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(54) Title: DSRNA COMPOSITIONS AND METHODS FOR TREATING HPV INFECTION

(57) Abstract: The invention relates to a double-stranded ribonucleic acid (dsRNA) for treating human papilloma virus (HPV) infection. The dsRNA comprises 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 an HPV Target gene selected from among HPV E1, HPV E6 and the human E6AP gene. The invention also relates to a pharmaceutical composition comprising the dsRNA together with a pharmaceutically acceptable carrier; methods for treating diseases caused by HPV infection and the expression of the E6AP gene using the pharmaceutical composition; and methods for inhibiting the expression of the HPV Target genes in a cell.

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dsRNA COMPOSITIONS AND METHODS FOR TREATING HPV INFECTION

Sequence Listing

The instant application contains a Sequence Listing which has been submitted on compact disc and is hereby incorporated by reference in its entirety. The sequence listing file was created on December 22, 2009, is named 154994AU_CRF_sequencelisting.txt, and is 775,941 bytes in size.

Field of the Invention

This invention relates to double-stranded ribonucleic acid (dsRNA), and its use in mediating RNA interference to treat pathological processes mediated by human papillomavirus (HPV) infection, such as cervical cancer, anal cancer, HPV associated precancerous lesions, and genital warts.

Background of the Invention

Papillomaviruses (PV) are non-enveloped DNA viruses that induce hyperproliferative lesions of the epithelia. The papillomaviruses are widespread in nature and have been recognized in higher vertebrates. Viruses have been characterized, amongst others, from humans, cattle, rabbits, horses, and dogs. The first papillomavirus was described in 1933 as cottontail rabbit papillomavirus (CRPV). Since then, the cottontail rabbit as well as bovine papillomavirus type 1 (BPV-1) have served as experimental prototypes for studies on papillomaviruses. Most animal papillomaviruses are associated with purely epithelial proliferative lesions, and most lesions in animals are cutaneous. In the human more than 100 types of papillomavirus (HPV) have been identified and they have been catalogued by site of infection: cutaneous epithelium and mucosal epithelium (oral and genital mucosa). The cutaneous-related diseases include flat warts, plantar warts, etc. The mucosal-related diseases include laryngeal papillomas and anogenital diseases comprising cervical carcinomas (Fields, 1996, Virology, 3rd ed. Lippincott--Raven Pub., Philadelphia, N.Y.; Bernard, H-U., 2005. J. Clin. Virol. 328: S1-S6).

Human papillomavirus (HPV) is one of the most prevalent sexually transmitted infections in the world. The majority of HPV infections are harmless. Some types of HPV cause genital warts, which appear as single or multiple bumps in the genital areas of men and women including the vagina, cervix, vulva (area outside of the vagina), penis, and rectum. Many people infected with HPV have no symptoms.

While most HPV subtypes result in benign lesions, certain subtypes are considered high-risk and can lead to more serious lesions, such as cervical and anal dysplasia. Fifteen HPV types were recently classified as high-risk types (Munoz, N. et al. 2003. N. Engl. J. Med. 348(6):518-27.) These high-risk subtypes are genetically diverse, demonstrating >10% sequence divergence at the L1 gene, a major virus capsid protein. (Bernard, H-U., 2005. J. Clin. Virol. 328: S1-S6).

Women having HPV infection are often asymptomatic and may only discover their lesion after cervical screening. Cervical screening is widely performed using the Pap test. A Pap test is a histological evaluation of cervical tissue which is used to identify abnormal cervical cells. As part of a Pap test, the presence of HPV infection and the specific subtype may be determined with the use of nucleic acid based assays such as PCR or the commercial Hybrid Capture II technique (HCII) (Digene, Gaithersburg, Maryland, U.S.A).

Abnormal cervical cells, if identified, are graded as LSIL (low-grade squamous intraepithelial lesions) having a low risk of progressing to cancer (including CIN-1 designated cells ("cervical intraepithelial neoplasia-1")); or HSIL (High-grade squamous intraepithelial lesions), including CIN-2 and CIN-3 designated cells, having a higher likelihood of progressing to cancer.

About 85% of low-grade lesions spontaneously regress, and the remainder either stay unchanged, or progress to high-grade lesions. About 10% of high-grade lesions, if left untreated, are expected to transform into cancerous tissues. HPV-16 and HPV-18 are most often associated with dysplasias, although several other transforming HPV subtypes are also associated with dysplasias.

Recent studies indicate that up to 89% of HIV positive homosexual males may be infected with these high-risk subtypes of HPV. HIV positive patients are also more likely to be infected with multiple subtypes of HPV at the same time, which is associated with a higher risk of dysplasia progression.

Evidence over the last two decades has led to a broad acceptance that HPV infection is necessary, though not sufficient, for the development of cervical cancer. The presence of HPV in

cervical cancer is estimated at 99.7%. Anal cancer is thought to have a similar association between HPV infection and the development of anal dysplasia and anal cancer as is the case with cervical cancer. In one study of HIV negative patients with anal cancer, HPV infection was found in 88% of anal cancers. In the US in 2003, 12,200 new cases of cervical cancer and 4,100 cervical-cancer deaths were predicted along with 4,000 new cases of anal cancer and 500 anal-cancer deaths. While the incidence of cervical cancer has decreased in the last four decades due to widespread preventive screening, the incidence of anal cancer is increasing. The increase in anal cancer incidence may be attributed in part to HIV infection since HIV positive patients have a higher incidence of anal cancer than the general population. While anal cancer has an incidence of 0.9 cases per 100,000 in the general population, anal cancer has an incidence of 35 cases per 100,000 in the homosexual male population and 70-100 cases per 100,000 in the HIV positive homosexual male population. In fact, due to the high prevalence of anal dysplasia among HIV-infected patients and a growing trend of anal cancers, the 2003 USPHA/IDSA Guidelines for the Treatment of Opportunistic Infections in HIV Positive Patients will include treatment guidelines for patients diagnosed with anal dysplasia.

There is no known cure for HPV infection. There are treatments for genital warts, although they often disappear even without treatment. The method of treatment depends on factors such as the size and location of the genital warts. Among the treatments used are Imiquimod cream, 20 percent podophyllin antimitotic solution, 0.5 percent podofilox solution, 5 percent 5-fluorouracil cream, and Trichloroacetic acid. The use of podophyllin or podofilox is not recommended for pregnant women because they are absorbed by the skin and may cause birth defects. The use of 5-fluorouracil cream is also not recommended for pregnant women. Small genital warts can be physically removed by freezing (cryosurgery), burning (electrocautery) or laser treatment. Large warts that do not responded to other treatment may have to be removed by surgery. Genital warts have been known to return following physical removal; in these instances α-interferon has been directly injected into these warts. However, α -interferon is expensive, and its use does not reduce the rate of return of the genital warts.

As such there exists an unmet need for effective HPV treatment. Surprisingly, compounds have been discovered that meet this need, and provide other benefits as well.

Recently, 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.

PCT Publication WO 03/008573 discloses a previous effort to develop a nucleic acid based medicament for the treatment of disease caused by HPV infection. This publication reports the use of two siRNAs directed to HPV mRNA to inhibit HPV replication in a cell based system; a related publication is found at Jiang, M. et al. 2005. N. A. R. 33(18): e151.

Despite significant advances in the field of RNAi and advances in the treatment of pathological processes mediated by HPV infection, there remains a need for agents that can inhibit the progression of HPV infection and that can treat diseases associated with HPV infection. The challenge is exacerbated because such agents must be designed to inhibit all the high-risk HPV subtypes, which together display a wide degree of genotypic diversity.

Summary of the Invention

The invention provides a solution to the problem of treating diseases associated with HPV infection, by using double-stranded ribonucleic acid (dsRNA) to silence gene expression essential for HPV propagation. E6AP is a conserved gene of the human host species required by HPV for proliferation.

The invention provides double-stranded ribonucleic acid (dsRNA), as well as compositions and methods for inhibiting the expression of the E6AP gene in a cell or mammal using such dsRNA. The invention also provides compositions and methods for treating

pathological conditions and diseases caused by the expression of the E6AP gene in connection with HPV infection, such as in cervical cancer and gential warts. 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 the E6AP gene.

In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of the E6AP gene. The dsRNA comprises at least two sequences that are complementary to each other. The dsRNA comprises a sense strand comprising a first sequence and an antisense strand comprising a second sequence. The antisense strand comprises a nucleotide sequence which is substantially complementary to at least part of an mRNA encoding E6AP, and the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length. The dsRNA, upon contacting with a cell expressing the E6AP, inhibits the expression of the E6AP gene 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 Table 1 and the second sequence is selected from the group consisting of the antisense sequences of Table 1. 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 Table 1 and a second sequence selected from the group consisting of the antisense sequences of Table 1.

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

In another embodiment, the invention provides a pharmaceutical composition for inhibiting the expression of the E6AP gene in an organism, generally a human subject, comprising one or more of the dsRNA of the invention and a pharmaceutically acceptable carrier or delivery vehicle.

In another embodiment, the invention provides a method for inhibiting the expression of the E6AP gene in a cell, comprising the following steps:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNA), wherein the dsRNA comprises at least two sequences that are complementary to each other. The dsRNA comprises a sense strand comprising a first sequence and an antisense strand comprising a second sequence. The antisense strand comprises a region of complementarity which is substantially complementary to at least a part of a mRNA encoding E6AP, and wherein the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and wherein the dsRNA, upon contact with a cell expressing the E6AP, inhibits expression of the E6AP gene 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 the E6AP gene, thereby inhibiting expression of the E6AP gene in the cell.

In another embodiment, the invention provides methods for treating, preventing or managing pathological processes mediated by HPV infection, e.g. cancer or gential warts, comprising administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of one or more of the dsRNAs of the invention.

In another embodiment, the invention provides vectors for inhibiting the expression of the E6AP gene in a cell, comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention.

In another embodiment, the invention provides a cell comprising a vector for inhibiting the expression of the E6AP gene in a cell. The vector comprises a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention.

Brief Description of the Figures

No Figures are presented

Detailed Description of the Invention

The invention provides a solution to the problem of treating diseases associated with HPV infection, by using double-stranded ribonucleic acid (dsRNA) to silence expression of genes essential for HPV proliferation. In particular, the dsRNA of the invention silence the HPV genes E1 or E6 or human E6AP, a conserved gene of the human host species required by HPV for proliferation. Herein, these genes are sometimes collectively called the HPV Target genes.

The invention provides double-stranded ribonucleic acid (dsRNA), as well as compositions and methods for inhibiting the expression of the E1, E6 or E6AP gene in a cell or mammal using the dsRNA. The invention also provides compositions and methods for treating pathological conditions and diseases in a mammal caused by the expression of the E1, E6 or E6AP gene in association with HPV 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 the HPV Target mRNA transcript. The use of these dsRNAs enables the targeted degradation of mRNAs of genes that are implicated in replication and/or maintenance of an HPV in mammals. Using cell-based and animal-based assays, the present inventors have demonstrated that very low dosages of these dsRNA can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of the E1, E6 or E6AP gene. Thus, the methods and compositions of the invention comprising these

dsRNAs are useful for treating pathological processes mediated by HPV infection by targeting a host factor gene involved in the HPV life cycle.

Description of the HPV Targets: HPV E1 and E6 and human E6AP

The cellular ubiquitin ligase E6AP of the human host is implicated in the replication of HPV, particularly integrated (non-episomal) forms of HPV, through its complex with the E6 protein of the virus. E6 binds to many proteins regulating cell proliferation pathways and often provokes their degradation (Chakrabarti, O. and Krishna, S. 2003. J. Biosci. 28:337-348). E6 complexes with E6AP to target the tumor suppressor p53 for degradation (Scheffner, M. et al., 1990. Cell. 63:1129-1136; and Scheffner, M. et al., 1993. Cell 75:495-505). By inactivating p53, the virus not only prevents p53-mediated apoptosis of the infected cells (Chakrabarti and Krishna, 2003) and facilitates the replication of its DNA that would otherwise be blocked by p53 (Lepik, D. et al. 1998. J. Virol. 72:6822-6831), but it also favors oncogenesis by decreasing p53-mediated control on genomic integrity (Thomas, M. et al. 1999. Oncogene. 18:7690-7700).

E1 and E6 are both described in considerable detail in "Papillomaviridae: The Viruses and Their Replication" by Peter M. Howley, pp. 947-978, in: Fundamental Virology, 3rd ed. Bernard N. Fields, David M. Knipe, and Peter M. Howley, eds. Lippincott-Raven Publishers, Philadelphia, 1996. The E1 ORF encodes a 68–76 kD protein essential for plasmid DNA replication. The full-length E1 product is a phosphorylated nuclear protein that binds to the origin of replication in the LCR of BPV1. E1 has also been shown to bind ATP and to bind in vitro to the full length E2 protein called the E2 transcription transactivator (E2TA), thereby enhancing viral transcription. Binding to E2 also strengthens the affinity of E1 for the origin of DNA replication. In HPV-16, E1 has indirect effects on immortalization.

E6 is a small basic cell-transforming protein (e.g., the HPV16 E6 comprises 151 amino acids), about 16–19 kD, which is localized to the nuclear matrix and non-nuclear membrane fraction. The E6 gene product contains four Cys–X–X–Cys motifs, indicating a potential for zinc binding; it may also act as a nucleic acid binding protein. In high-risk HPVs such as HPV-16, E6 and E7 proteins are necessary and sufficient to immortalize their hosts—squamous epithelial

cells. The E6 gene products of high-risk HPVs have been shown to complex with p53, and to promote its degradation.

The following detailed description discloses how to make and use the dsRNA and compositions containing dsRNA to inhibit the expression of the HPV Target genes, as well as compositions and methods for treating diseases and disorders caused by HPV infection, e.g. cervical cancer and genital warts. 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 an HPV Target gene, together with a pharmaceutically acceptable carrier. An embodiment of the invention is the employment of more than one dsRNA, optionally targeting different HPV Target genes, in combination in a pharmaceutical formulation.

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 one or more HPV Target genes, and methods of using the pharmaceutical compositions to treat diseases caused by HPV infection.

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, "E6AP" refers to the ubiquitin protein ligase E3A (ube3A, also referred to as E6-associated protein or E6AP) gene or protein. Human mRNA sequences to E6AP representing different isoforms are provided as GenBank Accession numbers NM_130838.1, NM_130839.1, and NM_000462.2.

As used herein, "E1" refers to the human papillomavirus type 16 (HPV16) E1 gene (GenBank accession number NC_001526, nucleotides 865 to 2813). As used herein, "E6" refers to the human papillomavirus type 16 (HPV16) E6 gene (GenBank accession number NC_001526, nucleotides 65 to 559). Many variants of the E1 and E6 genes have also been publicly disclosed. These and future published E1 and E6 gene variants are intended to be covered herein by the use of "E1" and "E6", unless specifically excluded by the context.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of one of the HPV Target genes, 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 E6AP). For example, a polynucleotide is complementary to at least a part of a E6AP mRNA if the sequence is substantially complementary to a non-interrupted portion of a mRNA encoding E6AP.

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 separate RNA molecules, such dsRNA are often referred to in the literature as siRNA ("short interfering RNA"). 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", "short hairpin RNA" or "shRNA". 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. In addition, as used in this specification, "dsRNA" may include chemical modifications to ribonucleotides, internucleoside linkages, end-groups, caps, and conjugated moieties, including substantial modifications at multiple nucleotides and including all types of modifications disclosed herein or known in the art. Any such modifications, as used in an siRNA type molecule, are encompassed by "dsRNA" for the purposes of this specification and claims.

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. For clarity, chemical caps or non-nucleotide

chemical moieties conjugated to the 3' end or 5' end of an siRNA are not considered in determining whether an siRNA has an overhang or is blunt ended.

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

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

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

For example, in certain instances, expression of the E6AP gene is suppressed by at least about 20%, 25%, 35%, or 50% by administration of the double-stranded oligonucleotide of the invention. In some embodiments, the E6AP gene is suppressed by at least about 60%, 70%, or 80% by administration of the double-stranded oligonucleotide of the invention. In some embodiments, the E6AP gene is suppressed by at least about 85%, 90%, or 95% by administration of the double-stranded oligonucleotide of the invention. Table 2 provides a wide range of values for inhibition of transcription obtained in an in vitro assay using various E6AP dsRNA molecules at various concentrations. Likewise, Table 6 provides a wide range of values for the inhibition of transcription of E1; and Table 8 provides a wide range of values for the inhibition of transcription of E6.

As used herein in the context of HPV infection, the terms "treat", "treatment", and the like, refer to relief from or alleviation of pathological processes mediated by HPV infection. Such description includes use of the therapeutic agents of the invention for prophylaxis or prevention of HPV infection, and relief from symptoms or pathologies caused by HPV 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 HPV infection), 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 HPV infection or an overt symptom of pathological processes mediated by HPV infection. 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 HPV infection, the patient's history and age, the stage of pathological processes mediated by HPV infection, and the administration of other anti-pathological agents.

As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of a dsRNA and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," "therapeutically effective amount" or simply "effective amount" refers to that amount of a dsRNA 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 the HPV Target gene in a cell or mammal, wherein the dsRNA comprises an antisense strand comprising a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of the HPV Target gene, and wherein the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and wherein said dsRNA, upon contact with a cell expressing said HPV Target gene, inhibits the expression of said HPV Target gene by at least 10%, 25%, or 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 the HPV Target gene, the other strand (the sense strand) comprises a region which is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. Generally, the duplex structure is between 15 and 30, more generally between 18 and 25, yet more generally between 19 and 24, and most generally between 19 and 21 base pairs in length. Similarly, the region of complementarity to the target sequence is between 15 and 30, more generally between 18 and 25, yet more generally between 19 and 24, and most generally between 19 and 21 nucleotides in length. The dsRNA of the invention may further comprise one or more single-stranded nucleotide overhang(s). The dsRNA can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc. In a preferred embodiment, the HPV Target gene is the human E6AP gene. In specific embodiments, the antisense strand of the dsRNA comprises a strand selected

from the sense sequences of Table 1 and a second sequence selected from the group consisting of the antisense sequences of Table 1. Alternative antisense agents that target elsewhere in the target sequence provided in Table 1 can readily be determined using the target sequence and the flanking E6AP sequence.

In further embodiments, the dsRNA comprises at least one nucleotide sequence selected from the groups of sequences provided in Table 1. 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 the E6AP gene. Generally, the dsRNA comprises two oligonucleotides, wherein one oligonucleotide is described as the sense strand in Table 1 and the second oligonucleotide is described as the antisense strand in Table 1. Table 1 provides a duplex name and sequence ID number for each preferred dsRNA.

In further embodiments, the dsRNA comprises at least one named duplex dsRNA selected from the groups of sequences provided in Table 5 (E1 dsRNA) or Table 7 (E6 dsRNA).

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 Table 1, Table 5 or Table 7, 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 Table 1, Table 5 or Table 7, 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 Table 1, Table 5 or Table 7, and differing in their ability to inhibit the expression of the HPV Target gene in a FACS assay or other 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 Table 1, Table 5 or Table 7 can readily be made using the reference sequence and the target sequence provided.

In addition, the RNAi agents provided in Table 1, Table 5 and Table 7 identify a site in the respective HPV Target 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 Table 1, Table 5 or Table 7 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in the HPV Target gene. For example, the last 15 nucleotides of SEQ ID NO:1 (minus the added AA sequences) combined with the next 6 nucleotides from the target E6AP gene produces a single strand agent of 21 nucleotides that is based on one of the sequences provided in Table 1.

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 the HPV Target gene, the dsRNA generally does not contain any mismatch within the central 13 nucleotides. The methods described within the invention can be used to determine whether a dsRNA containing a mismatch to a target sequence is effective in inhibiting the expression of the HPV Target gene. Consideration of the efficacy of dsRNAs with mismatches in inhibiting expression of the HPV Target gene is important, especially if the particular region of complementarity in the HPV Target gene is known to have polymorphic sequence variation in the virus (if E1 or E6) or within the human population (for E6AP).

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. Chemical modifications may include, but are not limited to 2' modifications, modifications at other sites of the sugar or base of an oligonucleotide, introduction of non-natural bases into the oligonucleotide chain, covalent attachment to a ligand or chemical moiety, and replacement of internucleotide phosphate linkages with alternate linkages such as thiophosphates. More than one such modification may be employed.

Chemical linking of the two separate dsRNA strands may be achieved by any of a variety of well-known techniques, for example by introducing covalent, ionic or hydrogen bonds; hydrophobic interactions, van der Waals or stacking interactions; by means of metal-ion coordination, or through use of purine analogues. Generally, the chemical groups that can be used to modify the dsRNA include, without limitation, methylene blue; bifunctional groups, generally bis-(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; and

psoralen. In one embodiment, the linker is a hexa-ethylene glycol linker. In this case, the dsRNA are produced by solid phase synthesis and the hexa-ethylene glycol linker is incorporated according to standard methods (e.g., Williams, D.J., and K.B. Hall, *Biochem.* (1996) 35:14665-14670). In a particular embodiment, the 5'-end of the antisense strand and the 3'-end of the sense strand are chemically linked via a hexaethylene glycol linker. In another embodiment, at least one nucleotide of the dsRNA comprises a phosphorothioate or phosphorodithioate groups. The chemical bond at the ends of the dsRNA is generally formed by triple-helix bonds. Table 1 provides examples of modified RNAi agents of the invention.

In yet another embodiment, the nucleotides at one or both of the two single strands may be modified to prevent or inhibit the degradation activities of cellular enzymes, such as, for example, without limitation, certain nucleases. Techniques for inhibiting the degradation activity of cellular enzymes against nucleic acids are known in the art including, but not limited to, 2'-amino modifications, 2'-amino sugar modifications, 2'-F sugar modifications, 2'-F modifications, 2'-alkyl sugar modifications, uncharged backbone modifications, morpholino modifications, 2'-O-methyl modifications, and phosphoramidate (see, e.g., Wagner, *Nat. Med.* (1995) 1:1116-8). Thus, at least one 2'-hydroxyl group of the nucleotides on a dsRNA is replaced by a chemical group, generally by a 2'-amino or a 2'-methyl group. Also, at least one nucleotide may be modified to form a locked nucleotide. Such locked nucleotide contains a methylene bridge that connects the 2'-oxygen of ribose with the 4'-carbon of ribose. Oligonucleotides containing the locked nucleotide are described in Koshkin, A.A., et al., *Tetrahedron* (1998), 54: 3607-3630) and Obika, S. et al., *Tetrahedron Lett.* (1998), 39: 5401-5404). Introduction of a locked nucleotide into an oligonucleotide improves the affinity for complementary sequences and increases the melting temperature by several degrees (Braasch, D.A. and D.R. Corey, *Chem. Biol.* (2001), 8:1-7).

Conjugating a ligand to a dsRNA can enhance its cellular absorption as well as targeting to a particular tissue or uptake by specific types of cells such as vaginal epithelium. In certain instances, a hydrophobic ligand is conjugated to the dsRNA to facilitate direct permeation of the cellular membrane. Alternatively, the ligand conjugated to the dsRNA is a substrate for receptor-mediated endocytosis. These approaches have been used to facilitate cell permeation of antisense

oligonucleotides as well as dsRNA agents. For example, cholesterol has been conjugated to various antisense oligonucleotides resulting in compounds that are substantially more active compared to their non-conjugated analogs. See M. Manoharan *Antisense & Nucleic Acid Drug Development* **2002**, *12*, 103. Other lipophilic compounds that have been conjugated to oligonucleotides include 1-pyrene butyric acid, 1,3-bis-O-(hexadecyl)glycerol, and menthol. One example of a ligand for receptor-mediated endocytosis is folic acid. Folic acid enters the cell by folate-receptor-mediated endocytosis. dsRNA compounds bearing folic acid would be efficiently transported into the cell via the folate-receptor-mediated endocytosis. Li and coworkers report that attachment of folic acid to the 3'-terminus of an oligonucleotide resulted in an 8-fold increase in cellular uptake of the oligonucleotide. Li, S.; Deshmukh, H. M.; Huang, L. *Pharm. Res.* **1998**, *15*, 1540. Other ligands that have been conjugated to oligonucleotides include polyethylene glycols, carbohydrate clusters, cross-linking agents, porphyrin conjugates, and delivery peptides.

In certain instances, conjugation of a cationic ligand to oligonucleotides results in improved resistance to nucleases. Representative examples of cationic ligands are propylammonium and dimethylpropylammonium. Interestingly, antisense oligonucleotides were reported to retain their high binding affinity to mRNA when the cationic ligand was dispersed throughout the oligonucleotide. See M. Manoharan *Antisense & Nucleic Acid Drug Development* **2002**, *12*, 103 and references therein.

The ligand-conjugated dsRNA of the invention may be synthesized by the use of a dsRNA that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the dsRNA. This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto. The methods of the invention facilitate the synthesis of ligand-conjugated dsRNA by the use of, in some preferred embodiments, nucleoside monomers that have been appropriately conjugated with ligands and that may further be attached to a solid-support material. Such ligand-nucleoside conjugates, optionally attached to a solid-support material, are prepared according to some preferred embodiments of the methods of the invention via reaction of a selected serum-binding ligand

with a linking moiety located on the 5' position of a nucleoside or oligonucleotide. In certain instances, an dsRNA bearing an aralkyl ligand attached to the 3'-terminus of the dsRNA is prepared by first covalently attaching a monomer building block to a controlled-pore-glass support via a long-chain aminoalkyl group. Then, nucleotides are bonded via standard solid-phase synthesis techniques to the monomer building-block bound to the solid support. The monomer building block may be a nucleoside or other organic compound that is compatible with solid-phase synthesis.

The dsRNA used in the conjugates of the invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

Teachings regarding the synthesis of particular modified oligonucleotides may be found in the following U.S. patents: U.S. Pat. Nos. 5,138,045 and 5,218,105, drawn to polyamine conjugated oligonucleotides; U.S. Pat. No. 5,212,295, drawn to monomers for the preparation of oligonucleotides having chiral phosphorus linkages; U.S. Pat. Nos. 5,378,825 and 5,541,307, drawn to oligonucleotides having modified backbones; U.S. Pat. No. 5,386,023, drawn to backbone-modified oligonucleotides and the preparation thereof through reductive coupling; U.S. Pat. No. 5,457,191, drawn to modified nucleobases based on the 3-deazapurine ring system and methods of synthesis thereof; U.S. Pat. No. 5,459,255, drawn to modified nucleobases based on N-2 substituted purines; U.S. Pat. No. 5,521,302, drawn to processes for preparing oligonucleotides having chiral phosphorus linkages; U.S. Pat. No. 5,539,082, drawn to peptide nucleic acids; U.S. Pat. No. 5,554,746, drawn to oligonucleotides having β-lactam backbones; U.S. Pat. No. 5,571,902, drawn to methods and materials for the synthesis of oligonucleotides; U.S. Pat. No. 5,578,718, drawn to nucleosides having alkylthio groups, wherein such groups may be used as linkers to other moieties attached at any of a variety of positions of the nucleoside; U.S. Pat. Nos. 5,587,361 and 5,599,797, drawn to oligonucleotides having phosphorothioate linkages of high chiral purity; U.S. Pat. No. 5,506,351, drawn to processes for the preparation of

2'-O-alkyl guanosine and related compounds, including 2,6-diaminopurine compounds; U.S. Pat. No. 5,587,469, drawn to oligonucleotides having N-2 substituted purines; U.S. Pat. No. 5,587,470, drawn to oligonucleotides having 3-deazapurines; U.S. Pat. No. 5,223,168, and U.S. Pat. No. 5,608,046, both drawn to conjugated 4'-desmethyl nucleoside analogs; U.S. Pat. Nos. 5,602,240, and 5,610,289, drawn to backbone-modified oligonucleotide analogs; U.S. Pat. Nos. 6,262,241, and 5,459,255, drawn to, inter alia, methods of synthesizing 2'-fluoro-oligonucleotides.

In the ligand-conjugated dsRNA and ligand-molecule bearing sequence-specific linked nucleosides of the invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. Oligonucleotide conjugates bearing a variety of molecules such as steroids, vitamins, lipids and reporter molecules, has previously been described (see Manoharan et al., PCT Application WO 93/07883). In a preferred embodiment, the oligonucleotides or linked nucleosides of the invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

The incorporation of a 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-allyl, 2'-O-aminoalkyl or 2'-deoxy-2'-fluoro group in nucleosides of an oligonucleotide confers enhanced hybridization properties to the oligonucleotide. Further, oligonucleotides containing phosphorothioate backbones have enhanced nuclease stability. Thus, functionalized, linked nucleosides of the invention can be augmented to include either or both a phosphorothioate backbone or a 2'-O-

methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-aminoalkyl, 2'-O-allyl or 2'-deoxy-2'-fluoro group. A summary listing of some of the oligonucleotide modifications known in the art is found at, for example, PCT Publication WO 200370918.

In some embodiments, functionalized nucleoside sequences of the invention possessing an amino group at the 5'-terminus are prepared using a DNA synthesizer, and then reacted with an active ester derivative of a selected ligand. Active ester derivatives are well known to those skilled in the art. Representative active esters include N-hydrosuccinimide esters, tetrafluorophenolic esters, pentafluorophenolic esters and pentachlorophenolic esters. The reaction of the amino group and the active ester produces an oligonucleotide in which the selected ligand is attached to the 5'-position through a linking group. The amino group at the 5'-terminus can be prepared utilizing a 5'-Amino-Modifier C6 reagent. In one embodiment, ligand molecules may be conjugated to oligonucleotides at the 5'-position by the use of a ligand-nucleoside phosphoramidite wherein the ligand is linked to the 5'-hydroxy group directly or indirectly via a linker. Such ligand-nucleoside phosphoramidites are typically used at the end of an automated synthesis procedure to provide a ligand-conjugated oligonucleotide bearing the ligand at the 5'-terminus.

Examples of modified internucleoside linkages or 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 United States Patents relating to the preparation of the above phosphorus-atom-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 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; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; and 5,697,248, each of which is herein incorporated by reference.

Examples of modified internucleoside linkages or backbones that do not include a phosphorus atom therein (i.e., oligonucleosides) have backbones that are formed by short chain alkyl or cycloalkyl intersugar linkages, mixed heteroatom and alkyl or cycloalkyl intersugar linkages, or one or more short chain heteroatomic or heterocyclic intersugar linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

Representative United States patents relating to 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; 5,264,562; 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; 5,610,289; 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 certain instances, the oligonucleotide may be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to oligonucleotides in order to enhance the activity, cellular distribution or cellular uptake of the oligonucleotide, 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., Biochem. 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 oligonucleotide conjugates have been listed above. Typical conjugation protocols involve the synthesis of oligonucleotides 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 oligonucleotide still bound to the solid support or following cleavage of the oligonucleotide in solution phase. Purification of the oligonucleotide conjugate by HPLC typically affords the pure conjugate. The use of a cholesterol conjugate is particularly preferred since such a moiety can increase targeting vaginal epithelium cells, a site of HPV infection.

The instant disclosure describes a wide variety of embodiments of dsRNA that are useful to silence HPV Target genes and thus to treat HPV associated disorders. While the design of the specific therapeutic agent can take a variety of forms, certain functional characteristics will distinguish preferred dsRNA from other dsRNA. In particular, features such as good serum stability, high potency, lack of induced immune response, and good drug like behaviour, all measurable by those skilled in the art, will be tested to identify preferred dsRNA of the invention. In some situations, not all of these functional aspects will be present in the preferred dsRNA. But those skilled in the art are able to optimize these variables and others to select preferred compounds of the invention.

While many nucleotide modifications are possible, the inventors have identified patterns of chemical modifications which provide significantly improved pharmacological, immunological and ulitimately therapeutic benefit. Table 9 sets out patterns of chemical

modifications preferred for use with the duplex dsRNA set out in Table 1, Table 5 and Table 7 of the invention. Some of these modifications are also illustrated in Table 3.

Table 9

Chemical Modification Series	Changes made to sense strand (5′-3′)	Changes made to antisense stand (5′-3′)
1 (single phosphorothioate at the ends of both strands)	dTsdT	dTsdT
2 (single phosphorothioate at the ends of both strands plus, 2'OMe sense strand modification of all pyrimidines and 2'Ome modification of all U's followed by and A and all C's followed by A)	dTsdT, 2'OMe@all Py	dTsdT, 2'OMe@uA, cA
3 (single phosphorothioate at the ends of both strands plus, 2'OMe sense strand modification of all pyrimidines and, 2'Ome of indicted bases all U's followed by an A, all C's followed by an A, all U's followed by a G and all U's followed by a U	dTsdT, 2'OMe@all Py	dTsdT,2'OMe@uA,cA,uG,uU

on the antisense strand)		
4 (same as 1 except addition of cholesterol conjugated to the sense strand)	Chol ("exo")	dTsdT ("exo")
5 (same as 2 except cholesterol conjugated to the sense strand)	Chol ("endo")	dTsdT, 2'OMe@uA, cA
6 (same as 3 except cholesterol conjugated to the sense strand)	Chol ("endo")	dTsdT,2'OMe@uA,cA,uG,uU

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 the HPV Target gene, such as pathological processes mediated by HPV infection. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for either topical administration in the cervix or systemic administration via parenteral delivery.

The pharmaceutical compositions of the invention are administered in dosages sufficient to inhibit expression of the HPV Target gene. The present inventors have determined that, because of their improved efficiency, compositions comprising the dsRNA of the invention can be administered at surprisingly low dosages. A dosage of 5 mg dsRNA per kilogram body weight of recipient per day is sufficient to inhibit or suppress expression of the HPV Target gene, and in the case of warts or cervical or anal treatment, may be applied directly to the infected tissue.

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 of vaginal gel. 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. Sustained release formulations are well known in the art and are particularly useful for vaginal delivery of agents, such as could be used with the agents of the present invention. 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.

The inventors recognize that for a variety of reasons, including the variability of HPV genotypes, it may be desireable to treat HPV infection with more than one dsRNA of the invention at the same time. In an embodiment, a combination of dsRNA are selected to target the widest range of HPV genotypes, with the least complex mixture of dsRNA. A pharmaceutical composition of the invention comprising more than one type of dsRNA would be expected to contain dosages of individual dsRNA as described herein.

Combinations of dsRNA may be provided together in a single dosage form pharmaceutical composition. Alternatively, combination dsRNA may be provided in separate dosage forms, in which case they may be administered at the same time or at different times, and possibly by different means. The invention therefore contemplates pharmaceutical compositions comprising the desired combinations of dsRNA of the invention; and it also contemplates

pharmaceutical compositions of single dsRNA which are intended to be provided as part of a combination regimen. In this latter case, the combination therapy invention is thereby a method of administering rather than a composition of matter.

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

Any method can be used to administer a dsRNA of the present invention to a mammal containing cells infected with HPV. For example, administration can be topical (e.g., vaginal, transdermal, etc); oral; or parenteral (e.g., by subcutaneous, intraventricular, intramuscular, or intraperitoneal injection, or by intravenous drip). Administration can be rapid (e.g., by injection), or can occur over a period of time (e.g., by slow infusion or administration of slow release formulations).

Typically, when treating a mammal having cells infected with HPV, the dsRNA molecules are administered topically in a vaginal gel or cream. For example, dsRNAs formulated with or without liposomes can be topically applied directly to the cervix, anal tract or HPV lesions such as genital warts. For topical administration, a dsRNA molecule can be formulated into compositions such as sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions in liquid or solid oil bases. Such solutions also can contain buffers, diluents, and other suitable additives. Compositions for topical administration can be formulated in the form of transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids, and powders. Gels and creams may be formulated using polymers and permeabilizers known in the art. Gels or creams containing the dsRNA and associated excipients may be applied to the cervix using a cervical cap, vaginal diaphragm, coated condom, glove, and the like. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners, and the like can be added.

For parenteral, intrathecal, or intraventricular administration, a dsRNA molecule can be formulated into compositions such as sterile aqueous solutions, which also can contain buffers, diluents, and other suitable additives (e.g., penetration enhancers, carrier compounds, and other pharmaceutically acceptable carriers).

In addition, dsRNA molecules can be administered to a mammal containing HPV-infected cells using non-viral methods, such as biologic or abiologic means as described in, for example, U.S. Pat. No. 6,271,359. Abiologic delivery can be accomplished by a variety of methods including, without limitation, (1) loading liposomes with a dsRNA acid molecule provided herein and (2) complexing a dsRNA molecule with lipids or liposomes to form nucleic acid-lipid or nucleic acid-liposome complexes. The liposome can be composed of cationic and neutral lipids commonly used to transfect cells in vitro. Cationic lipids can complex (e.g., charge-associate) with negatively charged nucleic acids to form liposomes. Examples of cationic liposomes include, without limitation, lipofectin, lipofectamine, lipofectace, DOTAP (1,2-dioleoyl-3-trimethylammonium propane), DOTMA (N-[1,2(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride), DOSPA (2,3-dioleoyloxy-N-[2-(sperminecarboxamido)ethyl]-N,N-deimethyl-1-propanaminium), DOGS (dioctadecyl amido glycil spermine), and DC-chol (3,[N-N^{1},N-dimethylethylenediamine)-carbamoyl]cholesterol).

Procedures for forming liposomes are well known in the art. Liposome compositions can be formed, for example, from phosphatidylcholine, dimyristoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, dimyristoyl phosphatidylglycerol, or dioleoyl phosphatidylethanolamine. Numerous lipophilic agents are commercially available, including Lipofectin.RTM. (Invitrogen/Life Technologies, Carlsbad, Calif.) and Effectene.TM. (Qiagen, Valencia, Calif.). In addition, systemic delivery methods can be optimized using commercially available cationic lipids such as DDAB or DOTAP, each of which can be mixed with a neutral lipid such as DOPE or cholesterol. In some cases, liposomes such as those described by Templeton et al. (Nature Biotechnology, 15: 647-652 (1997)) can be used. In other embodiments, polycations such as polyethyleneimine can be used to achieve delivery in vivo and ex vivo (Boletta et al., J. Am Soc. Nephrol. 7: 1728 (1996)). Additional information regarding the use of liposomes to deliver

nucleic acids can be found in U.S. Pat. No. 6,271,359, PCT Publication WO 96/40964 and Morrissey, D. et al. 2005. Nat Biotechnol. 23(8):1002-7.

Other non-viral methods of administering dsRNA molecules to a mammal containing HPV-infected cells include cationic lipid-based delivery systems (in addition to lipsomes) such as lipoplexes and nanoemulsions. Additionally, condensing polymeric delivery systems (i.e., DNA-polymer complexes, or "polyplexes") may be used, including but not limited to chitosans, poly(L-lysine)(PLL), polyethylenimine (PEI), dendrimers (e.g., polyamidoamine (PANAM) dendrimers), and poloxamines. Additionally, noncondensing polymeric delivery systems may be used, including but not limited to poloxamers, gelatin, PLGA (polylactic-co-glycolic acid), PVP (polyvinylpyrrolidone), and PVA (polyvinyl alcohol).

Procedures for the above-mentioned delivery or administration techniques are well known in the art. For instance, condensing polymeric delivery systems work by easily complexing with anionic DNA molecules; for example, poly(L-lysine)(PLL) works by forming a positively charged complex that interacts with negatively charged cell surface and subsequently undergoing rapid internalization.

Biologic delivery can be accomplished by a variety of methods including, without limitation, the use of viral vectors. For example, viral vectors (e.g., adenovirus and herpesvirus vectors) can be used to deliver dsRNA molecules to skin cells and cervical cells. Standard molecular biology techniques can be used to introduce one or more of the dsRNAs provided herein into one of the many different viral vectors previously developed to deliver nucleic acid to cells. These resulting viral vectors can be used to deliver the one or more dsRNAs to cells by, for example, infection.

dsRNAs of the present invention can be formulated in a pharmaceutically acceptable carrier or diluent. A "pharmaceutically acceptable carrier" (also referred to herein as an "excipient") is a pharmaceutically acceptable solvent, suspending agent, or any other pharmacologically inert vehicle. Pharmaceutically acceptable carriers can be liquid or solid, and can be selected with the planned manner of administration in mind so as to provide for the desired bulk, consistency, and other pertinent transport and chemical properties. Typical

pharmaceutically acceptable carriers include, by way of example and not limitation: water; saline solution; binding agents (e.g., polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose and other sugars, gelatin, or calcium sulfate); lubricants (e.g., starch, polyethylene glycol, or sodium acetate); disintegrates (e.g., starch or sodium starch glycolate); and wetting agents (e.g., sodium lauryl sulfate).

In addition, dsRNA that target the HPV Target gene can be formulated into compositions containing the dsRNA admixed, encapsulated, conjugated, or otherwise associated with other molecules, molecular structures, or mixtures of nucleic acids. For example, a composition containing one or more dsRNA agents that target the E6AP gene can contain other therapeutic agents such as anti-inflammatory drugs (e.g., nonsteroidal anti-inflammatory drugs and corticosteroids) and antiviral drugs (e.g., ribivirin, vidarabine, acyclovir, and ganciclovir). In some embodiments, a composition can contain one or more dsRNAs having a sequence complementary to the HPV Target gene in combination with a keratolytic agent. Keratolytic agents are agents that separate or loosen the horny layer of the epidermis. An example of a keratolytic agent includes, without limitation, salicylic acid. Other examples are provided in U.S. Pat. No. 5,543,417. Keratolytic agents can be used in an amount effective to enhance the penetration of dsRNAs, for example, into tissues such as skin. For example, a keratolytic agent can be used in an amount that allows a dsRNA applied to a genital wart to penetrate throughout the wart.

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 HPV infection. 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.

Combinations of dsRNA can be tested in vitro and in vivo using the same methods employed for identification of preferred single dsRNA. Such combinations may be selected based on a purely bioinformatics basis, wherein the minimum number of siRNA are selected which provide coverage over the widest range of genotypes. Alternatively, such combinations may be selected based on in vitro or in vivo evaluations along the lines of those described herein for single dsRNA agents. A preferred assay for testing combinations of dsRNA is to evaluate the phenotypic conseuqences of siRNA mediated HPV target knockdown in HPV16 positive cancer cell lines (e.g. SiHa or Caski, as described in, e.g., Hengstermann et al. (2005) Journal Vir. 79(14): 9296; and Butz et al. (2003) Oncogene 22: 5938), or in organotypic culture systems, as described in, e.g., Jeon et al. (1995) Journal Vir. 69(5):2989.

The inventors have identified certain preferred combinations of dsRNA which may be used to treat HPV infection. In the most general terms, the combination of dsRNA comprises more than one dsRNA selected from among Table 1, Table 3, Table 5 and Table 7. Thus the invention contemplates the use of 2, 3, 4, 5 or more dsRNA duplexes selected from among Table 1, Table 3, Table 5 and Table 7 in a combination therapy. In principle, the smallest number of dsRNA is preferred for simplicity of the therapeutic product. This forces the selection of dsRNA

which will cover the greatest number of deleterious or potentially deleterious HPV genotypes, and indeed may justify selection of a combination that does not necessarily cover all such HPV genotypes.

The following dsRNA are particularly amenable to combination:

From E1: ND-9072; ND-9142; ND-9092; ND-9162; ND-9097; ND-9167; ND-9066; ND-9123; AL-DP-8082; AL-DP-8095;

From E6: ND-8903; ND-8991; ND-8914; ND-9002; ND-8906; ND-8994; ND-8943; ND-9031; ND-9032; ND-8920; ND-8952; ND-8951; ND-9008; ND-9040; ND-9039; AL-DP-7783; AL-DP-7784;

From E6AP: AL-DP-7365; AL-DP-7371; AL-DP-7499; AL-DP-7545; AL-DP-7492; AL-DP-7473; AL-DP-7478; AL-DP-7554; AL-DP-7514; AL-DP-7397, ND-9300.

Methods for treating diseases caused by HPV infection

The methods and compositions described herein can be used to treat diseases and conditions caused by human papillomavirus, which can be the result of clinical or sub-clinical papillomavirus infections. Such diseases and conditions, herein sometimes called "HPV associated disorders" or "pathological processes mediated by HPV infection", include, e.g., epithelial malignancies, skin cancer (non-melanoma or melanoma), anogenital malignancies such as cervical cancer, HPV associated precancerous lesions (including LSIL or HSIL cervical tissue), anal carcinoma, malignant lesions, benign lesions, papillomacarcinomas, papilloadenocystomas, papilloma neuropathicum, papillomatosis, cutaneous and mucosal papillomas, condylomas, fibroblastic tumors, and other pathological conditions associated with papillomavirus.

For example, the compositions described herein can be used to treat warts caused by HPV. Such warts include, e.g., common warts (verruca vulgaris), for example, palmar, plantar, and periungual warts; flat and filiform warts; anal, oral, pharyngeal, laryngeal, and tongue

papillomas; and venereal warts (condyloma accuminata), also known as genital warts (for example, penile, vulvar, vaginal and cervical warts), which are one of the most serious manifestations of HPV infection. HPV DNA can be found in all grades of cervical intraepithelial neoplasia (CIN I-III), and a specific subset of HPV types can be found in carcinoma in situ of the cervix. Consequently, women with genital warts, containing specific HPV types, are considered to be at high risk for the development of cervical cancer.

The most common disease associated with papillomavirus infection is benign skin warts, or common warts. Common warts generally contain HPV types 1, 2, 3, 4 or 10. Other conditions caused by papillomavirus include, e.g., laryngeal papillomas, which are benign epithelial tumors of the larynx. Two papillomavirus types, HPV-6 and HPV-11, are most commonly associated with laryngeal papillomas. The compositions described herein can be used to treat these diseases and conditions.

The compositions described herein can also be used in the treatment of epidermodysplasia verruciformis (EV), a rare genetically transmitted disease characterized by disseminated flat warts that appear as small reddish macules.

In addition, the compositions described herein can be used to treat lesions resulting from cellular transformation for which HPV is an etiological agent, e.g., in the treatment of cervical cancer.

The compositions described herein can also be used in the treatment of HPV-induced dysplasias, e.g., penile, vulvar, cervical, vaginal oral, anal, and pharyngeal dysplasias, and in the treatment of HPV-induced cancers, e.g., penile, vulvar, cervical, vaginal, anal, oral, pharyngeal, and head and neck cancers.

The invention can also be practiced by including a specific dsRNA 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.

In a further alternative, the dsRNA targeting E6AP may be employed to treat neurological and behavioural disorders. E6AP has been implicated in neurological and behavioural disorders through the identification of E6AP mutations in patients having Angelman syndrome. Angelman syndrome (AS) is an imprinted neurobehavioral disorder characterized by mental retardation, absent speech, excessive laughter, seizures, ataxia, and a characteristic EEG pattern. (Hitchins, M.P. et al. 2004. Am J Med Genet A. 125(2):167-72.) It would not, presumably, be the intent of treatment to induce such conditions; rather, as observed in many hereditary defects, this evidence that E6AP has a critical role in neurological and behavioural conditions also indicates that this target may have a variety of roles in human pathologies and is likely a suitable target for other diseases in this class where silencing of E6AP will compensate for other biochemical defects or diseases. As used herein "E6AP associated disorders" include the HPV associated disorders noted above and other neurological and behavioural disorders.

Methods for inhibiting expression of the E6AP gene

In yet another aspect, the invention provides a method for inhibiting the expression of the E6AP gene in a mammal. The method comprises administering a composition of Table 1 of the invention to the mammal such that expression of the target E6AP gene is silenced. Because of their high specificity, such dsRNAs of the invention specifically target RNAs (primary or

processed) of the target E6AP gene. Compositions and methods for inhibiting the expression of these E6AP genes using such 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 the E6AP gene of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, vaginal and topical (including buccal and sublingual) administration. In preferred embodiments, the compositions are administered by topical/vaginal administration or 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

Gene Walking of the E6AP gene

siRNA design was carried out to identify siRNAs targeting human ubiquitin protein ligase E3A (ube3A, also referred to as E6AP). Human mRNA sequences to E6AP representing different isoforms (NM_130838.1, NM_130839.1, NM_000462.2) were used.

The ClustalW multiple alignment function (Thompson J.D., et al., Nucleic Acids Res. 1994, 22:4673) of the BioEdit software was used with all human E6AP isoforms to identify mRNA sequence NM_130838.1 as shortest sequence as well as to confirm sequence conservation from position 5 to 4491 (end position) of the reference sequence, a requirement for efficient targeting of all E6AP isoforms.

All possible overlapping 19mers (representing siRNA sense strand sequences) spanning E6AP reference sequence NM_130838.1 were identified, resulting in 4473 19mer candidate sequences. Combined, these candidate target sequences cover the 5'UTR, coding and 3'UTR domains of the E6AP mRNA, and the junction sites of these domains.

In order to rank and select siRNAs out of the pool of candidates, the 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) complementarity to a target gene in positions 2 to 9 (counting 5' to 3') of a strand (seed region) may be sufficient for interaction of that strand with the mRNA transcribed from the target gene and subsequent downregulation (Jackson AL, et al. Nat Biotechnol. 2003 Jun;21(6):635-7)

2) positions 1 and 19 of each strand are not relevant for off-target interactions

3) seed region may contribute more to off-target potential than rest of sequence

4) cleavage site region positions 10 and 11 (counting 5' to 3') of a strand may contribute more to off-target potential than the sequences 3' to the cleavage site (non-seed region), but not as much as the seed region

5) an off-target score can be calculated for each gene and each strand, based on complementarity of siRNA strand sequence to the gene's sequence and position of mismatches while considering assumptions 1 to 4

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

7) the off-target potential of an siRNA can be inferred from the gene displaying the highest homology according to our criteria (best off-target gene), thus can be expressed by the off-target score of the respective gene

To identify potential off-target genes, 19mer antisense sequences were subjected to a homology search against publicly available human mRNA sequences. To this purpose, fastA (version 3.4) searches were performed with all 19mer candidate antisense sequences against the human RefSeq database. A Perl script was used to generate antisense sequences from the candidate 19mer sequences (perl script 2). fastA search was executed with parameter/value pairs –g 30 –f 30 –L –i –H in order to take into account the homology over the full length of the 19mer and to format the output suitable for the script analysis in the next step. The search resulted in a list of potential off-target genes for candidate siRNAs.

Further, fastA search parameters were applied with values –E 15000 in order to make database entries with more than 8 contiguous nucleobases identical to the 19mer sense strand sequences very likely to be transferred to a fastA output file while displaying the homology of the complete 19mer length (see assumption 1).

In order to identify the best off-target gene and its off-target score, the fastA output file was analyzed. The following off-target properties for each 19mer input sequence were extracted for each potential off-target gene:

Number of mismatches in seed region

Number of mismatches in non-seed region

Number of mismatches in cleavage site region

The off-target score for each off-target gene was calculated as follows:

(number of seed mismatches multiplied by 10) + (number of cleavage site mismatches multiplied by 1.2) + number of non-seed mismatches

The lowest off-target score was extracted for each input 19mer sequence and successively written into an output file resulting in a list of off-target scores for all siRNAs corresponding to the input 19mer sequences.

In order to generate a ranking of siRNAs, off-target scores were entered into a result table. All siRNAs were finally sorted according descending to the off-target score and sequences containing stretches with more than 3 Gs in a row were excluded from selection.

The 156 siRNAs with an off-target score of >=3 were selected and synthesized (Table 1).

Table 1 dsRNA targeting E6AP

Target sequence of mRNA from human reference sequence NM_130838 (human iso3)	SEQ ID. NO.	Sense strand (target sequence) having double overhang	SEQ ID. NO.	antisense strand (guide sequence) having double overhang	SEQ ID. NO.	
sequence of total 19mer target site + AA at ends		sequence (5´-3´)		sequence (5´-3´)		duplex name
AAAUACGAUGAAUCUACAAAAAA	1	AUACGAUGAAUCUACAAAATT	157	UUUUGUAGAUUCAUCGUAUTT	313	AL-DP-7545
AAUGACUACAUUCUCAAUAAAAA	2	UGACUACAUUCUCAAUAAATT	158	UUUAUUGAGAAUGUAGUCATT	314	AL-DP-7558
AAAGCCUGCACGAAUGAGUUUAA	3	AGCCUGCACGAAUGAGUUUTT	159	AAACUCAUUCGUGCAGGCUTT	315	AL-DP-7548
AAGGAUUGUCGAAAACCACUUAA	4	GGAUUGUCGAAAACCACUUTT	160	AAGUGGUUUUCGACAAUCCTT	316	AL-DP-7509
AACUCUCGAGAUCCUAAUUAUAA	5	CUCUCGAGAUCCUAAUUAUTT	161	AUAAUUAGGAUCUCGAGAGTT	317	AL-DP-7492
AAAUGUGACUUACUUAACAGAAA	6	AUGUGACUUACUUAACAGATT	162	UCUGUUAAGUAAGUCACAUTT	318	AL-DP-7554
AAGUAUACUCUCGAGAUCCUAAA	7	GUAUACUCUCGAGAUCCUATT	163	UAGGAUCUCGAGAGUAUACTT	319	AL-DP-7557
AAAGGUUACCUACAUCUCAUAAA	8	AGGUUACCUACAUCUCAUATT	164	UAUGAGAUGUAGGUAACCUTT	320	AL-DP-7476
AAAGUACUUAUUCAGACCAGAAA	9	AGUACUUAUUCAGACCAGATT	165	UCUGGUCUGAAUAAGUACUTT	321	AL-DP-7514
AAAUCCUAAUUAUCUGAAUUUAA	10	AUCCUAAUUAUCUGAAUUUTT	166	AAAUUCAGAUAAUUAGGAUTT	322	AL-DP-7540
AAAAGGAUAGGUGAUAGCUCAAA	11	AAGGAUAGGUGAUAGCUCATT	167	UGAGCUAUCACCUAUCCUUTT	323	AL-DP-7397
AAGGAAGCCGGAAUCUAGAUUAA	12	GGAAGCCGGAAUCUAGAUUTT	168	AAUCUAGAUUCCGGCUUCCTT	324	AL-DP-7526
AAUGCUUCGAAGUGCUUGAAAAA	13	UGCUUCGAAGUGCUUGAAATT	169	UUUCAAGCACUUCGAAGCATT	325	AL-DP-7473
AAUGGAUUGUCGAAAACCACUAA	14	UGGAUUGUCGAAAACCACUTT	170	AGUGGUUUUCGACAAUCCATT	326	AL-DP-7478
AACGGCUAGAGAUGAUCGCUAAA	15	CGGCUAGAGAUGAUCGCUATT	171	UAGCGAUCAUCUCUAGCCGTT	327	AL-DP-7553
AAACAGUCGAAAUCUAGUGAAAA	16	ACAGUCGAAAUCUAGUGAATT	172	UUCACUAGAUUUCGACUGUTT	328	AL-DP-7395
AAGAUCAGACUGUGGUCUAAAAA	17	GAUCAGACUGUGGUCUAAATT	173	UUUAGACCACAGUCUGAUCTT	329	AL-DP-7522
AACUCGAGAUCCUAAUUAUCUAA	18	CUCGAGAUCCUAAUUAUCUTT	174	AGAUAAUUAGGAUCUCGAGTT	330	AL-DP-7499
AAUAUCGUAAUGGAGAAUAGAAA	19	UAUCGUAAUGGAGAAUAGATT	175	UCUAUUCUCCAUUACGAUATT	331	AL-DP-7527
AACUCAAAGUUAGACGUGACCAA	20	CUCAAAGUUAGACGUGACCTT	176	GGUCACGUCUAACUUUGAGTT	332	AL-DP-7544
AAAGGAUAGGUGAUAGCUCACAA	21	AGGAUAGGUGAUAGCUCACTT	177	GUGAGCUAUCACCUAUCCUTT	333	AL-DP-7489
AACACCUAACGUGGAAUGUGAAA	22	CACCUAACGUGGAAUGUGATT	178	UCACAUUCCACGUUAGGUGTT	334	AL-DP-7365
AAAAUCGUUCAUUCAUUUACAAA	23	AAUCGUUCAUUCAUUUACATT	179	UGUAAAUGAAUGAACGAUUTT	335	AL-DP-7390
AACUUGACGUAUCACAAUGUAAA	24	CUUGACGUAUCACAAUGUATT	180	UACAUUGUGAUACGUCAAGTT	336	AL-DP-7458
AAUGGUAUGUUCACAUACGAUAA	25	UGGUAUGUUCACAUACGAUTT	181	AUCGUAUGUGAACAUACCATT	337	AL-DP-7532
AAGAUAGGUGAUAGCUCACAGAA	26	GAUAGGUGAUAGCUCACAGTT	182	CUGUGAGCUAUCACCUAUCTT	338	AL-DP-7546
AACCGGCUAGAGAUGAUCGCUAA	27	CCGGCUAGAGAUGAUCGCUTT	183	AGCGAUCAUCUCUAGCCGGTT	339	AL-DP-7512

AACAUAGUACUGGGUCUGGCUAA	28	CAUAGUACUGGGUCUGGCUTT	184	AGCCAGACCCAGUACUAUGTT	340	AL-DP-7470
AAAAUGUAUACUCUCGAGAUCAA	29	AAUGUAUACUCUCGAGAUCTT	185	GAUCUCGAGAGUAUACAUUTT	341	AL-DP-7406
AAAACUUUUCGUGACUUGGGAAA	30	AACUUUUCGUGACUUGGGATT	186	UCCCAAGUCACGAAAAGUUTT	342	AL-DP-7382
AAAAAGUUAGACGUGACCAUAAA	31	AAAGUUAGACGUGACCAUATT	187	UAUGGUCACGUCUAACUUUTT	343	AL-DP-7547
AAUGAUUAGGGAGUUCUGGGAAA	32	UGAUUAGGGAGUUCUGGGATT	188	UCCCAGAACUCCCUAAUCATT	344	AL-DP-7490
AAUACGAUGAAUCUACACAAAUAA	33	UACGAUGAAUCUACACAAAUTT	189	AUUUUGUAGAUUCAUCGUATT	345	AL-DP-7493
AACUUGUCCGGCUAGAGAUGAAA	34	CUUGUCCGGCUAGAGAUGATT	190	UCAUCUCUAGCCGGACAAGTT	346	AL-DP-7529
AAUAUACUCUCGAGAUCCUAAAA	35	UAUACUCUCGAGAUCCUAATT	191	UUAGGAUCUCGAGAGUAUATT	347	AL-DP-7400
AAACUUGACGUAUCACAAUGUAA	36	ACUUGACGUAUCACAAUGUTT	192	ACAUUGUGAUACGUCAAGUTT	348	AL-DP-7391
AAAACAGUCGAAAUCUAGUGAAA	37	AACAGUCGAAAUCUAGUGATT	193	UCACUAGAUUUCGACUGUUTT	349	AL-DP-7393
AAUCAUUAUCGUAAUGGAGAAAA	38	UCAUUAUCGUAAUGGAGAATT	194	UUCUCCAUUACGAUAAUGATT	350	AL-DP-7511
AAAUAGUACUGGGUCUGGCUAAA	39	AUAGUACUGGGUCUGGCUATT	195	UAGCCAGACCCAGUACUAUTT	351	AL-DP-7454
AACCUAACGUGGAAUGUGACUAA	40	CCUAACGUGGAAUGUGACUTT	196	AGUCACAUUCCACGUUAGGTT	352	AL-DP-7450
AAUUGUCCGGCUAGAGAUGAUAA	41	UUGUCCGGCUAGAGAUGAUTT	197	AUCAUCUCUAGCCGGACAATT	353	AL-DP-7533
AAACCUAACGUGGAAUGUGACAA	42	ACCUAACGUGGAAUGUGACTT	198	GUCACAUUCCACGUUAGGUTT	354	AL-DP-7485
AAUUAACAGUCGAAAUCUAGUAA	43	UUAACAGUCGAAAUCUAGUTT	199	ACUAGAUUUCGACUGUUAATT	355	AL-DP-7495
AAUUGGCAUAGUACUGGGUCUAA	44	UUGGCAUAGUACUGGGUCUTT	200	AGACCCAGUACUAUGCCAATT	356	AL-DP-7456
AAGAACUUUUCGUGACUUGGGAA	45	GAACUUUUCGUGACUUGGGTT	201	CCCAAGUCACGAAAAGUUCTT	357	AL-DP-7538
AAGUCCGGCUAGAGAUGAUCGAA	46	GUCCGGCUAGAGAUGAUCGTT	202	CGAUCAUCUCUAGCCGGACTT	358	AL-DP-7377
AAGCCCUCGAGCUUUAUAAGAAA	47	GCCCUCGAGCUUUAUAAGATT	203	UCUUAUAAAGCUCGAGGGCTT	359	AL-DP-7405
AACUCGAGCUUUAUAAGAUUAAA	48	CUCGAGCUUUAUAAGAUUATT	204	UAAUCUUAUAAAGCUCGAGTT	360	AL-DP-7392
AAUGGCAUAGUACUGGGUCUGAA	49	UGGCAUAGUACUGGGUCUGTT	205	CAGACCCAGUACUAUGCCATT	361	AL-DP-7453
AAACGAAUGAGUUUUGUGCUUAA	50	ACGAAUGAGUUUUGUGCUUTT	206	AAGCACAAAACUCAUUCGUTT	362	AL-DP-7366
AAUUUCUUCGUAUGGAUAAUAAA	51	UUUCUUCGUAUGGAUAAUATT	207	UAUUAUCCAUACGAAGAAATT	363	AL-DP-7534
AAAGACGUGACCAUAUCAUAGAA	52	AGACGUGACCAUAUCAUAGTT	208	CUAUGAUAUGGUCACGUCUTT	364	AL-DP-7401
AAUAGUACUGGGUCUGGCUAUAA	53	UAGUACUGGGUCUGGCUAUTT	209	AUAGCCAGACCCAGUACUATT	365	AL-DP-7523
AACGUAUGGAUAAUAAUGCAGAA	54	CGUAUGGAUAAUAAUGCAGTT	210	CUGCAUUAUUAUCCAUACGTT	366	AL-DP-7555
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AAAAUUCGCAUGUACAGUGAAAA	56	AAUUCGCAUGUACAGUGAATT	212	UUCACUGUACAUGCGAAUUTT	368	AL-DP-7371
AAAAUAGAAUUCGCAUGUACAAA	57	AAUAGAAUUCGCAUGUACATT	213	UGUACAUGCGAAUUCUAUUTT	369	AL-DP-7372
AAUGGUAACCCAAUGAUGUAUAA	58	UGGUAACCCAAUGAUGUAUTT	214	AUACAUCAUUGGGUUACCATT	370	AL-DP-7370
AAAGCCGGAAUCUAGAUUUCCAA	59	AGCCGGAAUCUAGAUUUCCTT	215	GGAAAUCUAGAUUCCGGCUTT	371	AL-DP-7474
AAACUUUUCGUGACUUGGGAGAA	60	ACUUUUCGUGACUUGGGAGTT	216	CUCCCAAGUCACGAAAAGUTT	372	AL-DP-7452
AAAGCCCUCGAGCUUUAUAAGAA	61	AGCCCUCGAGCUUUAUAAGTT	217	CUUAUAAAGCUCGAGGGCUTT	373	AL-DP-7498

AAGAACGAAGAAUCACUGUUCAA	62	GAACGAAGAAUCACUGUUCTT	218	GAACAGUGAUUCUUCGUUCTT	374	AL-DP-7551
AAUAUUCUGACUACAUUCUCAAA	63	UAUUCUGACUACAUUCUCATT	219	UGAGAAUGUAGUCAGAAUATT	375	AL-DP-7552
AAGCAUCUAAUAGAACGCUACAA	64	GCAUCUAAUAGAACGCUACTT	220	GUAGCGUUCUAUUAGAUGCTT	376	AL-DP-7504
AAUCGAAAUCUAGUGAAUGAUAA	65	UCGAAAUCUAGUGAAUGAUTT	221	AUCAUUCACUAGAUUUCGATT	377	AL-DP-7467
AAAGUCGAAAUCUAGUGAAUGAA	66	AGUCGAAAUCUAGUGAAUGTT	222	CAUUCACUAGAUUUCGACUTT	378	AL-DP-7463
AAGAAAGGCGCUAGAAUUGAUAA	67	GAAAGGCGCUAGAAUUGAUTT	223	AUCAAUUCUAGCGCCUUUCTT	379	AL-DP-7399
AAAAAGGCGCUAGAAUUGAUUAA	68	AAAGGCGCUAGAAUUGAUUTT	224	AAUCAAUUCUAGCGCCUUUTT	380	AL-DP-7501
AAAGGCGCUAGAAUUGAUUUUAA	69	AGGCGCUAGAAUUGAUUUUTT	225	AAAAUCAAUUCUAGCGCCUTT	381	AL-DP-7385
AAAGCAUCUAAUAGAACGCUAAA	70	AGCAUCUAAUAGAACGCUATT	226	UAGCGUUCUAUUAGAUGCUTT	382	AL-DP-7480
AACAAAGCGAUGAGCAAGCUAAA	71	CAAAGCGAUGAGCAAGCUATT	227	UAGCUUGCUCAUCGCUUUGTT	383	AL-DP-7528
AACCAUGGUUGUCUACAGGAAAA	72	CCAUGGUUGUCUACAGGAATT	228	UUCCUGUAGACAACCAUGGTT	384	AL-DP-7535
AAAGAAAGGCGCUAGAAUUGAAA	73	AGAAAGGCGCUAGAAUUGATT	229	UCAAUUCUAGCGCCUUUCUTT	385	AL-DP-7403
AAAACGCUACUACCACCAGUUAA	74	AACGCUACUACCACCAGUUTT	230	AACUGGUGGUAGUAGCGUUTT	386	AL-DP-7380
AAGCGCUAGAAUUGAUUUAAAA	75	GCGCUAGAAUUGAUUUAATT	231	UUAAAAUCAAUUCUAGCGCTT	387	AL-DP-7364
AAGCACGUGAUCAGUGUUGCAAA	76	GCACGUGAUCAGUGUUGCATT	232	UGCAACACUGAUCACGUGCTT	388	AL-DP-7469
AAGAUAGUGUCCCAGUACAAAAA	77	GAUAGUGUCCCAGUACAAATT	233	UUUGUACUGGGACACUAUCTT	389	AL-DP-7518
AAUUUGCGUGAAAGUGUUACAAA	78	UUUGCGUGAAAGUGUUACATT	234	UGUAACACUUUCACGCAAATT	390	AL-DP-7464
AAAGUAUGUGCUACUUUUUUGAA	79	AGUAUGUGCUACUUUUUUGTT	235	CAAAAAAGUAGCACAUACUTT	391	AL-DP-7560
AAGUAUGUCGUCUUCAUGUGUAA	80	GUAUGUCGUCUUCAUGUGUTT	236	ACACAUGAAGACGACAUACTT	392	AL-DP-7461
AAGGUAGUCAAGCCUAUUGCAAA	81	GGUAGUCAAGCCUAUUGCATT	237	UGCAAUAGGCUUGACUACCTT	393	AL-DP-7472
AAACGUAACCUUCAAGUAUGUAA	82	ACGUAACCUUCAAGUAUGUTT	238	ACAUACUUGAAGGUUACGUTT	394	AL-DP-7459
AAACCACGUAACCUUCAAGUAAA	83	ACCACGUAACCUUCAAGUATT	239	UACUUGAAGGUUACGUGGUTT	395	AL-DP-7381
AACAGUAAAGCUGACCUGGAAAAA	84	CAGUAAAGCUGACCUGGAAATT	240	UUUCCAGGUCAGCUUACUGTT	396	AL-DP-7515
AAAGUAGGUUUACAUUACUGAAA	85	AGUAGGUUUACAUUACUGATT	241	UCAGUAAUGUAAACCUACUTT	397	AL-DP-7517
AAAAUGGUAGUCAAGCCUAUUAA	86	AAUGGUAGUCAAGCCUAUUTT	242	AAUAGGCUUGACUACCAUUTT	398	AL-DP-7521
AAGCCUAUUGCAACAAAGUUAAA	87	GCCUAUUGCAACAAAGUUATT	243	UAACUUUGUUGCAAUAGGCTT	399	AL-DP-7530
AAGACCACGUAACCUUCAAGUAA	88	GACCACGUAACCUUCAAGUTT	244	ACUUGAAGGUUACGUGGUCTT	400	AL-DP-7388
AAUGUUAAACGUUACUUUCAUAA	89	UGUUAAACGUUACUUUCAUTT	245	AUGAAAGUAACGUUUAACATT	401	AL-DP-7451
AAAUUGAAGCUAGCCGAAUGAAA	90	AUUGAAGCUAGCCGAAUGATT	246	UCAUUCGGCUAGCUUCAAUTT	402	AL-DP-7484
AAAUAUAACGAGGGAUAAAUUAA	91	AUAUAACGAGGGAUAAAUUTT	247	AAUUUAUCCCUCGUUAUAUTT	403	AL-DP-7376
AAACUUACUUAUUACCUAGAUAA	92	ACUUACUUAUUACCUAGAUTT	248	AUCUAGGUAAUAAGUAAGUTT	404	AL-DP-7500
AAUGUUCUCCGUUGUUGUUUAAA	93	UGUUCUCCGUUGUUGUUUATT	249	UAAAACAACAACGAGAACATT	405	AL-DP-7488
AAUUUUAAGGGUUAAAUCACUAA	94	UUUUAAGGGUUAAAUCACUTT	250	AGUGAUUUAACCCUUAAAATT	406	AL-DP-7541
AAAGUAACAGCACAACAAAUUAA	95	AGUAACAGCACAACAAAUUTT	251	AAUUUGUUGUGCUGUUACUTT	407	AL-DP-7550

AACAACUCCUGCUCUGAGAUAAA	96	CAACUCCUGCUCUGAGAUATT	252	UAUCUCAGAGCAGGAGUUGTT	408	AL-DP-7776
AAGAUGUGACUUACUUAACAGAA	97	GAUGUGACUUACUUAACAGTT	253	CUGUUAAGUAAGUCACAUCTT	409	AL-DP-7777
AACAUUAUCGUAAUGGAGAAUTT	98	CAUUAUCGUAAUGGAGAAUTT	254	AUUCUCCAUUACGAUAAUGTT	410	AL-DP-7510
AACCAUUUUAUCGAGGCACGUAA	99	CCAUUUUAUCGAGGCACGUTT	255	ACGUGCCUCGAUAAAAUGGTT	411	AL-DP-7507
AAAGUAGCCAAUCCUCUUUCUAA	100	AGUAGCCAAUCCUCUUUCUTT	256	AGAAAGAGGAUUGGCUACUTT	412	AL-DP-7479
AAUAAUAGAACGCUACUACCAAA	101	UAAUAGAACGCUACUACCATT	257	UGGUAGUAGCGUUCUAUUATT	413	AL-DP-7542
AACUUCGUGCAACUGUAGUCAAA	102	CUUCGUGCAACUGUAGUCATT	258	UGACUACAGUUGCACGAAGTT	414	AL-DP-7494
AAUCAUAUGGUGACCAAUGAAAA	103	UCAUAUGGUGACCAAUGAATT	259	UUCAUUGGUCACCAUAUGATT	415	AL-DP-7531
AAUUAAUCCGUGUUAUUGGAAAA	104	UUAAUCCGUGUUAUUGGAATT	260	UUCCAAUAACACGGAUUAATT	416	AL-DP-7373
AAAUACGCUACCUUGAUGAAAAA	105	AUACGCUACCUUGAUGAAATT	261	UUUCAUCAAGGUAGCGUAUTT	417	AL-DP-7508
AAAGCUAGCCGAAUGAAGCGAAA	106	AGCUAGCCGAAUGAAGCGATT	262	UCGCUUCAUUCGGCUAGCUTT	418	AL-DP-7487
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AAAAUAUAACGAGGGAUAAAUTT	108	AAUAUAACGAGGGAUAAAUTT	264	AUUUAUCCCUCGUUAUAUUTT	420	AL-DP-7462
AAGUUCUCGUUGUUGUUUUAAAA	109	GUUCUCGUUGUUGUUUUAATT	265	UUAAAACAACAACGAGAACTT	421	AL-DP-7513
AAUGAUUGACUGAUUGUUUUAAA	110	UGAUUGACUGAUUGUUUUATT	266	UAAAACAAUCAGUCAAUCATT	422	AL-DP-7455
AAUUUAAUCCGUGUUAUUGGAAA	111	UUUAAUCCGUGUUAUUGGATT	267	UCCAAUAACACGGAUUAAATT	423	AL-DP-7374
AAUGUCCGGCUAGAGAUGAUCAA	112	UGUCCGGCUAGAGAUGAUCTT	268	GAUCAUCUCUAGCCGGACATT	424	AL-DP-7475
AAGCUAGCCGAAUGAAGCGAGAA	113	GCUAGCCGAAUGAAGCGAGTT	269	CUCGCUUCAUUCGGCUAGCTT	425	AL-DP-7369
AAGUACAUACGCUACCUUGAUAA	114	GUACAUACGCUACCUUGAUTT	270	AUCAAGGUAGCGUAUGUACTT	426	AL-DP-7466
AAUCGAGCUUUAUAAGAUUAAAA	115	UCGAGCUUUAUAAGAUUAATT	271	UUAAUCUUAUAAAGCUCGATT	427	AL-DP-7491
AACUACCACCAGUUAACUGAGAA	116	CUACCACCAGUUAACUGAGTT	272	CUCAGUUAACUGGUGGUAGTT	428	AL-DP-7482
AAUUUUAUCGAGGCACGUGAUAA	117	UUUUAUCGAGGCACGUGAUTT	273	AUCACGUGCCUCGAUAAAATT	429	AL-DP-7398
AAAUUUUAUCGAGGCACGUGAAA	118	AUUUUAUCGAGGCACGUGATT	274	UCACGUGCCUCGAUAAAAUTT	430	AL-DP-7471
AAAACGUUACUUUCAUGUACUAA	119	AACGUUACUUUCAUGUACUTT	275	AGUACAUGAAAGUAACGUUTT	431	AL-DP-7383
AAAGAAUUCGCAUGUACAGUGAA	120	AGAAUUCGCAUGUACAGUGTT	276	CACUGUACAUGCGAAUUCUTT	432	AL-DP-7367
AAUCACGUAUGCCAAAGGAUUAA	121	UCACGUAUGCCAAAGGAUUTT	277	AAUCCUUUGGCAUACGUGATT	433	AL-DP-7386
AACUUUAAUCCGUGUUAUUGGAA	122	CUUUAAUCCGUGUUAUUGGTT	278	CCAAUAACACGGAUUAAAGTT	434	AL-DP-7525
AAACAUACGCUACCUUGAUGAAA	123	ACAUACGCUACCUUGAUGATT	279	UCAUCAAGGUAGCGUAUGUTT	435	AL-DP-7486
AAUCCUAGUCUUCUGUGUAUGAA	124	UCCUAGUCUUCUGUGUAUGTT	280	CAUACACAGAAGACUAGGATT	436	AL-DP-7539
AACAUUUUAUCGAGGCACGUGAA	125	CAUUUUAUCGAGGCACGUGTT	281	CACGUGCCUCGAUAAAAUGTT	437	AL-DP-7483
AAUUAACUGAUUACUGUAGAUAA	126	UUAACUGAUUACUGUAGAUTT	282	AUCUACAGUAAUCAGUUAATT	438	AL-DP-7503
AACCUUCGUGCAACUGUAGUCAA	127	CCUUCGUGCAACUGUAGUCTT	283	GACUACAGUUGCACGAAGGTT	439	AL-DP-7537
AAUGUUAGGGUACAUACGCUAAA	128	UGUUAGGGUACAUACGCUATT	284	UAGCGUAUGUACCCUAACATT	440	AL-DP-7396
AACACUGUUAGGGUACAUACGAA	129	CACUGUUAGGGUACAUACGTT	285	CGUAUGUACCCUAACAGUGTT	441	AL-DP-7404

AAUGUGGCACUUUUCACCAUAAA	130	UGUGGCACUUUUCACCAUATT	286	UAUGGUGAAAAGUGCCACATT	442	AL-DP-7543
AAAUCACGUAUGCCAAAGGAUAA	131	AUCACGUAUGCCAAAGGAUTT	287	AUCCUUUGGCAUACGUGAUTT	443	AL-DP-7379
AAAGGGUACAUACGCUACCUUAA	132	AGGGUACAUACGCUACCUUTT	288	AAGGUAGCGUAUGUACCCUTT	444	AL-DP-7502
AAACUAAGGUGAGACAUUGAUAA	133	ACUAAGGUGAGACAUUGAUTT	289	AUCAAUGUCUCACCUUAGUTT	445	AL-DP-7519
AAUGUCACCUAACGUGGAAUGAA	134	UGUCACCUAACGUGGAAUGTT	290	CAUUCCACGUUAGGUGACATT	446	AL-DP-7506
AAAUAGAAUUCGCAUGUACAGAA	135	AUAGAAUUCGCAUGUACAGTT	291	CUGUACAUGCGAAUUCUAUTT	447	AL-DP-7457
AAUUCGUGACUUGGGAGACUCAA	136	UUCGUGACUUGGGAGACUCTT	292	GAGUCUCCCAAGUCACGAATT	448	AL-DP-7468
AAUAGAAUUCGCAUGUACAGUAA	137	UAGAAUUCGCAUGUACAGUTT	293	ACUGUACAUGCGAAUUCUATT	449	AL-DP-7368
AAUGAGGGCGUUUUAUAUAAUAA	138	UGAGGGCGUUUUAUAUAAUTT	294	AUUAUAUAAAAACGCCCUCATT	450	AL-DP-7402
AAACACUGUUAGGGUACAUACAA	139	ACACUGUUAGGGUACAUACTT	295	GUAUGUACCCUAACAGUGUTT	451	AL-DP-7481
AAUUCUCGUUGUUUUUUAAGAA	140	UUCUCGUUGUUUUUUAAGTT	296	CUUAAAAACAACAACGAGAATT	452	AL-DP-7465
AACUGUUAGGGUACAUACGCUAA	141	CUGUUAGGGUACAUACGCUTT	297	AGCGUAUGUACCCUAACAGTT	453	AL-DP-7496
AAUUUAACUAAGGUGAGACAUAA	142	UUUAACUAAGGUGAGACAUTT	298	AUGUCUCACCUUAGUUAAATT	454	AL-DP-7549
AAUAACAGUCGAAAUCUAGUGAA	143	UAACAGUCGAAAUCUAGUGTT	299	CACUAGAUUUCGACUGUUATT	455	AL-DP-7394
AAUGUGGUCUAAAUACAAUGCAA	144	UGUGGUCUAAAUACAAUGCTT	300	GCAUUGUAUUUAGACCACATT	456	AL-DP-7477
AAUCACCUAACGUGGAAUGUGAA	145	UCACCUAACGUGGAAUGUGTT	301	CACAUUCCACGUUAGGUGATT	457	AL-DP-7516
AAUUCUUAGUAUAUGAAAGGAAA	146	UUCUUAGUAUAUGAAAGGATT	302	UCCUUUCAUAUACUAAGAATT	458	AL-DP-7556
AAAAAUAGAACGCUACUACCACAA	147	AAUAGAACGCUACUACCACTT	303	GUGGUAGUAGCGUUCUAUUTT	459	AL-DP-7387
AACUCAGGGCGUUUUAUAUAAAA	148	CUGAGGGCGUUUUAUAUAATT	304	UUAUAUAAAACGCCCUCAGTT	460	AL-DP-7524
AAAAAUCGUUCAUUCAUUUACAA	149	AAAUCGUUCAUUCAUUUACTT	305	GUAAAUGAAUGAACGAUUUTT	461	AL-DP-7378
AAUUUUCGUGACUUGGGAGACAA	150	UUUUCGUGACUUGGGAGACTT	306	GUCUCCCAAGUCACGAAAATT	462	AL-DP-7389
AAUCGUGCAACUGUAGUCAUCAA	151	UCGUGCAACUGUAGUCAUCTT	307	GAUGACUACAGUUGCACGATT	463	AL-DP-7384
AAUCAUACAGUAAGCUGACCUAA	152	UCAUACAGUAAGCUGACCUTT	308	AGGUCAGCUUACUGUAUGATT	464	AL-DP-7497
AACGUGCAACUGUAGUCAUCUAA	153	CGUGCAACUGUAGUCAUCUTT	309	AGAUGACUACAGUUGCACGTT	465	AL-DP-7559
AAUGCCAUUAGAAGGGUCUACAA	154	UGCCAUUAGAAGGGUCUACTT	310	GUAGACCCUUCUAAUGGCATT	466	AL-DP-7520
AAUUACAAUAACUGUAUACUGAA	155	UUACAAUAACUGUAUACUGTT	311	CAGUAUACAGUUAUUGUAATT	467	AL-DP-7505
AAUUCGCAUGUACAGUGAACGAA	156	UUCGCAUGUACAGUGAACGTT	312	CGUUCACUGUACAUGCGAATT	468	AL-DP-7460

dsRNA synthesis

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent may be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

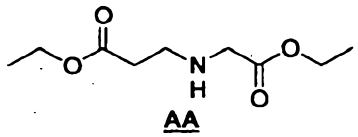
siRNA synthesis

Single-stranded RNAs were produced by solid phase synthesis on a scale of 1 µmole using an Expedite 8909 synthesizer (Applied Biosystems, Applera Deutschland GmbH, Darmstadt, Germany) and controlled pore glass (CPG, 500Å, Proligo Biochemie GmbH, Hamburg, Germany) as solid support. RNA and RNA containing *2'-O*-methyl nucleotides were generated by solid phase synthesis employing the corresponding phosphoramidites and *2'-O*-methyl phosphoramidites, respectively (Proligo Biochemie GmbH, Hamburg, Germany). These building blocks were incorporated at selected sites within the sequence of the oligoribonucleotide chain using standard nucleoside phosphoramidite chemistry such as described in Current protocols in nucleic acid chemistry, Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA. Phosphorothioate linkages were introduced by replacement of the iodine oxidizer solution with a solution of the Beaucage reagent (Chruachem Ltd, Glasgow, UK) in acetonitrile (1%). Further ancillary reagents were obtained from Mallinckrodt Baker (Griesheim, Germany).

Deprotection and purification of the crude oligoribonucleotides by anion exchange HPLC were carried out according to established procedures. Yields and concentrations were determined by UV absorption of a solution of the respective RNA at a wavelength of 260 nm using a spectral photometer (DU 640B, Beckman Coulter GmbH, Unterschleißheim, Germany). Double stranded RNA was generated by mixing an equimolar solution of complementary strands in annealing buffer (20 mM sodium phosphate, pH 6.8; 100 mM sodium chloride), heated in a water bath at 85 - 90°C for 3 minutes and cooled to room temperature over a period of 3 - 4 hours. The annealed RNA solution was stored at –20 °C until use.

For the synthesis of 3'-cholesterol-conjugated siRNAs (herein referred to as -Chol-3'), an appropriately modified solid support was used for RNA synthesis. The modified solid support was prepared as follows:

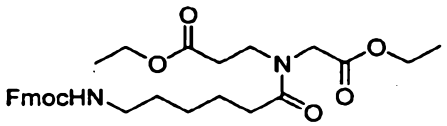
Diethyl-2-azabutane-1,4-dicarboxylate **AA**



AA

A 4.7 M aqueous solution of sodium hydroxide (50 mL) was added into a stirred, ice-cooled solution of ethyl glycinate hydrochloride (32.19 g, 0.23 mole) in water (50 mL). Then, ethyl acrylate (23.1 g, 0.23 mole) was added and the mixture was stirred at room temperature until completion of the reaction was ascertained by TLC. After 19 h the solution was partitioned with dichloromethane (3 x 100 mL). The organic layer was dried with anhydrous sodium sulfate, filtered and evaporated. The residue was distilled to afford AA (28.8 g, 61%).

3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonyl-amino)-hexanoyl]-amino}-propionic acid ethyl ester **AB**

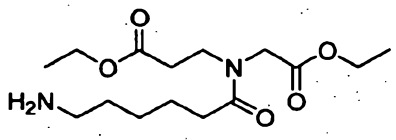


AB

Fmoc-6-amino-hexanoic acid (9.12 g, 25.83 mmol) was dissolved in dichloromethane (50 mL) and cooled with ice. Diisopropylcarbodiimde (3.25 g, 3.99 mL, 25.83 mmol) was added to the solution at 0°C. It was then followed by the addition of Diethyl-azabutane-1,4-dicarboxylate (5 g, 24.6 mmol) and dimethylamino pyridine (0.305 g, 2.5 mmol). The solution was brought to room temperature and stirred further for 6 h. Completion of the reaction was ascertained by TLC. The reaction mixture was concentrated under vacuum and ethyl acetate was added to precipitate diisopropyl urea. The suspension was filtered. The filtrate was washed with 5% aqueous

hydrochloric acid, 5% sodium bicarbonate and water. The combined organic layer was dried over sodium sulfate and concentrated to give the crude product which was purified by column chromatography (50 % EtOAC/Hexanes) to yield 11.87 g (88%) of AB.

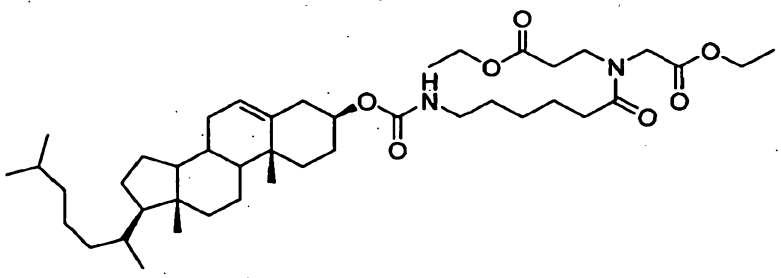
3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester **AC**



AC

3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonylamino)-hexanoyl]-amino}-propionic acid ethyl ester AB (11.5 g, 21.3 mmol) was dissolved in 20% piperidine in dimethylformamide at 0°C. The solution was continued stirring for 1 h. The reaction mixture was concentrated under vacuum, water was added to the residue, and the product was extracted with ethyl acetate. The crude product was purified by conversion into its hydrochloride salt.

3-({6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}ethoxycarbonylmethyl-amino)-propionic acid ethyl ester **AD**

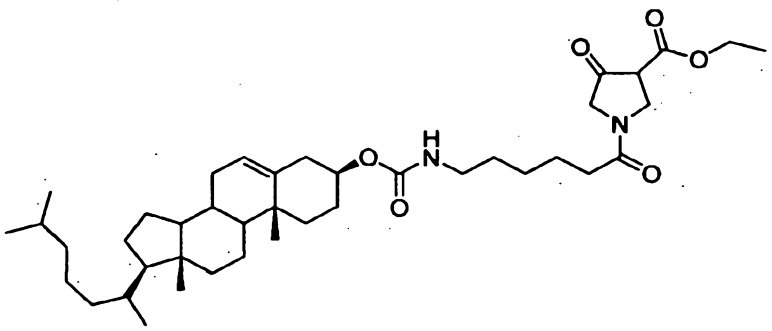


AD

The hydrochloride salt of 3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester AC (4.7 g, 14.8 mmol) was taken up in dichloromethane. The suspension was cooled to 0°C on ice. To the suspension diisopropylethylamine (3.87 g, 5.2 mL,

30 mmol) was added. To the resulting solution cholesteryl chloroformate (6.675 g, 14.8 mmol) was added. The reaction mixture was stirred overnight. The reaction mixture was diluted with dichloromethane and washed with 10% hydrochloric acid. The product was purified by flash chromatography (10.3 g, 92%).

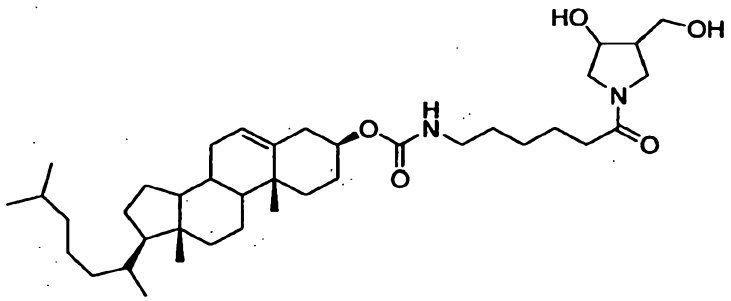
1-{6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a] phenanthren-3-yloxycarbonylamino]-hexanoyl}-4-oxo-pyrrolidine-3-carboxylic acid ethyl ester **AE**



AE

Potassium t-butoxide (1.1 g, 9.8 mmol) was slurried in 30 mL of dry toluene. The mixture was cooled to 0°C on ice and 5 g (6.6 mmol) of diester AD was added slowly with stirring within 20 mins. The temperature was kept below 5°C during the addition. The stirring was continued for 30 mins at 0°C and 1 mL of glacial acetic acid was added, immediately followed by 4 g of $NaH_2PO_4 \cdot H_2O$ in 40 mL of water The resultant mixture was extracted twice with 100 mL of dichloromethane each and the combined organic extracts were washed twice with 10 mL of phosphate buffer each, dried, and evaporated to dryness. The residue was dissolved in 60 mL of toluene, cooled to 0°C and extracted with three 50 mL portions of cold pH 9.5 carbonate buffer. The aqueous extracts were adjusted to pH 3 with phosphoric acid, and extracted with five 40 mL portions of chloroform which were combined, dried and evaporated to dryness. The residue was purified by column chromatography using 25% ethylacetate/hexane to afford 1.9 g of b-ketoester (39%).

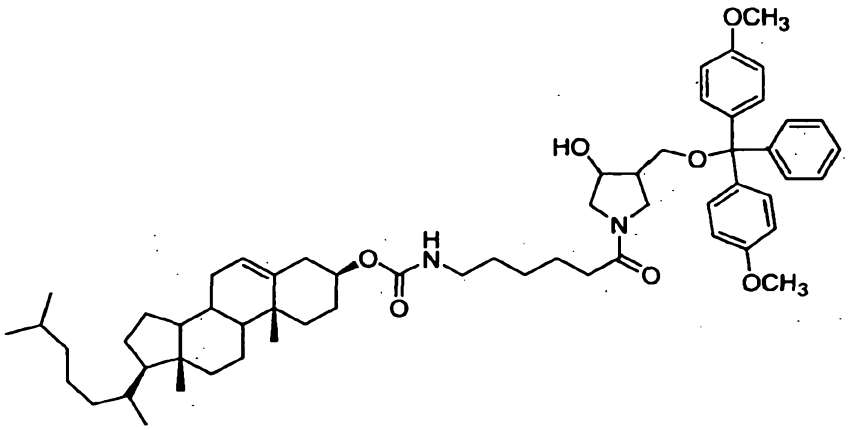
[6-(3-Hydroxy-4-hydroxymethyl-pyrrolidin-1-yl)-6-oxo-hexyl]-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AF**



AF

Methanol (2 mL) was added dropwise over a period of 1 h to a refluxing mixture of b-ketoester AE (1.5 g, 2.2 mmol) and sodium borohydride (0.226 g, 6 mmol) in tetrahydrofuran (10 mL). Stirring was continued at reflux temperature for 1 h. After cooling to room temperature, 1 N HCl (12.5 mL) was added, the mixture was extracted with ethylacetate (3 x 40 mL). The combined ethylacetate layer was dried over anhydrous sodium sulfate and concentrated under vacuum to yield the product which was purified by column chromatography (10% MeOH/$CHCl_3$) (89%).

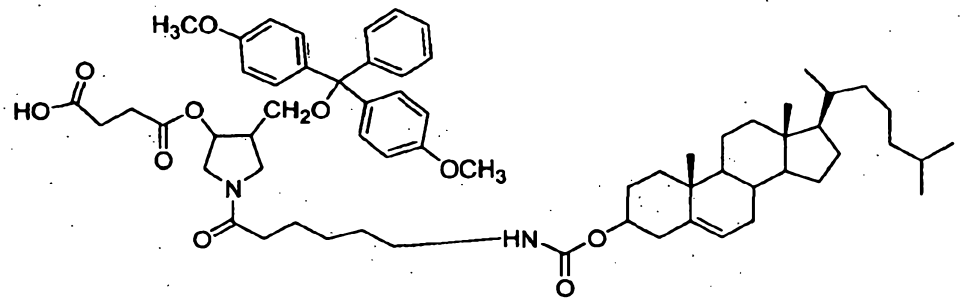
(6-{3-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-4-hydroxy-pyrrolidin-1-yl}-6-oxo-hexyl)-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AG**



AG

Diol AF (1.25 gm 1.994 mmol) was dried by evaporating with pyridine (2 x 5 mL) *in vacuo*. Anhydrous pyridine (10 mL) and 4,4'-dimethoxytritylchloride (0.724 g, 2.13 mmol) were added with stirring. The reaction was carried out at room temperature overnight. The reaction was quenched by the addition of methanol. The reaction mixture was concentrated under vacuum and to the residue dichloromethane (50 mL) was added. The organic layer was washed with 1M aqueous sodium bicarbonate. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residual pyridine was removed by evaporating with toluene. The crude product was purified by column chromatography (2% MeOH/Chloroform, Rf = 0.5 in 5% MeOH/$CHCl_3$) (1.75 g, 95%).

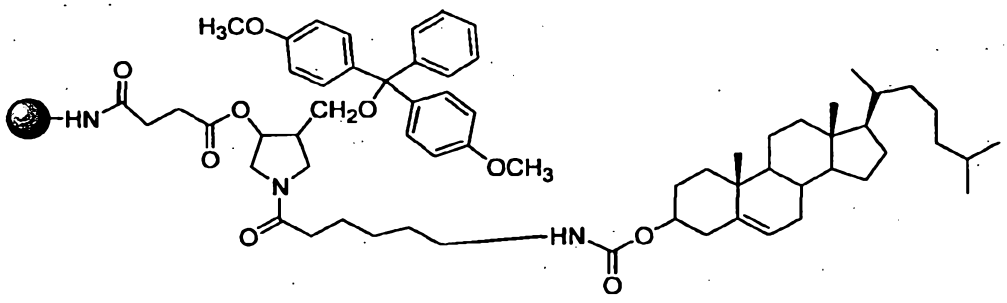
Succinic acid mono-(4-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-1-{6-[17-(1,5-dimethyl-hexyl)-10,13-dimethyl 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H cyclopenta[a]phenanthren-3-yloxycarbonylamino]-hexanoyl}-pyrrolidin-3-yl) ester **AH**



AH

Compound AG (1.0 g, 1.05 mmol) was mixed with succinic anhydride (0.150 g, 1.5 mmol) and DMAP (0.073 g, 0.6 mmol) and dried in a vacuum at 40°C overnight. The mixture was dissolved in anhydrous dichloroethane (3 mL), triethylamine (0.318 g, 0.440 mL, 3.15 mmol) was added and the solution was stirred at room temperature under argon atmosphere for 16 h. It was then diluted with dichloromethane (40 mL) and washed with ice cold aqueous citric acid (5 wt%, 30 mL) and water (2 X 20 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was used as such for the next step.

Cholesterol derivatised CPG **AI**



AI

Succinate AH (0.254 g, 0.242 mmol) was dissolved in a mixture of dichloromethane/acetonitrile (3:2, 3 mL). To that solution DMAP (0.0296 g, 0.242 mmol) in acetonitrile (1.25 mL), 2,2'-Dithio-bis(5-nitropyridine) (0.075 g, 0.242 mmol) in acetonitrile/dichloroethane (3:1, 1.25 mL) were added successively. To the resulting solution triphenylphosphine (0.064 g, 0.242 mmol) in acetonitrile (0.6 ml) was added. The reaction mixture turned bright orange in color. The solution was agitated briefly using a wrist-action shaker (5 mins). Long chain alkyl amine-CPG (LCAA-CPG) (1.5 g, 61 mM) was added. The suspension was agitated for 2 h. The CPG was filtered through a sintered funnel and washed with acetonitrile, dichloromethane and ether successively. Unreacted amino groups were masked using acetic anhydride/pyridine. The achieved loading of the CPG was measured by taking UV measurement (37 mM/g).

The synthesis of siRNAs bearing a 5'-12-dodecanoic acid bisdecylamide group (herein referred to as "5'-C32-") or a 5'-cholesteryl derivative group (herein referred to as "5'-Chol-") was performed as described in WO 2004/065601, except that, for the cholesteryl derivative, the oxidation step was performed using the Beaucage reagent in order to introduce a phosphorothioate linkage at the 5'-end of the nucleic acid oligomer.

dsRNA expression vectors

In another aspect of the invention, E6AP specific dsRNA molecules that modulate E6AP gene 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 E6AP gene or multiple E6AP genes 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 E6AP 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.

E6AP siRNA screening in HCT-116 cells

HCT-116 cells were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) (Braunschweig, Germany, cat. No. ACC 581) and cultured in McCoys (Biochrom AG, Berlin, Germany, cat. No. F1015) supplemented to contain 10% fetal calf serum (FCS), Penicillin 100 U/ml, Streptomycin 100 µg/ml and 2mM L-Glutamin at 37°C in an atmosphere with 5% CO_2 in a humidified incubator.

For transfection with siRNA, HCT-116 cells were seeded at a density of 2.0×10^4 cells/well in 96-well plates and transfected directly. Transfection of siRNA (30nM and 3nM for single dose screen) was carried out with lipofectamine 2000 (Invitrogen) as described by the manufacturer.

24 hours after transfection HCT-116 cells were lysed and E6AP mRNA expression levels were quantified with the Quantigene Explore Kit (Panomics, Inc. (Fremont, CA)(formerly Genospectra, Inc.)) according to the standard protocol. E6AP mRNA levels were normalized to GAP-DH mRNA. For each siRNA four individual datapoints were collected. siRNA duplexes unrelated to E6AP gene were used as control. The activity of a given E6AP specific siRNA duplex was expressed as percent E6AP mRNA concentration in treated cells relative to E6AP mRNA concentration in cells treated with the control siRNA duplex.

Table 2 below provides the results. Many active siRNA molecules that target the E6AP gene were identified.

Table 2 Activity of dsRNA targeting E6AP

duplex name	**mean activity at 30nM**	Standard deviation at 30nM	**mean activity at 3nM**	Standard deviation at 3nM
AL-DP-7545	9.35	3.36	14.04	3.82
AL-DP-7558	12.36	3.07	18.49	4.36
AL-DP-7548	12.55	5.85	18.92	4.72
AL-DP-7509	14.42	3.99	19.39	2.71
AL-DP-7492	11.25	2.53	19.61	7.89
AL-DP-7554	14.16	4.56	19.83	5.15
AL-DP-7557	16.00	6.50	19.97	7.04
AL-DP-7476	14.15	7.05	20.21	6.19
AL-DP-7514	24.01	12.46	20.54	6.13
AL-DP-7540	15.61	5.14	21.78	3.95
AL-DP-7397	13.05	5.68	22.03	11.42
AL-DP-7526	15.87	5.65	22.28	5.61
AL-DP-7473	17.22	6.09	22.65	6.64
AL-DP-7478	16.76	9.85	22.69	6.84
AL-DP-7553	23.50	5.15	23.19	3.34
AL-DP-7395	17.30	7.48	23.22	8.88
AL-DP-7522	26.16	10.71	23.51	8.18
AL-DP-7499	14.21	6.15	23.81	12.13
AL-DP-7527	24.11	5.05	23.98	8.89
AL-DP-7544	17.23	5.90	24.03	2.56
AL-DP-7489	23.56	10.21	24.54	7.57
AL-DP-7365	14.54	7.13	24.56	8.90
AL-DP-7390	16.44	6.37	24.74	6.73
AL-DP-7458	14.25	5.11	25.28	6.56
AL-DP-7532	21.47	4.18	25.48	6.40

AL-DP-7546	17.66	4.28	25.91	7.73
AL-DP-7512	27.88	6.58	26.22	5.07
AL-DP-7470	28.22	6.50	26.31	8.12
AL-DP-7406	20.23	6.01	26.62	6.35
AL-DP-7382	17.82	7.24	26.93	9.30
AL-DP-7547	24.63	6.66	28.80	10.23
AL-DP-7490	25.94	9.32	28.95	10.29
AL-DP-7493	12.53	5.36	29.56	12.54
AL-DP-7529	17.61	8.36	29.59	10.53
AL-DP-7400	21.03	14.86	30.04	12.58
AL-DP-7391	26.74	12.00	30.06	8.07
AL-DP-7393	22.40	9.77	30.69	8.51
AL-DP-7511	26.50	6.02	30.88	6.43
AL-DP-7454	25.16	14.85	31.09	8.75
AL-DP-7450	20.09	8.43	32.10	8.57
AL-DP-7533	26.93	5.86	33.91	5.52
AL-DP-7485	27.45	4.36	34.12	10.28
AL-DP-7495	28.51	13.42	34.45	11.20
AL-DP-7456	16.82	5.62	34.54	10.30
AL-DP-7538	29.04	5.12	34.71	6.42
AL-DP-7377	22.98	8.19	35.31	12.53
AL-DP-7405	21.93	10.30	35.66	15.22
AL-DP-7392	23.83	8.93	36.14	6.31
AL-DP-7453	25.78	12.10	36.98	5.22
AL-DP-7366	19.60	7.30	37.20	13.88
AL-DP-7534	26.35	5.24	37.69	8.49
AL-DP-7401	28.74	9.10	37.75	7.70
AL-DP-7523	33.88	6.85	39.81	9.45
AL-DP-7555	29.13	8.87	40.35	6.23
AL-DP-7536	32.33	3.49	41.08	8.00
AL-DP-7371	25.49	9.83	42.19	16.08
AL-DP-7372	21.83	12.03	42.87	17.78
AL-DP-7370	24.51	12.64	43.75	14.09
AL-DP-7474	32.57	13.13	44.40	7.78
AL-DP-7452	30.12	12.02	46.66	9.19
AL-DP-7498	32.38	11.81	54.11	12.74
AL-DP-7504	15.04	6.39	19.69	5.67
AL-DP-7467	19.81	6.42	21.66	8.12
AL-DP-7463	26.63	8.84	21.73	8.80
AL-DP-7399	15.62	8.32	22.98	7.65
AL-DP-7501	17.32	5.24	23.45	7.44
AL-DP-7385	17.60	5.11	28.00	11.84
AL-DP-7480	21.89	8.21	29.42	8.64
AL-DP-7528	26.47	2.94	30.76	10.87
AL-DP-7535	26.65	3.13	31.77	4.34

AL-DP-7403	24.10	6.21	38.79	14.41
AL-DP-7380	29.84	7.65	40.42	5.72
AL-DP-7469	17.18	7.41	21.13	6.29
AL-DP-7518	15.71	6.00	21.89	6.68
AL-DP-7464	29.18	12.30	22.13	8.99
AL-DP-7560	17.33	4.85	24.84	5.80
AL-DP-7461	30.55	8.26	25.62	9.48
AL-DP-7472	25.17	11.50	26.31	8.61
AL-DP-7459	29.60	7.71	27.27	9.68
AL-DP-7381	17.29	6.63	27.31	7.42
AL-DP-7515	32.18	10.22	29.76	6.01
AL-DP-7517	29.75	6.99	29.87	5.69
AL-DP-7521	28.60	8.06	31.68	5.72
AL-DP-7530	31.09	8.09	31.94	3.36
AL-DP-7388	22.81	3.80	32.28	6.23
AL-DP-7451	22.66	8.92	32.45	8.26
AL-DP-7484	26.77	13.00	32.84	6.95
AL-DP-7376	34.18	14.11	39.93	8.41
AL-DP-7500	32.69	8.47	41.55	13.32
AL-DP-7776	17.91	4.95	21.77	5.04
AL-DP-7777	21.10	7.60		
AL-DP-7510	34.70	5.83		
AL-DP-7507	35.11	6.78		
AL-DP-7479	35.29	13.76		
AL-DP-7542	36.32	5.00		
AL-DP-7494	38.34	12.68		
AL-DP-7531	38.58	14.26		
AL-DP-7373	39.04	16.08		
AL-DP-7508	39.95	12.87		
AL-DP-7487	40.48	15.20		
AL-DP-7375	41.19	15.06		
AL-DP-7462	41.61	17.23		
AL-DP-7513	41.69	9.15		
AL-DP-7455	43.35	12.72		
AL-DP-7374	43.37	12.26		
AL-DP-7475	43.68	11.45		
AL-DP-7369	43.99	15.44		
AL-DP-7466	44.27	15.53		
AL-DP-7491	45.06	10.32		
AL-DP-7482	45.06	12.37		
AL-DP-7398	45.79	9.50		
AL-DP-7471	46.11	13.53		
AL-DP-7383	46.87	20.08		

AL-DP-7367	46.96	16.88
AL-DP-7386	47.46	10.01
AL-DP-7525	49.60	14.11
AL-DP-7486	49.64	8.95
AL-DP-7539	49.97	12.73
AL-DP-7483	49.97	12.50
AL-DP-7503	51.28	7.08
AL-DP-7537	53.19	7.75
AL-DP-7396	54.11	13.02
AL-DP-7404	54.96	17.72
AL-DP-7543	55.48	9.23
AL-DP-7379	55.82	18.47
AL-DP-7502	56.15	16.52
AL-DP-7519	56.15	13.30
AL-DP-7506	57.24	21.04
AL-DP-7457	57.30	15.19
AL-DP-7468	57.83	15.40
AL-DP-7368	59.38	22.50
AL-DP-7402	59.57	13.42
AL-DP-7481	60.17	14.54
AL-DP-7465	61.44	28.49
AL-DP-7496	61.65	17.78
AL-DP-7549	61.90	12.36
AL-DP-7394	61.94	17.08
AL-DP-7477	63.20	14.74
AL-DP-7516	67.72	19.24
AL-DP-7556	69.49	13.89
AL-DP-7387	72.14	16.20
AL-DP-7524	72.52	19.76
AL-DP-7378	73.44	19.20
AL-DP-7389	73.74	23.83
AL-DP-7384	76.45	21.99
AL-DP-7497	77.66	22.60
AL-DP-7559	78.86	16.61
AL-DP-7520	85.45	14.83
AL-DP-7505	86.86	39.07
AL-DP-7460	100.95	22.69

Testing of Chemically Modified dsRNA Targeting E6AP

Chemically modified dsRNA were tested to identify their relative abilities to reduce the expression level of mRNA encoding E6AP in a cell. The assay conditions described above for HCT-116 cells were employed. The activity of a given E6AP specific siRNA duplex was expressed as percent E6AP mRNA concentration in treated cells relative to E6AP mRNA concentration in cells treated with the control siRNA duplex.

1. Chemically Modified dsRNA

Table 3 sets forth dsRNA compositions of the invention. In this table the unmodified sequence is followed by the same sequence containing one or more nucleotide modifications.

Table 3

Upper case letters: unmodified ribonucleotide (except for T which is an unmodified deoxyribonucleotide)
Lower case letters: ribonucloetide bearing 2'-O-methyl substituent on ribose moiety
s : Indicates position of phosphorothioate internucleoside linkage
chol : cholesterol moiety conjugated to 3' ribonucleotide.
'duplex name'means the name of the composition formed by specific hybridization of the indicated sense strand and the indicated antisense strand.

sense strand	SEQ ID	antisense strand	SEQ ID	
sequence (5'-3')	NO:	sequence (5'-3')	NO:	duplex name
AUACGAUGAAUCUACAAAATT	469	UUUUGUAGAUUCAUCGUAUTT	644	AL-DP-7545
AUACGAUGAAUCUACAAAATsT	470	UUUUGUAGAUUCAUCGUAUTsT	645	ND-8763
AuAcGAuGAAucuAcAAAATsT	471	UUUUGuAGAUUcAUCGuAUTsT	646	ND-8782
AuAcGAuGAAucuAcAAAATsT	472	uuuuGuAGAuUcAUCGuAUTsT	647	ND-8801

AUACGAUGAAUCUACAAAATTChol	473	UUUUGUAGAUUCAUCGUAUTsT	648	ND-8820
AuAcGAuGAAucuAcAAAATTchol	474	UUUUGuAGAUUcAUCGuAUTsT	649	ND-8845
AuAcGAuGAAucuAcAAAATTchol	475	uuuuGuAGAuUcAUCGuAUTsT	650	ND-8870
UGACUACAUUCUCAAUAAATT	476	UUUAUUGAGAAUGUAGUCATT	651	AL-DP-7558
UGACUACAUUCUCAAUAAATsT	477	UUUAUUGAGAAUGUAGUCATsT	652	ND-8764
uGAcuAcAuucucAAuAAATsT	478	UUuAUUGAGAAUGuAGUcATsT	653	ND-8783
uGAcuAcAuucucAAuAAATsT	479	uuuAuuGAGAAuGuAGUcATsT	654	ND-8802
UGACUACAUUCUCAAUAAATTChol	480	UUUAUUGAGAAUGUAGUCATsT	655	ND-8821
uGAcuAcAuucucAAuAAATTchol	481	UUuAUUGAGAAUGuAGUcATsT	656	ND-8846
uGAcuAcAuucucAAuAAATTchol	482	uuuAuuGAGAAuGuAGUcATsT	657	ND-8871
AGCCUGCACGAAUGAGUUUTT	483	AAACUCAUUCGUGCAGGCUTT	658	AL-DP-7548
AGCCUGCACGAAUGAGUUUTsT	484	AAACUCAUUCGUGCAGGCUTsT	659	ND-8765
AGccuGcAcGAAuGAGuuuTsT	485	AAACUcAUUCGUGcAGGCUTsT	660	ND-8784
AGccuGcAcGAAuGAGuuuTsT	486	AAACUcAuUCGuGcAGGCUTsT	661	ND-8803
AGCCUGCACGAAUGAGUUUTTChol	487	AAACUCAUUCGUGCAGGCUTsT	662	ND-8822
AGccuGcAcGAAuGAGuuuTTchol	488	AAACUcAUUCGUGcAGGCUTsT	663	ND-8847
AGccuGcAcGAAuGAGuuuTTchol	489	AAACUcAuUCGuGcAGGCUTsT	664	ND-8872
GGAUUGUCGAAAACCACUUTT	490	AAGUGGUUUUCGACAAUCCTT	665	AL-DP-7509
GGAUUGUCGAAAACCACUUTsT	491	AAGUGGUUUUCGACAAUCCTsT	666	ND-8766
GGAuuGucGAAAAccAcuuTsT	492	AAGUGGUUUUCGAcAAUCCTsT	667	ND-8785
GGAuuGucGAAAAccAcuuTsT	493	AAGuGGuUuUCGAcAAUCCTsT	668	ND-8804
GGAUUGUCGAAAACCACUUTTChol	494	AAGUGGUUUUCGACAAUCCTsT	669	ND-8823
GGAuuGucGAAAAccAcuuTTchol	495	AAGUGGUUUUCGAcAAUCCTsT	670	ND-8848
GGAuuGucGAAAAccAcuuTTchol	496	AAGuGGuUuUCGAcAAUCCTsT	671	ND-8873
CUCUCGAGAUCCUAAUUAUTT	497	AUAAUUAGGAUCUCGAGAGTT	672	AL-DP-7492
CUCUCGAGAUCCUAAUUAUTsT	498	AUAAUUAGGAUCUCGAGAGTsT	673	ND-8767
cucucGAGAuccuAAuuAuTsT	499	AuAAUuAGGAUCUCGAGAGTsT	674	ND-8786
cucucGAGAuccuAAuuAuTsT	500	AuAAuUAGGAUCUCGAGAGTsT	675	ND-8805
CUCUCGAGAUCCUAAUUAUTTChol	501	AUAAUUAGGAUCUCGAGAGTsT	676	ND-8824
cucucGAGAuccuAAuuAuTTchol	502	AuAAUuAGGAUCUCGAGAGTsT	677	ND-8849
cucucGAGAuccuAAuuAuTTchol	503	AuAAuUAGGAUCUCGAGAGTsT	678	ND-8874
AUGUGACUUACUUAACAGATT	504	UCUGUUAAGUAAGUCACAUTT	679	AL-DP-7554
AUGUGACUUACUUAACAGATsT	505	UCUGUUAAGUAAGUCACAUTsT	680	ND-8768
AuGuGAcuuAcuuAAcAGATsT	506	UCUGUuAAGuAAGUcAcAUTsT	681	ND-8787
AuGuGAcuuAcuuAAcAGATsT	507	UCuGuuAAGuAAGUcAcAUTsT	682	ND-8806
AUGUGACUUACUUAACAGATTChol	508	UCUGUUAAGUAAGUCACAUTsT	683	ND-8825
AuGuGAcuuAcuuAAcAGATTchol	509	UCUGUuAAGuAAGUcAcAUTsT	684	ND-8850
AuGuGAcuuAcuuAAcAGATTchol	510	UCuGuuAAGuAAGUcAcAUTsT	685	ND-8875
GUAUACUCUCGAGAUCCUATT	511	UAGGAUCUCGAGAGUAUACTT	686	AL-DP-7557

GUAUACUCUCGAGAUCCUATsT	512	UAGGAUCUCGAGAGUAUACTsT	687	ND-8769
GuAuAcucucGAGAuccuATsT	513	uAGGAUCUCGAGAGuAuACTsT	688	ND-8788
GuAuAcucucGAGAuccuATsT	514	uAGGAUCUCGAGAGuAuACTsT	689	ND-8788
GUAUACUCUCGAGAUCCUATTChol	515	UAGGAUCUCGAGAGUAUACTsT	690	ND-8826
GuAuAcucucGAGAuccuATTchol	516	uAGGAUCUCGAGAGuAuACTsT	691	ND-8851
GuAuAcucucGAGAuccuATTchol	517	uAGGAUCUCGAGAGuAuACTsT	692	ND-8851
AGGUUACCUACAUCUCAUATT	518	UAUGAGAUGUAGGUAACCUTT	693	AL-DP-7476
AGGUUACCUACAUCUCAUATsT	519	UAUGAGAUGUAGGUAACCUTsT	694	ND-8770
AGGuuAccuAcAucucAuATsT	520	uAUGAGAUGuAGGuAACCUTsT	695	ND-8789
AGGuuAccuAcAucucAuATsT	521	uAuGAGAuGuAGGuAACCUTsT	696	ND-8808
AGGUUACCUACAUCUCAUATTChol	522	UAUGAGAUGUAGGUAACCUTsT	697	ND-8827
AGGuuAccuAcAucucAuATTchol	523	uAUGAGAUGuAGGuAACCUTsT	698	ND-8852
AGGuuAccuAcAucucAuATTchol	524	uAuGAGAuGuAGGuAACCUTsT	699	ND-8877
AGUACUUAUUCAGACCAGATT	525	UCUGGUCUGAAUAAGUACUTT	700	AL-DP-7514
AGUACUUAUUCAGACCAGATsT	526	UCUGGUCUGAAUAAGUACUTsT	701	ND-8771
AGuAcuuAuucAGAccAGATsT	527	UCUGGUCUGAAuAAGuACUTsT	702	ND-8790
AGuAcuuAuucAGAccAGATsT	528	UCuGGUCuGAAuAAGuACUTsT	703	ND-8809
AGUACUUAUUCAGACCAGATTChol	529	UCUGGUCUGAAUAAGUACUTsT	704	ND-8828
AGuAcuuAuucAGAccAGATTchol	530	UCUGGUCUGAAuAAGuACUTsT	705	ND-8853
AGuAcuuAuucAGAccAGATTchol	531	UCuGGUCuGAAuAAGuACUTsT	706	ND-8878
AUCCUAAUUAUCUGAAUUUTT	532	AAAUUCAGAUAAUUAGGAUTT	707	AL-DP-7540
AUCCUAAUUAUCUGAAUUUTsT	533	AAAUUCAGAUAAUUAGGAUTsT	708	ND-8772
AuccuAAuuAucuGAAuuuTsT	534	AAAUUcAGAuAAUuAGGAUTsT	709	ND-8791
AuccuAAuuAucuGAAuuuTsT	535	AAAuUcAGAuAAuUAGGAUTsT	710	ND-8810
AUCCUAAUUAUCUGAAUUUTTChol	536	AAAUUCAGAUAAUUAGGAUTsT	711	ND-8829
AuccuAAuuAucuGAAuuuTTchol	537	AAAUUcAGAuAAUuAGGAUTsT	712	ND-8854
AuccuAAuuAucuGAAuuuTTchol	538	AAAuUcAGAuAAuUAGGAUTsT	713	ND-8879
AAGGAUAGGUGAUAGCUCATT	539	UGAGCUAUCACCUAUCCUUTT	714	AL-DP-7397
AAGGAUAGGUGAUAGCUCATsT	540	UGAGCUAUCACCUAUCCUUTsT	715	ND-8731
AAGGAuAGGuGAuAGcucATsT	541	UGAGCuAUcACCuAUCCUUTsT	716	ND-8743
AAGGAuAGGuGAuAGcucATsT	542	uGAGCuAUcACCuAUCCuuTsT	717	ND-8754
AAGGAUAGGUGAUAGCUCATTChol	543	UGAGCUAUCACCUAUCCUUTsT	718	ND-8839
AAGGAuAGGuGAuAGcucATTchol	544	UGAGCuAUcACCuAUCCUUTsT	719	ND-8864
AAGGAuAGGuGAuAGcucATTchol	545	uGAGCuAUcACCuAUCCuuTsT	720	ND-8889
GGAAGCCGGAAUCUAGAUUTT	546	AAUCUAGAUUCCGGCUUCCTT	721	AL-DP-7526
GGAAGCCGGAAUCUAGAUUTsT	547	AAUCUAGAUUCCGGCUUCCTsT	722	ND-8773
GGAAGccGGAAucuAGAuuTsT	548	AAUCuAGAUUCCGGCUUCCTsT	723	ND-8792
GGAAGccGGAAucuAGAuuTsT	549	AAUCuAGAuUCCGGCuUCCTsT	724	ND-8811
GGAAGCCGGAAUCUAGAUUTTChol	550	AAUCUAGAUUCCGGCUUCCTsT	725	ND-8830

GGAAGccGGAAucuAGAuuTTchol	551	AAUCuAGAUUCCGGCUUCCTsT	726	ND-8855
GGAAGccGGAAucuAGAuuTTchol	552	AAUCuAGAuUCCGGCuUCCTsT	727	ND-8880
UGCUUCGAAGUGCUUGAAATT	553	UUUCAAGCACUUCGAAGCATT	728	AL-DP-7473
UGCUUCGAAGUGCUUGAAATsT	554	UUUCAAGCACUUCGAAGCATsT	729	ND-8774
uGcuucGAAGuGcuuGAAATsT	555	UUUcAAGcACUUCGAAGcATsT	730	ND-8793
uGcuucGAAGuGcuuGAAATsT	556	uuUcAAGcACuUCGAAGcATsT	731	ND-8812
UGCUUCGAAGUGCUUGAAATTChol	557	UUUCAAGCACUUCGAAGCATsT	732	ND-8831
uGcuucGAAGuGcuuGAAATTchol	558	UUUcAAGcACUUCGAAGcATsT	733	ND-8856
uGcuucGAAGuGcuuGAAATTchol	559	uuUcAAGcACuUCGAAGcATsT	734	ND-8881
UGGAUUGUCGAAAACCACUTT	560	AGUGGUUUUCGACAAUCCATT	735	AL-DP-7478
UGGAUUGUCGAAAACCACUTsT	561	AGUGGUUUUCGACAAUCCATsT	736	ND-8775
uGGAuuGucGAAAAccAcuTsT	562	AGUGGUUUUCGAcAAUCcATsT	737	ND-8794
uGGAuuGucGAAAAccAcuTsT	563	AGuGGuuuUCGAcAAUCcATsT	738	ND-8813
UGGAUUGUCGAAAACCACUTTChol	564	AGUGGUUUUCGACAAUCCATsT	739	ND-8832
uGGAuuGucGAAAAccAcuTTchol	565	AGUGGUUUUCGAcAAUCcATsT	740	ND-8857
uGGAuuGucGAAAAccAcuTTchol	566	AGuGGuuuUCGAcAAUCcATsT	741	ND-8882
CGGCUAGAGAUGAUCGCUATT	567	UAGCGAUCAUCUCUAGCCGTT	742	AL-DP-7553
CGGCUAGAGAUGAUCGCUATsT	568	UAGCGAUCAUCUCUAGCCGTsT	743	ND-8776
cGGcuAGAGAuGAucGcuATsT	569	uAGCGAUcAUCUCuAGCCGTsT	744	ND-8795
cGGcuAGAGAuGAucGcuATsT	570	uAGCGAUcAUCUCuAGCCGTsT	745	ND-8795
CGGCUAGAGAUGAUCGCUATTChol	571	UAGCGAUCAUCUCUAGCCGTsT	746	ND-8833
cGGcuAGAGAuGAucGcuATTchol	572	uAGCGAUcAUCUCuAGCCGTsT	747	ND-8858
cGGcuAGAGAuGAucGcuATTchol	573	uAGCGAUcAUCUCuAGCCGTsT	748	ND-8858
ACAGUCGAAAUCUAGUGAATT	574	UUCACUAGAUUUCGACUGUTT	749	AL-DP-7395
ACAGUCGAAAUCUAGUGAATsT	575	UUCACUAGAUUUCGACUGUTsT	750	ND-8730
AcAGucGAAAucuAGuGAATsT	576	UUcACuAGAUUUCGACUGUTsT	751	ND-8742
AcAGucGAAAucuAGuGAATsT	577	uucACuAGAuuUCGACuGUTsT	752	ND-8753
ACAGUCGAAAUCUAGUGAATTChol	578	UUCACUAGAUUUCGACUGUTsT	753	ND-8840
AcAGucGAAAucuAGuGAATTchol	579	UUcACuAGAUUUCGACUGUTsT	754	ND-8865
AcAGucGAAAucuAGuGAATTchol	580	uucACuAGAuuUCGACuGUTsT	755	ND-8890
CUCGAGAUCCUAAUUAUCUTT	581	AGAUAAUUAGGAUCUCGAGTT	756	AL-DP-7499
CUCGAGAUCCUAAUUAUCUTsT	582	AGAUAAUUAGGAUCUCGAGTsT	757	ND-8777
cucGAGAuccuAAuuAucuTsT	583	AGAuAAUuAGGAUCUCGAGTsT	758	ND-8796
cucGAGAuccuAAuuAucuTsT	584	AGAuAAuUAGGAUCUCGAGTsT	759	ND-8815
CUCGAGAUCCUAAUUAUCUTTChol	585	AGAUAAUUAGGAUCUCGAGTsT	760	ND-8834
cucGAGAuccuAAuuAucuTTchol	586	AGAuAAUuAGGAUCUCGAGTsT	761	ND-8859
cucGAGAuccuAAuuAucuTTchol	587	AGAuAAuUAGGAUCUCGAGTsT	762	ND-8884
CACCUAACGUGGAAUGUGATT	588	UCACAUUCCACGUUAGGUGTT	763	AL-DP-7365
CACCUAACGUGGAAUGUGATsT	589	UCACAUUCCACGUUAGGUGTsT	764	ND-8724

cAccuAAcGuGGAAuGuGATsT	590	UcAcAUUCcACGUuAGGUGTsT	765	ND-8736
cAccuAAcGuGGAAuGuGATsT	591	UcAcAuuCcACGuuAGGuGTsT	766	ND-8748
CACCUAACGUGGAAUGUGATTChol	592	UCACAUUCCACGUUAGGUGTsT	767	ND-8841
cAccuAAcGuGGAAuGuGATTchol	593	UcAcAUUCcACGUuAGGUGTsT	768	ND-8866
cAccuAAcGuGGAAuGuGATTchol	594	UcAcAuuCcACGuuAGGuGTsT	769	ND-8891
AAUCGUUCAUUCAUUUACATT	595	UGUAAAUGAAUGAACGAUUTT	770	AL-DP-7390
AAUCGUUCAUUCAUUUACATsT	596	UGUAAAUGAAUGAACGAUUTsT	771	ND-8727
AAucGuucAuucAuuuAcATsT	597	UGuAAAUGAAUGAACGAUUTsT	772	ND-8739
AAucGuucAuucAuuuAcATsT	598	uGuAAAuGAAuGAACGAuuTsT	773	ND-8750
AAUCGUUCAUUCAUUUACATTChol	599	UGUAAAUGAAUGAACGAUUTsT	774	ND-8842
AAucGuucAuucAuuuAcATTchol	600	UGuAAAUGAAUGAACGAUUTsT	775	ND-8867
AAucGuucAuucAuuuAcATTchol	601	uGuAAAuGAAuGAACGAuuTsT	776	ND-8892
AACUUUUCGUGACUUGGGATT	602	UCCCAAGUCACGAAAAGUUTT	777	AL-DP-7382
AACUUUUCGUGACUUGGGATsT	603	UCCCAAGUCACGAAAAGUUTsT	778	ND-8726
AAcuuuucGuGAcuuGGGATsT	604	UCCcAAGUcACGAAAAGUUTsT	779	ND-8738
AAcuuuucGuGAcuuGGGATsT	605	UCCcAAGUcACGAAAAGuuTsT	780	ND-8749
AACUUUUCGUGACUUGGGATTChol	606	UCCCAAGUCACGAAAAGUUTsT	781	ND-8843
AAcuuuucGuGAcuuGGGATTchol	607	UCCcAAGUcACGAAAAGUUTsT	782	ND-8868
AAcuuuucGuGAcuuGGGATTchol	608	UCCcAAGUcACGAAAAGuuTsT	783	ND-8893
AACAGUCGAAAUCUAGUGATT	609	UCACUAGAUUUCGACUGUUTT	784	AL-DP-7393
AACAGUCGAAAUCUAGUGATsT	610	UCACUAGAUUUCGACUGUUTsT	785	ND-8729
AAcAGucGAAAucuAGuGATsT	611	UcACuAGAUUUCGACUGUUTsT	786	ND-8741
AAcAGucGAAAucuAGuGATsT	612	UcACuAGAuuUCGACuGuuTsT	787	ND-8752
AACAGUCGAAAUCUAGUGATTChol	613	UCACUAGAUUUCGACUGUUTsT	788	ND-8844
AAcAGucGAAAucuAGuGATTchol	614	UcACuAGAUUUCGACUGUUTsT	789	ND-8869
AAcAGucGAAAucuAGuGATTchol	615	UcACuAGAuuUCGACuGuuTsT	790	ND-8894
ACGAAUGAGUUUUGUGCUUTT	616	AAGCACAAAACUCAUUCGUTT	791	AL-DP-7366
ACGAAUGAGUUUUGUGCUUTsT	617	AAGCACAAAACUCAUUCGUTsT	792	ND-8778
AcGAAuGAGuuuuGuGcuuTsT	618	AAGcAcAAAACUcAUUCGUTsT	793	ND-8797
AcGAAuGAGuuuuGuGcuuTsT	619	AAGcAcAAAACUcAuUCGUTsT	794	ND-8816
ACGAAUGAGUUUUGUGCUUTTChol	620	AAGCACAAAACUCAUUCGUTsT	795	ND-8835
AcGAAuGAGuuuuGuGcuuTTchol	621	AAGcAcAAAACUcAUUCGUTsT	796	ND-8860
AcGAAuGAGuuuuGuGcuuTTchol	622	AAGcAcAAAACUcAuUCGUTsT	797	ND-8885
AAUUCGCAUGUACAGUGAATT	623	UUCACUGUACAUGCGAAUUTT	798	AL-DP-7371
AAUUCGCAUGUACAGUGAATsT	624	UUCACUGUACAUGCGAAUUTsT	799	ND-8779
AAuucGcAuGuAcAGuGAATsT	625	UUcACUGuAcAUGCGAAUUTsT	800	ND-8798
AAuucGcAuGuAcAGuGAATsT	626	uUcACuGuAcAuGCGAAuUTsT	801	ND-8817
AAUUCGCAUGUACAGUGAATTChol	627	UUCACUGUACAUGCGAAUUTsT	802	ND-8836
AAuucGcAuGuAcAGuGAATTchol	628	UUcACUGuAcAUGCGAAUUTsT	803	ND-8861

AAuucGcAuGuAcAGuGAATTchol	629	uUcACuGuAcAuGCGAAuUTsT	804	ND-8886
AAUAGAAUUCGCAUGUACATT	630	UGUACAUGCGAAUUCUAUUTT	805	AL-DP-7372
AAUAGAAUUCGCAUGUACATsT	631	UGUACAUGCGAAUUCUAUUTsT	806	ND-8780
AAuAGAAuucGcAuGuAcATsT	632	UGuAcAUGCGAAUUCuAUUTsT	807	ND-8799
AAuAGAAuucGcAuGuAcATsT	633	uGuAcAuGCGAAuUCuAuUTsT	808	ND-8818
AAUAGAAUUCGCAUGUACATTChol	634	UGUACAUGCGAAUUCUAUUTsT	809	ND-8837
AAuAGAAuucGcAuGuAcATTchol	635	UGuAcAUGCGAAUUCuAUUTsT	810	ND-8862
AAuAGAAuucGcAuGuAcATTchol	636	uGuAcAuGCGAAuUCuAuUTsT	811	ND-8887
UGGUAACCCAAUGAUGUAUTT	637	AUACAUCAUUGGGUUACCATT	812	AL-DP-7370
UGGUAACCCAAUGAUGUAUTsT	638	AUACAUCAUUGGGUUACCATsT	813	ND-8781
uGGuAAcccAAuGAuGuAuTsT	639	AuAcAUcAUUGGGUuACcATsT	814	ND-8800
uGGuAAcccAAuGAuGuAuTsT	640	AuAcAUcAuUGGGuUACcATsT	815	ND-8819
UGGUAACCCAAUGAUGUAUTTChol	641	AUACAUCAUUGGGUUACCATsT	816	ND-8838
uGGuAAcccAAuGAuGuAuTTchol	642	AuAcAUcAUUGGGUuACcATsT	817	ND-8863
uGGuAAcccAAuGAuGuAuTTchol	643	AuAcAUcAuUGGGuUACcATsT	818	ND-8888
cucGAGAuccuAAuuAucuTsT	1748	AGAuAAuuAGGAUCUCGAGTst	1749	ND-9300

Table 4 sets forth the results of testing of dsRNA listed in Table 3.

Table 4

Duplex name	Mean activity remaining after 30nM treatment	Standard deviation	Mean activity remaining after 100pM treatment	Standard deviation
AL-DP-7545	6.74	1.80	18.41	4.14
ND-8763	6.28	1.79	21.38	5.93
ND-8782	7.21	1.59	23.76	7.49
ND-8801	18.52	2.58	51.10	12.01
ND-8820	9.26	1.88	58.34	10.61
ND-8845	34.08	7.03	69.28	14.97
ND-8870	30.96	5.97	77.58	12.41
AL-DP-7558	11.93	1.66	25.71	3.57
ND-8764	8.97	1.53	25.81	7.79
ND-8783	27.33	3.10	51.35	6.52
ND-8802	28.82	4.39	91.90	14.32
ND-8821	8.96	2.36	71.92	10.68

ND-8846	75.94	17.07	88.87	7.90
ND-8871	58.02	9.96	91.79	13.92
AL-DP-7548	11.23	1.92	35.58	6.27
ND-8765	8.24	1.01	45.42	10.63
ND-8784	25.07	4.28	68.74	7.10
ND-8803	45.89	10.22	97.46	12.87
ND-8822	11.59	2.94	75.22	17.11
ND-8847	64.96	9.30	100.47	16.50
ND-8872	78.50	14.11	86.77	5.96
AL-DP-7509	17.62	2.26	21.58	2.98
ND-8766	15.26	1.22	25.45	2.92
ND-8785	19.66	3.35	43.13	4.50
ND-8804	21.66	2.34	50.36	8.66
ND-8823	15.66	2.09	48.14	4.88
ND-8848	27.84	3.58	95.42	20.53
ND-8873	29.97	3.32	91.79	13.36
AL-DP-7492	11.09	1.19	19.22	3.29
ND-8767	11.90	1.73	20.65	2.66
ND-8786	11.69	1.72	19.78	2.74
ND-8805	14.97	1.46	26.41	6.08
ND-8824	11.53	1.51	43.76	5.00
ND-8849	25.37	11.97	43.95	10.44
ND-8874	16.84	2.99	53.87	6.12
AL-DP-7554	15.01	1.22	23.48	4.39
ND-8768	14.46	1.30	26.79	4.77
ND-8787	15.20	2.47	24.76	4.44
ND-8806	15.01	2.02	33.77	10.43
ND-8825	17.00	3.82	72.33	14.34
ND-8850	29.25	7.49	93.94	19.23
ND-8875	23.33	3.94	79.79	9.03
AL-DP-7557	13.10	1.34	22.30	8.07
ND-8769	11.17	1.10	24.91	4.44
ND-8788	21.84	2.02	60.20	10.58
ND-8788	23.53	1.55	69.43	13.87
ND-8826	12.81	1.35	50.68	10.86
ND-8851	36.41	3.49	116.14	48.06
ND-8851	36.42	5.05	100.91	26.50
AL-DP-7476	17.11	2.75	25.33	7.43
ND-8770	13.36	1.65	30.58	8.25

ND-8789	46.06	6.35	76.12	14.80
ND-8808	43.15	5.55	98.81	21.90
ND-8827	14.76	2.03	56.08	13.96
ND-8852	70.35	13.51	107.70	22.62
ND-8877	58.73	8.08	90.83	10.87
AL-DP-7514	15.63	2.76	18.89	0.67
ND-8771	14.96	1.69	23.31	10.62
ND-8790	15.91	1.57	31.71	2.88
ND-8809	16.79	2.80	36.42	5.40
ND-8828	14.61	2.09	53.50	8.13
ND-8853	34.20	4.88	81.95	16.33
ND-8878	26.63	2.95	87.21	33.73
AL-DP-7540	18.18	3.06	32.59	5.25
ND-8772	19.31	2.99	36.01	5.41
ND-8791	35.43	4.60	55.34	7.39
ND-8810	17.83	2.64	25.48	7.36
ND-8829	18.93	3.20	68.53	14.55
ND-8854	50.71	6.95	89.19	9.26
ND-8879	21.76	5.10	62.43	16.86
AL-DP-7397	17.10	2.37	22.44	4.36
ND-8731	17.09	2.86	31.25	8.34
ND-8743	15.89	2.29	27.33	4.67
ND-8754	19.53	2.97	41.57	9.22
ND-8839	18.18	2.95	66.39	13.77
ND-8864	19.51	3.79	59.13	5.60
ND-8889	19.91	2.30	92.91	14.85
AL-DP-7526	17.67	2.32	41.09	7.63
ND-8773	15.59	1.57	42.07	6.55
ND-8792	19.42	2.08	46.87	6.99
ND-8811	34.56	7.82	72.57	9.85
ND-8830	19.49	3.09	69.87	7.25
ND-8855	27.49	4.52	85.38	13.45
ND-8880	38.04	6.41	87.78	13.97
AL-DP-7473	15.81	2.07	31.86	6.43
ND-8774	15.81	2.61	30.89	8.60
ND-8793	14.04	1.44	25.98	2.91
ND-8812	21.16	3.28	49.59	8.12
ND-8831	19.07	3.60	75.06	16.79
ND-8856	17.86	5.51	65.08	11.14

ND-8881	28.56	6.18	83.97	12.49
AL-DP-7478	16.41	2.58	33.38	6.20
ND-8775	16.74	1.63	31.52	2.92
ND-8794	19.05	3.19	24.88	3.34
ND-8813	17.04	2.34	26.53	3.90
ND-8832	16.40	2.16	66.67	15.18
ND-8857	26.53	6.20	69.13	9.30
ND-8882	20.04	2.43	68.67	8.67
AL-DP-7553	20.83	2.66	28.97	4.93
ND-8776	21.10	2.76	29.95	5.15
ND-8795	26.00	3.54	79.53	11.78
ND-8795	25.14	3.95	80.83	12.02
ND-8833	21.76	3.23	52.28	7.24
ND-8858	33.25	7.09	92.18	20.40
ND-8858	31.50	5.36	84.22	13.01
AL-DP-7395	18.01	2.33	25.01	4.17
ND-8730	18.63	2.22	35.55	6.30
ND-8742	18.04	2.92	29.24	6.48
ND-8753	19.03	3.21	50.35	10.66
ND-8840	24.81	3.87	81.78	17.12
ND-8865	27.65	3.29	72.55	12.44
ND-8890	22.03	1.60	105.32	26.89
AL-DP-7499	12.40	1.94	25.24	3.83
ND-8777	12.78	2.14	25.07	6.35
ND-8796	11.28	0.83	21.19	2.68
ND-8815	10.85	1.12	27.56	7.33
ND-8834	9.88	1.77	48.81	8.56
ND-8859	38.05	5.09	56.68	8.15
ND-8884	38.13	7.42	75.98	15.04
AL-DP-7365	15.72	2.57	23.60	5.58
ND-8724	14.88	2.37	27.95	11.09
ND-8736	71.51	11.99	81.07	19.08
ND-8748	71.98	14.80	82.12	16.76
ND-8841	18.39	3.01	66.82	19.67
ND-8866	79.40	15.36	80.86	15.81
ND-8891	73.79	17.04	86.53	21.21
AL-DP-7390	17.45	3.14	30.46	4.87
ND-8727	17.98	3.47	44.60	4.60
ND-8739	23.47	4.83	53.99	8.89

ND-8750	25.98	3.55	83.20	10.09
ND-8842	21.10	2.77	109.29	34.23
ND-8867	44.74	4.83	91.06	22.68
ND-8892	57.70	9.50	96.07	23.52
AL-DP-7382	16.90	3.54	30.39	3.91
ND-8726	17.17	3.84	38.93	6.26
ND-8738	19.51	2.77	41.20	3.80
ND-8749	17.03	3.66	34.11	8.30
ND-8843	26.36	4.99	83.57	8.12
ND-8868	26.78	3.25	88.44	7.96
ND-8893	22.70	2.05	86.71	12.41
AL-DP-7393	24.38	3.02	38.04	7.48
ND-8729	29.07	4.34	59.65	11.35
ND-8741	68.38	7.91	87.12	8.74
ND-8752	50.68	7.27	86.26	11.15
ND-8844	36.14	5.29	102.26	16.83
ND-8869	71.02	12.42	97.57	17.41
ND-8894	52.86	8.43	106.24	17.77
AL-DP-7366	18.69	2.05	44.08	7.35
ND-8778	18.46	2.08	41.29	5.42
ND-8797	15.49	2.21	36.71	5.29
ND-8816	13.61	1.76	33.13	6.21
ND-8835	22.00	3.84	76.17	11.70
ND-8860	17.81	4.03	68.48	8.32
ND-8885	15.76	2.33	70.03	10.95
AL-DP-7371	18.77	2.20	52.94	11.86
ND-8779	19.88	2.86	56.27	9.68
ND-8798	24.79	4.89	59.87	8.65
ND-8817	26.06	2.89	85.76	15.79
ND-8836	33.17	7.60	87.15	20.65
ND-8861	78.78	18.21	88.22	14.97
ND-8886	70.66	10.62	96.55	14.35
AL-DP-7372	19.23	3.22	46.80	10.62
ND-8780	19.94	0.97	62.29	12.87
ND-8799	42.73	4.67	81.61	10.68
ND-8818	88.99	6.53	104.90	8.74
ND-8837	25.18	3.70	99.41	12.93
ND-8862	79.80	15.30	92.36	10.28
ND-8887	78.27	16.96	92.55	14.48

AL-DP-7370	20.04	1.52	46.57	9.68
ND-8781	16.68	1.83	62.43	12.82
ND-8800	41.69	5.44	77.39	12.71
ND-8819	35.98	3.15	78.56	17.49
ND-8838	21.49	3.65	84.71	30.30
ND-8863	59.44	19.74	91.91	17.80
ND-8888	49.74	16.09	97.57	15.46

Design of siRNA Targeting HPV E1 Gene Expression

Table 5 sets forth dsRNA compositions of the invention.

Table 5

Upper case letters: unmodified ribonucleotide (except for T which is an unmodified deoxyribonucleotide)
Lower case letters: ribonucloetide bearing 2'-O-methyl substituent on ribose moiety
s : Indicates position of phosphorothioate internucleoside linkage
chol : cholesterol moiety conjugated to 3' ribonucleotide.
'duplex name'means the name of the composition formed by specific hybridization of the indicated sense strand and the indicated antisense strand.

Target sequence of mRNA from HPV E1 reference sequence (sequence of total 19mer target site + AA at both ends)	SEQ ID. NO.	Sense strand (target sequence) having double TT overhang (5'-3')	SEQ ID. NO.	antisense strand (guide sequence) having double TT overhang (5'-3')	SEQ ID. NO.	duplex name
AAAAAUCAACGUGUUGCGAUUAA	819	AAAUCAACGUGUUGCGAUUTT	945	AAUCGCAACACGUUGAUUUTT	1141	ND-9061
AAGAGCCUCCAAAAUUGCGUAAA	820	GAGCCUCCAAAAUUGCGUATT	946	UACGCAAUUUUGGAGGCUCTT	1142	ND-9062
AAUCAACGUGUUGCGAUUGGUAA	821	UCAACGUGUUGCGAUUGGUTT	947	ACCAAUCGCAACACGUUGATT	1143	ND-9063
AAUCCAAAAUUGCGUAGUACAAA	822	UCCAAAAUUGCGUAGUACATT	948	UGUACUACGCAAUUUUGGATT	1144	ND-9064
AAAAUCAACGUGUUGCGAUUGAA	823	AAUCAACGUGUUGCGAUUGTT	949	CAAUCGCAACACGUUGAUUTT	1145	ND-9065
AACCUCCAAAAUUGCGUAGUAAA	824	CCUCCAAAAUUGCGUAGUATT	950	UACUACGCAAUUUUGGAGGTT	1146	ND-9066
AAAGAGCCUCCAAAAUUGCGUAA	825	AGAGCCUCCAAAAUUGCGUTT	951	ACGCAAUUUUGGAGGCUCUTT	1147	ND-9067
AACAACGUGUUGCGAUUGGUGAA	826	CAACGUGUUGCGAUUGGUGTT	952	CACCAAUCGCAACACGUUGTT	1148	ND-9068
AAAUAGAUGUGAUAGGGUAGAAA	827	AUAGAUGUGAUAGGGUAGATT	953	UCUACCCUAUCACAUCUAUTT	1149	ND-9069
AAGGGAAGAGGGUACGGGAUGAA	828	GGGAAGAGGGUACGGGAUGTT	954	CAUCCCGUACCCUCUUCCCTT	1150	ND-9070
AAAGAUUAAGUUUGCACGAGGAA	829	AGAUUAAGUUUGCACGAGGTT	955	CCUCGUGCAAACUUAAUCUTT	1151	ND-9071
AAGGUAUCAAGGUGUAGAGUUAA	830	GGUAUCAAGGUGUAGAGUUTT	956	AACUCUACACCUUGAUACCTT	1152	ND-9072

AAACUUAGUGAUAUUAGUGGAAA	831	ACUUAGUGAUAUUAGUGGATT	957	UCCACUAAUAUCACUAAGUTT	1153	ND-9073
AAGAGAUUAUUUGAAAGCGAAAA	832	GAGAUUAUUUGAAAGCGAATT	958	UUCGCUUUCAAAUAAAUCUCTT	1154	ND-9074
AAAACACCAUGUAGUCAGUAUAA	833	AACACCAUGUAGUCAGUAUTT	959	AUACUGACUACAUGGUGUUTT	1155	ND-9075
AAAGCCUCCCAAAAUUGCGUAGAA	834	AGCCUCCCAAAAUUGCGUAGTT	960	CUACGCAAUUUUGGAGGCUTT	1156	ND-9076
AAGCCUCCCAAAAUUGCGUAGUAA	835	GCCUCCCAAAAUUGCGUAGUTT	961	ACUACGCAAUUUUGGAGGCTT	1157	ND-9077
AAGUGUAUGGAGACACGCCAGAA	836	GUGUAUGGAGACACGCCAGTT	962	CUGGCGUGUCUCCAUACACTT	1158	ND-9078
AAGUACAAUGGGCCUACGAUAAA	837	GUACAAUGGGCCUACGAUATT	963	UAUCGUAGGCCCAUUGUACTT	1159	ND-9079
AAUACAAUGGGCCUACGAUAAAA	838	UACAAUGGGCCUACGAUAATT	964	UUAUCGUAGGCCCAUUGUATT	1160	ND-9080
AAUGACAUAGUAGACGAUAGUAA	839	UGACAUAGUAGACGAUAGUTT	965	ACUAUCGUCUACUAUGUCATT	1161	ND-9081
AAGACAUAGUAGACGAUAGUGAA	840	GACAUAGUAGACGAUAGUGTT	966	CACUAUCGUCUACUAUGUCTT	1162	ND-9082
AAACUCUUUGCCAACGUUUAAAA	841	ACUCUUUGCCAACGUUUAATT	967	UUAAACGUUGGCAAAGAGUTT	1163	ND-9083
AAAUAAUGACAUAGUAGACGAAA	842	AUAAUGACAUAGUAGACGATT	968	UCGUCUACUAUGUCAUUAUTT	1164	ND-9084
AAAAGUAUUUGGGUAGUCCACAA	843	AAGUAUUUGGGUAGUCCACTT	969	GUGGACUACCCAAAUACUUTT	1165	ND-9085
AAACGUGUUGCGAUUGGUGUAAA	844	ACGUGUUGCGAUUGGUGUATT	970	UACACCAAUCGCAACACGUTT	1166	ND-9086
AACGAAAGUAUUUGGGUAGUCAA	845	CGAAAGUAUUUGGGUAGUCTT	971	GACUACCCAAAUACUUUCGTT	1167	ND-9087
AACUCCAAAAUUGCGUAGUACAA	846	CUCCAAAAUUGCGUAGUACTT	972	GUACUACGCAAUUUUGGAGTT	1168	ND-9088
AAUGGUACAAUGGGCCUACGAAA	847	UGGUACAAUGGGCCUACGATT	973	UCGUAGGCCCAUUGUACCATT	1169	ND-9089
AAUAAUGACACUAGUAGACGAUA	848	UAAUGACACUAGUAGACGAUTT	974	AUCGUCUACUAUGUCAUUATT	1170	ND-9090
AAACAUAGUAGACGAUAGUGAAA	849	ACAUAGUAGACGAUAGUGATT	975	UCACUAUCGUCUACUAUGUTT	1171	ND-9091
AAGUGUAGACAUUAUAAACGAAA	850	GUGUAGACAUUAUAAACGATT	976	UCGUUUAUAAUGUCUACACTT	1172	ND-9092
AAUAGACAUUAUAAACGAGCAAA	851	UAGACAUUAUAAACGAGCATT	977	UGCUCGUUUAUAAUGUCUATT	1173	ND-9093
AAUUGCGAUUGGUGUAUUGCUAA	852	UUGCGAUUGGUGUAUUGCUTT	978	AGCAAUACACCAAUCGCAATT	1174	ND-9094
AAUUGGCAGACACUAAUAGUAAA	853	UUGGCAGACACUAAUAGUATT	979	UACUAUUAGUGUCUGCCAATT	1175	ND-9095
AAUUCAGAAUUAGUAAGACCAAA	854	UUCAGAAUUAGUAAGACCATT	980	UGGUCUUACUAAUUCUGAATT	1176	ND-9096
AAGGAGAUUAUUUGAAAGCGAAA	855	GGAGAUUAUUUGAAAGCGATT	981	UCGCUUUCAAAUAAUCUCCTT	1177	ND-9097
AAACCAUGUAGUCAGUAUAGUAA	856	ACCAUGUAGUCAGUAUAGUTT	982	ACUAUACUGACUACAUGGUTT	1178	ND-9098
AAGAAGAGGGUACGGGAUGUAAA	857	GAAGAGGGUACGGGAUGUATT	983	UACAUCCCGUACCCUCUUCTT	1179	ND-9099
AAAUAAAUCAACGUGUUGCGAAA	858	AUAAAUCAACGUGUUGCGATT	984	UCGCAACACGUUGAUUUAUTT	1180	ND-9100

AACGUGUUGCGAUUGGUGUAUAA	859	CGUGUUGCGAUUGGUGUAUTT	985	AUACACCAAUCGCAACACGTT	1181	ND-9101
AAUGGGCCUACGAUAAUGACAAA	860	UGGGCCUACGAUAAUGACATT	986	UGUCAUUAUCGUAGGCCCATT	1182	ND-9102
AAUGCAUAUUACUAUAUGGUGAA	861	UGCAUAUUACUAUAUGGUGTT	987	CACCAUAUAGUAAUAUGCATT	1183	ND-9103
AACCAGAUUAAGUUUGCACGAAA	862	CCAGAUUAAGUUUGCACGATT	988	UCGUGCAAACUUAAUCUGGTT	1184	ND-9104
AAAGAUUAUUUGAAAGCGAAGAA	863	AGAUUAUUUGAAAGCGAAGTT	989	CUUCGCUUUCAAAUAAUCUTT	1185	ND-9105
AAGUUACAGGUAGAAGGGCGCAA	864	GUUACAGGUAGAAGGGCGCTT	990	GCGCCCUUCUACCUGUAACTT	1186	ND-9106
AAGCCAAAAUAGGUAUGUUAGAA	865	GCCAAAAUAGGUAUGUUAGTT	991	CUAACAUACCUAUUUUGGCTT	1187	ND-9107
AAGCAUAGACCAUUGGUACAAAA	866	GCAUAGACCAUUGGUACAATT	992	UUGUACCAAUGGUCUAUGCTT	1188	ND-9108
AAAAAUUGCGUAGUACAGCAGAA	867	AAAUUGCGUAGUACAGCAGTT	993	CUGCUGUACUACGCAAUUUTT	1189	ND-9109
AAAGUAUUUGGGUAGUCCACUAA	868	AGUAUUUGGGUAGUCCACUTT	994	AGUGGACUACCCAAAUACUTT	1190	ND-9110
AAAUGACAUAGUAGACGAUAGAA	869	AUGACAUAGUAGACGAUAGTT	995	CUAUCGUCUACUAUGUCAUTT	1191	ND-9111
AAGGUAGUCCACUUAGUGAUAAA	870	GGUAGUCCACUUAGUGAUATT	996	UAUCACUAAGUGGACUACCTT	1192	ND-9112
AAAGCAUAGACCAUUGGUACAAA	871	AGCAUAGACCAUUGGUACATT	997	UGUACCAAUGGUCUAUGCUTT	1193	ND-9113
AAUUUCAGAAUUAGUAAGACCAA	872	UUUCAGAAUUAGUAAGACCTT	998	GGUCUUACUAAUUCUGAAATT	1194	ND-9114
AAUGCGAUUGGUGUAUUGCUGAA	873	UGCGAUUGGUGUAUUGCUGTT	999	CAGCAAUACACCAAUCGCATT	1195	ND-9115
AACCAAAAUUGCGUAGUACAGAA	874	CCAAAAUUGCGUAGUACAGTT	1000	CUGUACUACGCAAUUUUGGTT	1196	ND-9116
AAGACAGCACAUGCGUUGUUUAA	875	GACAGCACAUGCGUUGUUUTT	1001	AAACAACGCAUGUGCUGUCTT	1197	ND-9117
AAUGUUACAGGUAGAAGGGCGAA	876	UGUUACAGGUAGAAGGGCGTT	1002	CGCCCUUCUACCUGUAACATT	1198	ND-9118
AAAGACAAUAAUAUUAGUCCUAA	877	AGACAAUAAUAUUAGUCCUTT	1003	AGGACUAAUAUUAUUGUCUTT	1199	ND-9119
AAAUGAUUAUUUAACACAGGCAA	878	AUGAUUAUUUAACACAGGCTT	1004	GCCUGUGUUAAAUAAUCAUTT	1200	ND-9120
AAAGAUGUGAUAGGGUAGAUGAA	879	AGAUGUGAUAGGGUAGAUGTT	1005	CAUCUACCCUAUCACAUCUTT	1201	ND-9121
AACUGCAAGGGUCUGUAAUAUAA	880	CUGCAAGGGUCUGUAAUAUTT	1006	AUAUUACAGACCCUUGCAGTT	1202	ND-9122
AAUCAGAUGACGAGAACGAAAAA	881	UCAGAUGACGAGAACGAAATT	1007	UUUCGUUCUCGUCAUCUGATT	1203	ND-9123
AACACCAUGUAGUCAGUAUAGAA	882	CACCAUGUAGUCAGUAUAGTT	1008	CUAUACUGACUACAUGGUGTT	1204	ND-9124
AAAGACAGCACAUGCGUUGUUAA	883	AGACAGCACAUGCGUUGUUTT	1009	AACAACGCAUGUGCUGUCUTT	1205	ND-9125
AAAGAGGGUACGGGAUGUAAUAA	884	AGAGGGUACGGGAUGUAAUTT	1010	AUUACAUCCCGUACCCUCUTT	1206	ND-9126
AAAAAAGUAAUAAAUCAACGUAA	885	AAAAGUAAUAAAUCAACGUTT	1011	ACGUUGAUUUAUUACUUUUTT	1207	ND-9127
AAAAAGCAUAGACCAUUGGUAAA	886	AAAGCAUAGACCAUUGGUATT	1012	UACCAAUGGUCUAUGCUUUTT	1208	ND-9128

AAUUGUACAUUUGAAUUAUCAAA	887	UUGUACAUUUGAAUUAUCATT	1013	UGAUAAUUCAAAUGUACAATT	1209	ND-9129
AAGUAAAGCAUAGACCAUUGGAA	888	GUAAAGCAUAGACCAUUGGTT	1014	CCAAUGGUCUAUGCUUUACTT	1210	ND-9130
		AAAucAAcGuGuuGcGAuuTsT	1015	AAUCGcAAcACGUUGAUUUTsT	1211	ND-9131
		GAGccucccAAAAuuGcGuATsT	1016	uACGcAAUUUUGGAGGCUCTsT	1212	ND-9132
		ucAAcGuGuuGcGAuuGGuTsT	1017	ACcAAUCGcAAcACGUUGATsT	1213	ND-9133
		uccAAAAuuGcGuAGuAcATsT	1018	UGuACuACGcAAUUUUGGATsT	1214	ND-9134
		AAucAAcGuGuuGcGAuuGTsT	1019	cAAUCGcAAcACGUUGAUUTsT	1215	ND-9135
		ccuccAAAAuuGcGuAGuATsT	1020	uACuACGcAAUUUUGGAGGTsT	1216	ND-9136
		AGAGccucccAAAAuuGcGuTsT	1021	ACGcAAUUUUGGAGGCUCUTsT	1217	ND-9137
		cAAcGuGuuGcGAuuGGuGTsT	1022	cACcAAUCGcAAcACGUUGTsT	1218	ND-9138
		AuAGAuGuGAuAGGGuAGATsT	1023	UCuACCCuAUcAcAUCuAUTsT	1219	ND-9139
		GGGAAGAGGGuAcGGGAuGTsT	1024	cAUCCCGuACCCUCUUCCCTsT	1220	ND-9140
		AGAuuAAGuuuGcAcGAGGTsT	1025	CCUCGUGcAAACUuAAUCUTsT	1221	ND-9141
		GGuAucAAGGuGuAGAGuuTsT	1026	AACUCuAcACCUUGAuACCTsT	1222	ND-9142
		AcuuAGuGAuAuuAGuGGATsT	1027	UCcACuAAuAUcACuAAGUTsT	1223	ND-9143
		GAGAuuAuuuGAAAGcGAATsT	1028	UUCGCUUUcAAAuAAUCUCTsT	1224	ND-9144
		AAcAccAuGuAGucAGuAuTsT	1029	AuACUGACuAcAUGGUGUUTsT	1225	ND-9145
		AGccucccAAAAuuGcGuAGTsT	1030	CuACGcAAUUUUGGAGGCUTsT	1226	ND-9146
		GccucccAAAAuuGcGuAGuTsT	1031	ACuACGcAAUUUUGGAGGCTsT	1227	ND-9147
		GuGuAuGGAGAcAcGccAGTsT	1032	CUGGCGUGUCUCCcAuAcACTsT	1228	ND-9148
		GuAcAAuGGGccuAcGAuATsT	1033	uAUCGuAGGCCCcAUUGuACTsT	1229	ND-9149
		uAcAAuGGGccuAcGAuAATsT	1034	UuAUCGuAGGCCcAUUGuATsT	1230	ND-9150
		uGAcAuAGuAGAcGAuAGuTsT	1035	ACuAUCGUCuACuAUGUcATsT	1231	ND-9151
		GAcAuAGuAGAcGAuAGuGTsT	1036	cACuAUCGUCuACuAUGUCTsT	1232	ND-9152
		AcucuuuGccAAcGuuuAATsT	1037	UuAAACGUUGGcAAAGAGUTsT	1233	ND-9153
		AuAAuGAcAuAGuAGAcGATsT	1038	UCGUCuACuAUGUcAUuAUTsT	1234	ND-9154
		AAGuAuuuGGGuAGuccAcTsT	1039	GUGGACuACCcAAAuACUUTsT	1235	ND-9155
		AcGuGuuGcGAuuGGuGuATsT	1040	uAcACcAAUCGcAAcACGUTsT	1236	ND-9156

cGAAAGuAuuuGGGuAGucTsT	1041	GACuACCcAAAuACUUUCGTsT	1237	ND-9157
cuccAAAAuuGcGuAGuAcTsT	1042	GuACuACGcAAUUUUGGAGTsT	1238	ND-9158
uGGuAcAAuGGGccuAcGATsT	1043	UCGuAGGCCcAUUGuACcATsT	1239	ND-9159
uAAuGAcAuAGuAGAcGAuTsT	1044	AUCGUCuACuAUGUcAUuATsT	1240	ND-9160
AcAuAGuAGAcGAuAGuGATsT	1045	UcACuAUCGUCuACuAUGUTsT	1241	ND-9161
GuGuAGAcAuuAuAAAcGATsT	1046	UCGUUuAuAAUGUCuAcACTsT	1242	ND-9162
uAGAcAuuAuAAAcGAGcATsT	1047	UGCUCGUUuAuAAUGUCuATsT	1243	ND-9163
uuGcGAuuGGuGuAuuGcuTsT	1048	AGcAAuAcACcAAUCGcAATsT	1244	ND-9164
uuGGcAGAcAcuAAuAGuATsT	1049	uACuAUuAGUGUCUGCcAATsT	1245	ND-9165
uucAGAAuuAGuAAGAccATsT	1050	UGGUCUuACuAAUUCUGAATsT	1246	ND-9166
GGAGAuuAuuuGAAAGcGATsT	1051	UCGCUUUcAAAuAAUCUCCTsT	1247	ND-9167
AccAuGuAGucAGuAuAGuTsT	1052	ACuAuACUGACuAcAUGGUTsT	1248	ND-9168
GAAGAGGGuAcGGGAuGuATsT	1053	uAcAUCCCCGuACCCUCUUCTsT	1249	ND-9169
AuAAAucAAcGuGuuGcGATsT	1054	UCGcAAcACGUUGAUUuAUTsT	1250	ND-9170
cGuGuuGcGAuuGGuGuAuTsT	1055	AuAcACcAAUCGcAAcACGTsT	1251	ND-9171
uGGGccuAcGAuAAuGAcATsT	1056	UGUcAUuAUCGuAGGCCCcATsT	1252	ND-9172
uGcAuAuuAcuAuAuGGuGTsT	1057	cACcAuAuAGuAAuAUGcATsT	1253	ND-9173
ccAGAuuAAGuuuGcAcGATsT	1058	UCGUGcAAACUuAAUCUGGTsT	1254	ND-9174
AGAuuAuuuGAAAGcGAAGTsT	1059	CUUCGCUUUcAAAuAAUCUTsT	1255	ND-9175
GuuAcAGGuAGAAGGGcGcTsT	1060	GCGCCCUUCuACCUGuAACTsT	1256	ND-9176
GccAAAAuAGGuAuGuuAGTsT	1061	CuAAcAuACCuAUUUUGGCTsT	1257	ND-9177
GcAuAGAccAuuGGuAcAATsT	1062	UUGuACcAAUGGUCuAUGCTsT	1258	ND-9178
AAAuuGcGuAGuAcAGcAGTsT	1063	CUGCUGuACuACGcAAUUUTsT	1259	ND-9179
AGuAuuuGGGuAGuccAcuTsT	1064	AGUGGACuACCcAAAuACUTsT	1260	ND-9180
AuGAcAuAGuAGAcGAuAGTsT	1065	CuAUCGUCuACuAUGUcAUTsT	1261	ND-9181
GGuAGuccAcuuAGuGAuATsT	1066	uAUcACuAAGUGGACuACCTsT	1262	ND-9182
AGcAuAGAccAuuGGuAcATsT	1067	UGuACcAAUGGUCuAUGCUTsT	1263	ND-9183
uuucAGAAuuAGuAAGAccTsT	1068	GGUCUuACuAAUUCUGAAATsT	1264	ND-9184

uGcGAuuGGuGuAuuGcuGTsT	1069	cAGcAAuAcACcAAUCGcATsT	1265	ND-9185		
ccAAAAuuGcGuAGuAcAGTsT	1070	CUGuACuACGcAAUUUUGGTsT	1266	ND-9186		
GAcAGcAcAuGcGuuGuuuTsT	1071	AAAcAACGcAUGUGCUGUCTsT	1267	ND-9187		
uGuuAcAGGuAGAAGGGcGTsT	1072	CGCCCUUCuACCUGuAAcATsT	1268	ND-9188		
AGAcAAuAAuAuuAGuccuTsT	1073	AGGACuAAuAUuAUUGUCUTsT	1269	ND-9189		
AuGAuuAuuuAAcAcAGGcTsT	1074	GCCUGUGUuAAAuAAUcAUTsT	1270	ND-9190		
AGAuGuGAuAGGGuAGAuGTsT	1075	cAUCuACCCuAUcAcAUCUTsT	1271	ND-9191		
cuGcAAGGGucuGuAAuAuTsT	1076	AuAUuAcAGACCCUUGcAGTsT	1272	ND-9192		
ucAGAuGAcGAGAAcGAAATsT	1077	UUUCGUUCUCGUcAUCUGATsT	1273	ND-9193		
cAccAuGuAGucAGuAuAGTsT	1078	CuAuACUGACuAcAUGGUGTsT	1274	ND-9194		
AGAcAGcAcAuGcGuuGuuTsT	1079	AAcAACGcAUGUGCUGUCUTsT	1275	ND-9195		
AGAGGGuAcGGGAuGuAAuTsT	1080	AUuAcAUCCCGuACCCUCUTsT	1276	ND-9196		
AAAAGuAAuAAAucAAcGuTsT	1081	ACGUUGAUUuAUuACUUUUTsT	1277	ND-9197		
AAAGcAuAGAccAuuGGuATsT	1082	uACcAAUGGUCuAUGCUUUTsT	1278	ND-9198		
uuGuAcAuuuGAAuuAucATsT	1083	UGAuAAUUcAAAUGuAcAATsT	1279	ND-9199		
GuAAAGcAuAGAccAuuGGTsT	1084	CcAAUGGUCuAUGCUUuACTsT	1280	ND-9200		
AAAUAUCAAAUAUUAGUGAAGAA	889	AUAUCAAAUAUUAGUGAAGTT	1085	CUUCACUAAUAUUUGAUAUTT	1281	AL-DP-8042
AAUAUCAAAUAUUAGUGAAGUAA	890	UAUCAAAUAUUAGUGAAGUTT	1086	ACUUCACUAAUAUUUGAUATT	1282	AL-DP-8043
AAGGGUAUGGCAAUACUGAAGAA	891	GGGUAUGGCAAUACUGAAGTT	1087	CUUCAGUAUUGCCAUACCCTT	1283	AL-DP-8044
AACAACGUUUAAAUGUGUGUCAA	892	CAACGUUUAAAUGUGUGUCTT	1088	GACACACAUUUAAACGUUGTT	1284	AL-DP-8045
AAAACGUUUAAAUGUGUGUCAAA	893	AACGUUUAAAUGUGUGUCATT	1089	UGACACACAUUUAAACGUUTT	1285	AL-DP-8046
AAGAAAACGAUGGAGACUCUUAA	894	GAAAACGAUGGAGACUCUUTT	1090	AAGAGUCUCCAUCGUUUUCTT	1286	AL-DP-8047
AAGCGGGUAUGGCAAUACUGAAA	895	GCGGGUAUGGCAAUACUGATT	1091	UCAGUAUUGCCAUACCCGCTT	1287	AL-DP-8048
AACGGGUAUGGCAAUACUGAAAA	896	CGGGUAUGGCAAUACUGAATT	1092	UUCAGUAUUGCCAUACCCGTT	1288	AL-DP-8049
AAUUAUAAACGAGCAGAAAAAAA	897	UUAUAAACGAGCAGAAAAATT	1093	UUUUUCUGCUCGUUUAUAATT	1289	AL-DP-8050
AAACAAUGUGUAGACAUUAUAAA	898	ACAAUGUGUAGACAUUAUATT	1094	UAUAAUGUCUACACAUUGUTT	1290	AL-DP-8051
AAAUUAUAAACGAGCAGAAAAAA	899	AUUAUAAACGAGCAGAAAATT	1095	UUUUCUGCUCGUUUAUAAUTT	1291	AL-DP-8052
AAUGUGUAGACAUUAUAAACGAA	900	UGUGUAGACAUUAUAAACGTT	1096	CGUUUAUAAUGUCUACACATT	1292	AL-DP-8053

AAUGUGUGUCAGGACAAAAUAAA	901	UGUGUGUCAGGACAAAAUATT	1097	UAUUUUGUCCUGACACACATT	1293	AL-DP-8054
AAACAUUAUAAAACGAGCAGAAAA	902	ACAUUAUAAAACGAGCAGAATT	1098	UUCUGCUCGUUUAUAAAUGUTT	1294	AL-DP-8055
AAAGACAGCGGGUAUGGCAAUAA	903	AGACAGCGGGUAUGGCAAUTT	1099	AUUGCCAUACCCGCUGUCUTT	1295	AL-DP-8056
AAGACAGCGGGUAUGGCAAUAAA	904	GACAGCGGGUAUGGCAAUATT	1100	UAUUGCCAUACCCGCUGUCTT	1296	AL-DP-8057
AAACAGCGGGUAUGGCAAUACAA	905	ACAGCGGGUAUGGCAAUACTT	1101	GUAUUGCCAUACCCGCUGUTT	1297	AL-DP-8058
AAAGCGGGUAUGGCAAUACUGAA	906	AGCGGGUAUGGCAAUACUGTT	1102	CAGUAUUGCCAUACCCGCUTT	1298	AL-DP-8059
AAAACAAUGUGUAGACAUUAUAA	907	AACAAUGUGUAGACAUUAUTT	1103	AUAAUGUCUACACAUUGUUTT	1299	AL-DP-8060
AACAUUAUAAACGAGCAGAAAAA	908	CAUUAUAAACGAGCAGAAATT	1104	UUUCUGCUCGUUUAUAAAUGTT	1300	AL-DP-8061
AAACGUUUAAAUGUGUGUCAGAA	909	ACGUUUAAAUGUGUGUCAGTT	1105	CUGACACACAUUUAAACGUTT	1301	AL-DP-8062
AAAAAUGUGUGUCAGGACAAAAA	910	AAAUGUGUGUCAGGACAAATT	1106	UUUGUCCUGACACACAUUUTT	1302	AL-DP-8063
AAGGUUCUAAAACGAAAGUAUAA	911	GGUUCUAAAACGAAAGUAUTT	1107	AUACUUUCGUUUUAGAACCTT	1303	AL-DP-8064
AAAUGUGUAGACAUUAUAAACAA	912	AUGUGUAGACAUUAUAAACTT	1108	GUUUAUAAUGUCUACACAUTT	1304	AL-DP-8065
AAAGUAAGACCAUUUAAAAGUAA	913	AGUAAGACCAUUUAAAAGUTT	1109	ACUUUUAAAUGGUCUUACUTT	1305	AL-DP-8066
AAUAGUAAGACCAUUUAAAAGAA	914	UAGUAAGACCAUUUAAAAGTT	1110	CUUUUAAAUGGUCUUACUATT	1306	AL-DP-8067
AAGAAUUAGUAAGACCAUUUAAA	915	GAAUUAGUAAGACCAUUUATT	1111	UAAAUGGUCUUACUAAUUCTT	1307	AL-DP-8068
AAAAUUAGUAAGACCAUUUAAAA	916	AAUUAGUAAGACCAUUUAATT	1112	UUAAAUGGUCUUACUAAUUTT	1308	AL-DP-8069
AAAUUAGUAAGACCAUUUAAAAA	917	AUUAGUAAGACCAUUUAAATT	1113	UUUAAAUGGUCUUACUAAUTT	1309	AL-DP-8070
AAUUAGUAAGACCAUUUAAAAAA	918	UUAGUAAGACCAUUUAAAATT	1114	UUUUAAAUGGUCUUACUAATT	1310	AL-DP-8071
AAAAUACUGAAGUGGAAACUCAA	919	AAUACUGAAGUGGAAACUCTT	1115	GAGUUUCCACUUCAGUAUTT	1311	AL-DP-8072
AAAUACUGAAGUGGAAACUCAAA	920	AUACUGAAGUGGAAACUCATT	1116	UGAGUUUCCACUUCAGUATT	1312	AL-DP-8073
AACAAUACUGAAGUGGAAACUCAA	921	CAAUACUGAAGUGGAAACUTT	1117	AGUUUCCACUUCAGUAUUGTT	1313	AL-DP-8074
AAUACUGAAGUGGAAACUCAGAA	922	UACUGAAGUGGAAACUCAGTT	1118	CUGAGUUUCCACUUCAGUATT	1314	AL-DP-8075
AAACUGAAGUGGAAACUCAGCAA	923	ACUGAAGUGGAAACUCAGCTT	1119	GCUGAGUUUCCACUUCAGUTT	1315	AL-DP-8076
AACUGAAGUGGAAACUCAGCAAA	924	CUGAAGUGGAAACUCAGCATT	1120	UGCUGAGUUUCCACUUCAGTT	1316	AL-DP-8077
AAUGAAGUGGAAACUCAGCAGAA	925	UGAAGUGGAAACUCAGCAGTT	1121	CUGCUGAGUUUCCACUUCATT	1317	AL-DP-8078
AAGAAGUGGAAACUCAGCAGAAA	926	GAAGUGGAAACUCAGCAGATT	1122	UCUGCUGAGUUUCCACUUCTT	1318	AL-DP-8079
AAAAGUGGAAACUCAGCAGAUAA	927	AAGUGGAAACUCAGCAGAUTT	1123	AUCUGCUGAGUUUCCACUUTT	1319	AL-DP-8080
AAAGUGGAAACUCAGCAGAUGAA	928	AGUGGAAACUCAGCAGAUGTT	1124	CAUCUGCUGAGUUUCCACUTT	1320	AL-DP-8081

AAAUGGCAAUACUGAAGUGGAAA	929	AUGGCAAUACUGAAGUGGATT	1125	UCCACUUCAGUAUUGCCAUTT	1321 AL-DP-8082
AAAAAUCCUUUUUUCUCAAGGAAA	930	AAAUCCUUUUUUCUCAAGGATT	1126	UCCUUGAGAAAAAGGAUUUTT	1322 AL-DP-8083
AAUCCUUUUUCUCAAGGACGUAA	931	UCCUUUUUCUCAAGGACGUTT	1127	ACGUCCUUGAGAAAAAGGATT	1323 AL-DP-8084
AAAUCCUUUUUCUCAAGGACGAA	932	AUCCUUUUUCUCAAGGACGTT	1128	CGUCCUUGAGAAAAAGGAUTT	1324 AL-DP-8085
AAGGCAAUACUGAAGUGGAAAAA	933	GGCAAUACUGAAGUGGAAATT	1129	UUUCCACUUCAGUAUUGCCTT	1325 AL-DP-8086
AACUUUUUCUCAAGGACGUGGAA	934	CUUUUUCUCAAGGACGUGGTT	1130	CCACGUCCUUGAGAAAAAGTT	1326 AL-DP-8087
AACCUUUUUCUCAAGGACGUGAA	935	CCUUUUUCUCAAGGACGUGTT	1131	CACGUCCUUGAGAAAAAGGTT	1327 AL-DP-8088
AAUUUUUCUCAAGGACGUGGUAA	936	UUUUUCUCAAGGACGUGGUTT	1132	ACCACGUCCUUGAGAAAAATT	1328 AL-DP-8089
AAUGGAAAUCCUUUUUCUCAAAA	937	UGGAAAUCCUUUUUCUCAATT	1133	UUGAGAAAAAGGAUUUCCATT	1329 AL-DP-8090
AAGGAAAUCCUUUUUCUCAAGAA	938	GGAAAUCCUUUUUCUCAAGTT	1134	CUUGAGAAAAAGGAUUUCCTT	1330 AL-DP-8091
AAGAAAUCCUUUUUCUCAAGGAA	939	GAAAUCCUUUUUCUCAAGGTT	1135	CCUUGAGAAAAAGGAUUUCTT	1331 AL-DP-8092
AAAAUCCUUUUUCUCAAGGACAA	940	AAUCCUUUUUCUCAAGGACTT	1136	GUCCUUGAGAAAAAGGAUUTT	1332 AL-DP-8093
AAUAUGGCAAUACUGAAGUGGAA	941	UAUGGCAAUACUGAAGUGGTT	1137	CCACUUCAGUAUUGCCAUATT	1333 AL-DP-8094
AAUGGCAAUACUGAAGUGGAAAA	942	UGGCAAUACUGAAGUGGAATT	1138	UUCCACUUCAGUAUUGCCATT	1334 AL-DP-8095
AAGCAAUACUGAAGUGGAAACAA	943	GCAAUACUGAAGUGGAAACTT	1139	GUUUCCACUUCAGUAUUGCTT	1335 AL-DP-8096
AAAAUGUGUAGACAUUAUAAAAA	944	AAUGUGUAGACAUUAUAAATT	1140	UUUAUAAUGUCUACACAUUTT	1336 AL-DP-8097

Testing of siRNA Targeting HPV E1 Gene Expression

Unmodified and chemically modified dsRNA were tested to identify their relative abilities to reduce the expression level of mRNA encoding HPV E1 gene in a cell.

The assay conditions employed were as follows: C33A cells were obtained from ATCC. Sequences encoding HPV16 E6 and E1 were cloned into the pNAS-055 vector (Husken et al,. Nucleic Acids Research, 31:e102, 2003), for expression as YFP fusion transcripts. The resulting plasmids were transfected into C33A cells, and stable lines expressing these fusion transcripts were derived by Zeocin selection, as per the manufacturer's protocol (Invitrogen). For transfection with siRNA against HPV16 E6 or HPV16 E1, respective cells were seeded at a density of 2.0×10^4 cells/well in 96-well plates and transfected directly. Transfection of siRNA (30nM, 3nM or 300pm as indicated) was carried out in a single dose with lipofectamine 2000® (Invitrogen) as described by the manufacturer.

24 hours after transfection,cells were lysed and fusion YFP mRNA expression levels were quantified with the Quantigene Explore Kit (Panomics, Inc. (Fremont, CA)(formerly Genospectra, Inc.)) using a probe directed against YFP, according to the standard protocol. Fusion-YFP mRNA levels were normalized to GAP-DH mRNA. For each siRNA four individual datapoints were collected. siRNA duplexes unrelated to the HPV16 E1 or E6 genes were used as control. The activity of a given siRNA duplex was expressed as percent fusion-YFP mRNA concentration in treated cells relative to concentration of the same transcript in cells treated with the control siRNA duplex.

Table 6 shows the results of testing the E1 dsRNA of the invention.

Duplex dsRNA	%mRNA remaining after treatement at 300pM	S.D.
ND-9061	41.45	10.69
ND-9062	30.67	10.43
ND-9063	61.87	22.99
ND-9064	40.79	22.73
ND-9065	68.58	28.46
ND-9066	23.51	7.60
ND-9067	37.13	13.60
ND-9068	34.50	17.21
ND-9069	40.61	12.42
ND-9070	32.61	8.73
ND-9071	30.68	11.65
ND-9072	24.38	7.47
ND-9073	76.28	15.06
ND-9074	29.11	10.42
ND-9075	27.20	11.56
ND-9076	42.06	17.88
ND-9077	51.19	9.09
ND-9078	43.42	16.63
ND-9079	25.79	4.85
ND-9080	29.33	5.67
ND-9081	36.66	4.51
ND-9082	48.67	10.47
ND-9083	39.51	12.70
ND-9084	44.28	7.54
ND-9085	55.73	9.77
ND-9086	28.90	7.93
ND-9087	28.88	5.47
ND-9088	45.35	11.67
ND-9089	49.13	12.46
ND-9090	41.76	5.88
ND-9091	31.35	9.16

ND-9092	23.79	8.74
ND-9093	47.62	9.89
ND-9094	91.33	29.84
ND-9095	43.33	8.69
ND-9096	63.53	11.44
ND-9097	30.51	4.48
ND-9098	40.76	10.57
ND-9099	37.61	9.94
ND-9100	106.18	30.69
ND-9101	37.75	16.37
ND-9102	41.98	14.66
ND-9103	98.17	14.30
ND-9104	29.61	11.44
ND-9105	29.71	6.48
ND-9106	51.42	14.12
ND-9107	78.38	28.72
ND-9108	34.69	4.19
ND-9109	97.63	14.18
ND-9110	47.58	7.48
ND-9111	65.14	15.02
ND-9112	30.24	7.33
ND-9113	31.69	10.80
ND-9114	108.54	7.17
ND-9115	87.16	14.74
ND-9116	56.35	14.69
ND-9117	33.79	8.42
ND-9118	65.12	19.60
ND-9119	33.37	12.37
ND-9120	70.98	18.74
ND-9121	39.37	10.06
ND-9122	33.24	14.79
ND-9123	20.37	7.53
ND-9124	30.47	5.18
ND-9125	26.22	5.56
ND-9126	29.86	5.15
ND-9127	84.95	22.37
ND-9128	35.14	6.10
ND-9129	49.41	15.75
ND-9130	51.54	12.31

ND-9131	45.51	7.96
ND-9132	81.48	16.52
ND-9133	46.79	13.27
ND-9134	63.22	32.12
ND-9135	118.82	19.88
ND-9136	47.83	12.16
ND-9137	65.11	15.44
ND-9138	92.31	36.27
ND-9139	42.01	10.70
ND-9140	40.54	7.24
ND-9141	101.31	24.39
ND-9142	33.83	7.06
ND-9143	86.43	16.50
ND-9144	33.94	11.74
ND-9145	41.93	12.85
ND-9146	118.24	29.81
ND-9147	69.90	30.13
ND-9148	40.74	6.28
ND-9149	65.26	10.10
ND-9150	36.62	4.85
ND-9151	27.83	4.48
ND-9152	88.99	9.86
ND-9153	66.45	33.75
ND-9154	45.42	8.86
ND-9155	63.55	8.36
ND-9156	53.00	7.71
ND-9157	32.74	7.39
ND-9158	102.06	26.87
ND-9159	59.47	10.16
ND-9160	31.23	7.52
ND-9161	84.78	36.89
ND-9162	24.83	5.17
ND-9163	26.64	5.90
ND-9164	77.97	10.06
ND-9165	59.95	25.75
ND-9166	69.74	8.15
ND-9167	23.04	5.43
ND-9168	46.16	12.02
ND-9169	62.24	11.73

ND-9170	92.69	14.72
ND-9171	46.55	6.56
ND-9172	49.39	16.23
ND-9173	98.36	37.53
ND-9174	44.90	13.73
ND-9175	69.98	18.22
ND-9176	60.73	13.02
ND-9177	70.93	10.18
ND-9178	62.53	7.70
ND-9179	76.68	31.77
ND-9180	66.35	10.48
ND-9181	78.42	12.70
ND-9182	72.09	28.88
ND-9183	58.97	28.59
ND-9184	97.06	8.62
ND-9185	85.29	16.92
ND-9186	77.52	18.17
ND-9187	60.16	36.16
ND-9188	58.61	39.92
ND-9189	69.35	30.11
ND-9190	71.87	36.13
ND-9191	81.64	18.99
ND-9192	52.76	14.33
ND-9193	25.18	8.23
ND-9194	50.69	12.78
ND-9195	40.01	10.21
ND-9196	47.41	15.85
ND-9197	94.68	24.60
ND-9198	103.12	27.52
ND-9199	50.82	15.18
ND-9200	97.72	24.20
AL-DP-8042	117.14	34.54
AL-DP-8043	131.44	38.69
AL-DP-8044	28.60	11.52
AL-DP-8045	120.81	36.35
AL-DP-8046	93.19	17.57
AL-DP-8047	66.27	5.06
AL-DP-8048	33.70	8.18
AL-DP-8049	34.31	7.16

AL-DP-8050	60.60	19.36
AL-DP-8051	66.49	12.36
AL-DP-8052	45.46	12.49
AL-DP-8053	121.92	29.06
AL-DP-8054	45.00	4.56
AL-DP-8055	51.64	9.55
AL-DP-8056	35.51	4.67
AL-DP-8057	45.89	8.82
AL-DP-8058	38.47	4.44
AL-DP-8059	34.97	7.85
AL-DP-8060	66.44	14.39
AL-DP-8061	52.17	12.80
AL-DP-8062	100.52	25.88
AL-DP-8063	43.83	8.22
AL-DP-8064	26.25	5.84
AL-DP-8065	107.74	32.53
AL-DP-8066	94.13	13.45
AL-DP-8067	107.09	17.49
AL-DP-8068	48.99	10.40
AL-DP-8069	68.14	19.39
AL-DP-8070	60.42	11.52
AL-DP-8071	71.76	13.75
AL-DP-8072	62.25	6.16
AL-DP-8073	31.33	7.21
AL-DP-8074	47.97	11.55
AL-DP-8075	51.35	14.67
AL-DP-8076	50.40	17.25
AL-DP-8077	38.99	8.15
AL-DP-8078	50.93	11.54
AL-DP-8079	32.27	10.82
AL-DP-8080	33.91	10.48
AL-DP-8081	31.45	6.72
AL-DP-8082	26.41	7.99
AL-DP-8083	86.75	6.66
AL-DP-8084	112.73	25.79
AL-DP-8085	112.33	22.53
AL-DP-8086	39.84	12.22
AL-DP-8087	104.24	29.47
AL-DP-8088	59.29	13.99

AL-DP-8089	114.08	24.06
AL-DP-8090	35.69	6.75
AL-DP-8091	47.28	12.14
AL-DP-8092	92.85	19.28
AL-DP-8093	102.59	15.83
AL-DP-8094	87.51	18.86
AL-DP-8095	27.99	8.27
AL-DP-8096	31.74	7.52
AL-DP-8097	40.29	9.18

Design of dsRNA Targeting HPV E6 Gene Expression

Table 7 sets forth dsRNA compositions of the invention.

Table 7

Upper case letters: unmodified ribonucleotide (except for T which is an unmodified deoxyribonucleotide)
Lower case letters: ribonucloetide bearing 2'-O-methyl substituent on ribose moiety
s : Indicates position of phosphorothioate internucleoside linkage
chol : cholesterol moiety conjugated to 3' ribonucleotide.
'duplex name'means the name of the composition formed by specific hybridization of the indicated sense strand and the indicated antisense strand.

Target sequence of mRNA from HPV E6 reference sequence (sequence of total 19mer target site + AA at both ends)	SEQ ID. NO.	Sense strand (target sequence) having double TT overhang (5'-3')	SEQ ID. NO.	antisense strand (guide sequence) having double TT overhang (5'-3')	SEQ ID. NO.	duplex name
AAUCGGUGGACCGGUCGAUGUAA	1336	UCGGUGGACCGGUCGAUGUTT	1424	ACAUCGACCGGUCCACCGATT	1586	ND-8899
AAGGUCGGUGGACCGGUCGAUAA	1337	GGUCGGUGGACCGGUCGAUTT	1425	AUCGACCGGUCCACCGACCTT	1587	ND-8900
AACGGUGGACCGGUCGAUGUAAA	1338	CGGUGGACCGGUCGAUGUATT	1426	UACAUCGACCGGUCCACCGTT	1588	ND-8901
AAGUCGGUGGACCGGUCGAUGAA	1339	GUCGGUGGACCGGUCGAUGTT	1427	CAUCGACCGGUCCACCGACTT	1589	ND-8902
AAAUCAUCAAGAACACGUAGAAA	1340	AUCAUCAAGAACACGUAGATT	1428	UCUACGUGUUCUUGAUGAUTT	1590	ND-8903
AACAACAGUUACUGCGACGUGAA	1341	CAACAGUUACUGCGACGUGTT	1429	CACGUCGCAGUAACUGUUGTT	1591	ND-8904
AACAAUACAACAAACCGUUGUAA	1342	CAAUACAACAAACCGUUGUTT	1430	ACAACGGUUUGUUGUAUUGTT	1592	ND-8905
AAGCUGCAAACAACUAUACAUAA	1343	GCUGCAAACAACUAUACAUTT	1431	AUGUAUAGUUGUUUGCAGCTT	1593	ND-8906
AAGGUGGACCGGUCGAUGUAUAA	1344	GGUGGACCGGUCGAUGUAUTT	1432	AUACAUCGACCGGUCCACCTT	1594	ND-8907
AAAAAUUAGUGAGUAUAGACAAA	1345	AAAUUAGUGAGUAUAGACATT	1433	UGUCUAUACUCACUAAUUUTT	1595	ND-8908
AAUCAUCAAGAACACGUAGAGAA	1346	UCAUCAAGAACACGUAGAGTT	1434	CUCUACGUGUUCUUGAUGATT	1596	ND-8909
AAAUACAACAAACCGUUGUGUAA	1347	AUACAACAAACCGUUGUGUTT	1435	ACACAACGGUUUGUUGUAUTT	1597	ND-8910
AAUGGACCGGUCGAUGUAUGUAA	1348	UGGACCGGUCGAUGUAUGUTT	1436	ACAUACAUCGACCGGUCCATT	1598	ND-8911

AAUACAACAAACCGUUGUGUGAA	1349	UACAACAAACCGUUGUGUGTT	1437	CACACAACGGUUUGUUGUATT	1599	ND-8912
AAAGAUUCCAUAAUAUAAGGGAA	1350	AGAUUCCAUAAUAUAAGGGTT	1438	CCCUUAUAUUAUGGAAUCUTT	1600	ND-8913
AACAAGCAACAGUUACUGCGAAA	1351	CAAGCAACAGUUACUGCGATT	1439	UCGCAGUAACUGUUGCUUGTT	1601	ND-8914
AAGUUAAUUAGGUGUAUUAACAA	1352	GUUAAUUAGGUGUAUUAACTT	1440	GUUAAUACACCUAAUUAACTT	1602	ND-8915
AAUUUGCUUUUCGGGAUUUAUAA	1353	UUUGCUUUUCGGGAUUUAUTT	1441	AUAAAUCCCGAAAAGCAAATT	1603	ND-8916
AAACUUUGCUUUUCGGGAUUUAA	1354	ACUUUGCUUUUCGGGAUUUTT	1442	AAAUCCCGAAAAGCAAAGUTT	1604	ND-8917
AACUGCAAACAACUAUACAUGAA	1355	CUGCAAACAACUAUACAUGTT	1443	CAUGUAUAGUUGUUUGCAGTT	1605	ND-8918
AAAUGACUUUGCUUUUCGGGAAA	1356	AUGACUUUGCUUUUCGGGATT	1444	UCCCGAAAAGCAAAGUCAUTT	1606	ND-8919
AACGACCCAGAAAGUUACCACAA	1357	CGACCCAGAAAGUUACCACTT	1445	GUGGUAACUUUCUGGGUCGTT	1607	ND-8920
AAUUACUGCGACGUGAGGUAUAA	1358	UUACUGCGACGUGAGGUAUTT	1446	AUACCUCACGUCGCAGUAATT	1608	ND-8921
AAGUUACUGCGACGUGAGGUAAA	1359	GUUACUGCGACGUGAGGUATT	1447	UACCUCACGUCGCAGUAACTT	1609	ND-8922
AAUGCGACGUGAGGUAUAUGAAA	1360	UGCGACGUGAGGUAUAUGATT	1448	UCAUAUACCUCACGUCGCATT	1610	ND-8923
AAGUCGAUGUAUGUCUUGUUGAA	1361	GUCGAUGUAUGUCUUGUUGTT	1449	CAACAAGACAUACAUCGACTT	1611	ND-8924
AACGACGUGAGGUAUAUGACUAA	1362	CGACGUGAGGUAUAUGACUTT	1450	AGUCAUAUACCUCACGUCGTT	1612	ND-8925
AAGACUUUGCUUUUCGGGAUUAA	1363	GACUUUGCUUUUCGGGAUUTT	1451	AAUCCCGAAAAGCAAAGUCTT	1613	ND-8926
AAUUAGGUGUAUUAACUGUCAAA	1364	UUAGGUGUAUUAACUGUCATT	1452	UGACAGUUAAUACACCUAATT	1614	ND-8927
AAUUACCACAGUUAUGCACAGAA	1365	UUACCACAGUUAUGCACAGTT	1453	CUGUGCAUAACUGUGGUAATT	1615	ND-8928
AAGCAACAGUUACUGCGACGUAA	1366	GCAACAGUUACUGCGACGUTT	1454	ACGUCGCAGUAACUGUUGCTT	1616	ND-8929
AAUGCUUUUCGGGAUUUAUGCAA	1367	UGCUUUUCGGGAUUUAUGCTT	1455	GCAUAAAUCCCGAAAAGCATT	1617	ND-8930
AAUUAGUGAGUAUAGACAUUAAA	1368	UUAGUGAGUAUAGACAUUATT	1456	UAAUGUCUAUACUCACUAATT	1618	ND-8931
AAUAAUUAGGUGUAUUAACUGAA	1369	UAAUUAGGUGUAUUAACUGTT	1457	CAGUUAAUACACCUAAUUATT	1619	ND-8932
AAGAUGUAUGUCUUGUUGCAGAA	1370	GAUGUAUGUCUUGUUGCAGTT	1458	CUGCAACAAGACAUACAUCTT	1620	ND-8933
AACCGGUCGAUGUAUGUCUUGAA	1371	CCGGUCGAUGUAUGUCUUGTT	1459	CAAGACAUACAUCGACCGGTT	1621	ND-8934

AAGGAGCGACCCAGAAAGUUAAA	1372	GGAGCGACCCAGAAAGUUATT	1460	UAACUUUCUGGGUCGCUCCTT	1622	ND-8935
AAGAGCGACCCAGAAAGUUACAA	1373	GAGCGACCCAGAAAGUUACTT	1461	GUAACUUUCUGGGUCGCUCTT	1623	ND-8936
AAUGAGUAUAGACAUUAUUGUAA	1374	UGAGUAUAGACAUUAUUGUTT	1462	ACAAUAAUGUCUAUACUCATT	1624	ND-8937
AAAAUACAACAAACCGUUGUGAA	1375	AAUACAACAAACCGUUGUGTT	1463	CACAACGGUUUGUUGUAUUTT	1625	ND-8938
AAGUAUGUCUUGUUGCAGAUCAA	1376	GUAUGUCUUGUUGCAGAUCTT	1464	GAUCUGCAACAAGACAUACTT	1626	ND-8939
AACUUUGCUUUUCGGGAUUUAAA	1377	CUUUGCUUUUCGGGAUUUATT	1465	UAAAUCCCGAAAAGCAAAGTT	1627	ND-8940
AAAUUAGUGAGUAUAGACAUUAA	1378	AUUAGUGAGUAUAGACAUUTT	1466	AAUGUCUAUACUCACUAAUTT	1628	ND-8941
AAAAGAUUCCAUAAUAUAAGGAA	1379	AAGAUUCCAUAAUAUAAGGTT	1467	CCUUAUAUUAUGGAAUCUUTT	1629	ND-8942
AAGGUCGAUGUAUGUCUUGUUAA	1380	GGUCGAUGUAUGUCUUGUUTT	1468	AACAAGACAUACAUCGACCTT	1630	ND-8943
AACAUCAAGAACACGUAGAGAAA	1381	CAUCAAGAACACGUAGAGATT	1469	UCUCUACGUGUUCUUGAUGTT	1631	ND-8944
AAAACAGUUACUGCGACGUGAAA	1382	AACAGUUACUGCGACGUGATT	1470	UCACGUCGCAGUAACUGUUTT	1632	ND-8945
AAACAGUUACUGCGACGUGAGAA	1383	ACAGUUACUGCGACGUGAGTT	1471	CUCACGUCGCAGUAACUGUTT	1633	ND-8946
AAGUGUGAUUUGUUAAUUAGGAA	1384	GUGUGAUUUGUUAAUUAGGTT	1472	CCUAAUUAACAAAUCACACTT	1634	ND-8947
AAAUCAAGAACACGUAGAGAAAA	1385	AUCAAGAACACGUAGAGAATT	1473	UUCUCUACGUGUUCUUGAUTT	1635	ND-8948
AAUUUCGGGAUUUAUGCAUAGAA	1386	UUUCGGGAUUUAUGCAUAGTT	1474	CUAUGCAUAAAUCCCGAAATT	1636	ND-8949
AAACCCACAGGAGCGACCCAGAA	1387	ACCCACAGGAGCGACCCAGTT	1475	CUGGGUCGCUCCUGUGGGUTT	1637	ND-8950
AAAGAUGGGAAUCCAUAUGCUAA	1388	AGAUGGGAAUCCAUAUGCUTT	1476	AGCAUAUGGAUUCCCAUCUTT	1638	ND-8951
AAUAGUGAGUAUAGACAUUAUAA	1389	UAGUGAGUAUAGACAUUAUTT	1477	AUAAUGUCUAUACUCACUATT	1639	ND-8952
AAUGUGUGAUUUGUUAAUUAGAA	1390	UGUGUGAUUUGUUAAUUAGTT	1478	CUAAUUAACAAAUCACACATT	1640	ND-8953
AAUUAAUUAGGUGUAUUAACUAA	1391	UUAAUUAGGUGUAUUAACUTT	1479	AGUUAAUACACCUAAUUAATT	1641	ND-8954
AAAUAUGACUUUGCUUUUCGGAA	1392	AUAUGACUUUGCUUUUCGGTT	1480	CCGAAAAGCAAAGUCAUAUTT	1642	ND-8955
AACGGUCGAUGUAUGUCUUGUAA	1393	CGGUCGAUGUAUGUCUUGUTT	1481	ACAAGACAUACAUCGACCGTT	1643	ND-8956
AACAGGACCCACAGGAGCGACAA	1394	CAGGACCCACAGGAGCGACTT	1482	GUCGCUCCUGUGGGUCCUGTT	1644	ND-8957

AAUUUUCGGGAUUUAUGCAUAAA	1395	UUUUCGGGAUUUAUGCAUATT	1483	UAUGCAUAAAUCCCGAAAATT	1645	ND-8958
AAAAACAACUAUACAUGAUAUAA	1396	AAACAACUAUACAUGAUAUTT	1484	AUAUCAUGUAUAGUUGUUUTT	1646	ND-8959
AAUCCAUAUGCUGUAUGUGAUAA	1397	UCCAUAUGCUGUAUGUGAUTT	1485	AUCACAUACAGCAUAUGGATT	1647	ND-8960
AAUAUUCUAAAAUUAGUGAGUAA	1398	UAUUCUAAAAUUAGUGAGUTT	1486	ACUCACUAAUUUUAGAAUATT	1648	ND-8961
AAUAUGGAACAACAUUAGAACAA	1399	UAUGGAACAACAUUAGAACTT	1487	GUUCUAAUGUUGUUCCAUATT	1649	ND-8962
AAGUCUUGUUGCAGAUCAUCAAA	1400	GUCUUGUUGCAGAUCAUCATT	1488	UGAUGAUCUGCAACAAGACTT	1650	ND-8963
AAUAUUAACUGUCAAAAGCCAAA	1401	UAUUAACUGUCAAAAGCCATT	1489	UGGCUUUUGACAGUUAAUATT	1651	ND-8964
AAACCAAAAGAGAACUGCAAUAA	1402	ACCAAAAGAGAACUGCAAUTT	1490	AUUGCAGUUCUCUUUUGGUTT	1652	ND-8965
AAAAUUAGUGAGUAUAGACAUAA	1403	AAUUAGUGAGUAUAGACAUTT	1491	AUGUCUAUACUCACUAAUUTT	1653	ND-8966
AACAGAUCAUCAAGAACACGUAA	1404	CAGAUCAUCAAGAACACGUTT	1492	ACGUGUUCUUGAUGAUCUGTT	1654	ND-8967
AAUAUGCAUAGUAUAUAGAGAAA	1405	UAUGCAUAGUAUAUAGAGATT	1493	UCUCUAUAUACUAUGCAUATT	1655	ND-8968
AAAGAGAUGGGAAUCCAUAUGAA	1406	AGAGAUGGGAAUCCAUAUGTT	1494	CAUAUGGAUUCCCAUCUCUTT	1656	ND-8969
AAAGUGAGUAUAGACAUUAUUAA	1407	AGUGAGUAUAGACAUUAUUTT	1495	AAUAAUGUCUAUACUCACUTT	1657	ND-8970
AAUUCUAAAAUUAGUGAGUAUAA	1408	UUCUAAAAUUAGUGAGUAUTT	1496	AUACUCACUAAUUUUAGAATT	1658	ND-8971
AAAUGCAUAGUAUAUAGAGAUAA	1409	AUGCAUAGUAUAUAGAGAUTT	1497	AUCUCUAUAUACUAUGCAUTT	1659	ND-8972
		ucGGuGGAccGGucGAuGuTsT	1498	AcAUCGACCGGUCcACCGATsT	1660	ND-8987
		GGucGGuGGAccGGucGAuTsT	1499	AUCGACCGGUCcACCGACCTsT	1661	ND-8988
		cGGuGGAccGGucGAuGuATsT	1500	uAcAUCGACCGGUCcACCGTsT	1662	ND-8989
		GucGGuGGAccGGucGAuGTsT	1501	cAUCGACCGGUCcACCGACTsT	1663	ND-8990
		AucAucAAGAAcAcGuAGATsT	1502	UCuACGUGUUCUUGAUGAUTsT	1664	ND-8991
		cAAcAGuuAcuGcGAcGuGTsT	1503	cACGUCGcAGuAACUGUUGTsT	1665	ND-8992
		cAAuAcAAcAAAccGuuGuTsT	1504	AcAACGGUUUGUUGuAUUGTsT	1666	ND-8993
		GcuGcAAAcAAcuAuAcAuTsT	1505	AUGuAuAGUUGUUUGcAGCTsT	1667	ND-8994

GGuGGAccGGucGAuGuAuTsT	1506	AuAcAUCGACCGGUCcACCTsT	1668	ND-8995
AAAuuAGuGAGuAuAGAcATsT	1507	UGUCuAuACUcACuAAUUUTsT	1669	ND-8996
ucAucAAGAAcAcGuAGAGTsT	1508	CUCuACGUGUUCUUGAUGATsT	1670	ND-8997
AuAcAAcAAAccGuuGuGuTsT	1509	AcAcAACGGUUUGUUGuAUTsT	1671	ND-8998
uGGAccGGucGAuGuAuGuTsT	1510	AcAuAcAUCGACCGGUCcATsT	1672	ND-8999
uAcAAcAAAccGuuGuGuGTsT	1511	cAcAcAACGGUUUGUUGuATsT	1673	ND-9000
AGAuuccAuAAuAuAAGGGTsT	1512	CCCUuAuAUuAUGGAAUCUTsT	1674	ND-9001
cAAGcAAcACGuuAcuGcGATsT	1513	UCGcAGuAACUGUUGCUUGTsT	1675	ND-9002
GuuAAuuAGGuGuAuuAAcTsT	1514	GUuAAuAcACCuAAUuAACTsT	1676	ND-9003
uuuGcuuuucGGGAuuuAuTsT	1515	AuAAAUCCCGAAAAGcAAATsT	1677	ND-9004
AcuuuGcuuuucGGGAuuuTsT	1516	AAAUCCCGAAAAGcAAAGUTsT	1678	ND-9005
cuGcAAAcAAcuAuAcAuGTsT	1517	cAUGuAuAGUUGUUUGcAGTsT	1679	ND-9006
AuGAcuuuGcuuuucGGGATsT	1518	UCCCGAAAAGcAAAGUcAUTsT	1680	ND-9007
cGAcccAGAAAGuuAccAcTsT	1519	GUGGuAACUUUCUGGGUCGTsT	1681	ND-9008
uuAcuGcGAcGuGAGGuAuTsT	1520	AuACCUcACGUCGcAGuAATsT	1682	ND-9009
GuuAcuGcGAcGuGAGGuATsT	1521	uACCUcACGUCGcAGuAACTsT	1683	ND-9010
uGcGAcGuGAGGuAuAuGATsT	1522	UcAuAuACCUcACGUCGcATsT	1684	ND-9011
GucGAuGuAuGucuuGuuGTsT	1523	cAAcAAGAcAuAcAUCGACTsT	1685	ND-9012
cGAcGuGAGGuAuAuGAcuTsT	1524	AGUcAuAuACCUcACGUCGTsT	1686	ND-9013
GAcuuuGcuuuucGGGAuuTsT	1525	AAUCCCGAAAAGcAAAGUCTsT	1687	ND-9014
uuAGGuGuAuuAAcuGucATsT	1526	UGAcAGUuAAuAcACCuAATsT	1688	ND-9015
uuAccAcAGuuAuGcAcAGTsT	1527	CUGUGcAuAACUGUGGuAATsT	1689	ND-9016
GcAAcAGuuAcuGcGAcGuTsT	1528	ACGUCGcAGuAACUGUUGCTsT	1690	ND-9017

uGcuuuucGGGAuuuAuGcTsT	1529	GcAuAAAUCCCGAAAAGcATsT	1691	ND-9018
uuAGuGAGuAuAGAcAuuATsT	1530	uAAUGUCuAuACUcACuAATsT	1692	ND-9019
uAAuuAGGuGuAuuAAcuGTsT	1531	cAGUuAAuAcACCuAAUuATsT	1693	ND-9020
GAuGuAuGucuuGuuGcAGTsT	1532	CUGcAAcAAGAcAuAcAUCTsT	1694	ND-9021
ccGGucGAuGuAuGucuuGTsT	1533	cAAGAcAuAcAUCGACCGGTsT	1695	ND-9022
GGAGcGAcccAGAAAGuuATsT	1534	uAACUUUCUGGGUCGCUCCTsT	1696	ND-9023
GAGcGAcccAGAAAGuuAcTsT	1535	GuAACUUUCUGGGUCGCUCTsT	1697	ND-9024
uGAGuAuAGAcAuuAuuGuTsT	1536	AcAAuAAUGUCuAuACUcATsT	1698	ND-9025
AAuAcAAcAAAccGuuGuGTsT	1537	cAcAACGGUUUGUUGuAUUTsT	1699	ND-9026
GuAuGucuuGuuGcAGAucTsT	1538	GAUCUGcAAcAAGAcAuACTsT	1700	ND-9027
cuuuGcuuuucGGGAuuuATsT	1539	uAAAUCCCGAAAAGcAAAGTsT	1701	ND-9028
AuuAGuGAGuAuAGAcAuuTsT	1540	AAUGUCuAuACUcACuAAUTsT	1702	ND-9029
AAGAuuccAuAAuAuAAGGTsT	1541	CCUuAuAUuAUGGAAUCUUTsT	1703	ND-9030
GGucGAuGuAuGucuuGuuTsT	1542	AAcAAGAcAuAcAUCGACCTsT	1704	ND-9031
cAucAAGAAcAcGuAGAGATsT	1543	UCUCuACGUGUUCUUGAUGTsT	1705	ND-9032
AAcAGuuAcuGcGAcGuGATsT	1544	UcACGUCGcAGuAACUGUUTsT	1706	ND-9033
AcAGuuAcuGcGAcGuGAGTsT	1545	CUcACGUCGcAGuAACUGUTsT	1707	ND-9034
GuGuGAuuuGuuAAuuAGGTsT	1546	CCuAAUuAAcAAAUcAcACTsT	1708	ND-9035
AucAAGAAcAcGuAGAGAATsT	1547	UUCUCuACGUGUUCUUGAUTsT	1709	ND-9036
uuucGGGAuuuAuGcAuAGTsT	1548	CuAUGcAuAAAUCCCGAAATsT	1710	ND-9037
AcccAcAGGAGcGAcccAGTsT	1549	CuGGGUCGCUCCuGuGGGUTsT	1711	ND-9038
AGAuGGGAAuccAuAuGcuTsT	1550	AGcAuAUGGAUUCCcAUCUTsT	1712	ND-9039
uAGuGAGuAuAGAcAuuAuTsT	1551	AuAAUGUCuAuACUcACuATsT	1713	ND-9040

uGuGuGAuuuGuuAAuuAGTsT	1552	CuAAUuAAcAAAUcAcAcATsT	1714	ND-9041
uuAAuuAGGuGuAuuAAcuTsT	1553	AGUuAAuAcACCuAAUuAATsT	1715	ND-9042
AuAuGAcuuuGcuuuucGGTsT	1554	CCGAAAAGcAAAGUcAuAUTsT	1716	ND-9043
cGGucGAuGuAuGucuuGuTsT	1555	AcAAGAcAuAcAUCGACCGTsT	1717	ND-9044
cAGGAcccAcAGGAGcGAcTsT	1556	GUCGCUCCuGuGGGUCCUGTsT	1718	ND-9045
uuuucGGGAuuuAuGcAuATsT	1557	uAUGcAuAAAUCCCGAAAATsT	1719	ND-9046
AAAcAAcuAuAcAuGAuAuTsT	1558	AuAUcAUGuAuAGUUGUUUTsT	1720	ND-9047
uccAuAuGcuGuAuGuGAuTsT	1559	AUcAcAuAcAGcAuAUGGATsT	1721	ND-9048
uAuucuAAAAuuAGuGAGuTsT	1560	ACUcACuAAUUUuAGAAuATsT	1722	ND-9049
uAuGGAAcAAcAuuAGAAcTsT	1561	GUUCuAAUGUUGUUCcAuATsT	1723	ND-9050
GucuuGuuGcAGAucAucATsT	1562	UGAUGAUCUGcAAcAAGACTsT	1724	ND-9051
uAuuAAcuGucAAAAGccATsT	1563	UGGCUUUUGAcAGUuAAuATsT	1725	ND-9052
AccAAAAGAGAAcuGcAAuTsT	1564	AUUGcAGUUCUCUUUUGGUTsT	1726	ND-9053
AAuuAGuGAGuAuAGAcAuTsT	1565	AUGUCuAuACUcACuAAUUTsT	1727	ND-9054
cAGAucAucAAGAAcAcGuTsT	1566	ACGuGUUCUuGAuGAUCuGTsT	1728	ND-9055
uAuGcAuAGuAuAuAGAGATsT	1567	UCUCuAuAuACuAUGcAuATsT	1729	ND-9056
AGAGAuGGGAAuccAuAuGTsT	1568	cAuAUGGAUUCCcAUCUCUTsT	1730	ND-9057
AGuGAGuAuAGAcAuuAuuTsT	1569	AAuAAUGUCuAuACUcACUTsT	1731	ND-9058
uucuAAAAuuAGuGAGuAuTsT	1570	AuACUcACuAAUUUuAGAATsT	1732	ND-9059
AuGcAuAGuAuAuAGAGAuTsT	1571	AUCUCuAuAuACuAUGcAUTsT	1733	ND-9060
AAGUGAUUUGUUAAUUAGGUGAA	1410	GUGAUUUGUUAAUUAGGUGTT	1572	CACCUAAUUAACAAAUCACTT	1734	AL-DP-7778
AAUGAUUUGUUAAUUAGGUGUAA	1411	UGAUUUGUUAAUUAGGUGUTT	1573	ACACCUAAUUAACAAAUCATT	1735	AL-DP-7779
AAGAUUUGUUAAUUAGGUGUAAA	1412	GAUUUGUUAAUUAGGUGUATT	1574	UACACCUAAUUAACAAAUCTT	1736	AL-DP-7780
AAAUUUGUUAAUUAGGUGUAUAA	1413	AUUUGUUAAUUAGGUGUAUTT	1575	AUACACCUAAUUAACAAAUTT	1737	AL-DP-7781

AAUGUGAUUUGUUAAUUAGGUAA	1414	UGUGAUUUGUUAAUUAGGUTT	1576	ACCUAAUUAACAAAUCACATT	1738	AL-DP-7782
AAUGUAUGGAACAACAUUAGAAA	1415	UGUAUGGAACAACAUUAGATT	1577	UCUAAUGUUGUUCCAUACATT	1739	AL-DP-7783
AAGUAUGGAACAACAUUAGAAAA	1416	GUAUGGAACAACAUUAGAATT	1578	UUCUAAUGUUGUUCCAUACTT	1740	AL-DP-7784
AAUGUGUACUGCAAGCAACAGAA	1417	UGUGUACUGCAAGCAACAGTT	1579	CUGUUGCUUGCAGUACACATT	1741	AL-DP-7803
AAACUGCGACGUGAGGUAUAUAA	1418	ACUGCGACGUGAGGUAUAUTT	1580	AUAUACCUCACGUCGCAGUTT	1742	7804
AAGAGGUAUAUGACUUUGCUUAA	1419	GAGGUAUAUGACUUUGCUUTT	1581	AAGCAAAGUCAUAUACCUCTT	1743	AL-DP-7805
AAAUGCUGUAUGUGAUAAAUGAA	1420	AUGCUGUAUGUGAUAAAUGTT	1582	CAUUUAUCACAUACAGCAUTT	1744	AL-DP-7807
AAUUUAUUCUAAAAUUAGUGAAA	1421	UUUAUUCUAAAAUUAGUGATT	1583	UCACUAAUUUUAGAAUAAATT	1745	AL-DP-7808
AACUGCGACGUGAGGUAUAUGAA	1422	CUGCGACGUGAGGUAUAUGTT	1584	CAUAUACCUCACGUCGCAGTT	1746	AL-DP-7810
AAACCGUUGUGUGAUUUGUUAAA	1423	ACCGUUGUGUGAUUUGUUATT	1585	UAACAAAUCACACAACGGUTT	1747	AL-DP-7812

Testing of siRNA Targeting HPV E6 Gene Expression

Unmodified and chemically modified dsRNA were tested to identify their relative abilities to reduce the expression level of mRNA encoding HPV E6 gene in a cell.

The assay conditions employed were as follows: C33A cells were obtained from ATCC. Sequences encoding HPV16 E6 and E1 were cloned into the pNAS-055 vector (Husken et al,. Nucleic Acids Research, 31:e102, 2003), for expression as YFP fusion transcripts. The resulting plasmids were transfected into C33A cells, and stable lines expressing these fusion transcripts were derived by Zeocin selection, as per the manufacturer's protocol (Invitrogen). For transfection with siRNA against HPV16 E6 or HPV16 E1, respective cells were seeded at a density of 2.0 x 10^4 cells/well in 96-well plates and transfected directly. Transfection of siRNA (30nM, 3nM or 300pm as indicated) was carried out in a single dose with lipofectamine 2000® (Invitrogen) as described by the manufacturer.

24 hours after transfection,cells were lysed and fusion YFP mRNA expression levels were quantified with the Quantigene Explore Kit (Panomics, Inc. (Fremont, CA)(formerly Genospectra, Inc.)) using a probe directed against YFP, according to the standard protocol. Fusion-YFP mRNA levels were normalized to GAP-DH mRNA. For each siRNA four individual datapoints were collected. siRNA duplexes unrelated to the HPV16 E1 or E6 genes were used as control. The activity of a given siRNA duplex was expressed as percent fusion-YFP mRNA concentration in treated cells relative to concentration of the same transcript in cells treated with the control siRNA duplex.

Table 8 shows the results of testing the E6 dsRNA of the invention.

Table 8

duplex name	Mean activity remaining after 30 nM treatment	sd	Mean activity remaining after 300 pM treatment	sd
ND-8899	15.23	3.19	31.29	9.57
ND-8900	11.61	2.88	26.80	10.23
ND-8901	10.88	3.54	24.77	5.19
ND-8902	20.19	7.36	43.46	6.89
ND-8903	10.38	2.51	22.95	5.47
ND-8904	13.71	4.67	22.11	5.50
ND-8905	13.81	4.29	24.69	4.62
ND-8906	8.35	2.17	24.23	6.62
ND-8907	13.88	3.12	36.94	6.13
ND-8908	14.47	3.48	45.15	7.92
ND-8909	19.99	3.67	49.36	9.80
ND-8910	36.96	9.77	74.18	15.82
ND-8911	18.66	4.19	45.51	6.82
ND-8912	47.42	6.99	76.11	12.97
ND-8913	55.53	16.75	76.63	15.44
ND-8914	9.69	2.50	19.91	6.63
ND-8915	49.02	7.97	93.38	6.83
ND-8916	11.88	2.94	49.78	8.49
ND-8917	14.00	2.04	50.36	8.31
ND-8918	13.70	3.43	29.01	6.51
ND-8919	10.31	2.44	42.51	10.89
ND-8920	10.29	2.72	25.20	9.73
ND-8921	20.23	3.71	37.17	10.15
ND-8922	11.64	2.31	24.95	8.99
ND-8923	12.43	1.97	24.39	8.13

ND-8924	15.19	4.52	32.09	7.01
ND-8925	14.24	1.87	34.21	5.61
ND-8926	10.17	2.85	19.04	3.68
ND-8927	20.77	4.89	41.40	9.23
ND-8928	95.02	20.87	92.24	15.19
ND-8929	17.51	5.27	19.86	6.81
ND-8930	13.58	2.65	61.16	11.03
ND-8931	13.78	2.00	37.55	8.11
ND-8932	105.07	21.10	91.19	12.68
ND-8933	14.88	3.07	43.06	8.64
ND-8934	13.03	3.75	24.32	5.92
ND-8935	13.19	2.88	21.87	4.17
ND-8936	10.04	1.94	21.98	6.92
ND-8937	15.39	3.44	42.70	11.35
ND-8938	55.90	5.56	93.49	10.41
ND-8939	11.51	2.04	29.57	10.38
ND-8940	12.80	2.94	29.67	6.73
ND-8941	19.46	2.91	43.13	3.64
ND-8942	96.02	29.93	85.34	4.57
ND-8943	13.44	3.90	19.03	5.18
ND-8944	14.35	2.09	20.03	4.68
ND-8945	11.45	1.98	23.80	8.36
ND-8946	15.43	2.27	43.12	13.34
ND-8947	13.32	2.20	53.58	18.04
ND-8948	12.85	3.18	23.22	6.79
ND-8949	86.23	23.43	75.99	7.17
ND-8950	29.49	7.99	47.19	14.70
ND-8951	10.51	2.85	20.21	6.23
ND-8952	12.10	2.74	28.82	9.06
ND-8953	41.13	11.23	77.64	9.46
ND-8954	46.52	8.41	81.61	14.93
ND-8955	38.40	8.46	83.38	16.32
ND-8956	12.13	2.23	21.94	9.54

ND-8957	28.39	6.18	53.84	12.53
ND-8958	36.41	9.92	55.67	8.77
ND-8959	33.95	11.23	63.63	5.53
ND-8960	13.63	2.39	26.49	9.24
ND-8961	80.42	18.39	89.13	8.61
ND-8962	33.00	4.18	82.57	9.01
ND-8963	16.67	1.85	28.39	9.50
ND-8964	14.17	2.58	43.74	14.37
ND-8965	17.23	5.15	48.03	10.97
ND-8966	23.01	5.10	53.86	10.10
ND-8967	18.68	5.13	23.30	6.20
ND-8968	10.99	1.83	28.22	9.33
ND-8969	13.75	2.67	32.62	10.53
ND-8970	11.02	2.68	29.14	10.73
ND-8971	21.71	3.11	57.75	10.54
ND-8972	17.10	3.20	52.10	10.84
ND-8987	40.36	7.25	91.12	9.07
ND-8988	20.54	5.27	30.76	12.23
ND-8989	36.60	7.23	74.41	8.61
ND-8990	17.55	8.33	61.02	11.13
ND-8991	11.29	2.87	19.03	5.98
ND-8992	14.49	3.37	44.53	14.74
ND-8993	18.45	5.75	48.07	6.79
ND-8994	13.16	1.80	25.92	7.94
ND-8995	52.21	5.93	90.43	4.86
ND-8996	32.77	6.96	57.54	7.12
ND-8997	14.45	1.50	20.63	4.28
ND-8998	137.83	33.37	90.09	14.65
ND-8999	82.01	13.74	85.69	10.39
ND-9000	69.77	21.32	83.16	14.34
ND-9001	54.71	18.91	74.70	8.87
ND-9002	12.15	2.05	22.98	6.98
ND-9003	76.52	11.49	98.54	7.10

ND-9004	62.23	16.29	84.38	8.22
ND-9005	38.12	6.77	64.57	6.57
ND-9006	12.96	3.15	26.03	4.76
ND-9007	18.24	4.88	42.16	7.87
ND-9008	21.06	4.60	20.01	6.00
ND-9009	35.15	5.62	79.96	7.01
ND-9010	13.71	2.83	53.80	12.21
ND-9011	38.04	3.56	60.45	10.19
ND-9012	44.63	37.28	67.30	8.30
ND-9013	13.31	1.81	31.12	6.40
ND-9014	12.69	3.66	27.50	7.48
ND-9015	16.26	3.61	21.18	4.80
ND-9016	29.49	8.14	66.50	15.07
ND-9017	16.98	2.22	27.17	7.64
ND-9018	35.62	7.31	86.49	7.60
ND-9019	23.48	2.57	60.66	13.05
ND-9020	113.04	21.57	88.75	12.94
ND-9021	38.45	5.44	68.21	9.53
ND-9022	14.21	2.86	53.78	13.38
ND-9023	21.84	3.72	41.95	11.93
ND-9024	117.68	33.94	86.00	6.55
ND-9025	86.38	19.82	81.09	9.82
ND-9026	113.52	9.02	95.62	10.60
ND-9027	13.61	2.09	51.98	15.63
ND-9028	14.49	4.02	45.08	11.80
ND-9029	20.16	3.25	39.00	8.28
ND-9030	104.95	34.72	76.74	10.03
ND-9031	19.90	6.09	26.32	9.90
ND-9032	16.43	3.38	19.10	5.44
ND-9033	100.99	24.54	86.16	11.95
ND-9034	13.77	2.84	33.36	13.56
ND-9035	13.54	1.58	57.07	19.24
ND-9036	12.91	3.20	21.78	6.03

ND-9037	30.90	8.30	74.12	12.35
ND-9038	121.49	24.79	87.65	7.07
ND-9039	10.19	3.13	23.32	9.60
ND-9040	11.45	2.34	22.86	8.27
ND-9041	33.73	8.63	82.99	13.62
ND-9042	18.21	3.81	60.07	13.85
ND-9043	36.15	3.87	71.81	12.23
ND-9044	13.77	3.59	30.27	10.81
ND-9045	56.81	19.55	85.99	9.99
ND-9046	26.03	6.18	51.21	10.14
ND-9047	100.23	24.53	85.98	5.59
ND-9048	21.82	4.07	44.44	12.82
ND-9049	82.93	21.46	87.79	7.07
ND-9050	18.51	3.33	40.70	10.96
ND-9051	22.80	3.37	42.44	14.86
ND-9052	12.61	3.78	37.58	13.35
ND-9053	19.88	4.32	53.11	3.23
ND-9054	33.65	8.32	59.71	6.42
ND-9055	22.61	7.41	27.44	7.04
ND-9056	16.61	3.38	34.34	13.22
ND-9057	25.51	6.29	51.45	10.10
ND-9058	27.60	4.56	54.99	13.52
ND-9059	23.83	4.36	84.76	13.88
ND-9060	17.12	3.29	44.54	15.68
AL-DP-7778	19.35	8.95	63.31	14.21
AL-DP-7779	41.30	9.51	65.96	7.82
AL-DP-7780	24.01	7.52	59.43	8.85
AL-DP-7781	13.69	3.41	53.58	9.31
AL-DP-7782	31.35	5.31	65.84	10.41
AL-DP-7783	14.46	2.85	38.92	10.30
AL-DP-7784	13.52	1.52	25.09	7.89
AL-DP-7803	39.68	4.75	66.72	11.32
7804	12.56	3.96	26.81	6.28

AL-DP-7805	13.92	2.22	35.87	8.95
AL-DP-7807	35.54	4.95	70.94	11.01
AL-DP-7808	81.47	9.77	96.18	10.87
AL-DP-7810	15.14	2.12	37.66	16.19
AL-DP-7812	12.89	1.99	25.18	12.05

Those skilled in the art are familiar with methods and compositions in addition to those specifically set out in the instant disclosure which will allow them to practice this invention to the full scope of the claims hereinafter appended.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a human E6AP gene 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 15 contiguous nucleotides of SEQ ID NO: 1, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said E6AP, inhibits expression of said E6AP gene by at least 40%.

2. The dsRNA of claim 1, wherein the antisense strand comprises the sequence set forth in SEQ ID NO: 313 .

3. The dsRNA according to claim 1 or 2, wherein the antisense strand consists of the sequence set forth in SEQ ID NO: 313.

4. The dsRNA according to any one of claims 1 to 3, wherein the sense strand comprises the sequence set forth in SEQ ID NO: 157.

5. The dsRNA according to any one of claims 1 to 3, wherein the sense strand consists of the sequence set forth in SEQ ID NO: 157.

6. The dsRNA according to any one of claims 1 to 5, the antisense strand consists of the sequence set forth in SEQ ID NO: 313 and the sense strand consists of the sequence set forth in SEQ ID NO: 157.

7. The dsRNA according to any one of claims 1 to 6, wherein the dsRNA comprises at least one modified nucleotide.

8. The dsRNA according to claim 7, wherein the modified nucleotide is selected from the group consisting of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-

phosphorothioate group, a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group, 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.

9. The dsRNA according to claim 7 or 8, wherein the sense strand comprises a sequence selected from the group consisting of SEQ ID NO: 470, SEQ ID NO: 471, SEQ ID NO: 472, SEQ ID NO: 473, SEQ ID NO: 474 and SEQ ID NO: 475.

10. The dsRNA according to claim 7 or 8, wherein the sense strand consists of a sequence selected from the group consisting of SEQ ID NO: 470, SEQ ID NO: 471, SEQ ID NO: 472, SEQ ID NO: 473, SEQ ID NO: 474 and SEQ ID NO: 475.

11. The dsRNA according to any one of claims 7 to 10, wherein the sense strand comprises a sequence selected from the group consisting of SEQ ID NO: 645, SEQ ID NO: 646, SEQ ID NO: 647, SEQ ID NO: 648, SEQ ID NO: 649 and SEQ ID NO: 650.

12. The dsRNA according to any one of claims 7 to 10, wherein the sense strand consists of a sequence selected from the group consisting of SEQ ID NO: 645, SEQ ID NO: 646, SEQ ID NO: 647, SEQ ID NO: 648, SEQ ID NO: 649 and SEQ ID NO: 650.

13. A vector for inhibiting the expression of the E6AP gene in a cell, said vector comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a dsRNA according to any one of claims 1 to 12.

14. An isolated cell comprising the dsRNA according to any one of claims 1 to 12 or the vector according to claim 13.

15. A pharmaceutical composition for inhibiting the expression of the E6AP gene in an organism, said composition comprising the dsRNA according to any one of claims 1 to 12 and a pharmaceutically acceptable carrier.

16. The pharmaceutical composition according to claim 15, wherein said composition further comprises at least one additional dsRNA that inhibits expression of the human E6AP gene in a cell, and wherein the additional dsRNA comprises complementary sense and antisense strand sequences selected from the sequences set forth in Table 1, Table 3, Table 5 or Table 7 hereof, and subject to the proviso that the antisense strand of the additional dsRNA is not complementary to at least 15 contiguous nucleotides of SEQ ID NO: 1.

17. A method for inhibiting the expression of the E6AP gene in a cell, the method comprising:

(a) introducing into a cell that expresses said E6AP gene the double-stranded ribonucleic acid (dsRNA) according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 15 or 16 such that the dsRNA, upon contact with said cell expressing said E6AP, inhibits expression of said E6AP gene by at least 40%; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of an mRNA transcript of the E6AP gene, thereby inhibiting expression of the E6AP gene in the cell.

18. The method according to claim 17, wherein the dsRNA is contacted with the mRNA transcript of the E6AP gene *in vitro* at a concentration of 30 nM or less.

19. A method of treating, preventing or managing pathological processes mediated by HPV infection, said method comprising administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of the dsRNA according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 15 or 16such that the dsRNA, upon contact with a cell expressing said E6AP, inhibits expression of said E6AP gene by at least 40%.

20. A method of treating an HPV associated disorder, said method comprising administering to a patient in need of such treatment a therapeutically effective amount of a dsRNA according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 15 or 16.

21. A method of treating an E6AP-associated disorder, said method comprising administering to a patient in need of such treatment a therapeutically effective amount of a dsRNA according to any one of claims 1 to 12 or the pharmaceutical composition according to claim 15 or 16.

22. Use of the dsRNA according to any one of claims 1 to 12 in the preparation of a medicament for inhibiting the expression of an E6AP gene in a cell.

23. Use of the dsRNA according to any one of claims 1 to 12 in the preparation of a medicament for treating, preventing or managing a pathological process mediated by HPV infection.

24. Use of the dsRNA according to any one of claims 1 to 12 in the preparation of a medicament for treating an HPV associated disorder.

25. Use of the dsRNA according to any one of claims 1 to 12 in the preparation of a medicament for treating an E6AP-associated disorder.

26. The use according to any one of claims 22 to 25 wherein the medicament further comprises at least one additional dsRNA that inhibits expression of the human E6AP gene in a cell, and wherein the additional dsRNA comprises complementary sense and antisense strand sequences selected from the sequences set forth in Table 1, Table 3, Table 5 or Table 7 hereof, and subject to the proviso that the antisense strand of the additional dsRNA is not complementary to at least 15 contiguous nucleotides of SEQ ID NO: 1.

27. The dsRNA according to any one of claims 1 to 12 or the vector according to claim 13 or the isolated cell according to claim 14 or the pharmaceutical composition according to claim 15 or 16 or the method according to any one of claims 17 to 21 or the use according to any one of claims 22 to 26 substantially as hereinbefore described with reference to eh accompanying Examples and/or Drawings and/or the Sequence Listing.

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By patent attorneys for the applicant:

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EDITORIAL NOTE

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It should be noted that the Gene Sequence is on disc and can be viewed by contacting IP Australia.