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(54) Title: OLIGONUCLEOTIDES WITH ANTI-RESPIRATORY SYNCYTIAL VIRUS ACTIVITY

(57) Abstract

Disclosed are oligonucleotides which hybridize to a portion of respiratory syncytial virus (RSV) genomic RNA under physiological conditions, and, in doing so, inhibit viral replication. Also disclosed are pharmaceutical compositions and methods useful for inhibiting and treating RSV infection and including at least one or two of these oligonucleotides, or at least one of these oligonucleotides and ribavirin.

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OLIGONUCLEOTIDES WITH ANTI-RESPIRATORY SYNCYTIAL VIRUS ACTIVITY

BACKGROUND OF THE INVENTION

This invention relates to respiratory syncytial virus infection. More particularly, this invention relates to oligonucleotides complementary to portions of the genome of the virus which, when hybridized to it, inhibit viral replication.

Respiratory syncytial virus (RSV) is a major cause of pneumonia, bronchiolitis, and other respiratory diseases in infants, children, and even adults (Hruska et al. (1980) *Antimicrob. Ag. Chemother.* **17**:770-775; Kim et al. (1973) *Am. J. Epidem.* **98**:216-225; McIntosh et al. (1985) *Virol.* **1985**:1285-1304). Mortality resulting from RSV infection has been estimated as 0.5% in infected children (Report to the Medical Research Council (1978) *Br. Med. J.* **2**:796-798), but this may increase significantly in immunocompromised infants (Hall et al. (1981) *Pediatr.* **15**:613) or those with congenital heart disease (MacDonald et al. (1982) *N. Engl. J. Med.* **307**:397-400).

RSV is a member of the Paramyxoviridae family and comprises the genus *Pneumovirus*. It is an enveloped virus approximately 150 to 300 nm in size, so named because virus replication leads to fusion of neighboring cells into large

multinucleated syncytia (Chanock et al. (1957) *Am. J. Hyg.* **66**:281-290). The single-stranded RNA genome codes for ten virus-specific proteins. This negative stranded genome is contained in a helical nucleocapsid surrounded by a lipid envelope bearing two glycoproteins, one of which is the fusion protein which facilitates entry of virus into the cell by fusing host and viral membranes.

Upon entry, portions of the RSV genomic RNA are transcribed and translated, yielding viral proteins that mediate subsequent steps in virus reproduction. The next step is replication of the genome, which provides additional templates for transcription and further genome replication, amplifying the populations of virus-specific macromolecules within the cell, and supplying progeny genomes in large amounts for incorporation into new virus particles.

In genome replication, the entire base sequence of the negative-strand RNA template must be conserved as a single entity. Therefore, the sequences that are not expressed as mRNA during transcriptive RNA synthesis must be incorporated into the positive-strand RNA (antigenome) that serves as an intermediate template for the synthesis of progeny negative-strand genomes. This requires that the RNA synthesizing machinery enter an antitermination mode, ignoring all of the signals at gene boundaries and at the boundary

between the leader RNA template and various viral genes.

At present, the only clinically recognized treatment for RSV infection is the broad-spectrum antiviral drug ribavirin (1-ß-D-ribofuranosyl-1,2,4-triazole-3-carb-oxamide), a synthetic nucleoside resembling guanosine. It has been proposed that this drug functions by decreasing the intracellular concentration of GTP due to competitive inhibition of IMP dehydrogenase and two virus-specific actions, by inhibiting the function of virus-coded RNA polymerases necessary to prime and elongate viral mRNAs (reviewed in Gilbert et al. (1986) *Antimicrob. Ag. Chemother.* **30**:201-205). When administered in aerosol form, ribavirin has been reported to reduce the severity of illness and the amount of virus shed (Hall et al. (1983) ***N. Eng. J. Med.*** **308**:1443-1447). However, there is as yet no evidence that it decreases the duration of hospitalization or diminishes the need for supportive therapies.

Studies in experimental animals suggest that it may be possible to use immunotherapy for the prevention or treatment of serious RSV lower respiratory tract disease of infants and young children (Prince et al. (1985) *Virus Res.* **3**:193-206; Prince et al. (1987) *J. Virol.* **61**:1851-1854; McIntosh et al., "Respiratory Syncytial Virus" in *Virology* (2d ed.) Raven Press, New York (1990) pp.1045-1072). However, the unusual age distribution of RSV bronchiolitis and pneumonia

constitutes a major obstacle to the development of an effective vaccine for prevention of serious disease during very early infancy. If a vaccine is to have a major impact on RSV infection, it must be capable of stimulating effective resistance by the second month of life, since the peak incidence of disease occurs at this time (Parrott et al. (1973) *Am. J. Epidemiol.* **98**:289-290). Unfortunately in this age group immunosuppression by maternally derived antibodies and immunologic immaturity act to decrease vaccine efficacy (Murphy et al. (1986) *J. Clin. Microbiol.* **23**:1009-1014; Murphy (1988) *J. Virol.* **62**:3907-3910). Vaccine safety and reactogenicity, especially in the case of live vaccines, may also pose special problems in this group. Hence, alternative antiviral strategies are needed.

More recently, chemotherapeutic agents have been developed which are capable of modulating cellular and foreign gene expression. These agents, called antisense oligonucleotides, bind to a target single-stranded nucleic acid molecules according to the Watson-Crick or the Hoogsteen rule of base pairing, and in doing so, disrupt the function of the target by one of several mechanisms: by preventing the binding of factors required for normal transcription, splicing, or translation; by triggering the enzymatic destruction of mRNA by RNase H, or by destroying the target via reactive groups attached directly to the antisense oligonucleotide.

Antisense oligodeoxynucleotides have been designed to specifically inhibit the expression of HIV-1, influenza, and other viruses (see, e.g., Agrawal et al., U.S. Patent No. 5,194,428; Pederson et al., U.S. Patent Nos. 5,149,797; Agrawal (1992) *Trends in Biotechnology* **10**:152-158; Agrawal et al. in *Gene Regulation: Biology of Antisense RNA and DNA* (Erickson and Izant, eds.) Raven Press Ltd., New York (1992) pp. 273-283); Matsukura et al. in *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*, Wiley-Liss, Inc. (1992) pp. 159-178; and Agrawal (1991) in *Prospects for Antisense Nucleic Acid Therapy for Cancer and AIDS*, (Wickstrom, ed.) Liss, New York, pp. 145-148). A need remains for the development of oligonucleotides that are capable of inhibiting the replication or propagation of respiratory syncytial virus and whose uses are accompanied by low or no cellular toxicity.

SUMMARY OF THE INVENTION

The present invention provides synthetic oligonucleotides which hybridize under normal physiological conditions to a portion of respiratory syncytial virus genomic RNA (vRNA) or antigenomic replicative intermediate. The specific portions of the RSV RNA targeted by the oligonucleotides of the invention are the recognition sites for the RSV RNA polymerase. As used herein, the term "synthetic oligonucleotide" includes chemically synthesized polymers of three or up to 100 and preferably from about 12 to about 50 ribonucleotide and/or deoxyribonucleotide monomers connected together or linked by at least one 5′ to 3′ internucleotide linkage.

In some aspects of the invention, the oligonucleotides are modified. The term "modified oligonucleotide" is used herein as an oligonucleotide in which at least two of its nucleotides are covalently linked via a synthetic linkage, i.e., a linkage other than a phosphodiester between the 5′ end of one nucleotide and the 3′ end of another nucleotide in which the 5′ nucleotide phosphate has been replaced with any number of chemical groups. Preferable synthetic linkages include alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, phosphoramidates, phosphate esters, carbamates, carbonates, phosphate triesters, acetamidate, and carboxymethyl esters. In one preferred embodiment

of the invention, the oligonucleotide comprises at least one phosphorothioate linkage. The term "modified oligonucleotide" also encompasses oligonucleotides with a modified base and/or sugar. For example, a 3', 5'-substituted oligonucleotide is a modified oligonucleotide having a sugar which, at both its 3' and 5' positions is attached to a chemical group other than a hydroxyl group (at its 3' position) and other than a phosphate group (at its 5' position). A modified oligonucleotide may also be a capped species. In addition, unoxidized or partially oxidized oligonucleotides having a substitution in one nonbridging oxygen per nucleotide in the molecule are also considered to be modified oligonucleotides. Also considered as modified oligonucleotides are oligonucleotides having nuclease resistance-conferring bulky substituents at their 3' and/or 5' end(s) and/or various other structural modifications not found *in vivo* without human intervention are also considered herein as modified.

The oligonucleotides of the invention are complementary to a portion of the vRNA or antigenomic RNA and hybridize to the vRNA or antigenomic RNA under normal physiological conditions existing within a cell harboring the RNA. Such conditions include pH, temperature, and ionic conditions.

The portion of the RNA to which the oligonucleotides of the invention are

complementary includes any region of the genome containing an RSV viral polymerase recognition site. This region is at least 6, and preferably 12 to 60 nucleotides in length. In some preferred embodiments, the oligonucleotides are complementary to 15 to 25 nucleotides of the RSV RNA. In some embodiments, the region is at least a portion of the leader, NS1, NS2, and P RSV genes such as nucleotides (starting from the 3' end of vRNA) 1-20 (leader region), 31-50 or 41-60 (NS1/leader), and 590-609 (NS2/P). Oligonucleotides complementary to these regions have the nucleotides sequences set forth herein as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, respectively.

In some aspects, the invention provides an oligonucleotide which has antiviral activity against respiratory syncytial virus effected by hybridization with a portion of the vRNA. In others, a pharmaceutical composition including at least one of the oligonucleotides of the invention, and in some embodiments, at least two different oligonucleotides of the invention, and a pharmaceutically acceptable carrier are provided. In yet other embodiments, the composition further includes ribavirin.

The pharmaceutical composition is used in a method of inhibiting, preventing, and/or reducing RSV replication in a cell. In this method, a therapeutic amount of the pharmaceutical composition is administered to the cell which is

to be protected from infection or treated for an existing infection. The oligonucleotide (or oligonucleotides) in the pharmaceutical composition enters the cell, wherein it (they) hybridize(s) to RSV RNA, thereby inhibiting RSV replication. Ribavirin in ribavirin-containing compositions inhibits replication of vRNA in the cell in three possible ways: it causes a decrease in the intracellular concentration of GTP; it inhibits 5'-cap formation of mRNAs; and/or it inhibits the function of virus-coded RNA polymerases. The pharmaceutical composition is also utilized in a method of treating respiratory syncytial virus infection wherein the composition is administered to an infected mammal or cell.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects of the present invention, the various features thereof, as well as the invention itself may be more fully understood from the following description, when read together with the accompanying drawings in which:

FIG. 1 is a diagrammatic representation of the genomic map of RSV (3' to 5'), wherein gene boundaries are indicated by vertical lines and gene lengths of intergenic, gene overlap, and proposed leader and trailer regions are indicated in number of bases beneath the map;

FIG. 2 is a schematic representation of the partial nucleotide sequences of the RSV genes NS1, NS2, N, P, M, SH, G, F, 22K, and L;

FIG. 3 is a graphic representation of the effect of various oligonucleotides on RSV replication *in vitro*: -□-, unrelated oligonucleotide, -◇-, vRNA 1-20 (SEQ ID NO:1); -o-, vRNA 590-609 (SEQ ID NO:4); -Δ-, vRNA 41-60 (SEQ ID NO:3);

FIG. 4 is a graphic representation of the effect of ribavirin (-□-) and various oligonucleotides: -◇-, NS2 1-20 (SEQ ID NO:6); -o-, NS2 aug (SEQ ID NO:5); -Δ-, vRNA 31-50 (SEQ ID NO:2) on RSV replication *in vitro*;

FIG. 5 is a graphic representation of the effect of ribavirin (-□-) and various oligonucleotides: -◇-, vRNA 590-609 (SEQ ID NO:4); -o-, vRNA 41-60 (SEQ ID NO:3); -Δ-, vRNA 31-50 (SEQ ID NO:2); and (-□-), unrelated oligonucleotide on RSV replication *in vitro*;

FIG. 6 is a graphic representation of the percent difference between the expected inhibition of RSV replication and the measured inhibition of RSV replication obtained when two different oligonucleotides of the invention are used in combination; and

FIG. 7 is a graphic representation of the percent difference between the expected inhibition of RSV replication and the measured inhibition of RSV replication obtained when one oligonucleotide of the invention and ribavirin are used in combination.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. The issued U.S. patent and allowed applications and references cited herein are hereby incorporated by reference.

The present invention relates to oligonucleotides which are sufficiently complementary to regions of the RSV genome such that, under normal physiological conditions existing in the cell, they hybridize to those regions, rendering the genome unavailable to serve as a template for production of another genome strand or mRNA. As a result, RSV replication is inhibited.

The oligonucleotides may be targeted to any region of the RNA, such as those depicted in the simplified genomic map of FIG. 1, and schematically in FIG. 2. Examples of such oligonucleotides include those complementary to a portion of the leader region including nucleotides 1-20 (from the 3' end of the vRNA), portions of the leader plus the NS1 gene, including nucleotides 31-50, and 41-60, and portions of the intergenic region (between NS1 and NS2) as well as the NS2 gene, including nucleotides 590-609. The base sequence between nucleotides 590-609 is also found at the end of the N gene and beginning of the P gene, between nucleotides 2323-2342 (the N/P

gene region); consequently, an oligonucleotide designed to bind to nucleotides 590-609 can bind to a second region on the viral genome. Each of these regions of the RSV RNA corresponds to a recognition site for the viral polymerase. These oligonucleotides are shown in TABLE 1 below and have the nucleotides sequences set forth herein as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, respectively.

RSV contains a nonsegmented negative stranded RNA genome of approximately 15000 nucleotides, encoding 10 genes. In addition, a 44 base leader sequence and 155 base trailer sequence are found at the 3' and 5' ends of the genome, respectively. After RSV infection, transcription of RSV mRNAs is mediated by proteins contained in the viral nucleocapsid. mRNA transcription is believed to occur processively along the genome; the transcriptional machinery recognizes individual gene start and gene stop sequences in order to generate individual mRNAs. In addition, replication of the genome to a full length antigenome (positive strand) intermediate occurs, indicating that the transcriptional machinery can ignore the gene start and gene stop sequences. Finally, the antigenome is replicated to produce the full length genome which is packaged into virion particles.

Viral polymerase recognition sites occur throughout the RSV genome. For example, such recognition sites comprise RNA sequences at the 3'

end of the genome and antigenome. Additional viral polymerase recognition sites occur at the gene or transcription start and gene or transcription stop sites for each RSV gene. The viral polymerase recognition site sequences are important recognition sequences for the replication and transcription of RSV RNA. The leader, portions of the NS1 gene within nucleotides 45-86; portions of the NS2 gene within nucleotides 585-615; portions of the N gene within nucleotides 1117-1144; portions of the P gene within nucleotides 2318-2348; portions of the M gene within nucleotides 3243-3271; portions of the SH gene within nucleotides 4211-4238; portions of the G gene within nucleotides 4664-4692; portions of the F gene within nucleotides 5640-5667; portions of the 22k gene within nucleotides 7588-7607; portions of the L gene within nucleotides 8480-8565;, and portions of the trailer sequence within nucleotides 15191-15222 are all RSV viral polymerase recognition sites in accordance with the invention, each of which is potentially required for RNA replication and packaging of the genome. These sequences must be recognized by the viral polymerase and structural proteins, and are potential targets for the antisense oligonucleotides of the invention. The minimum RSV proteins required for viral RNA replication are RSV polymerase (L), nucleocapsid (N), and phosphoprotein (P), and all of these genes are potential targets for the antisense oligonucleotides of the invention. More efficient transcription occurs when all viral proteins are

present after RSV infection, indicating that additional RSV proteins participate in RSV transcription, and that additional targets exist for the antisense oligonucleotides of the invention.

TABLE 2 below lists oligonucleotides of the invention which are specific for various viral polymerase recognition sites of the RSV genes. For example, the oligonucleotide of SEQ ID NO:8 specifically targets nucleotides 45-64 of the NS1 gene transcription start site. The oligonucleotides of SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11 specifically target portions of the NS2 gene transcription start site. The oligonucleotides of SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14 specifically target portions of the N gene transcription start site. The oligonucleotides of SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 specifically target portions of the P gene transcription start site. The oligonucleotides of SEQ ID NO:18 and SEQ ID NO:19 specifically target portions of the M gene transcription start site. The oligonucleotides of SEQ ID NO:20 and SEQ ID NO:21 specifically target portions of the SH gene transcription start site. The oligonucleotides of SEQ ID NO:22 and SEQ ID NO:23 specifically target portions of the G gene transcription start site. The oligonucleotides of SEQ ID NO:24 and SEQ ID NO:25 specifically target portions of the F gene transcription start site. The oligonucleotides of SEQ ID NO:26 and SEQ ID NO:27 specifically

target portions of the 22k gene transcription start site. The oligonucleotides of SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:31 specifically target portions of the L gene transcription start site. The oligonucleotides of SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34 specifically target portions of the complement or antigenome of the trailer region of the RSV genome. Additional antisense oligonucleotides of the invention may vary in length or may overlap within the gene regions described above.

TABLE 1

			EC_{50}		
Name	Sequence	SEQ ID NO.	Expt.# 1	2	3
ribavarin	N/A	--	10.0	11.0	7.0
vRNA1-20	ACGCGAAAAAATGCGTACAA	1	16	5	3.2
vRNA31-50	TAAACCAAAAAAATGGGGCA	2	1.1	1.1	0.7
vRNA41-60	AAATGGGGCAAATAAGAATT	3	1.1	1.1	1
vRNA590-609	AAAAATGGGGCAAATAAATC	4	1.2	1.1	1
NS1 aug	CTCAATGAATTGCTGCCCAT	5	>30	>30	--
NS2 (1-15)	TGATTTATTTGCCCC	6	25	30	--
NS2 (1-20)	TGAATTGATTTATTTGCCCC	7	>30	30	--

TABLE 2

Target	Sequence	RSV Genome Region	SEQ ID NO.
vNS1	GGGGCAAATAAGAATTTGAT	45-64	8
vNS2	AAGACAAAAATGGGGCAAAT	585-604	9
vNS2	TGGGGCAAATAAATCAATTC	595-614	10
vNS2	GGGGCAAATAAATCAATTCA	596-615	11
vN	TAGAAGATGGGGCAAATACA	1117-1136	12
vN	TGGGGCAAATACAAAGATGG	1124-1143	13
vN	GGGGCAAATACAAAGATGGC	1125-1144	14
vP	TAATAAAAAATGGGGCAAAT	2318-2337	15
vP	TGGGGCAAATAAATCATCAT	2328-2347	16
vP	GGGGCAAATAAATCATCATG	2329-2348	17
vM	GGAAAGGGTGGGGCAAATAT	3243-3262	18
vM	GGGGCAAATATGGAAACATA	3252-3271	19
vSH	ATACACATGGGGCAAATAAT	4211-4230	20
vSH	GGGGCAAATAATCATTGGAG	4219-4238	21
vG	AATAACATTGGGGCAAATGC	4664-4683	22
vG	GGGGCAAATGCAAACATGTC	4673-4692	23
vF	TAAACTCTGGGGCAAATAAC	5640-5659	24
vF	GGGGCAAATAACAATGGAGT	5648-5667	25
v22k	ATGAAAACTGGGGCAAATAT	7588-7607	26
v22k	GGGGCAAATATGTCACGAAG	7597-7616	27
vL	TTCAAGTTGTGGGACAAAAT	8480-8499	28
vL	TGGGACAAAATGGATCCCAT	8489-8508	29
vL	GGGACAAAATGGATCCCATT	8490-8509	30
vL	AGTTATTTAAAAGGTGTTAT	8546-8565	31

Target	Sequence	RSV Genome Region	SEQ ID NO.
trailer	ACGAGAAAAAAAGTGTCAAA	15203-15222	32
trailer	GTGTCAAAAACTAATATCTC	15191-15210	33
trailer	AAAAAGTGTCAAAAACTAAT	15196-15215	34

"RSV Genome Region" refers to the nucleotide number of the RSV viral genome.

Oligonucleotides of the invention are composed of deoxyribonucleotides, ribonucleotides, or a combination of both, with the 5' end of one nucleotide and the 3' end of another nucleotide being covalently linked. These oligonucleotides are at least 6 nucleotides in length, but are preferably 12 to 60 nucleotides long, with 15 to 25mers being the most common. They can be prepared by art-recognized methods such as phosphoramidate or H-phosphonate chemistry which can be carried out manually or by an automated synthesizer as described in Uhlmann et al. (*Chem. Rev.* (1990) **90**:534-583).

The oligonucleotides of the invention may also be modified in a number of ways without compromising their ability to hybridize to RSV vRNA. For example, the oligonucleotides may contain other than phosphodiester internucleotide linkages between the 5' end of one nucleotide and the 3' end of another nucleotide in which the 5' nucleotide phosphate has been replaced with any

number of chemical groups. Examples of such chemical groups include alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. Oligonucleotides with these linkages can be prepared according to known methods (see, e.g., Uhlmann et al. (1990) *Chem. Rev.* **90**:543-583).

Other modifications include those which are internal or at the end(s) of the oligonucleotide molecule and include additions to the molecule of the internucleoside phosphate linkages, such as cholesteryl or diamine compounds with varying numbers of carbon residues between the amino groups and terminal ribose, deoxyribose and phosphate modifications which cleave, or crosslink to the opposite chains or to associated enzymes or other proteins which bind to the viral genome. Examples of such modified oligonucleotides include oligonucleotides with a modified base and/or sugar such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide having a sugar which, at both its 3' and 5' positions is attached to a chemical group other than a hydroxyl group (at its 3' position) and other than a phosphate group (at its 5' position). Other modified oligonucleotides are capped with a nuclease resistance-conferring bulky substituent at their 3' and/or 5' end(s) , or have a substitution in one nonbridging oxygen per nucleotide.

Modifications of the oligonucleotides can be at some or all of the internucleoside linkages, as well as at either or both ends of the oligonucleotide and/or in the interior of the molecule.

The preparation of these modified and unmodified oligonucleotides is well known in the art (reviewed in Agrawal et al. (1992) *Trends Biotechnol.* **10**:152-158; Agrawal in Protocols for Oligonucleotides and Analogs, Synthesis and Properties (Agrawal, ed.), Humana Press, Totowa, New Jersey (1993), Chapter 20). For example, nucleotides can be covalently linked using art-recognized techniques such as phosphoramidate, H-phosphonate chemistry, or methylphosphoramidate chemistry (*see, e.g.*, Uhlmann et al. (1990) Chem. Rev. **90**:543-584; Agrawal et al. (1987) *Tetrahedron. Lett.* **28**:(31):3539-3542); Caruthers et al. (1987) *Meth. Enzymol.* **154**:287-313; U.S. Patent 5,149,798). Oligomeric phosphorothioate analogs can be prepared using methods well known in the field such as methoxyphosphoramidite (*see, e.g.*, Agrawal et al. (1988) *Proc. Natl. Acad. Sci.* (USA) **85**:7079-7083) or H-phosphonate (*see, e.g.*, Froehler (1986) *Tetrahedron Lett.* **27**:5575-5578) chemistry. The synthetic methods described in Bergot et al. (*J. Chromatog.* (1992) **559**:35-42) can also be used.

The present invention further provides therapeutic compositions having antiviral activity

against RSV, and including the synthetic oligonucleotides of the present invention, along with a physiologically acceptable carrier. The therapeutic compositions of the invention comprise at least one synthetic oligonucleotide which is capable of specifically down-regulating expression of at least one RSV gene or RSV RNA, and a pharmaceutically acceptable carrier or diluent. It is preferred that a synthetic oligonucleotide used in the therapeutic composition of the invention be complementary to at least a portion of the RSV viral leader region, a portion of the NS1/leader region, a portion of the NS1 gene, a portion of the NS2 gene, a portion of the P gene, and a portion of the N/P region.

As used herein, a "pharmaceutically or physiologically acceptable carrier" includes any and all solvents (including but not limited to lactose), dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional medium or agent is incompatible with the active ingredient, its use in the therapeutic compositions of the invention is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The therapeutic composition of the invention may be used to inhibit, reduce or prevent the

replication of RSV in cells through the activity of the synthetic oligonucleotides in the composition. RSV-infected cells are treated with the therapeutic composition of the invention in an amount sufficient to enable the binding of the synthetic oligonucleotide to the RNA in the infected cells. In this way, the binding of the synthetic oligonucleotide to the RSV RNA inhibits the expression and replication of the virus. Thus the synthetic oligonucleotides of the invention can be used to control RSV infection in infected cell cultures, thus allowing cultures to be used for other purposes.

The synthetic oligonucleotides of the invention may be used to inhibit RSV proliferation *in vitro* or *in vivo* or to treat RSV infection in mammals. A preferred therapeutic composition of the invention suitable for inhibiting RSV proliferation *in vitro* or *in vivo* or for treating RSV infection in mammals, including humans, in accordance with the methods of the invention comprises from about 25 to 75 mg of at least one lyophilized synthetic oligonucleotide(s) having SEQ ID NOS:1 through 7 and 20-75 mg lactose, USP, which is reconstituted with sterile normal saline to the therapeutically effective amounts or dosages described herein.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the therapeutic composition or method that is sufficient to show a meaningful

subject or patient benefit, i.e., a reduction in symptomatology, a reduction in the number of RSV virions produced, a reduction in the expression of RSV proteins, or a reduction in the level of RSV antigens or RSV-specific antibodies detected in the biological fluid of an infected mammal. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

A "therapeutically effective manner" refers to a route, duration, and frequency of administration of the therapeutic composition which ultimately results in meaningful patient benefit, as described above. In some embodiments of the invention, the therapeutic composition is administered via injection, sublingually, rectally, intradermally, orally, enterally, intranasally, or by aerosol, in bolus, continuous, intermittent, or continuous, followed by intermittent regimens.

The therapeutically effective amount of synthetic oligonucleotide in the therapeutic composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patent has undergone. Effective dosages of the synthetic oligonucleotide

and modes of its administration in the treatment of RSV infections can be determined by routine experimentation. Ultimately, the attending physician will decide the amount of synthetic oligonucleotide with which to treat each individual patient. Initially, the attending physician will administer low doses of the synthetic oligonucleotide and observe the patient's response, for example, by measuring the number of RSV virions, by measuring the RSV proteins, RSV antigens, or RSV-specific antibodies produced in the patient's bodily fluids, using an assay specific for RSV, for one or more RSV proteins, for one or more RSV antigens, or for one or more RSV-specific antibodies. Larger doses of synthetic oligonucleotide may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the dosages of the therapeutic compositions administered in the method of the present invention should contain about 0.1 to 30.0 mg synthetic oligonucleotide per kg body weight per day, and preferably 0.1 to 2.0 mg synthetic oligonucleotide per kg body weight per day. When administered systemically, the therapeutic composition is preferably administered at a sufficient dosage to attain a blood level of synthetic oligonucleotide from about 0.01 μM to about 10 μM. Preferably, the concentration of synthetic oligonucleotide at the site of viral gene expression should be from about 0.01 μM to about 10 μM, and more preferably from about 0.05

μM to about 5 μM. However, for localized administration, much lower concentrations than this may be effective, and much higher concentrations may be tolerated. It may be desirable to administer simultaneously or sequentially a therapeutically effective amount of one or more of the therapeutic compositions of the invention to the individual as a single treatment episode.

Administration of therapeutic compositions in accordance with invention or to practice the methods of the present invention can be carried out in a variety of conventional ways, such as by oral ingestion, enteral, rectal, or transdermal administration, inhalation (intranasal or by aerosol), sublingual administration, or cutaneous, subcutaneous, intramuscular, intraocular, intraperitoneal, or intravenous injection, or any other route of administration known in the art for administering therapeutic agents.

When the composition is to be administered orally, sublingually, or by any non-injectable route, the therapeutic formulation will preferably include a physiologically acceptable carrier, such as an inert diluent or an assimilable edible carrier with which the composition is administered. Suitable formulations that include pharmaceutically acceptable excipients for introducing compounds to the bloodstream by other than injection routes can be found in *Remington's Pharmaceutical Sciences* (18th ed.) (Genarro, ed. (1990)

Mack Publishing Co., Easton, PA). The synthetic oligonucleotide and other ingredients may be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. The therapeutic compositions may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. When the therapeutic composition is administered orally, it may be mixed with other food forms and pharmaceutically acceptable flavor enhancers. When the therapeutic composition is administered enterally, they may be introduced in a solid, semi-solid, suspension, or emulsion form and may be compounded with any number of well-known, pharmaceutically acceptable additives. Sustained release oral delivery systems and/or enteric coatings for orally administered dosage forms are also contemplated such as those described in U.S. Patent Nos. 4,704,295, 4,556,552, 4,309,404, and 4,309,406.

When a therapeutically effective amount of composition of the invention is administered by injection, the synthetic oligonucleotide will preferably be in the form of a pyrogen-free, parenterally-acceptable, aqueous solution. The preparation of such parenterally-acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred therapeutic composition for injection should contain, in addition to at least

one synthetic oligonucleotide, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The therapeutic composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile. It must be stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents. Prolonged absorption of the injectable therapeutic agents can be brought about by the use of the compositions of agents delaying absorption. Sterile injectable solutions are prepared by incorporating the synthetic oligonucleotide in the required amount in the appropriate solvent, followed by filtered sterilization.

The therapeutic composition can be administered in bolus, continuous, or intermittent

dosages, or in a combination of continuous and intermittent dosages, as determined by the physician and the degree and/or stage of illness of the patient. In the method of the invention, the therapeutic composition may be administered once in a therapeutically effective amount or repeatedly in less than therapeutic amounts. The duration of therapy using the therapeutic composition of the present invention will vary, depending on the unique characteristics of the synthetic oligonucleotide and the particular therapeutic effect to be achieved, the limitations inherent in the art of preparing such a therapeutic formulation for the treatment of humans, the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the therapeutic composition of the present invention.

A demonstration of the antiviral effect of individual synthetic oligonucleotides of the invention is described below in the examples and is depicted in FIGS. 3 - 5. Briefly, the phosphorothioate oligonucleotides targeted to mRNA and vRNA as set forth in TABLE 1 below were synthesized and administered at several different concentrations to human epidermal carcinoma cells which had been preinfected with RSV. Virus was removed and infected cells were incubated for 3 days with either an oligonucleotide, ribavirin, or

no additional treatment. The effect of the treatment was determined by plaque assay, ELISA, or virus yield assay. The ELISA results are shown in TABLE 1, where "EC_{50}" is the ribavirin or oligonucleotide concentration which inhibits virus replication by 50%. The oligonucleotides referred to as "NS1 aug," "NS2 (1-15)," and "NS2 (1-20)" are complementary to mRNA transcribed from the NS1 and NS2 genes. The vRNA oligonucleotides are complementary to genomic RNA nucleotides 1-20 of the leader sequences and those regions encoding portions of the NS1, NS2, and P genes.

As shown in the table above, EC_{50} values measured for mRNA-targeted oligonucleotides were greater than or equal to 10 μM. Antisense oligonucleotides targeted to vRNA were more potent inhibitors (EC_{50} values were about 1 μM). Under the same experimental conditions, ribavirin inhibited RSV replication with an EC_{50} of about 10 μM. These results indicate that the oligonucleotides of the invention targeted to RSV vRNA can effectively inhibit RSV replication. Under experimental conditions where the multiplicity of infection (MOI) was 10 fold lower, the antiviral activity of the oligonucleotides increased (see FIG. 5).

In addition, two different oligonucleotides or one oligonucleotide and ribavirin were tested in combination. Data was analyzed according to the dissimilar site inhibitor model of Prichard et al. (*Antiviral Res.* (1990) **14**:181-206). Three

dimensional graphs of percent inhibition above expected for all drug combinations were generated. The results are shown in FIGS. 6 and 7, which represent the average data from two individual experiments. These figures show that oligonucleotides of the invention can interact synergistically with ribavirin (FIG. 7) or another oligonucleotide (FIG. 6). At low concentrations, two oligonucleotides together produce as much as 50% more inhibition than expected by adding the inhibition of the individual oligonucleotides (FIG. 6). At higher concentrations, the interactions are somewhat antagonistic. Similarly, when ribavirin was combined with an oligonucleotide, 20-30% more inhibition than expected was detected (FIG. 7). At higher concentrations, the interactions were additive. These results suggest that combinations of oligonucleotides or one oligonucleotide plus ribavirin have a therapeutic advantage over the individual drugs alone.

The following examples illustrate the preferred modes of making and practicing the present invention, but are not meant to limit the scope of the invention since alternative methods may be utilized to obtain similar results.

EXAMPLES

1. Synthesis and Purification of Oligonucleotides

Phosphodiester-linked oligonucleotides and oligonucleotide phosphorothioates were synthesized on an automated synthesizer (Gene Assembler, Pharmacia Biotech, Inc., Piscataway, NJ). They were assembled by phosphoramidate chemistry. The synthesis and purification of were carried out by the method of Agrawal et al.(Proc. Natl. Acad. Sci. (USA) (1989) **86**:7790-7794). Oligonucleotide concentrations were determined by absorbance at 260 nm, taking into account the molar extinction coefficient of the nucleotides present in each sequence (Ausubel et al., eds. (1987) *Current Protocols in Molecular Biology* (Wiley, New York)).

2. Cells

HEp-2 cells, obtained from the American Type Culture Collection (Rockville, MD, ATCC No. CLL23) were cultured in Minimum Essential Medium with Earl's balanced salts (EMEM) (JRH Biosciences, Lenexa, KA) supplemented with 10% (volume:volume) fetal bovine serum (FBS) AT 37°C in 5% CO_2.

3. Virus

Respiratory syncytial virus (RSV) strain A2- obtained from American Type Culture Collection, (Rockville, MD, ATCC No. VR-1302) was grown in HEp-2 cells. Virus is harvested from supernatant 4 to 6 days after infection of the cells. The supernatant was stored at -80°C and used as stock in all experiments.

4. Ribavirin

The compound was obtained from Pharmacia, Inc. (Alachua, FL).

5. Inhibition Studies with Single Drug

HEp-2 cells (20,000 cell/well of a microtiter plate) were incubated with RSV at a multiplicity of infection (moi) of 0.01 for 60 to 90 minutes at 37°C. Virus was removed and oligonucleotides (diluted in culture medium) or ribavirin are/is added to the infected cells. Oligonucleotides were tested at 4 to 6 concentrations from 30 to 0.01 μM. Infected cells were incubated for 3 days at 37°C.

6. Inhibition Studies with Drug Combinations

Two oligonucleotides or one oligonucleotide and ribavirin (individually or collectively called "drugs") were evaluated in combination according to the methods of Prichard et al. (*Antiviral Res.* (1990) **14**:181-206). HEp-2 cells were infected with RSV as described above. Drugs were added to the RSV-infected HEp-2 cells in a 96 well plate. The same concentration of one drug was added to 10 wells in the top row of the plate. Serial dilutions of the drug were made into the other rows of the plate. Serial dilutions of the second drug were prepared in the columns of another 96 well plate, then transferred to the plate containing the RSV-infected cells. This

established a checkerboard matrix of concentrations on the plate, containing 9 concentrations of one drug in the presence of 7 concentrations of the second drug (a total of 63 different drug combinations). In addition, the drugs were titrated alone to determine their individual effects on virus replication.

Data from drug combinations were analyzed according to the following equation for dissimilar site inhibitors as described by Prichard et al. (*ibid.*)

$$Z = X + Y(1-X)$$

This equation states that the total inhibition (Z) produced by a combination of drugs is equal to the sum of the inhibition produced by one drug (X) plus the inhibition by a second drug (Y) on the populations unaffected by the first drug (1-X).

Using this equation, the inhibition data for the individual drugs at each concentration was used to calculate expected levels of inhibition for each combination. The difference between measured inhibition and expected inhibition was determined. The percent difference between measured and expected was plotted as a function of both drugs in a 3- dimensional graph. The results are shown in FIGS. 5 and 6. Values greater than 0 suggest synergistic interaction between drugs. Values less than 0 suggest that two drugs are

antagonistic. Values equal to 0 suggest that the interactions are additive.

7. Plaque Assay

Plate HEp-2 cells were seeded in 24-well plate(s) at 250,000 cells/well and incubated at 37°C overnight. Cells were washed in serum-free media. Virus was diluted in media with 2% FBS at half-log increments. Each dilution of virus (50 μl) was added to replicate wells and incubated 1-2 hours at 37°C, 5% CO_2. Each well was overlayed with 1 ml of 0;8% methylcellulose in media with 2% FBS and incubated at 37°C, 5% CO_2. When plaques developed (3-4 days), overlay was discarded and cells were stained with 5% glutaraldehyde/0.1% crystal violet (0.5 ml/well). Cells were incubated at room temperature for 45 minutes. Stain was discarded and plaques were counted under a dissecting microscope.

8. ELISA

The enzyme immunoassay procedure is based on the method of Kang and Pai (A.J.C.P. (March, 1989) 323-326) which is a modification of the methods of Berkowitz et al. (*Antimicrob. Agents Chemother.* (1985) **28**:207-210)) and Rabalais et al. (*Antimicrob. Agents Chemother.* (1987) **31**:946-948), and used monoclonal antibodies specific for RSV F glycoprotein (provided by the FDA). These RSV antibodies were diluted 1:10,000 in 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) and reacted

for 1 hour at 37°C. Secondary antibody (horse radish peroxidase labelled goat anti-mouse IgG) was also diluted 1:10,000. Color was developed with 3, 3', 5, 5'-tetramethyl-benzidine (TMB) substrate, stopped with 0.1 N H_2SO_4, and the color read at 450 nm with a 620 nm reference filter. Virus replication as determined by this ELISA in "drug"-treated cultures was compared to untreated, infected cultures to obtain the per cent inhibition. The EC_{50}, or the drug concentration which inhibits viral replication by 50%, was determined graphically.

9. Virus Yield Assay

Supernatant from RSV-infected HEp-2 cells was titrated onto uninfected HEp-2 cells. The cells were then cultured for 3 to 7 days (or until syncytia were present in the culture), and the virus measured by the immunoassay described in Example 7 above. Virus titer of oligonucleotide or ribavirin-treated cells was compared to that of untreated cells.

10. *In Vivo* Studies

At day 0, twenty cotton rats were inoculated intranasally with approximately 100 median cotton rat infectious doses of RSV strain A2 (see, Wyde et al. (1993) *Antiviral Res.* **20**, 145-154). On days 1 through 3, groups of animals (four animals per treatment group) were treated with: distilled water; an oligonucleotide control at a dosage of

30 mg/kg/day; or the oligonucleotide of SEQ ID NO:4 in distilled water administered intranasally to at dosages of 3 mg/kg/day, 10 mg/kg/day, or 30 mg/kg/day. At day 4, animals were sacrificed, lung tissue was harvested and prepared as in Wyde et al., *supra*. Dilutions of lung preparations were cultured in the presence of HEp2 cells, and the cells were observed for appearance of syncytia. Complete inhibition of RSV was observed in three of four animals receiving the 30 mg/kg/day dosage.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Brown-Vargas, Patrick E.

(ii) TITLE OF INVENTION: OLIGONUCLEOTIDES WITH ANTI-RESPIRATORY SYNCYTIAL VIRUS ACTIVITY

(iii) NUMBER OF SEQUENCES: 34

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(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/544,130
(B) FILING DATE: 17-OCT-1995

(viii) ATTORNEY/AGENT INFORMATION:
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(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617-330-1300
(B) TELEFAX: 617-330-1311

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACGCGAAAAA ATGCGTACAA 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TAAACCAAAA AAATGGGGCA 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAATGGGGCA AATAAGAATT 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAAAATGGGG CAAATAAATC 20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCAATGAAT TGCTGCCCAT 20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGATTTATTT GCCCC 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGAATTGATT TATTTGCCCC 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGGGCAAATA AGAATTTGAT 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AAGACAAAAA TGGGGCAAAT 20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGGGGCAAAT AAATCAATTC 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGGGCAAATA AATCAATTCA 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TAGAAGATGG GGCAAATACA 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGGGGCAAAT ACAAAGATGG 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGGGCAAATA CAAAGATGGC 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TAATAAAAAA TGGGGCAAAT 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGGGGCAAAT AAATCATCAT 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGGGCAAATA AATCATCATG
20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGAAAGGGTG GGGCAAATAT 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGGCAAATA TGGAAACATA 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATACACATGG GGCAAATAAT 20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGGGCAAATA ATCATTGGAG 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AATAACATTG GGGCAAATGC 20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGGGCAAATG CAAACATGTC 20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TAAACTCTGG GGCAAATAAC 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGGGCAAATA ACAATGGAGT 20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATGAAAACTG GGGCAAATAT 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGGGCAAATA TGTCACGAAG 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TTCAAGTTGT GGGACAAAAT 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TGGGACAAAA TGGATCCCAT 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GGGACAAAAT GGATCCCATT 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AGTTATTTAA AAGGTGTTAT 20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ACGAGAAAAA AAGTGTCAAA 20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTGTCAAAAA CTAATATCTC 20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc =
"oligodeoxynucleotide"

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AAAAAGTGTCAAAAACTAAT 20

What is claimed is:

1. A synthetic oligonucleotide comprising from 12 to 60 covalently linked nucleotides capable of inhibiting transcription of respiratory syncytial virus genomic RNA and having sequence complementarity with a portion of a recognition site for a respiratory syncytial virus RNA polymerase.

2. The oligonucleotide of claim 1, wherein the recognition site is selected from the group consisting of a portion of the NS1 gene, a portion of the intergenic region between the NS1 and NS2 genes including a portion of the NS2 gene, a portion of the NS2 gene, a portion of the N gene, a portion of the N/P gene region, a portion of the P gene, a portion of the M gene, a portion of the SH gene, a portion of the G gene, a portion of the F gene, a portion of the 22k gene, a portion of the L gene, and a portion of the trailer region.

3. The oligonucleotide of claim 2, wherein at least one internucleotide linkage between the covalently linked nucleotides comprises a modification selected from the group consisting of an alkylphosphonate linkage, a phosphorothioate linkage, a phosphorodithioate linkage, an alkylphosphonothioate linkage, a phosphoramidate linkage, a phosphate ester linkage, a carbamate linkage, a carbonate linkage, a phosphate triester

linkage, an acetamidate linkage, and a carboxymethyl ester linkage.

4. The oligonucleotide of claim 3 wherein at least one internucleotide linkage is a phosphorothioate linkage.

5. The oligonucleotide of claim 1 which comprises at least one deoxyribonucleotide.

6. The oligonucleotide of claim 1 which comprises at least one ribonucleotide.

7. The oligonucleotide of claim 5 which comprises at least one ribonucleotide.

8. The oligonucleotide of claim 1 which comprises at least one deoxyribonucleotide and at least one ribonucleotide.

9. The oligonucleotide of claim 2 having a nucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO: 23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28, SEQ ID NO: 29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34.

10. The oligonucleotide of claim 9 wherein the nucleotide sequence is SEQ ID NO:10.

11. The oligonucleotide of claim 9 wherein the nucleotide sequence is SEQ ID NO:13.

12. The oligonucleotide of claim 9 wherein the nucleotide sequence is SEQ ID NO:15.

13. The oligonucleotide of claim 9 wherein the nucleotide sequence is SEQ ID NO:32.

14. The oligonucleotide of claim 9 wherein the nucleotide sequence is SEQ ID NO:33.

15. The oligonucleotide of claim 9 wherein the nucleotide sequence is SEQ ID NO:34.

16. A therapeutic composition comprising the oligonucleotide of claim 1 and a pharmaceutically acceptable carrier.

17. The therapeutic composition of claim 16, comprising at least one oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO: 23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28, SEQ ID NO: 29, SEQ ID NO:30, SEQ

ID NO:31, SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34.

18. The therapeutic composition of claim 17, wherein the oligonucleotide has the nucleotide sequence of SEQ ID NO:10.

19. The therapeutic composition of claim 15, wherein the oligonucleotide has the nucleotide sequence of SEQ ID NO:13.

20. The therapeutic composition of claim 15, wherein the oligonucleotide has the nucleotide sequence of SEQ ID NO:15.

21. The therapeutic composition of claim 15, wherein the oligonucleotide has the nucleotide sequence of SEQ ID NO:32.

22. The therapeutic composition of claim 15, wherein the oligonucleotide has the nucleotide sequence of SEQ ID NO:33.

23. The therapeutic composition of claim 15, wherein the oligonucleotide has the nucleotide sequence of SEQ ID NO:34.

24. The pharmaceutical composition of claim 16 further comprising ribavirin.

25. A therapeutic composition comprising at least two oligonucleotides of claim 1 and a pharmaceutically acceptable carrier.

26. A method of inhibiting RSV replication in a cell comprising the step of administering to the cell a therapeutically effective amount of the composition of claim 16.

27. A method of inhibiting RSV replication in a cell comprising the step of administering to the cell a therapeutically effective amount of the composition of claim 24.

28. A method of treating respiratory syncytial virus infection comprising the step of administering to an infected cell a therapeutically effective amount of the composition of claim 16.

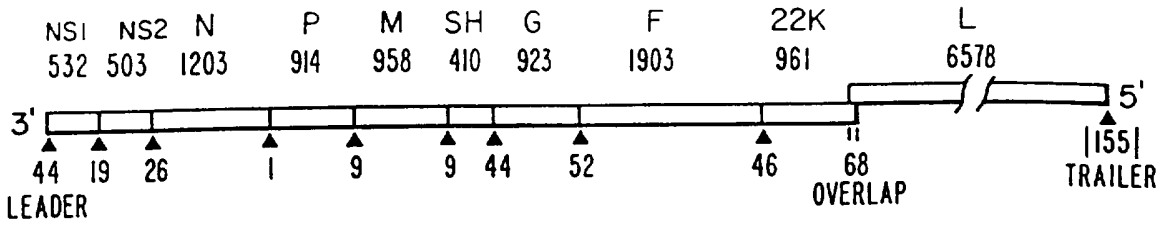
NS1 NS2 N P M SH G F 22K L
532 503 1203 914 958 410 923 1903 961 6578
3'
5'
44 19 26 1 9 9 44 52 46 68 |155|
LEADER
OVERLAP
TRAILER

FIG. 1

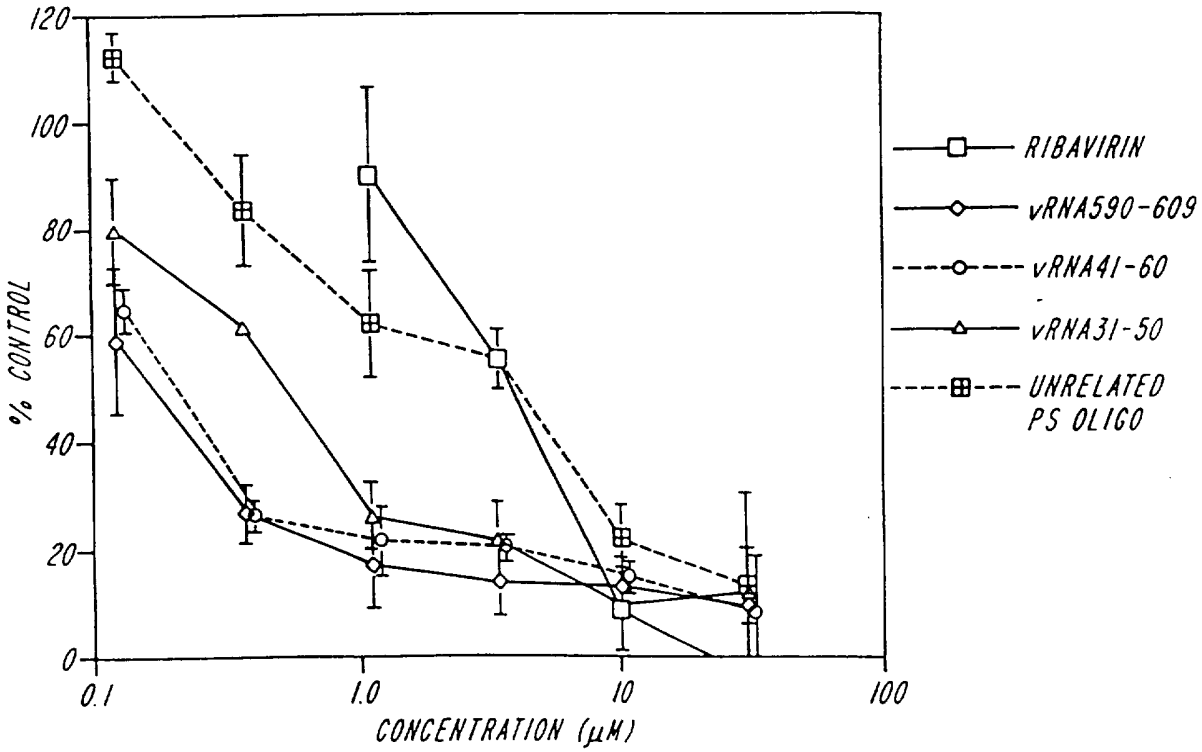
120
100
80
60
40
20
0
% CONTROL
0.1
1.0
10
100
CONCENTRATION (μM)
RIBAVIRIN
vRNA590-609
vRNA41-60
vRNA31-50
UNRELATED PS OLIGO

FIG. 5

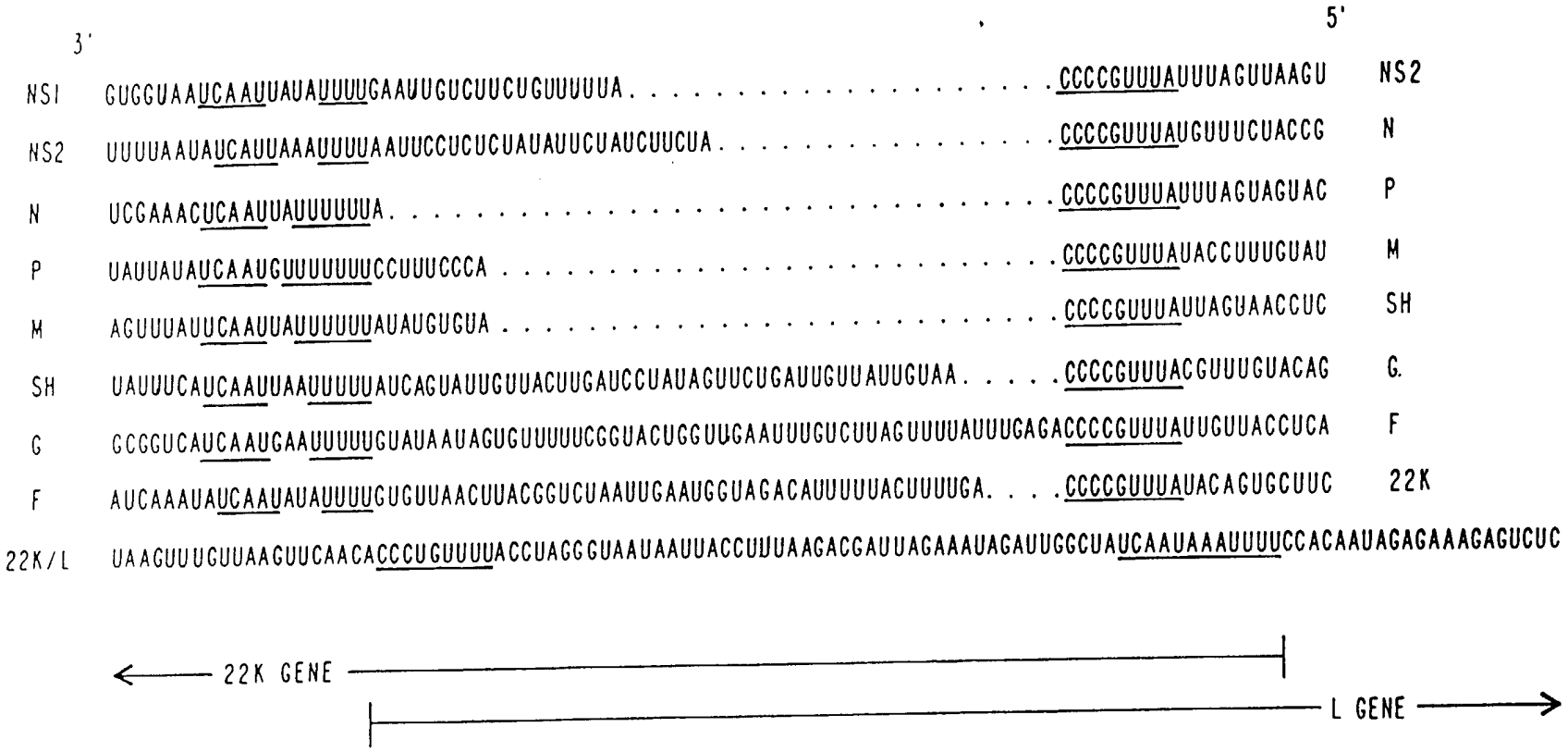
3'
5'
NS1 GUGGUAAUCAAUUAUAUUUUGAAUUGUCUUCUGUUUUUA . . . . . CCCCGUUUAUUUAGUUAAGU NS2
NS2 UUUUAAUAUCAUUAAAUUUUAAUUCCUCUCUAUAUUCUAUCUUCUA . . . . . CCCCGUUUAUGUUUCUACCG N
N UCGAAACUCAAUUAUUUUUUA . . . . . CCCCGUUUAUUUAGUAGUAC P
P UAUUAUAUCAAUGUUUUUUUCCUUUCCCA . . . . . CCCCGUUUAUACCUUUGUAU M
M AGUUUAUUCAAUUAUUUUUUAUAUGUGUA . . . . . CCCCGUUUAUUAGUAACCUC SH
SH UAUUUCAUCAAUUAAUUUUUAUCAGUAUUGUUACUUGAUCCUAUAGUUCUGAUUGUUAUUGUAA . . . . . CCCCGUUUACGUUUGUACAG G
G GCGGUCAUCAAUGAAUUUUUGUAUAAUAGUGUUUUUCGGUACUGGUUGAAUUUGUCUUAGUUUUAUUUGAGACCCCGUUUAUUGUUACCUCA F
F AUCAAAUAUCAAUAUAUUUUGUGUUAACUUACGGUCUAAUUGAAUGGUAGACAUUUUUACUUUUGA . . . . CCCCGUUUAUACAGUGCUUC 22K
22K/L UAAGUUUGUUAAGUUCAACACCCUGUUUUACCUAGGGUAAUAAUUACCUUUAAGACGAUUAGAAAAUAGAUUGGCUAUCAAUAAAUUUUCCACAAUAGAGAAAGAGUCUC
22K GENE
L GENE

FIG. 2

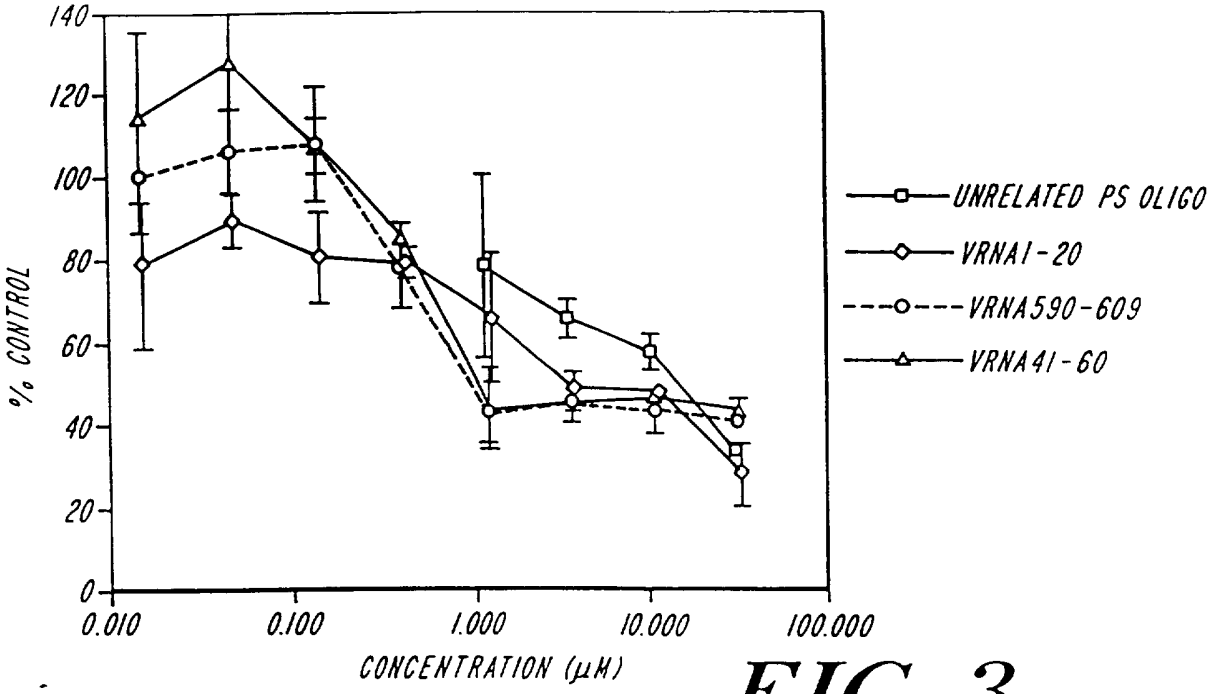
140
120
100
80
60
40
20
0
% CONTROL
0.010
0.100
1.000
10.000
100.000
CONCENTRATION (μM)
UNRELATED PS OLIGO
VRNA1-20
VRNA590-609
VRNA41-60

FIG. 3

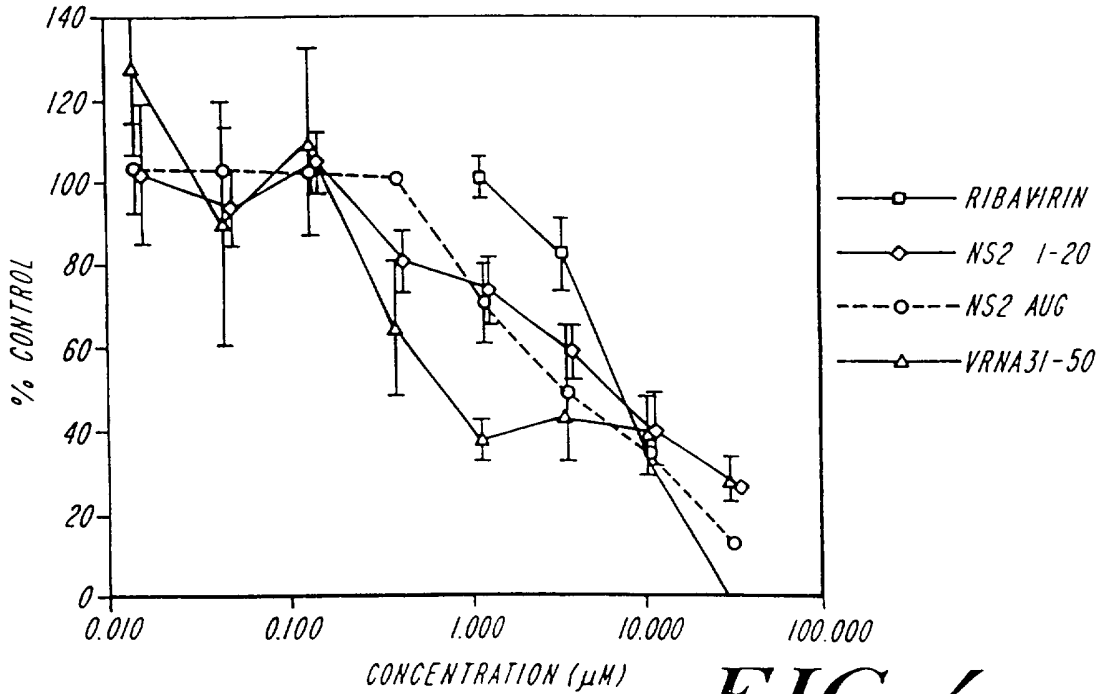
140
120
100
80
60
40
20
0
% CONTROL
0.010
0.100
1.000
10.000
100.000
CONCENTRATION (μM)
RIBAVIRIN
NS2 1-20
NS2 AUG
VRNA31-50

FIG. 4

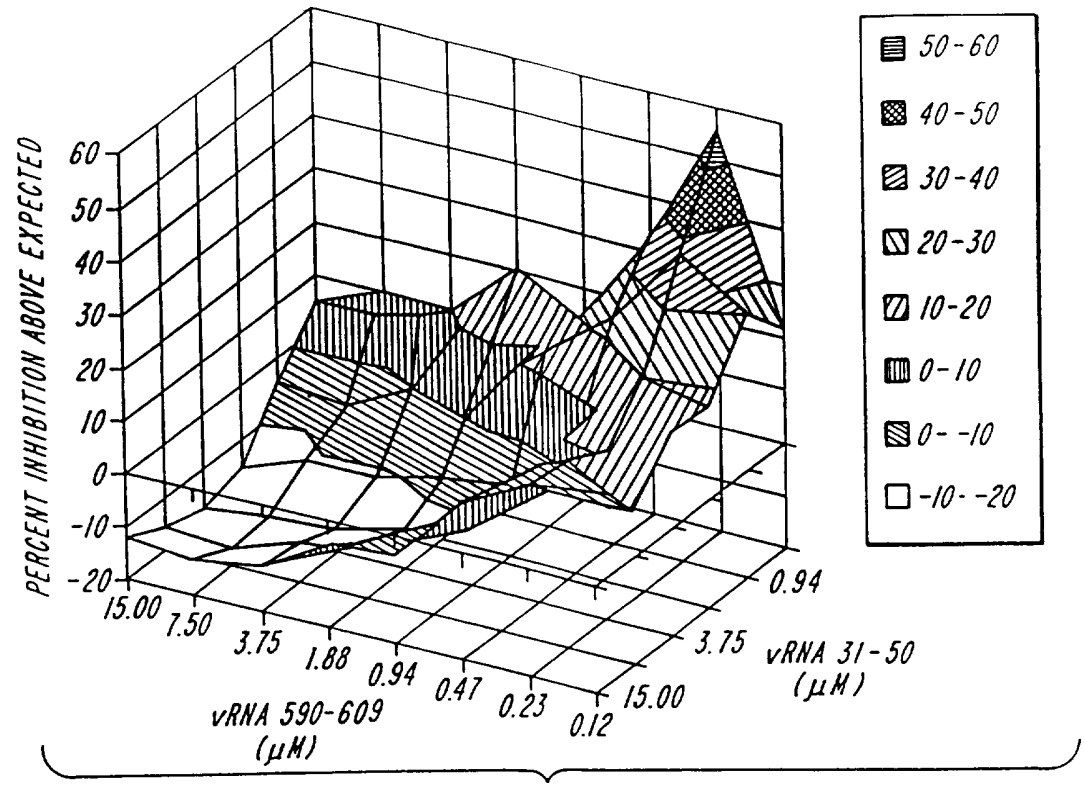
PERCENT INHIBITION ABOVE EXPECTED
60
50
40
30
20
10
0
-10
-20
15.00
7.50
3.75
1.88
0.94
0.47
0.23
0.12
vRNA 590-609 (μM)
0.94
3.75
15.00
vRNA 31-50 (μM)
50-60
40-50
30-40
20-30
10-20
0-10
0--10
-10--20

FIG. 6

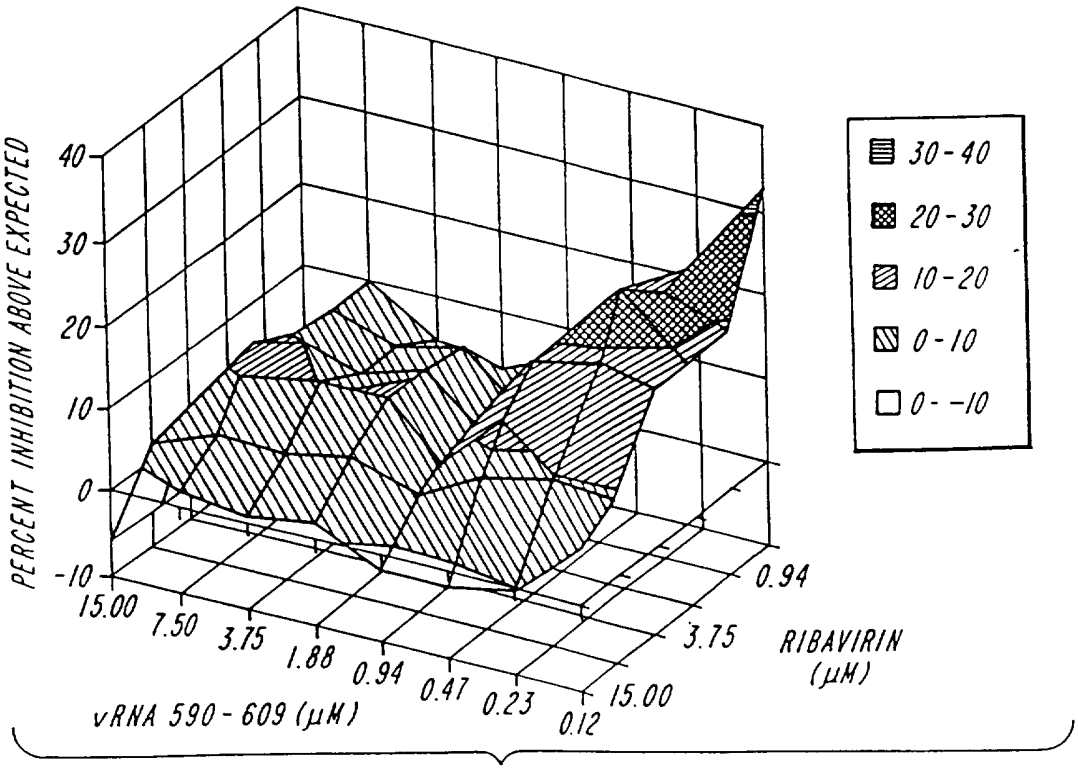
PERCENT INHIBITION ABOVE EXPECTED
40
30
20
10
0
-10
15.00
7.50
3.75
1.88
0.94
0.47
0.23
0.12
vRNA 590-609 (μM)
0.94
3.75
15.00
RIBAVIRIN (μM)
30-40
20-30
10-20
0-10
0--10

FIG. 7