC under denaturing conditions. Samples were transferred to single autosampler vials. IEX-HPLC analysis was performed under the following conditions: Dionex DNAPac PA200 (4x250 mm analytical column), temperature of

45°C (denaturing conditions by pH=11), flow rate of 1 ml/min, injection volume of 50 ul, and detection wavelength of 260 nm with 1 nm bandwidth (reference wavelength 600 nm). In addition, the gradient conditions were as follows with HPLC Eluent A: 20mM Na_3PO_4 in 10% ACN; pH=11 and HPLC Eluent B: 1 M NaBr in HPLC Eluent A:

Time	%A	%B
0.00 min	75	25
1.00 min	75	25
19.0 min	38	62
19.5 min	0	100
21.5 min	0	100
22.0 min	75	25
24.0 min	75	25

Under the above denaturing IEX-HPLC conditions, the duplexes eluted as two separated single strands. All chromatograms were integrated automatically by the Dionex Chromeleon 6.60 HPLC software, and were adjusted manually as necessary. The area under the peak for each strand was calculated and the %-values for each intact full length product (FLP) for each time points were calculated by the following equation:

$$\%\text{-FLP}_{(s/as;\ t=x)} = (\text{PeakArea}_{s/as;t=x}/\text{PeakArea}_{s/as;\ t=0\min}) * 100\%$$

All values were normalized to FLP at t=0 min. Table 6 provides the results after 48 hours of incubation in human CSF at 37°C. At least 75% of both antisense and sense strands of ten JCV siRNAs (AD-12622, AD-12724, AD-12767, AD-12769, AD-12795, AD-12813, AD-12818, AD-12823, AD-12824, AD-12842) were recovered, demonstrating that these siRNAs are highly stable in human CSF at 37°C. For AD-12821, 59% of the antisense and 97% of the sense strand

was recovered after 48h of incubation in human CSF at 37°C, showing that this siRNA has a half-life of greather than 48h in human CSF at 37°C.

Table 6: Stability in human CSF

	% full length material after 48 hours	
Duplex name	antisense	sense
AD-12622	93	105
AD-12724	90	106
AD-12767	85	104
AD-12769	100	104
AD-12795	86	109
AD-12813	94	98
AD-12818	75	99
AD-12821	59	97
AD-12823	98	98
AD-12824	84	98
AD-12842	87	102

dsRNA expression vectors

In another aspect of the invention, JC virus specific dsRNA molecules that modulate JC virus genome expression activity are expressed from transcription units inserted into DNA or RNA vectors (see, e.g., Couture, A, et al., *TIG.* (1996), **12**:5-10; Skillern, A., et al., International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, US Pat. No. 6,054,299). These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be incorporated and inherited as a transgene integrated into the host genome. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, et al., *Proc. Natl. Acad. Sci. USA* (1995) **92**:1292).

The individual strands of a dsRNA can be transcribed by promoters on two separate expression vectors and co-transfected into a target cell. Alternatively each individual strand of the dsRNA can be transcribed by promoters both of which are located on the same expression

plasmid. In a preferred embodiment, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

The recombinant dsRNA expression vectors are generally DNA plasmids or viral vectors. dsRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus (for a review, see Muzyczka, et al., *Curr. Topics Micro. Immunol.* (1992) **158**:97-129)); adenovirus (see, for example, Berkner, et al., BioTechniques (1998) 6:616), Rosenfeld et al. (1991, Science 252:431-434), and Rosenfeld et al. (1992), *Cell* 68:143-155)); or alphavirus as well as others known in the art. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, in vitro and/or in vivo (see, e.g., Eglitis, et al., *Science* (1985) **230**:1395-1398; Danos and Mulligan, *Proc. Natl. Acad. Sci. USA* (1998) **85**:6460-6464; Wilson et al., 1988, Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano et al., 1990, Proc. Natl. Acad. Sci. USA 87:61416145; Huber et al., 1991, Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry et al., 1991, Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury et al., 1991, Science 254:1802-1805; van Beusechem. et al., 1992, Proc. Nad. Acad. Sci. USA 89:7640-19 ; Kay et al., 1992, Human Gene Therapy 3:641-647; Dai et al., 1992, Proc. Natl.Acad. Sci. USA 89:10892-10895; Hwu et al., 1993, J. Immunol. 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573). Recombinant retroviral vectors capable of transducing and expressing genes inserted into the genome of a cell can be produced by transfecting the recombinant retroviral genome into suitable packaging cell lines such as PA317 and Psi-CRIP (Comette et al., 1991, Human Gene Therapy 2:5-10; Cone et al., 1984, Proc. Natl. Acad. Sci. USA 81:6349). Recombinant adenoviral vectors can be used to infect a wide variety of cells and tissues in susceptible hosts (e.g., rat, hamster, dog, and chimpanzee) (Hsu et al., 1992, J. Infectious Disease, 166:769), and also have the advantage of not requiring mitotically active cells for infection.

The promoter driving dsRNA expression in either a DNA plasmid or viral vector of the invention may be a eukaryotic RNA polymerase I (e.g. ribosomal RNA promoter), RNA polymerase II (e.g. CMV early promoter or actin promoter or U1 snRNA promoter) or generally RNA polymerase III promoter (e.g. U6 snRNA or 7SK RNA promoter) or a prokaryotic

promoter, for example the T7 promoter, provided the expression plasmid also encodes T7 RNA polymerase required for transcription from a T7 promoter. The promoter can also direct transgene expression to the pancreas (see, e.g. the insulin regulatory sequence for pancreas (Bucchini et al., 1986, Proc. Natl. Acad. Sci. USA 83:2511-2515)).

In addition, expression of the transgene can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, e.g., circulating glucose levels, or hormones (Docherty et al., 1994, FASEB J. 8:20-24). Such inducible expression systems, suitable for the control of transgene expression in cells or in mammals include regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 -thiogalactopyranoside (EPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the dsRNA transgene.

Generally, recombinant vectors capable of expressing dsRNA molecules are delivered as described below, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of dsRNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the dsRNAs bind to target RNA and modulate its function or expression. Delivery of dsRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

dsRNA expression DNA plasmids are typically transfected into target cells as a complex with cationic lipid carriers (e.g. Oligofectamine) or non-cationic lipid-based carriers (e.g. Transit-TKO™). Multiple lipid transfections for dsRNA-mediated knockdowns targeting different regions of a single JC virus genome or multiple JC virus genomes over a period of a week or more are also contemplated by the invention. Successful introduction of the vectors of the invention into host cells can be monitored using various known methods. For example, transient transfection. can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection. of ex vivo cells can be ensured using markers that

provide the transfected cell with resistance to specific environmental factors (e.g., antibiotics and drugs), such as hygromycin B resistance.

The JC virus specific dsRNA molecules can also be inserted into vectors and used as gene therapy vectors for human patients. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

CLAIMS

We claim:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a human JC virus genome in a cell, wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding JC virus, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said JC virus, inhibits expression of said JC virus genome.

2. The dsRNA of claim 1, wherein said first sequence is selected from the group consisting of Tables 1a and b and said second sequence is selected from the group consisting of Tables 1a and b.

3. The dsRNA of claim 1, wherein said dsRNA comprises at least one modified nucleotide.

4. The dsRNA of claim 2, wherein said dsRNA comprises at least one modified nucleotide.

5. The dsRNA of claims 3 or 4, wherein said modified nucleotide is chosen from the group of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

6. The dsRNA of claims 3 or 4, wherein said modified nucleotide is 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.

7. The dsRNA of claims 3 or 4, wherein said first sequence is selected from the group consisting of Tables 1a and b and said second sequence is selected from the group consisting of Tables 1a and b.

8. The dsRNA of claims 6 or 7, wherein said first sequence is selected from the group consisting of Tables 1a and b and said second sequence is selected from the group consisting of Tables 1a and b.

9. A cell comprising the dsRNA of claim 1.

10. A pharmaceutical composition for inhibiting the expression of a gene from the JC Virus in an organism, comprising a dsRNA and a pharmaceutically acceptable carrier, wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding JC virus, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said JC virus, inhibits expression of said JC virus genome.

11. The pharmaceutical composition of claim 10, wherein said first sequence of said dsRNA is selected from the group consisting of Tables 1a and b and said second sequence of said dsRNA is selected from the group consisting of Tables 1a and b.

12. The pharmaceutical composition of claim 10, wherein said first sequence of said dsRNA is selected from the group consisting of Tables 1a and b and said second sequence of said dsRNA is selected from the group consisting of Tables 1a and b.

13. A method for inhibiting the expression of a gene from the JC Virus in a cell, the method comprising:

(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 and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding JC virus, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said JC virus, inhibits expression of said JC virus genome; and

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

14. A method of treating, preventing or managing pathological processes mediated by JC virus expression comprising administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of a dsRNA, wherein the dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding JC virus, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said JC virus, inhibits expression of said JC virus genome.

15. A vector for inhibiting the expression of a gene from the JC Virus 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 substantially complementary to at least a part of a mRNA encoding JC virus and wherein said dsRNA is less than 30 base pairs in length and wherein said dsRNA, upon contact with a cell expressing said JC virus, inhibits the expression of said JC virus genome.

16. A cell comprising the vector of claim 15.