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(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING GROWTH OR REPLICATION OF VIRUSES

(57) Abstract

The invention relates to compositions and methods for inhibiting the growth or replication of microbes, viruses or self-replicating nucleic acids. Antisense oligonucleotides that bind to strategic sites in the microbe, virus of self-replicating nucleic acid genome find particular utility in preventing proliferation and pathogenesis; and in detecting microbe, virus or self-replicating nucleic acid. Therapeutic compositions comprising at least two oligonucleotides and methods using the compositions are effective in inhibiting the growth or replication of homologous and heterologous microbes, viruses or self-replicating nucleic acids.

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COMPOSITIONS AND METHODS FOR INHIBITING GROWTH OR REPLICATION OF VIRUSES

The invention described and claimed herein was supported in part by grants from the Department of Health and Human Services, National Institutes of Health.

FIELD OF THE INVENTION

The invention relates to oligonucleotides; medicaments and pharmaceutic compositions comprising the oligonucleotides; methods of detecting microbes, viruses and self-replicating nucleic acids using the oligonucleotides; methods of inhibiting growth or replication of microbes, viruses and self-replicating nucleic acids using the oligonucleotides; and therapeutic methods using the oligonucleotides to inhibit homologous and heterologous growth or replication of microbes, viruses or self-replicating nucleic acids.

BACKGROUND OF THE INVENTION

Antisense RNA that can inhibit selectively gene expression at the level of translation or mRNA processing has been proposed as a possible genetic approach for the prevention and treatment of disease. Antisense RNA introduced directly into cells or expressed off transfected DNA has been shown to repress the expression of endogenous eukaryotic genes. However, the inherent properties of RNA and problems of working with RNA, such as the near ubiquitous presence of RNases in cells, reagents and supplies, has stimulated a search for

alternative means of genetically repressing the expression of specific genes using antisense oligonucleotides.

One such development is non-ionic nucleic acid analogues that contain a 3'-5' methylphosphonate group in place of the negatively charged phosphodiester group found normally in oligonucleotides. The analogues are resistant to nuclease hydrolysis and penetrate the plasma membrane of cells in culture. Other means of improvement include the use of terminal blocking groups, intercalating agents or phosphorothioates.

Antisense oligonucleotides have found limited success in inhibiting the growth of Rous sarcoma virus, vesicular stomatitis virus, simian virus 40, influenza virus and human immunodeficiency virus.

SUMMARY OF THE INVENTION

Accordingly, one object of the instant invention is to provide oligonucleotides that inhibit herpesvirus growth or replication in a superior manner.

A second object of the invention is to provide novel compositions and methods for inhibiting growth or replication of microbes, viruses or self-replicating nucleic acids.

Another object of the invention relates to the use of multiple oligonucleotides directed to non-overlapping sites in a microbe, virus or self-replicating nucleic acid genome to inhibit growth or replication thereof.

A fourth object of the invention is to provide novel compositions and methods for inhibiting growth or replication of a primary microbe, virus or self-replicating nucleic acid, such as a herpesvirus, and of a secondary microbe, virus or self-replicating nucleic acid, such as human immunodeficiency virus, using one or more

oligonucleotides hybridizable with nucleic acids of said primary microbe, virus or self-replicating nucleic acid.

These and other objects have been attained by providing novel oligonucleotides that hybridize to specific target sites in the genome of a microbe, virus or self-replicating nucleic acid.

The invention provides a method of detecting herpesvirus comprising the steps of:

(i) obtaining a biologic sample containing nucleic acids;

(ii) treating said sample so the nucleic acids contained therein are made single stranded;

(iii) exposing said treated sample to a labelled oligonucleotide wherein said oligonucleotide is hybridizable with nucleic acids of herpesvirus; and

(iv) detecting hybridized sequences.

In one aspect, the invention provides a composition for inhibiting herpesvirus growth or replication comprising an oligonucleotide hybridizable with nucleic acids of said herpesvirus.

In another embodiment, the invention provides a method for inhibiting herpesvirus growth or replication comprising the step of contacting nucleic acids of herpesvirus with an oligonucleotide hybridizable with said nucleic acids of herpesvirus.

In yet another embodiment, the invention provides therapeutic compositions for inhibiting

herpesvirus growth or replication comprising an oligonucleotide, or pharmaceutically acceptable salts thereof, hybridizable with nucleic acids of herpesvirus and a pharmaceutically acceptable carrier.

In an even further embodiment, the invention provides a therapeutic method for inhibiting herpesvirus growth or replication in a host carrying herpesvirus comprising administering a pharmaceutically effective amount of a therapeutic composition comprising an oligonucleotide, or pharmaceutically acceptable salts thereof, hybridizable with nucleic acids of herpesvirus.

In another embodiment the invention provides a composition for inhibiting growth or replication of a microbe, virus or self-replicating nucleic acid comprising at least two oligonucleotides hybridizable with non-overlapping sites in nucleic acids of said microbe, virus or self-replicating nucleic acid.

In a further embodiment the invention provides a method for inhibiting growth or replication of a microbe, virus or self-replicating nucleic acid comprising the step of contacting nucleic acids of a microbe, virus or self-replicating nucleic acid with at least two oligonucleotides hybridizable with non-overlapping sites in nucleic acids of said microbe, virus or self-replicating nucleic acid.

In an even further embodiment, the invention provides therapeutic compositions for inhibiting microbe, virus or self-replicating nucleic acid growth or replication comprising at least two oligonucleotides, or pharmaceutically acceptable salts thereof, hybridizable with non-overlapping sites in nucleic acids of said microbe, virus or self-replicating nucleic acid and a pharmaceutically acceptable carrier.

In another embodiment, the invention provides a therapeutic method for inhibiting microbe, virus or self-replicating nucleic acid growth or replication in a host carrying a microbe, virus or self-replicating nucleic acid comprising administering pharmaceutically effective amounts of at least two oligonucleotides, or pharmaceutically acceptable salts thereof, hybridizable with non-overlapping sites in nucleic acids of said microbe, virus or self-replicating nucleic acid.

In yet a further embodiment, the invention provides a composition for inhibiting human immunodeficiency virus growth or replication in a specimen containing human immunodeficiency virus and a second virus comprising at least one oligonucleotide hybridizable with nucleic acids of said second virus.

The invention further provides a method for inhibiting human immunodeficiency virus growth or replication in a specimen containing human immunodeficiency virus and a second virus comprising the step of contacting nucleic acids of said second virus with at least one oligonucleotide hybridizable with nucleic acids of said second virus.

Another embodiment of the invention is a therapeutic composition for inhibiting human immunodeficiency virus growth or replication in a host carrying human immunodeficiency virus and a second virus comprising at least one oligonucleotide, or pharmaceutically acceptable salts thereof, hybridizable with nucleic acids of said second virus and a pharmacuetically acceptable carrier.

Finally, the invention provides a therapeutic method for inhibiting human immunodeficiency virus growth or replication in a host carrying human

immunodeficiency virus and a second virus comprising administering a pharmaceutically effective amount of at least one oligonucleotide, or pharmaceutically acceptable salts thereof, hybridizable with nucleic acids of said second virus.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic representation of the herpes simplex virus (HSV) genome with several genes preferred as targets for antisense oligonucleotides highlighted. In the figure, U_L denotes the long unique sequence segment flanked by the terminal, TR_L, and internal, IR_L, repeat sequences. U_S denotes the short unique sequence segment flanked by the internal, IR_S, and terminal, TR_S, repeat sequences. Above the gene map is a relative scale wherein 1 represents the entire HSV genome.

Figure 2 is another schematic representation of the HSV genome showing other relevant genes. The long region is divided into functional domains as determined by restriction enzymes and denoted as C, F, E and D. ORF size is the predicted open reading frame size in kilobases (K). In the relative scale above the gene map, the solid boxes represent the long segment flanking repeats and the hollow boxes represent the short segment flanking repeats. The genes are identified numerically, such as UL5, or according to function and include dbp which encodes the major DNA binding protein, which is involved in DNA unwinding, and pol which encodes DNA polymerase.

Figure 3 depicts dose response curves of HSV-1 growth in cells exposed to antisense oligonucleotides. Vero cells were infected with 10

pfu per cell of HSV-1 and treated with increasing concentrations of oligonucleotide at the time of infection. The cultures were assayed for infectious virus 24 hours later. Results are expressed as the percent inhibition of virus titers compared to untreated HSV-1-infected cells. In the figure, the solid circles represent the oligonucleotide TTCCTCCTGCGG; the X's represent the oligonucleotide TCCTCCTG; the hollow squares represent the oligonucleotide GCGGGAAGGCAC; the solid squares represent the oligonucleotide TCCTGCGGGAAG; the solid triangles represent the oligonucleotide TTCCTCCT; and the hollow triangles represent the oligonucleotide TCCTGCGG.

Figure 4 depicts activation of HSV by the IE110 gene product and inhibition of IE110-induced activation by specific oligonucleotides. Vero cells were transfected with pICP10-cat, a plasmid carrying an HSV promoter and the CAT structural gene, and activating IE110. In the graph, the open bar represents cells grown in the absence of an oligonucleotide; the hatched bars denoted as (a) represent cells grown in the presence of the oligonucleotide TTCCTCCTGCGC; the stippled bars denoted as (b) represent cells grown in the presence of the oligonucleotide GCGGGGCTCCAT; and the dotted bars denoted as (c) represent cells grown in the presence of the oligonucleotide AGTCTGCTGCAA.

Figure 5 depicts dose response and inhibition of HSV-1 growth in cells by antisense oligonucleotides, as in Figure 3. In the figure solid circles represent the oligonucleotide TTCCTCCTGCGG; solid triangles represent the oligonucleotide GCTTACCCGTGC; X's represent the oligonucleotide GCGGGGCTCCAT; and hollow circles represent the oligonucleotide AGTCTGCTGCAA.

Figure 6 shows the effect of virus m.o.i. (multiplicity of infection) on dose response inhibition of HSV-1 growth by an oligonucleotide complementary to the translation initiation site of HSV-1 IE mRNA 1 with the sequence GCGGGGCTCCAT, designated IE110. In the figure solid circles represent Vero cells infected with 0.1 pfu per cell of HSV-1 and hollow circles represent cells infected with 10 pfu per cell of HSV-1.

Figures 7A and 7B depict dose response inhibition of HSV-1 growth in Vero cells exposed to antisense oligonucleotides.

In Figure 7A, HSV-1 growth in Vero cells infected with 0.1 pfu per cell of HSV-1 was monitored. In the figure, X's represent the oligonucleotide GCGGGGCTCCAT (designated as IE110); circles represent the oligonucleotide TTCCTCCTGCGG (designated as IE4,5); and squares designate the use of both oligonucleotides simultaneously.

In Figure 7B, the synergistic effect of IE110 and IE4,5 oligomers on the inhibition of HSV-2 replication is depicted. The concentration of each oligonucleotide was covaried and inhibition of plaque formation was determined. The term FIC denotes the ratio of the concentration of IE4,5 required to inhibit plaque formation by 60% in the presence of a fixed concentration of IE110 to the concentration required in the absence of IE110. The units of the X axis are the ratio of the fixed concentration of IE110 to the concentration of IE110 that produced 60% inhibition of plaque formation in the absence of IE4,5. The diagonal line (the X's) shows the theoretical plot for each oligonucleotide used alone. The solid circles represent the synergistic effect of the combination.

Figure 8 depicts dose response curves of HSV-1 growth in cells exposed to antisense oligonucleotides. In the figure, the small circles represent oligomer specific for the IE5 initiation site and designated IE12 with the sequence GGCCCACGACAT. IE12 does not show significant inhibition. Triangles represent the oligonucleotide AATGTCGGCCAT (designated as IE68 and specific for the IE4 initiation site). The large circles represent inhibition obtained by combining the two oligonucleotides. At all points, the combination of oligonucleotides achieves a higher level of inhibition at a lower concentration of oligonucleotides than when either is used alone. In the combination treatment, the points represent total concentration of both oligonucleotides.

Figure 9 depicts inhibition of HIV activation by a herpesvirus-specific oligonucleotide. Cells were cotransfected with a plasmid carrying the HIV promoter upstream of the CAT structural gene and a plasmid encoding the HSV-1 IE110 protein. The transformants were then exposed to varying concentrations of oligonucleotides. Oligomer to the initiation site of IE110 is GCGGGGCTCCAT, to the second splice acceptor site of IE110 is AGTCTGCTGCAA and to the splice acceptor site of IE4,5 is TTCCTCCTGCGG.

Figure 10 depicts inhibition of HIV activation by a herpesvirus-specific oligonucleotide, GCGGGGCTCCAT (designated as IE110 oligomer); and activation by the IE110 protein of HSV-1 and the homologous gene product from cytomegalovirus (designated as CMV). The IE110 protein and the oligonucleotide were combined in one treatment. The curve denoted PBR is a control using pBR322. IFU is infectious forming unit.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to inhibiting growth or replication of microbes, viruses or self-replicating nucleic acids, or the translation or expression of microbe, virus or self-replicating nucleic acid genes that are essential for growth and proliferation, by interfering with the normal means of expression of critical genes by using antisense oligonucleotides that are complementary to specific targeted sites in the microbe, virus or self-replicaing nucleic acid genome.

The compositions and methods of the instant invention find utility in the detection, diagnosis and manipulation of the herpesviruses and in the treatment of disease caused directly or indirectly by the herpesviruses.

Particular advantages are obtained with any microbe, virus or self-replicating nucleic acid when more than one oligonucleotide is used wherein the oligonucleotides are directed to non-overlapping sites in the microbe, virus or self-replicating nucleic acid genome, that is, to separate target sites.

The term "oligonucleotide" is used herein to include oligomers comprised of deoxyribose and ribose, the sugars found normally in nucleic acids, and modified analogs thereof, such as 2'-O-methyl ribose. The oligonucleotide may bind to single-stranded nucleic acids or to double-stranded nucleic acids.

The term "self-replicating nucleic acids" is used herein to include nucleic acids found in organelles, such as chloroplasts and mitochondria, and alone, such as plasmids, viroids and the like.

The term "virus" is used herein to include intracellular agents that are characterized by a

lack of independent metabolism and by the ability to replicate only within living host cells.

The term "microbe" is used herein to include prokaryotes, such as bacteria, actinomycetes, chlamydiae, mycoplasmae and the like; and eukaryotes, such as protozoa, fungi, parasites and the like.

For example, the herpesviruses, whose natural host is the human species, comprise seven species, herpes simplex type 1 (HSV-1) and type 2 (HSV-2), varicella zoster (VSV), Epstein-Barr (EBV), cytomegalovirus (HCMV), human herpesvirus 6 (HHV-6) and 7 (HHV-7). (As used herein, the term "herpesvirus" includes the seven species described above.) They are DNA viruses with large genomes. Common genetic features include repeat sequences, long and short unique sequences and immediate-early regulatory genes that control the transcription and expression of downstream genes.

The oligonucleotides can be produced in any of a variety of art-recognized methods, for example the commonly used solid phase triester or phosphoramidite chemistry. Nucleic acid synthesis in vitro has become highly automated using equipment that is readily available to the artisan. Furthermore, customized oligonucleotides can be obtained under contract from a variety of commercial sources. Alternatively, the antisense nucleotides can be synthesized in situ by transfecting cells with an appropriate cloned sequence.

The oligonucleotides must be capable of bypassing membranous barriers, of resisting the degradative means present in the milieu and hybridizing the nucleic acids of the microbe, virus or self-replicating nucleic acid. Thus, unmodified oligodeoxyribonucleotides and oligoribonucleotides,

and other oligonucleotides can be used if introduced directly into cells by methods including electroporation, microimmunization, liposome fusion, precipitation and microparticle bombardment.

The antisense oligonucleotides also can be produced endogenously in host cells following transfection by an appropriate recombinant. Sequences encoding the antisense molecule are cloned into an appropriate site in an expression cassette carrying the necessary host RNA polymerase binding sites of a vector. The recombinant is then introduced into cells using standard techniques, such as those described above.

Chemically modified oligonucleotides (or as used herein "modified oligonucleotides or modified oligonucleosides or modified nucleosides") may be used, for example, oligonucleoside methylphosphonates as described in U.S. Patent No. 4,469,863 or 4,511,713; phosphorothioate analogs as described in Matsukura et al. (PNAS 84, 7706-7710, 1987); oligonucleotides carrying terminal groups and oligomers that are dervitized, such as with psoralen. Modified nucleotides offer the advantages of increased hydrophobicity or nuclease resistance or both. Thus, oligonucleotides carrying certain modified bases are capable of penetrating the mammalian cell plasma membrane. Also, the termini of the oligonucleotide can be modified to render the oligomer resistant to intracellular exonucleases. The use of chemically modified oligonucleotides is preferred in practicing the instant invention, and especially perferred is the use of oligonucleoside alkyl or aryl phosphonates.

The oligonucleotides can vary in size depending upon the specificity desired, expected

intracellular stability and technical considerations. Generally, the longer the oligonucleotide the greater the specificity, at least in the range of 8 to 20 nucleotides. However, if the targeted sequences are part of a small microbe, virus or self-replicating nucleic acid genome, then the effective concentration of the driver DNA, that is the microbe, virus or self-replicating nucleic acid genome, in the hybridization reaction between genome target site and oligonucleotide is high thereby enabling one to achieve adequate specificity using shorter oligonucleotides. Oligonucleotides of 8 to 20 bases are preferred, and those of 12-20 bases are particularly preferred. When introduced by means of an antisense vector, the oligonucleotides can be as large as 50 to 60 bases, if not larger. It is possible, in order to span a larger region, to employ two or more oligomers that hybridize to adjacent sites, for example two 15mers to span a 30 base pair site in situ.

Any means of contacting oligonucleotides with nucleic acids of a microbe, virus or self-replicating nucleic acid is suitable for practicing the instant invention. In a therapeutic setting, a preferred means is to use oligonucleotides that alone or in a composition are stable in the peripheral circulation and can penetrate the cell membrane. The means of introducing the oligonucleotides in a therapeutically effective concentration can be any of those used currently for delivering biologicals, such as immunoglobulin, peptides and enzymes, for example, intravenous administration, intramuscular immunization, implants, intranasal administration, contained in liposomes, attached to a cell-binding agent, such as an antibody, attached to ribozymes and other

parenteral routes. Furthermore, because many microbes, viruses or self-replicating nucleic acids produce epithelial lesions, the oligonucleotide may be delivered in a vehicle comprising a cream or an ointment.

Suitable concentrations of oligonucleotides needed to inhibit growth or replication of a microbe, virus or self-replicating nucleic acid can be determined readily by the skilled artisan. For example, the animal studies disclosed herein provide a basis for extrapolating dosage in other species based on weight comparisons between the mouse and other species. Or the amounts used herein can provide a reference point for use in other species. The concentration required will depend in part, for example, on the chemistry of the oligonucleotides; having to traverse physiologic barriers, such as the blood-brain or blood-testis barrier; toxicity; route of administration, such as oral, transdermal and nasal; the disease requiring treatment, such as a verruca, an outbreak of shingles, an intestinal ascaritic infestation and bacteremia; and half life in the peripheral circulation. Examples of suitable therapeutic doses in terms of internal doses (that is the dose at the required site in the body) are between 10 nM and 10 μM.

Depending on the intended mode of administration, the oligonucleotides can be delivered in a variety of pharmaceutically accepted ways. The compositions may be in the form of a solid or semisolid, but it is most likely that the oligomers will be admininistered in liquid form. The compositions will include the oligomers, or pharmaceutically acceptable salts thereof, conventional excipients, such as a sterile buffered saline solution, and other agents, carriers,

adjuvants, diluents etc. for increasing the stability, enhancing wettability and the like of the composition.

Actual methods and doses are known, or will be apparent to those skilled in the art, for example see Remington's Pharmaceutical Sciences, Mack Publ. Co., Easton, PA.

The invention also contemplates the use of at least two oligonucleotides directed to non-overlapping sites in the microbe, virus or self-replicating nucleic acid genome. Because targeted sites are not overlapping and the three-dimensional folding of the microbe, virus or self-replicating nucleic acid genome in a host cell or in situ may not be predictable, each oligonucleotide of a plurality of oligonucleotides contains sequences that are complementary to a single site. An unexpected enhanced inhibition of microbe, virus or self-replicating nucleic acid growth or replication is obtained when more than one oligonucleotide is used, the oligonucleotides being hybridizable to different sites in a single gene or in separate genes.

According to the instant invention, there are certain regions of the microbe, virus or self-replicating nucleic acid genome that are preferred as targets for antisense oligonucleotides.

Preferred targets are those that have direct or indirect control over basic mechanisms in microbe, virus or self-replicating nucleic acid growth. Thus, regulatory genes expressed early in development that trigger major developmental pathways are suitable (known as master regulatory genes) as are other regulatory genes that control the expression of a smaller number of genes, as in an operon. Other suitable targets are genes involved with microbe, virus or self-replicating nucleic acid replication, such as DNA polymerase

and reverse transcriptase. Another set of suitable targets are genes that are involved in pathogenesis per se such as those that confer tissue or cell specificity, virulence and latency.

Important sites within the targets are transcription initiation sites (also known as promoter or polymerase binding site), translation initiation sites, splice donor and acceptor sites, messenger cap sites and transcription initiation sites. For example in HSV-1 an oligonucleotide that is hybridizable to the IE2 translation initiation site, such as 5'-ATATCAATGTCAGTCGCCAT-3' (hereinafter all the sequences are in the 5' to 3' orientation), is suitable, as is an oligonucleotide hybridizable to the IE5 translation initiation site, such as GGCCCACGACAT; oligonucleotides hybridizable to the IE4 mRNA cap site, such as GCGGGCGTCGGT, GGCGCCGTCTGC and GTGGCCGGCGCC; oligonucleotides hybridizable to the IE5 mRNA cap site, such as GCGGGCGTCGGT, GGCGCCGTCTGC and GTGGCCGGCGCC; an oligonucleotide hybridizable to the IE4,5 splice acceptor site, such as TTCCTCCTGCGG; an oligonucleotide hybridizable to the IE4,5 splice donor site, such as GCTTACCCGTGC; an oligonucleotide hybridizable to the IE1 translation initiation site, such as GCGGGGCTCCAT; an oligonucleotide hybridizable to the IE3 translation initiation site, such as GTTCTCCGACGCCAT; an oligonucleotide hybridizable to the IE3 cap site, such as CGCTCCGTGTGG; and an oligonucleotide hybridizable to the IE4 translation initiation site, such as AATGTCGGCCAT.

As mentioned above and will be exemplified further in the Examples following, the size of the oligonucleotide may vary and the oligonucleotide may include bases complementary to the target genomic site that flank the sequences recited

herein or lack bases of the sequences recited herein. Thus, the IE4 translation initiation site sequence AATGTCGGCCAT may be extended with complementary, and thus hybridizable bases in either the 5' or 3' direction to obtain an oligonucleotide of more than 12 bases; may be truncated by one or more bases to yield an effective oligonucleotide of fewer than 12 bases; may be extended in either the 5' or 3' direction with a concommitant deletion of one or more bases from the non-extended end to yield an oligonucleotide containing only part of the recited sequence etc., so long as the oligonucleotide is capable of interfering with the normal transcription or translation of IE4.

Ideal targeting about a splice site can be achieved with an oligonucleotide that, as equally as possible, spans the exon/intron junction. Ideal targeting about a translation initiation site can be achieved with an oligonucleotide that in the complementary 3' to 5' orientation, begins at the ATG initiation codon and extends into the coding region (thus the oligonucleotide in the 5' to 3' orientation will have as the last three bases CAT).

Genes that are essential for synthesizing nucleic acids of a microbe, virus or self-replicating nucleic acid are preferred targets for antisense oligonucleotides (Figure 2). Examples of such genes are those that encode DNA binding proteins, gyrases, enzymes that relate to the synthesis of nucleotide triphosphates, DNA nucleases required for DNA replication and polymerases. Also included are transcrition initiation sites or promoter sequences. In the exemplary organism, HSV-1, suitable genes include the DNA polymerase gene, thymidine kinase gene (TK), ribonucleotide reductase gene (RR1 locus,

also known as 1CP6, and RR2 locus, also known as 38K) and genes encoding enzyme stabilizing proteins such as the 180K for ribonucletide reductase. An oligonucleotide hybridizable to the polymerase gene is CCGCCGCCACCGGAAAAGAT; an oligonucleotide hybridizable to TK is TGGCAGGGGTACGAAGCCAT; and an oligonucleotide hybridizable to RR1 is GCGGCTGGGCGGCTGGCCAT.

Also preferred as targets are sequences which are crucial to transcription, such as promoter sequences. For example, in HSV there is a consensus sequence common to the promoters of the IE genes with the sequence TAATGARAT, wherein R can be any base including A, T, G, C, U or functional equivalents thereof, wherein a functional equivalent is a nitrogenous base that can be substituted and retain complementarity. Examples include inosine and 5-methyl-cytosine.

Furthermore, regulatory genes, many of which are not translated, that govern the orderly expression of downstream genes are especially suitable as targets for antisense oligonucleotides. Many of these early expressed regulatory genes are termed master genes. In the HSV-1 genome, the VmW65 locus is one such master gene. An oligonucleotide hybridizable to the VmW65 initiation site would be suitable, such as TCGTCGACCAAGAGGTCCAT, CAAACAGCTCGT, CCATGTCGGCAA, GTCCGCGTCCAT, GCCGTCCGCGTC and TGGCGAAGCGCC; and an oligonucleotide hybridizable to the VmW65 mRNA cap site would suffice, such as ACAGCCCGTGGT, CCGAGGAATGAC and CCGTTCCCGAGG.

Another set of genes that are preferred for targeting are those that are essential for pathogenesis. In a virus, for example, such genes are those involved in latency, cell tropism, the choice of specific cell to infect or determining

virulence. In HSV-1, one such gene is LAT (latency-associated transcript) and suitable oligonucleotides are those hybridizable to the initiation site of LAT ORF-1, such as CACTGGCATGGA, GCATCCTGCCAC, CCCCGAAAGCAT, GATCCCCGAAAG and CTGACCACCGAT; and those hybridizable to the initiation site of LAT ORF-2, such as GCAGGCTCTGGT, CCATGTTGGGCA, TGGGGGTGCCAT, GAGTGGGGGTGC and GGTGCGTGGGAG.

As mentioned previously, IE genes are not restricted to HSV-1 and are a feature common to the herpesviruses. The IE gene homologues in related herpesviruses are suitable targets for antisense oligonucleotides. Thus in HCMV, oligonucleotides hybridizable to the HCMV IE2 initiation site are suitable, such as CAAGGACGGTGA, CCATCGTGTCAA, AGAGGACTCCAT, GGCAGAGGACTC and CTTTCTCTTGGC; as are those hybridizable to the IE2 homologue mRNA cap site, such as GACGGTTCACTA, CAGGCGATCTGA and CGTCTCCAGGCG.

In VZV, the HSV-1 IE110 gene homologue is known as gene 61. Oligonucleotides which are suitable are those that are hybridizable to the 61 translation initiation site, such as CAACTGGCTGTA, CCATGGTAACAA, TATGGTATCCAT, TAATATGGTATC and ACCGCCCGCTAA. The VZV 62 gene is the homologue of the HSV-1 IE3 gene. Oligonucleotides which are suitable are those that are hybridizable to the 62 translation initiation site, such as TGGGGTGAATTT, CCATCGCACTGG, CGGCGTATCCAT, CGGCGGCGTATC and GCGCTGCATCGG.

Especially preferred is to target two genes that complement the expression and/or function of each other, for example two genes that encode proteins of a metabolic pathway, two genes of a developmental pathway or two master regulatory genes that each control the expression of different

developmental programs, or combinations thereof. Knowing the strategy, the skilled artisan can determine readily suitable portions of the microbe, virus or self-replicating nucleic acid to target and hence suitable combinations of oligonucleotides.

Although in a preferred embodiment the invention contemplates the use of two or more oligonucleotides, there are instances where a single oligonucleotide is more than 90% effective. For example, the oligonucleotides TTCCTCCTGCGG, GCTTACCCGTGC, GCGGGGCTCCAT and AATGTCGGCCAT each are effective in inhibiting HSV-1 growth when used alone. However, the activity of the oligonucleotides is enhanced, that is additive or synergistic, when used in combination with another oligonucleotide. The phenomenon is manifest as higher level of inhibition or effectiveness at a lower dose or both.

The nature in which an oligonucleotide can influence normal gene expression includes binding to existing single-stranded regions in the nucleic acids of a microbe, virus or self-replicating nucleic acid, which includes naturally occurring single-stranded nucleic acids and regions of double-stranded nucleic acids that are melted partially, and binding to double-stranded nucleic acids to form a nucleic acid triplex structure, which includes binding to cloverleaf and stem-loop structures commonly found amongst single-stranded nucleic acids that self base pair or fold back upon themselves. For a review of the topic see Uhlmann & Peyman (Chem Rev 90, 544-579, 1990).

Of related interest is the observation that many people infected with HIV (human immunodeficiency virus) do not develop AIDS (acquired immune deficiency syndrome). It is

believed that factors other than HIV are essential for activation of HIV thereby enhancing its replication to the levels required to cause AIDS. Among those factors are the viruses including JC, adenoviruses and the herpesviruses including HSV-1, HSV-2, HCMV, VZV, EBV, HHV6 and HHV7. Specific genes of those viruses activate the expression of HIV either directly at the level of virus replication or indirectly by activating expression of a reporter gene driven by the HIV promoter. Thus, inhibition of herpesvirus growth or replication using herpes-specific antisense oligonucleotides prevents HIV activation and may indirectly forestall the progression of events from HIV exposure to the clinical manifestation of AIDS. HSV-1 genes involved in HIV activation include IE110 (encoded by IE1).

The invention will now be described in further detail by way of the following non-limiting examples.

REFERENCE EXAMPLE

The following materials and methods were employed in the experiments described in the Examples.

Vero (African green monkey kidney) cells were grown in Eagle's minimal essential medium (MEM) supplemented with 25 mM HEPES and 10% (v/v) fetal bovine serum. HEp-2 (human epidermoid carcinoma No. 2) cells were grown in medium 199 with 10% calf serum. U937 (human histiocytic lymphoma) cells were grown in RPMI 1640 with heat-inactivated fetal calf serum.

Oligo(nucleoside-methylphosphonate)s were synthesized, for example, as described in either of Miller et al. (Biochem 18, 5134-5143, 1979), Miller

et al. (Nucl Acids Res 10, 979-991, 1982), Miller et al. (Biochem 25, 5092-5097, 1986), U.S. Patent No. 4,469,863 or U.S. Patent No. 4,511,713. Briefly, the process is a solid phase technique using, for example polystyrene as a support. The process consists of esterifying a 5'-O-protected nucleoside having a 3'-hydroxyl group with an alkyl or arylphosphonic acid in the presence of a condensing agent, such as mesitylenesulfonyl tetrazolide. The result is a 5'-O-protected nucleoside-3'-O-alkyl or arylphosphonate. That resulting compound is then esterified with a 3'-O-protected nucleoside having a 5'-hydroxyl group in the presence of a condensing agent to form a fully protected dinucleoside alkyl or arylphosphonate. The protecting groups are removed from the dinucleoside compound to form the dinucleoside alkyl or arylphosphonate. For example, in the case of dA-containing oligomers bearing N-benzoyl protecting groups, the protected dinucleoside was treated with hydrazine. The 3'-O-acetyl and 5'-O-dimethoxytrityl protecting groups were removed by sequential treatment with ammonium hydroxide and 80% acetic acid.

The process is repeated the requisite number of steps using the appropriate protected nucleosides to form an oligonucleotide of defined length and sequence.

Alternatively, the process can also be carried out by esterifying a 3'-O-protected nucleoside having a 5'-hydroxyl group with an alkyl or arylphosphonate in the presence of an activating agent to form a 3'-O-protected nucleoside-5'-O-alkyl or arylphosphonate. That compound is then esterified with a 5'-O-protected nucleoside having a 3'-hydroxyl group in the presence of an activating agent to form a fully protected

dinucleoside alkyl or arylphosphonate. As described above, the protecting groups are removed to form the dinucleoside alkyl or arylphosphonate. Further details can be obtained by referring to one of the above-mentioned references.

HSV-1 was grown and titered on Vero or HEp-2 cells as described in Strnad & Aurelian (Virol 87, 401-415, 1978).

Cytoplasmic RNA was extracted from virus-infected cells. In some cases, infected cells were treated with cycloheximide (50 μg/ml) to enhance mRNA production. At six hours postinfection, cells were washed and harvested in 100 mM tris/HCl (pH 7.4) with 150 mM NaCl. The cells at a concentration of 2×10^7 per ml were resuspended in lysis buffer comprising 10 mM tris/HCl (pH 7.5)/150 mM NaCl/1.5 mM $MgCl_2$/0.5% Nonidet P-40/20 mM vanadyl ribonucleoside complex. The suspension was vortexed gently and placed on ice for 10 minutes. After lysis, the suspension was centrifuged at 400 x g for 5 minutes, the supernatant was removed and added to 3 volumes of buffer containing 10 mM tris/HCl (pH 7.5), 5 mM EDTA and 0.5% sodium dodecyl sulphate. The solution was extracted with phenol/chloroform followed by chloroform, made 0.3 M in sodium acetate (pH 5.0) and precipitated with 2.5 volumes of cold ethanol.

S1 nuclease analysis was performed by co-precipitating a labelled viral genomic probe (Kulka et al. PNAS 86, 6868-6872, 1989) with 10 μg of cytoplasmic RNA obtained as described above. The virus-infected cells were either treated or not treated with one or more oligonucleotides. The DNA/RNA pellet was resuspended in 20 μl of 90% (v/v) formamide in 0.4 M NaCl/40 mM PIPES, pH 6.8/1 mM EDTA. The mixture was heated at 90°C for 3 minutes, incubated at 57.5°C for 16 hours and then

rapidly chilled on ice. Nuclease S1 digestion was performed at 28°C for 1 hour in 300 µl of 0.25 M NaCl/30 mM sodium acetate, pH 4.5/2 mM $ZnSO_4$ with 60 units of S1 nuclease. The S1-digested hybrids were extracted with phenol/chloroform and precipitated with ethanol. The products were analyzed by electrophoresis (room temperature, 16 hours, 50V) on 1.5% agarose gels under nondenaturing (90 mM tris/90 mM boric acid, pH 8.3/1 mM EDTA) or alkaline (30 mM NaOH/2 mM EDTA) conditions. Gels were dried on DE-18 paper and radioactivity visualized by autoradiography (at room temperature) using Kodak X-Omat-S film. Relative proportions of spliced and unspliced mRNA were determined by densitometric scanning on a UV/visible spectrophotometer.

Cell proteins were metabolically labelled with [^{35}S] methionine (150 µCi/per ml) from 6 to 7 hours post-infection. The cells were harvested, washed in cold PBS (phosphate-buffered saline) and resuspended in lysis buffer (10 mM tris, pH 8.0/150 mM NaCl/1% Nonidet P-40/1% deoxycholate/0.1% sodium dodecyl sulphate/1 mM phenylmethylsulfonyl fluoride) at a concentration of 1 x 10^5 cells per 25 µl. Suspensions were placed on ice for 15 minutes and then subjected to 5 rounds of freeze-thaw. The suspensions were cleared of cell debris by centrifugation at 5000 x g for 10 minutes. Electrophoresis was carried out under reducing conditions in 8.5% polyacrylamide gels.

For <u>in vivo</u> assays the antiviral activity of an oligonucleotide was examined in the mouse. Mice were anesthetized using, for example, ether or intraperitoneal injection of sodium pentobarbital. Animals were infected with about 2 x 10^3 to 1 x 10^6 pfu of HSV-1. Animals were ear injected with a volume of approximately 10 µl or in the foot pad

with about the same volume. Mice in experimental groups were treated dermally with oligomer in a PEG base at the site of injection on day 0 or on days 0-5 after infection or injected with oligomer at infection. Tissues were examined for virus titer on day 6 after infection. Virus titers were determined on Vero cells.

For chloramphenicol acetyl transferase (CAT) assay, constructs were prepared using the plasmid pCATB' and method described in Wymer et al. (J. Virol 63, 2773-2784, 1989). The plasmid contains the CAT structural gene without eukaryotic promoter sequences. The construct pICP10-cat contains a 649 base pair fragment of HSV-2 DNA, the ICP10 promoter, inserted 5' to the CAT structural gene. A plasmid carrying the HIV promoter (HIV-LTR) driving the CAT gene was also used. With the appropriate transcription signals, the CAT mRNA is produced and translation of the bacterial CAT occurs.

CAT activity was monitored using an art-recognized assay. Briefly, 0.2 μCi of [^{14}C] chloramphenicol substrate is mixed with the test solution and incubated for 1 hour. Radioactivity was monitored in a liquid scintillation counter. Generally, Vero cells were cotransfected with the target plasmid pICP10-cat and a transactivating IE110 containing plasmid (pIGA-15) as described in Wymer et al., supra. The transfection mixtures contain 1 mg of target plasmid and 0.1 mg of transactivator DNA. Parallel cotransfections employ pBR322 as non-specific DNA to equalize concentration effects. The recombinant plasmid pSV2CAT was used as a positive control and the plasmid pCATB' was used as a negative control. Transfected cells were incubated in the presence or absence of increasing concentrations (0-250 μM) of

oligomer added immediately after transfection (0 hours) and harvested 40-44 hours later.

HIV titers were determined by a syncytium formation assay. Briefly AA2 cells (2×10^4) were grown in 96-well flat bottom microliter plates in RPMI 1640 with 10% heat-inactivated fetal calf serum, non-essential amino acids, 1 mM pyruvate and 1.5 μg/ml polybrene (Sigma). HTLV-III_B was obtained by clarifying the supernatant of an infected MOLT-3 cell line grown in RPMI 1640 with 10% heat-inactivated fetal calf serum. Two-fold dilutions of MOLT-3 supernatant in 100 μl volumes were added to the AA2 cultures and incubated for 5-8 days. Titers, expressed as infectious units, represent the reciprocal of the highest dilution that gives rise to syncytium formation.

EXAMPLE 1

Vero cells were exposed to the dodecamer, TTCCTCCTGCGG (0-100 μM) at the time of infection with HSV-1 (10 pfu per cell) and virus titers were determined 24 hours later. Other oligomers were used in similar fashion. The results of those experiments are shown in Figure 3 and summarized in Table 1 on the following page.

Cultures exposed to the octomer TCCTCCTG and dodecamer showed a dose dependent decrease in HSV-1 titer. Significant reduction in virus growth (90-98%) was seen in cultures exposed to the dodecamer in a concentration of 100 μM.

IE mRNA's 4 and 5 are colinear on the HSV-1 and HSV-2 genomes with 65% base sequence homology at the splice acceptor type. As shown in Table 1, some of the oligonucleotides were effective in both strains whereas other oligonucleotides were specific for HSV-1.

TABLE 1

Effect of the oligo(deoxynucleoside methylphosphonate sequence) on HSV growth

Oligomer		% inhibition	
Exon	Intron	HSV-1	HSV-2
(3' AAGGAG	GACGCC 5')		
TCCTC	CTG	85	40
TTCCTC	CT	14	ND
GCGTTCCTC	CTG	30	0
GTTCCTC	CTGCG	26	0
TTCCTC	CTGCGG	98	0
TC	CTGCGG	0	0
TC	CTGCGGGAAG	9	12
	GCGGGAAGGCAC	0	0
TCCCT	CTG	0	0

HSV-1- or HSV-2-infected Vero cells were treated with the respective oligomers (100 μM) at 0 hour postinfection. Infectious virus was determined by plaque assay at 24 hour postinfection. Results are the average of three to six experiments for each oligomer. They are expressed as the percent inhibition of virus titers compared to the appropriate control (no oligomer). HSV-1 IE mRNA 4 acceptor splice site is shown in parentheses. The HSV-2 IE mRNA 4 acceptor splice site is GGGCCG GACGCT.

EXAMPLE 2

Cytoplasmic RNA (10 μg) extracted from HSV-1-infected HEp-2 cells untreated or treated with 100 μM of the dodecamer TTCCTCCTGCGG at the time of infection was hybridized with a labelled HSV probe and then digested with S1 nuclease.

On neutral gels the band pattern of nuclease-resistant products from untreated cells consisted of two major bands which were interpreted to represent the 5' leader to the HindIII site (1200 base pairs, bp) and the splice junction to the HindIII site (930 bp).

Nuclease-resistant products from the oligomer-treated cells consisted of three bands. They included 930 bp and 1200 bp products, equivalent to those identified in untreated cells, and an additional band that consisted of unspliced mRNA and the 145 bp intron joined to the 1200 bp leader.

Analysis of S1 nuclease-resistant products on alkaline gels confirmed the presence of unspliced IE mRNA 4 in oligomer-treated cells. Thus, one band was observed in untreated HSV-1-infected cells representing a 3' terminal transcript extending from the splice junction to the HindIII site (about 900 bp).

The nuclease-resistant products from oligomer-treated cells revealed two bands including the 900 bp band and an additional 1300 bp band that probably corresponded to an RNA transcript extending from the 5' leader through the intron to the 3' HindIII site.

Approximately 16-20% of the IE mRNA 4 from oligomer-treated cells was present as unspliced message, as determined by densitometric scanning. The result was about a 20% decrease in the levels of spliced mRNA.

EXAMPLE 3

HEp-2 cells were infected with HSV-1 (25 pfu per cell) or mock infected with PBS in the presence of 2.8 mM L-canavanine. (L-canavanine restricts gene expression to IE(α) and a subclass of E(β) proteins.) The cells were then treated with 100 mM of the dodecamer, TTCCTCCTGCGG. The cells were labelled with [^{35}S] methionine (150 μCi/ml) at 6-7 hours postinfection. Cell extracts were adjusted to the same protein concentration and analyzed on sodium dodecyl sulphate polyacrylamide gels. The gel analysis revealed that the synthesis of viral but not cellular proteins was reduced significantly by oligomer treatment.

EXAMPLE 4

In two independent experiments for each oligomer, treatment of HSV-1-infected cells with the dodecamer GCGGGGCTCCAT, which is complementary to the translation initiation site of IE110, and the dodecamer AGTCTGCTGCAA, which is complementary to the IE110 second splice acceptor site, resulted in a dose dependent inhibition of IE110-mediated transactivation in a CAT assay. In these experiments Vero cells were co-transfected with 1 mg of pICP10-cat DNA and 0.1 mg activating IE110 DNA. The cells were grown for 40-44 hours in the absence or presence of increasing concentrations of oligomers. The cells were harvested and were assayed for CAT activity.

Maximal inhibition of 100% was observed in cells exposed to 250 mM of GCGGGGCTCCAT. The other dodecamer was somewhat less effective. Inhibition was specific, the dodecamer TTCCTCCTGCGG that is complementary to the IE mRNA 4,5 splice acceptor

site did not inhibit IE110-mediated transactivation (Figure 4).

EXAMPLE 5

The effect of an oligomer to the donor splice site of IE mRNA4,5 on HSV-1 growth was determined in comparison to the effect of the oligomer to the acceptor splice site shown in Example 1.

Vero cells were infected with 10 pfu per cell of HSV-1 and treated with four different dodecamers at a concentration of 0-200 μM. The dodecamers included the two of Example 4, the dodecamer of Example 1, and the dodecamer complementary to the IE4,5 mRNA splice donor site, GCTTACCCGTGC. Virus titers were determined 24 hours later.

As depicted in Figure 5, both of the dodecamers complementary to the IE mRNA4,5 acceptor and donor splice sites resulted in 85-98% inhibition of virus growth in the concentration range of 100-200 mM. However at the m.o.i. of 10, the dodecamer complementary to the IE mRNA1 translation initiation site resulted in a maximum of about 15-20% inhibition of virus growth and the dodecamer complementary to the IE mRNA 1 second splice acceptor site resulted in no inhibition.

EXAMPLE 6

Although the dodecamers of Example 4 showed a dose dependent inhibition of IE110-mediated transactivation of CAT activity, the oligomers had minimal or no affect on HSV-1 growth at a m.o.i. of 10.

The role of virus concentration and ability of the IE110 oligomers to inhibit HSV-1 growth were examined. Vero cells infected with either 10 pfu

or 0.1 pfu per cell of HSV-1 were exposed to the oligomer GCGGGGCTCCAT in a concentration range of 0-200 mM at the time of infection. Virus titers were determined 24 hours later.

The results are summarized in Figure 6 for two independent experiments. The dodecamer resulted in significant inhibition of virus growth in Vero cells infected at a low m.o.i.

EXAMPLE 7

The effectiveness of using a plurality of oligonucleotides for the inhibition of virus growth in Vero cells was studied. Vero cells were exposed to the dodecamer GCGGGGCTCCAT and TTCCTCCTGCGG at a concentration of 0-200 mM at the time of infection with 0.1 pfu per cell of HSV-1. Virus titers were determined 24 hours later.

The results are summarized in Figures 7A and 7B. Either of the two oligonucleotides, denoted in the figure as IE110 and IE4,5, caused inhibition of virus growth in a dose dependent fashion similar to that found in Examples 1-6 above, with a maximal inhibition of 90-98% at 100-200 mM. When infected cells, however, were treated with both oligomers simultaneously, the effective concentration range for virus inhibition is greater (25-200 mM) than for treatment with either oligonucleotide alone. Additionally, treatment with a plurality of nucleotides resulted in significant inhibition (80-99.8%) at 25-75 mM and a 50% decrease in virus titers at 10 mM.

In Figure 7B, the results of the data were calculated and presented according to formulae that verify synergistic interactions beteen the two oligomers. The data presented in this fashion reveal in a rather dramatic way that a combination

of the two oligonucleotides resulted in a synergistic inhibition of viral replication.

EXAMPLE 8

In a similar study, oligonucleotide complementary to the initiation site of IE4 (designated in Figure 8 as IE68) and oligonucleotide complementary to the IE5 initiation site (designated in Figure 8 as IE12) were tested as described above.

The data summarized in Figure 8 reveal that the IE12 oligonucleotide was ineffective in inhibiting virus growth whereas the other nucleotides resulted in a 85-95% inhibition of viral growth over a range of 25-200 mM of the oligonucleotides. Mixing the two oligonucleotides did not diminish the binding and inhibitory activity of the IE5 initiation site oligonucleotide.

EXAMPLE 9

Mice were infected with HSV-1 (2000 pfu), treated with oligomer on day 0 or on days 0-5 postinfection and the tissues were examined for virus titers on day 6 postinfection. While single treatment with oligomer (500 μM) had little effect on virus growth (27% inhibition), daily application resulted in 82% inhibition of virus titers. With a higher concentration of virus per dose, 2×10^6 pfu, animals treated twice daily (receiving a total amount of 1000 μM per day) with TTCCTCCTGCGG did not develop lesions (0/5 as compared to 5/5 in untreated animals).

Consistent with these studies, animals which were inoculated in the foot pad with HSV-1 (1×10^6

pfu) and then treated twice (days 1 and 2 post-immunization) with 50 μM of dodecamer, showed about an 86% reduction in virus titer.

EXAMPLE 10

Although it is unclear why not all people infected with HIV develop AIDS, it is becoming evident that many factors other than HIV are essential for activation of the virus. Notable among the factors that may enhance HIV activation are viruses including JC, adenoviruses and the herpesviruses including HSV-1, HSV-2, HCMV, VZV, EBV, HHV-6 and HHV-7. Specific genes of the herpesviruses activate the expression of HIV either directly at the level of viral replication or by activating expression of a reporter gene driven by the HIV promoter.

U937 cells were cotransfected with a construct carrying the HIV-LTR promoter upstream to the CAT structural gene and a construct carrying sequences encoding HSV-1 IE110. Those same transfected cells were then treated with oligomers complementary to the initiation or splice sites of the IE110 gene. After 40 hours of culture, the presence or absence of CAT was determined.

Results of a representative experiment are presented in Figure 9. In the experiment, three different oligomers were tested, two are complementary to IE110 sequences and the oligomer denoted as IE4,5 is complementary to an unrelated gene. The oligomers were used at two different concentrations. The two oligomers complementary to IE110 sequences resulted in a dose-related inhibition of the HIV promoter with the oligomer complementary to the initiation site showing a higher level of activity than the oligomer

complementary to the second splice site. The effect is specific as the IE4,5 oligomer does not inhibit HIV activation to a significant degree.

In another experiment that monitored HIV activation by assaying virus growth (Figure 10), it was found that growth of HIV was enhanced 2500-fold by the addition of HSV IE110 and the activation was reduced significantly by the addition of the IE110 oligomer complementary to the initiation site.

In the figure, it will be appreciated that untreated cells and cells that were treated with the oligomer showed no HIV activation after 8 days whereas cells treated with the HSV gene product showed remarkable proliferation.

When the cells were treated with a combination of IE110 and the oligomer at a concentration of 150 mM, a moderate degree of HIV growth was noted. The level of virus growth did not differ however from the degree of HIV activation obtained when the cells were exposed to the control plasmid pBR322.

As testimony to the relatedness of the herpesviruses, U937 cells were exposed also to the homologue IE gene product of cytomegalovirus. Although the degree of activation was not as great as that found with the HSV gene product, there was a significant enhancement of HIV growth by the CMV gene product.

EXAMPLE 11

Additional animal studies revealed that dermal application of oligonucleotides was effective in inhibiting virus replication.

Mice were infected with HSV-1 and injected interdermally with the oligonucleotide TTCCTCCTGCGG at the time of infection. Then once daily thereafter the oligonucleotide suspended in a PEG

(polyethylene glycol) cream was applied to the site of infection. The concentration of oligonucleotide in the cream was 500 μM which is 5-fold higher than used for parenteral administration.

The results are summarized in Table 2.

TABLE 2

Effect of IE4,$_{\wedge}$5 oligomer on virus clearance

Group (n = 10)	Av. virus titer/ear (day 3)	% Inhibition
Virus dose (1 x 10^6 PFU)		
Vehicle (no oligomer)	2 x 10^4	
Oligomer (500 μM)	5.0 x 10^3	75
Virus dose (1 x 10^4 PFU)		
Vehicle (no oligomer)	8 x 10^2	
Oligomer (500 μM)	6.8 x 10^1	91.5

At either of the two doses, virus titers were reduced significantly, however, somewhat better inhibition was obtained at an initial low virus dose of 1 x 10^4 pfu.

All publications cited in the specification are herein incorporated by reference.

It will be appreciated by one of ordinary skill in the art that the compositions and methods of the instant invention are capable of being incorporated in the form of variety of embodiments, only a few of which have been illustrated and described above. The invention may be embodied in other specific forms without departing from the spirit or the central characteristics of said invention. The described embodiments are to be considered in all respects only as illustrative and not restrictive. All changes that come within the

meaning and range of equivalency of the claims are to be embraced within the scope of the claims in view of the teachings in the specification.

IN THE CLAIMS:

1. An oligonucleotide selected from the group consisting of:

(a) T T C C T C C T G C G G;
(b) G C T T A C C C G T G C;
(c) G C G G G G C T C C A T;
(d) A A T G T C G G C C A T;
(e) A T A T C A A T G T C A G T C G C C A T;
(f) G G C C C A C G A C A T;
(g) G C G G G C G T C G G T;
(h) G G C G C C G T C T G C;
(i) G T G G C C G G C G C C;
(j) G C G G G C G T C G G T;
(k) G G C G C C G T C T G C;
(l) G T G G C C G G C G C C;
(m) G T T C T C C G A C G C C A T;
(n) C G C T C C G T G T G G;
(o) C C G C C G C C A C C G G A A A A C A T;
(p) T G G C A G G G G T A C G A A G C C A T;
(q) G C G G C T G G G C G G C T G G C C A T;
(r) T C G T C G A C C A A G A G G T C C A T;
(s) C A A A C A G C T C G T;
(t) C C A T G T C G G C A A;
(u) G T C C G C G T C C A T;
(v) G C C G T C C G C G T C;
(w) T G G C G A A G C G C C;
(x) A C A G C C C G T G G T;
(y) C C G A G G A A T G A C;
(z) C C G T T C C C G A G G;
(aa) C A C T G G C A T G G A;
(bb) G C A T C C T G C C A C;
(cc) C C C C G A A A G C A T;

(dd) G A T C C C C G A A A G;
(ee) C T G A C C A C C G A T;
(ff) G C A G G C T C T G G T;
(gg) C C A T G T T G G G C A;
(hh) T G G G G G T G C C A T;
(ii) G A G T G G G G G T G C;
(jj) G G T G C G T G G G A G;
(kk) C A A G G A C G G T G A;
(ll) C C A T C G T G T C A A;
(mm) A G A G G A C T C C A T;
(nn) G G C A G A G G A C T C;
(oo) C T T T C T C T T G G C;
(pp) G A C G G T T C A C T A;
(qq) C A G G C G A T C T G A;
(rr) C G T C T C C A G G C G;
(ss) C A A C T G G C T G T A;
(tt) C C A T G G T A A C A A;
(uu) T A T G G T A T C C A T;
(vv) T A A T A T G G T A T C;
(ww) A C C G C C C G C T A A;
(xx) T G G G G T G A A T T T;
(yy) C C A T C G C A C T G G;
(zz) C G G C G T A T C C A T;
(a') C G G C G G C G T A T C;
(b') G C G C T G C A T C G G;
(c') A G T C T G C T G C A A; and
(d') T A A T G A R A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine, and R is A, T, G, C, U or functional equivalents thereof.

2. The oligonucleotide or ribose containing oligonucleotide of claim 1 selected from the group consisting of:

(a) T T C C T C C T G C G G;
(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T;
(d) A A T G T C G G C C A T;
(e) A T A T C A A T G T C A G
T C G C C A T;
(f) G G C C C A C G A C A T;
(g) G C G G G C G T C G G T;
(h) G G C G C C G T C T G C;
(i) G T G G C C G G C G C C;
(j) G C G G G C G T C G G T;
(k) G G C G C C G T C T G C;
(l) G T G G C C G G C G C C;
(m) G T T C T C C G A C G C C A T;
(n) C G C T C C G T G T G G;
(o) C C G C C G C C A C C G G
A A A A C A T;
(p) T G G C A G G G G T A C G
A A G C C A T;
(q) G C G G C T G G G C G G C
T G G C C A T;
(r) T C G T C G A C C A A G A
G G T C C A T;
(s) C A A A C A G C T C G T;
(t) C C A T G T C G G C A A;
(u) G T C C G C G T C C A T;
(v) G C C G T C C G C G T C;
(w) T G G C G A A G C G C C;
(x) A C A G C C C G T G G T;
(y) C C G A G G A A T G A C;
(z) C C G T T C C C G A G G;
(aa) C A C T G G C A T G G A;
(bb) G C A T C C T G C C A C;
(cc) C C C C G A A A G C A T;
(dd) G A T C C C C G A A A G;
(ee) C T G A C C A C C G A T;
(ff) G C A G G C T C T G G T;
(gg) C C A T G T T G G G C A;
(hh) T G G G G G T G C C A T;

(ii) G A G T G G G G G T G C;

(jj) G G T G C G T G G G A G;

(kk) A G T C T G C T G C A A; and

(ll) T A A T G A R A T.

3. The oligonucleotide or ribose containing oligonucleotide of claim 2 selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T.

4. The oligonucleotide or ribose containing oligonucleotide of claim 1 selected from the group consisting of:

(a) C A A G G A C G G T G A;

(b) C C A T C G T G T C A A;

(c) A G A G G A C T C C A T;

(d) G G C A G A G G A C T C;

(e) C T T T C T C T T G G C;

(f) G A C G G T T C A C T A;

(g) C A G G C G A T C T G A; and

(h) C G T C T C C A G G C G.

5. The oligonucleotide or ribose containing oligonucleotide of claim 1 selected from the group consisting of:

(a) C A A C T G G C T G T A;

(b) C C A T G G T A A C A A;

(c) T A T G G T A T C C A T;

(d) T A A T A T G G T A T C;

(e) A C C G C C C G C T A A;

(f) T G G G G T G A A T T T;

(g) C C A T C G C A C T G G;

(h) C G G C G T A T C C A T;

(i) C G G C G G C G T A T C; and

(j) G C G C T G C A T C G G.

6. The oligonucleotide or ribose containing oligonucletide of claims 1, 2, 3, 4, or 5 which comprises one or more modified nucleosides.

7. The oligonucleotide of claim 6 wherein said modified nucleoside is an aryl or alkyl phosphonate nucleoside.

8. The oligonucleotide of claim 7 wherein said aryl or alkyl phosphonate nucleoside is methylphosphonate nucleoside.

9. A composition for inhibiting herpesvirus growth or replication comprising an oligonucleotide hybridizable with nucleic acids of said herpesvirus.

10. The composition of claim 9 wherein said oligonucleotide hybridizes to a cap site, a transcription initiation site, a translation initiation site or a splice site.

11. The composition of claim 10 wherein said oligonucleotides hybridizable to a splice site contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

12. The composition of claim 10 wherein said oligonucleotides hybridizable to a translation initiation site are complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

13. The composition of claims 9, 10, 11 or 12 wherein said oligonucleotide hybridizes to a regulatory gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

14. The composition of claim 13 wherein said regulatory gene is an immediate-early (IE) gene.

15. The composition of claim 13 wherein said regulatory gene is Vmw65.

16. The composition of claim 13 wherein said oligonucleotide is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

17. The composition of claim 9 wherein said oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

18. The composition of claim 17 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

19. The composition of claim 16 wherein said oligonucleotide or ribose containing oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

20. The composition of claim 19 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

21. A method of inhibiting herpesvirus growth or replication comprising the step of contacting nucleic acids of said herpesvirus with an oligonucleotide hybridizable with said nucleic acids of herpesvirus.

22. The method of claim 21 wherein said oligonucleotide hybridizes to a cap site, a transcription initiation site, a translation initiation site or a splice site.

23. The method of claim 22 wherein said oligonucleotides hybridizable to a splice site

contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

24. The method of claim 22 wherein said oligonucleotides hybridizable to a translation initiation site are complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

25. The method of claims 21, 22, 23 or 24 wherein said oligonucleotide hybridizes to a regulatory gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

26. The method of claim 25 wherein said regulatory gene is an immediate-early (IE) gene.

27. The method of claim 25 wherein said regulatory gene is Vmw65.

28. The method of claim 25 wherein said oligonucleotide is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

29. The method of claim 21 wherein said oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

30. The method of claim 29 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

31. The method of claim 28 wherein said oligonucleotide or ribose containing oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

32. The method of claim 31 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

33. A therapeutic composition for inhibiting herpesvirus growth or replication comprising an oligonucleotide, or pharmaceutically acceptable salts thereof, hybridizable with nucleic acids of said herpesvirus and a pharmaceutically acceptable carrier.

34. The therapeutic composition of claim 33 wherein said oligonucleotide hybridizes to a cap site, a transcription initiation site, a translation initiation site or a splice site.

35. The therapeutic composition of claim 34 wherein said oligonucleotides hybridizable to a splice site contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

36. The therapeutic composition of claim 34 wherein said oligonucleotides hybridizable to a translation initiation site are complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

37. The therapeutic composition of claims 33, 34, 35 or 36 wherein said oligonucleotide hybridizes to a regulatory gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

38. The therapeutic composition of claim 37 wherein said regulatory gene is an immediate-early (IE) gene.

39. The therapeutic composition of claim 37 wherein said regulatory gene is Vmw65.

40. The therapeutic composition of claim 37 wherein said oligonucleotide is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

41. The therapeutic composition of claim 33 wherein said oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

42. The therapeutic composition of claim 41 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

43. The therapeutic composition of claim 40 wherein said oligonucleotide or ribose containing oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

44. The therapeutic composition of claim 43 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

45. A therapeutic method of inhibiting herpesvirus growth or replication comprising administering to a host carrying herpesvirus a pharmaceutically effective amount of a composition comprising an oligonucleotide, or pharmaceutically acceptable salt thereof, hybridizable with nucleic acids of said herpesvirus.

46. The therapeutic method of claim 45 wherein said oligonucleotide hybridizes to a cap site, a transcription initiation site, a translation initiation site or a splice site.

47. The therapeutic method of claim 46 wherein said oligonucleotides hybridizable to a splice site contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

48. The therapeutic method of claim 46 wherein said oligonucleotides hybridizable to a translation initiation site are complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

49. The therapeutic method of claims 45, 46, 47 or 48 wherein said oligonucleotide hybridizes to a regulatory gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

50. The therapeutic method of claim 49 wherein said regulatory gene is an immediate-early (IE) gene.

51. The therapeutic method of claim 49 wherein said regulatory gene is Vmw65.

52. The therapeutic method of claim 49 wherein said oligonucleotide is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

53. The therapeutic method of claim 45 wherein said oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

54. The therapeutic method of claim 53 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

55. The therapeutic method of claim 52 wherein said oligonucleotide or ribose containing oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

56. The therapeutic method of claim 55 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

57. A method of detecting herpesvirus comprising the steps of:

(i) obtaining a biologic sample containing nucleic acids;

(ii) treating said sample so the nucleic acids contained therein are made single stranded;

(iii) exposing said treated sample to a labelled oligonucleotide wherein said oligonucleotide is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T;

(d) A A T G T C G G C C A T;

(e) A T A T C A A T
G T C A G T C G
C C A T;

(f) G G C C C A C G A C A T;

(g) G C G G G C G T C G G T;

(h) G G C G C C G T C T G C;

(i) G T G G C C G G C G C C;
(j) G C G G G C G T C G G T;
(k) G G C G C C G T C T G C;
(l) G T G G C C G G C G C C;
(m) G T T C T C C G A C G C C A T;
(n) C G C T C C G T G T G G;
(o) C C G C C G C C
A C C G G A A A
A G A T;
(p) T G G C A G G G
G T A C G A A G
C C A T;
(q) G C G G C T G G
G C G G C T G G
C C A T;
(r) T C G T C G A C
C A A G A G G T
C C A T;
(s) C A A A C A G C T C G T;
(t) C C A T G T C G G C A A;
(u) G T C C G C G T C C A T;
(v) G C C G T C C G C G T C;
(w) T G G C G A A G C G C C;
(x) A C A G C C C G T G G T;
(y) C C G A G G A A T G A C;
(z) C C G T T C C C G A G G;
(aa) C A C T G G C A T G G A;
(bb) G C A T C C T G C C A C;
(cc) C C C C G A A A G C A T;
(dd) G A T C C C C G A A A G;
(ee) C T G A C C A C C G A T;
(ff) G C A G G C T C T G G T;
(gg) C C A T G T T G G G C A;
(hh) T G G G G G T G C C A T;
(ii) G A G T G G G G G T G C;
(jj) G G T G C G T G G G A G;
(kk) C A A G G A C G G T G A;

(ll) C C A T C G T G T C A A;
(mm) A G A G G A C T C C A T;
(nn) G G C A G A G G A C T C;
(oo) C T T T C T C T T G G C;
(pp) G A C G G T T C A C T A;
(qq) C A G G C G A T C T G A;
(rr) C G T C T C C A G G C G;
(ss) C A A C T G G C T G T A;
(tt) C C A T G G T A A C A A;
(uu) T A T G G T A T C C A T;
(vv) T A A T A T G G T A T C;
(ww) A C C G C C C G C T A A;
(xx) T G G G G T G A A T T T;
(yy) C C A T C G C A C T G G;
(zz) C G G C G T A T C C A T;
(a') C G G C G G C G T A T C;
(b') G C G C T G C A T C G G;
(c') A G T C T G C T G C A A; and
(d') T A A T G A R A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine, and R is A, T, G, C, U or functional equivalents thereof; and

(iv) detecting hybridized sequences.

58. The method of claim 57 wherein said oligonucleotide or ribose containing oligonucletide comprises one or more modified nucleosides.

59. The method of claim 58 wherein said modified nucleoside is an aryl or alkyl phosphonate nucleoside.

60. The method of claim 59 wherein said aryl or alkyl phosphonate is methylphosphonate nucleoside.

61. A composition for inhibiting microbe, virus or self-replicating nucleic acid growth or

replication comprising at least two oligonucleotides hybridizable to non-overlapping sites in the nucleic acids of said microbe, virus or self-replicating nucleic acid.

62. The composition of claim 61 wherein at least one of said oligonucleotides is hybridizable to a cap site, a transcription initiation site, a translation initiation site or a splice site.

63. The composition of claim 62 wherein said oligonucleotides hybridizable to a splice site contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

64. The composition of claim 62 wherein said oligonucleotides hybridizable to a translation initiation site are complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

65. The composition of claims 61, 62, 63 or 64 wherein at least one of said oligonucleotides is hybridizable to a regulatory gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

66. The composition of claim 65 wherein one of said oligonucleotides is hybridizable to a first portion of nucleic acid of said microbe, virus or self-replicating nucleic acid in which the expression or function of said first portion is required for expression or function of a second portion of nucleic acid of said microbe, virus or self-replicating nucleic acid and another of said oligonucleotides is hybridizable to said second portion.

67. The composition of claim 66 wherein said virus is a herpesvirus.

68. The composition of claim 67 wherein said regulatory gene is an immediate-early (IE) gene.

69. The composition of claim 67 wherein said regulatory gene is Vmw65.

70. The composition of claim 67 wherein at least one of said oligonucleotides is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

71. The composition of claim 61 wherein at least two of said oligonucleotides comprise one or more alkyl or arylphosphonate nucleosides.

72. The composition of claim 71 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

73. The composition of claim 70 wherein said oligonucleotide or ribose containing oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

74. The composition of claim 73 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

75. A method for inhibiting microbe, virus or self-replicating nucleic acid growth or replication comprising the step of contacting nucleic acids of said virus or microbe with at least two oligonucleotides that are hybridizable with non-overlapping sites in said nucleic acids of said microbe, virus or self-replicating nucleic acid.

76. The method of claim 75 wherein at least one of said oligonucleotides is hybridizable to a cap site, a transcription initiation site, a translation initiation site or a splice site.

77. The method of claim 76 wherein said oligonucleotides hybridizable to a splice site contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

78. The method of claim 76 wherein said oligonucleotide hybridizable to a translation initiation site is complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

79. The method of claims 75, 76, 77 or 78 wherein at least one of said oligonucleotides is hybridizable to a regulatory gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

80. The method of claim 79 wherein one of said oligonucleotides is hybridizable to a first portion of nucleic acids of said microbe, virus or self-replicating nucleic acid in which the expression or function of said first portion is required for expression or function of a second portion of nucleic acids of said microbe, virus or self-replicating nucleic acid and another of said oligonucleotides is hybridizable to said second portion.

81. The method of claim 80 wherein said virus is a herpesvirus.

82. The method of claim 81 wherein said regulatory gene is an immediate-early (IE) gene.

83. The method of claim 81 wherein said regulatory gene is Vmw65.

84. The method of claim 81 wherein at least one of said oligonucleotides is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

85. The method of claim 75 wherein at least two of said oligonucleotides comprise one or more alkyl or arylphosphonate nucleosides.

86. The method of claim 85 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

87. The method of claim 84 wherein said oligonucleotide or ribose containing oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

88. The method of claim 87 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

89. A therapeutic composition for inhibiting microbe, virus or self-replicating nucleic acid growth or replication comprising at least two oligonucleotides, or pharmaceutically acceptable salts thereof, that are hybridizable with non-overlapping sites in nucleic acids of said microbe, virus or self-replicating nucleic acid and a pharmaceutically acceptable carrier.

90. The therapeutic composition of claim 89 wherein at least one of said oligonucleotides is hybridizable to a cap site, a transcription

initiation site, a translation initiation site or a splice site.

91. The therapeutic composition of claim 90 wherein said oligonucleotides hybridizable to a splice site contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

92. The therapeutic composition of claim 90 wherein said oligonucleotides hybridizable to a translation initiation site are complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

93. The therapeutic composition of claims 89, 90, 91 or 92 wherein at least one of said oligonucleotides is hybridizable to a regulatory gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

94. The therapeutic composition of claim 93 wherein one of said oligonucleotides is hybridizable to a first portion of nucleic acids of said microbe, virus or self-replicating nucleic acid in which the expression or function of said first portion is required for expression or function of a second portion of nucleic acids of said microbe, virus or self-replicating nucleic acid and another of said oligonucleotides is hybridazable to said second portion.

95. The therapeutic composition of claim 94 wherein said virus is a herpesvirus.

96. The therapeutic composition of claim 95 wherein said regulatory gene is an immediate-early (IE) gene.

97. The therapeutic composition of claim 95 wherein said regulatory gene is Vmw65.

98. The therapeutic composition of claim 95 wherein at least one of said oligonucleotides is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

99. The therapeutic composition of claim 89 wherein at least two of said oligonucleotides comprise one or more alkyl or arylphosphonate nucleosides.

100. The method of claim 99 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

101. The method of claim 98 wherein said oligonucleotide or ribose containing oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

102. The method of claim 101 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

103. A therapeutic method of inhibiting microbe, virus or self-replicating nucleic acid growth or replication comprising administering to a host in need of therapy a therapeutic composition comprising at least two oligonucleotides, or pharmaceutically acceptable salts thereof, that are hybridizable with non-overlapping sites in nucleic acids of said microbe, virus or self-replicating nucleic acid.

104. The therapeutic method of claim 103 wherein at least one of said oligonucleotides is hybridizable to a cap site, a transcription

initiation site, a translation initiation site or a splice site.

105. The therapeutic method of claim 104 wherein said oligonucleotides hybridizable to a splice site contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

106. The therapeutic method of claim 104 wherein said oligonucleotides hybridizable to a translation initiation site are complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

107. The therapeutic method of claims 103, 104, 105 or 106 wherein at least one of said oligonucleotides is hybridizable to a regulatory gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

108. The therapeutic method of claim 107 wherein one of said oligonucleotides is hybridizable to a first portion of nucleic acids of said microbe, virus or self-replicating nucleic acids in which the expression or function of said first portion is required for expression or function of a second portion of said nucleic acids of said microbe, virus or self-replicating nucleic acid and another of said oligonucleotides is hybridizable to said second portion.

109. The therapeutic method of claim 108 wherein said virus is a herpesvirus.

110. The therapeutic method of claim 109 wherein said regulatory gene is an immediate-early (IE) gene.

111. The therapeutic method of claim 109 wherein said regulatory gene is Vmw65.

112. The therapeutic method of claim 109 wherein at least one of said oligonucleotides is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

113. The therapeutic method of claim 103 wherein at least two of said oligonucleotides comprise one or more alkyl or arylphosphonate nucleosides.

114. The method of claim 113 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

115. The method of claim 112 wherein said oligonucleotide or ribose containing oligonucleotide comprises one or more alkyl or arylphosphonate nucleosides.

116. The method of claim 115 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

117. A method for inhibiting human immunodeficiency virus growth or replication in a specimen containing human immunodeficiency virus and a second virus comprising the step of contacting nucleic acids of said specimen with at least one oligonucleotide that is hybridizable with nucleic acids of said second virus.

118. The method of claim 117 wherein at least one of said oligonucleotides is hybridizable to a cap site, a transcription initiation site, a translation initiation site or a splice site.

119. The method of claim 118 wherein said oligonucleotides hybridizable to a splice site contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

120. The method of claim 118 wherein said oligonucleotides hybridizable to a translation initiation site are complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

121. The method of claims 117, 118, 119 or 120 wherein at least one of said oligonucleotides is hybridizable to a regulatory gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

122. The method of claim 121 wherein said second virus is herpesvirus.

123. The method of claim 122 wherein said regulatory gene is an immediate-early (IE) gene.

124. The method of claim 122 wherein said regulatory gene is Vmw65.

125. The method of claim 122 wherein at least one of said oligonucleotides is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

126. The method of claim 117 wherein at least one of said oligonucleotides comprise one or more alkyl or arylphosphonate nucleosides.

127. The method of claim 126 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

128. The method of claim 125 wherein at least one of said oligonucleotides or ribose containing oligonucleotides comprise one or more alkyl or arylphosphonate nucleosides.

129. The method of claim 128 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

130. The method of claim 117 wherein said specimen is contacted with at least two oligonucleotides.

131. A therapeutic composition for inhibiting human immunodeficiency virus growth or replication in a host carrying human immunodeficiency virus and a second virus comprising at least one oligonucleotide, or pharmaceutically acceptable salts thereof, that is hybridizable with nucleic acids of said second virus and a pharmaceutically acceptable carrier.

132. The therapeutic composition of claim 131 wherein at least one of said oligonucleotides is hybridizable to a cap site, a transcription initiation site, a translation initiation site or a splice site.

133. The therapeutic composition of claim 132 wherein said oligonucleotides hybridizable to a splice site contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

134. The therapeutic composition of claim 132 wherein said oligonucleotides hybridizable to a

translation initiation site are complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

135. The therapeutic composition of claims 131, 132, 133 or 134 wherein at least one of said oligonucleotides is hybridizable to a regulatory gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

136. The therapeutic composition of claim 135 wherein said second virus is herpesvirus.

137. The therapeutic composition of claim 136 wherein said regulatory gene is an immediate-early (IE) gene.

138. The therapeutic composition of claim 136 wherein said regulatory gene is Vmw65.

139. The therapeutic composition of claim 136 wherein said oligonucleotide is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

140. The therapeutic composition of claim 131 wherein at least one of said oligonucleotides comprise one or more alkyl or arylphosphonate nucleosides.

141. The therapeutic composition of claim 140 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

142. The therapeutic composition of claim 139 wherein at least one of said oligonucleotides or

ribose containing oligonucleotides comprise one or more alkyl or arylphosphonate nucleosides.

143. The therapeutic composition of claim 142 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

144. The therapeutic composition of claim 131 that comprises at least two oligonucleotides.

145. A therapeutic method for inhibiting human immunodeficiency virus growth or replication in a host carrying human immunodeficiency virus and a second virus comprising administering to said host a therapeutic composition comprising at least one oligonucleotide, or pharmaceutically acceptable salt thereof, that is hybridizable with nucleic acids of said second virus.

146. The therapeutic method of claim 145 wherein at least one of said oligonucleotides is hybridizable to a cap site, a transcription initiation site, a translation initiation site or a splice site.

147. The therapeutic method of claim 146 wherein said oligonucleotides hybridizable to a splice site contain exon and intron sequences and the difference in the number of bases containing exon sequences and the number of bases containing intron sequences is 0 or 1.

148. The therapeutic method of claim 146 wherein said oligonucleotide hybridizable to a translation initiation site are complementary to the ATG initiation codon and adjacent 3' bases in the coding region, so that the last three bases of said oligonucleotide in the 5' to 3' orientation are C, A and T, in that order.

149. The therapeutic method of claims 145, 146, 147 or 148 wherein at least one of said oligonucleotides is hybridizable to a regulatory

gene, a gene required for nucleic acid replication or a gene involved in pathogenesis.

150. The therapeutic method of claim 149 wherein said second virus is herpesvirus.

151. The therapeutic method of claim 150 wherein said regulatory gene is an immediate-early (IE) gene.

152. The therapeutic method of claim 150 wherein said regulatory gene is Vmw65.

153. The therapeutic method of claim 150 wherein said oligonucleotide is selected from the group consisting of:

(a) T T C C T C C T G C G G;

(b) G C T T A C C C G T G C;

(c) G C G G G G C T C C A T; and

(d) A A T G T C G G C C A T; or

ribose containing oligonucleotides corresponding thereto, wherein T is replaced by U; and where A, T, G, U and C are adenine, thymidine, guanine, uracil and cytosine.

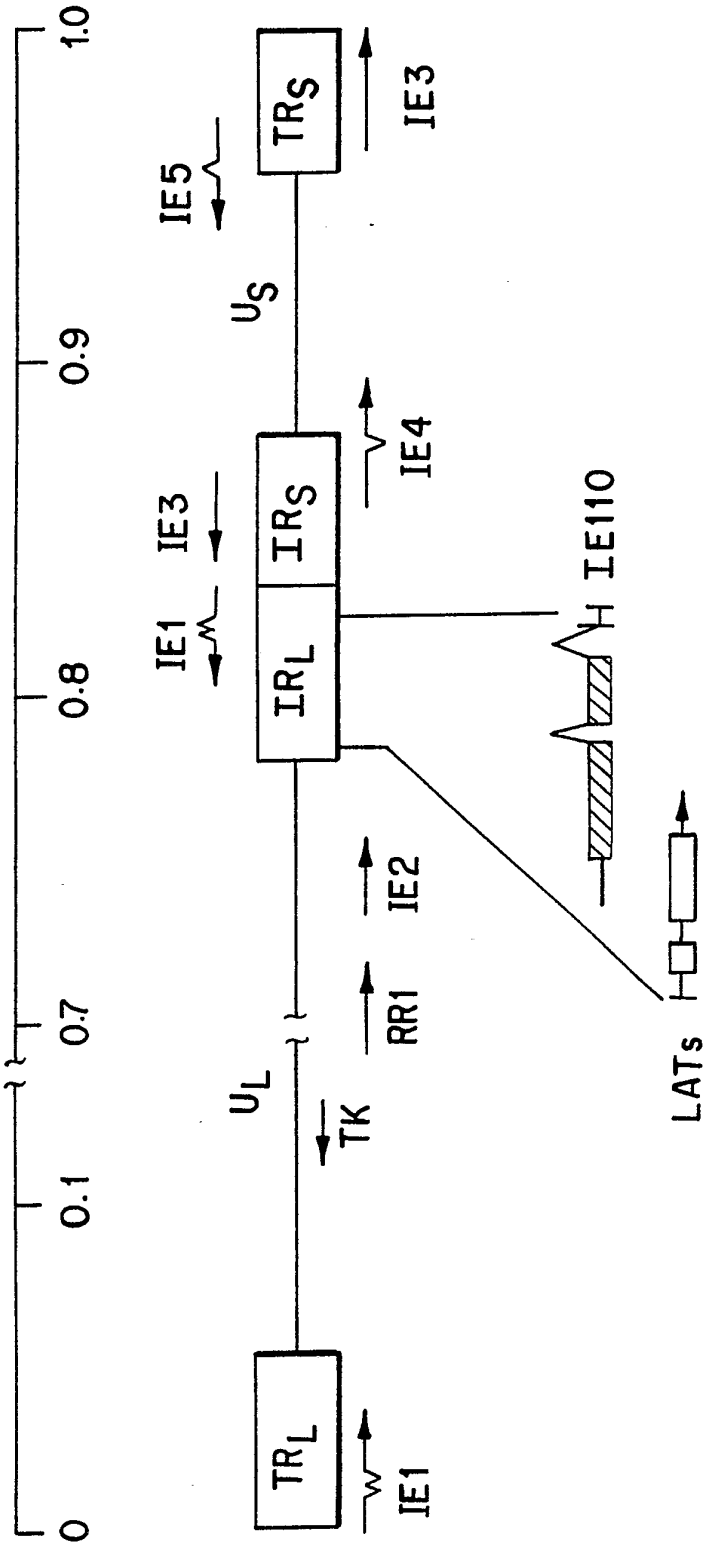
154. The therapeutic method of claim 153 wherein at least one of said oligonucleotides comprise one or more alkyl or arylphosphonate nucleosides.

155. The therapeutic method of claim 154 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

156. The therapeutic method of claim 145 wherein at least one of said oligonucleotides or ribose containing oligonucleotides comprise one or more alkyl or arylphosphonate nucleosides.

157. The therapeutic method of claim 156 wherein said alkyl or arylphosphonate nucleoside is methylphosphonate nucleoside.

158. The therapeutic method of claim 145 wherein said cell is contacted with at least two oligonucleotides.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0.8
0.7
0.1
0
TRS
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IE5
US
IE4
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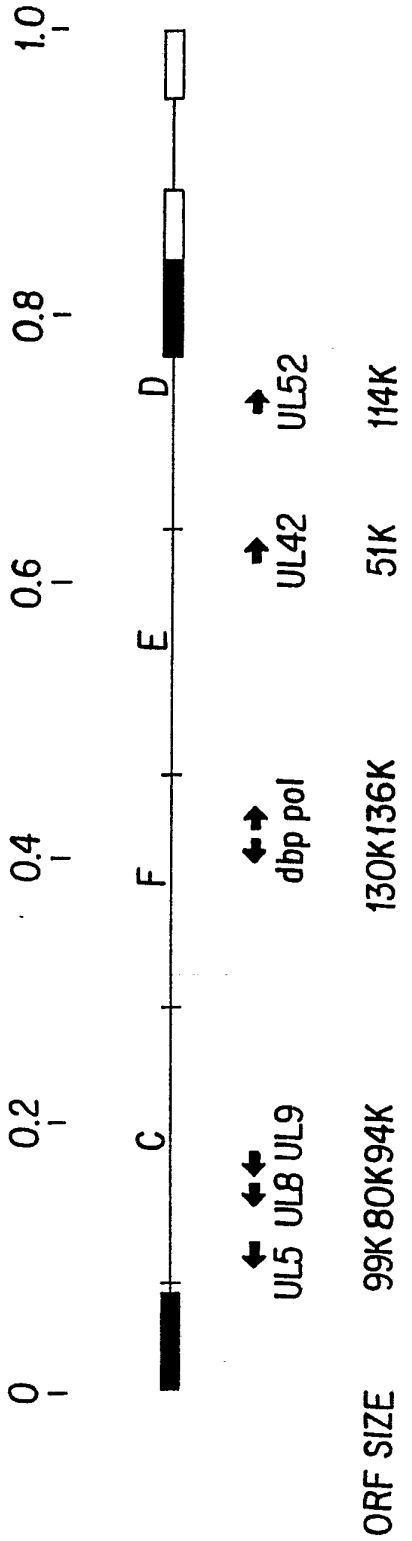
IE3
IE1
IRL
IE110
IE2
RR1
UL
TK
LATs
TRL
IE1

FIG. 1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D
E
F
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51K
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ORF SIZE

FIG. 2

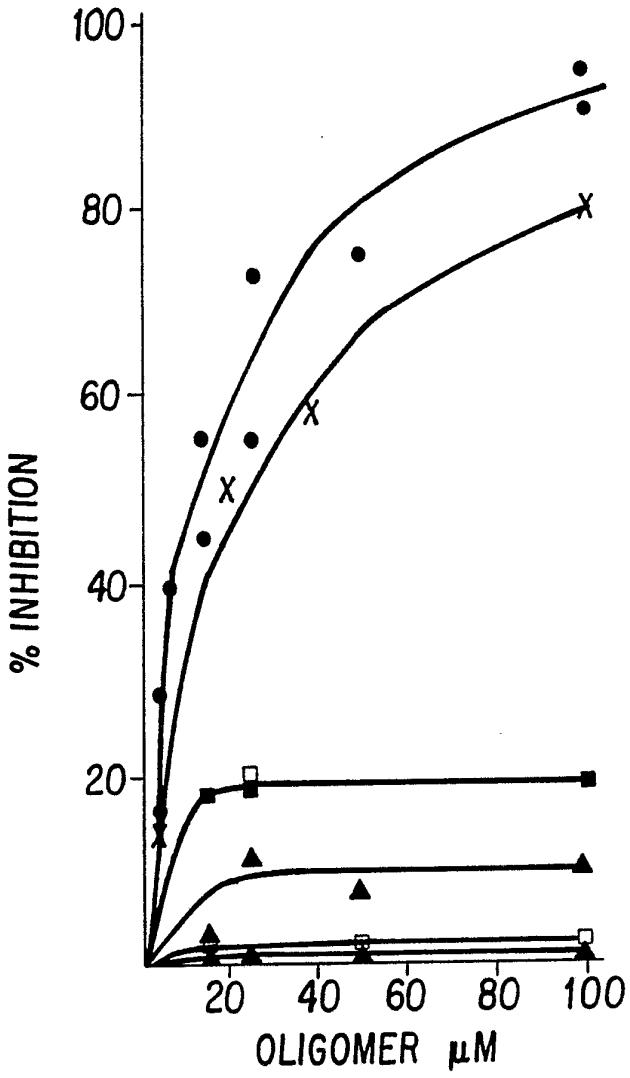

FIG. 3

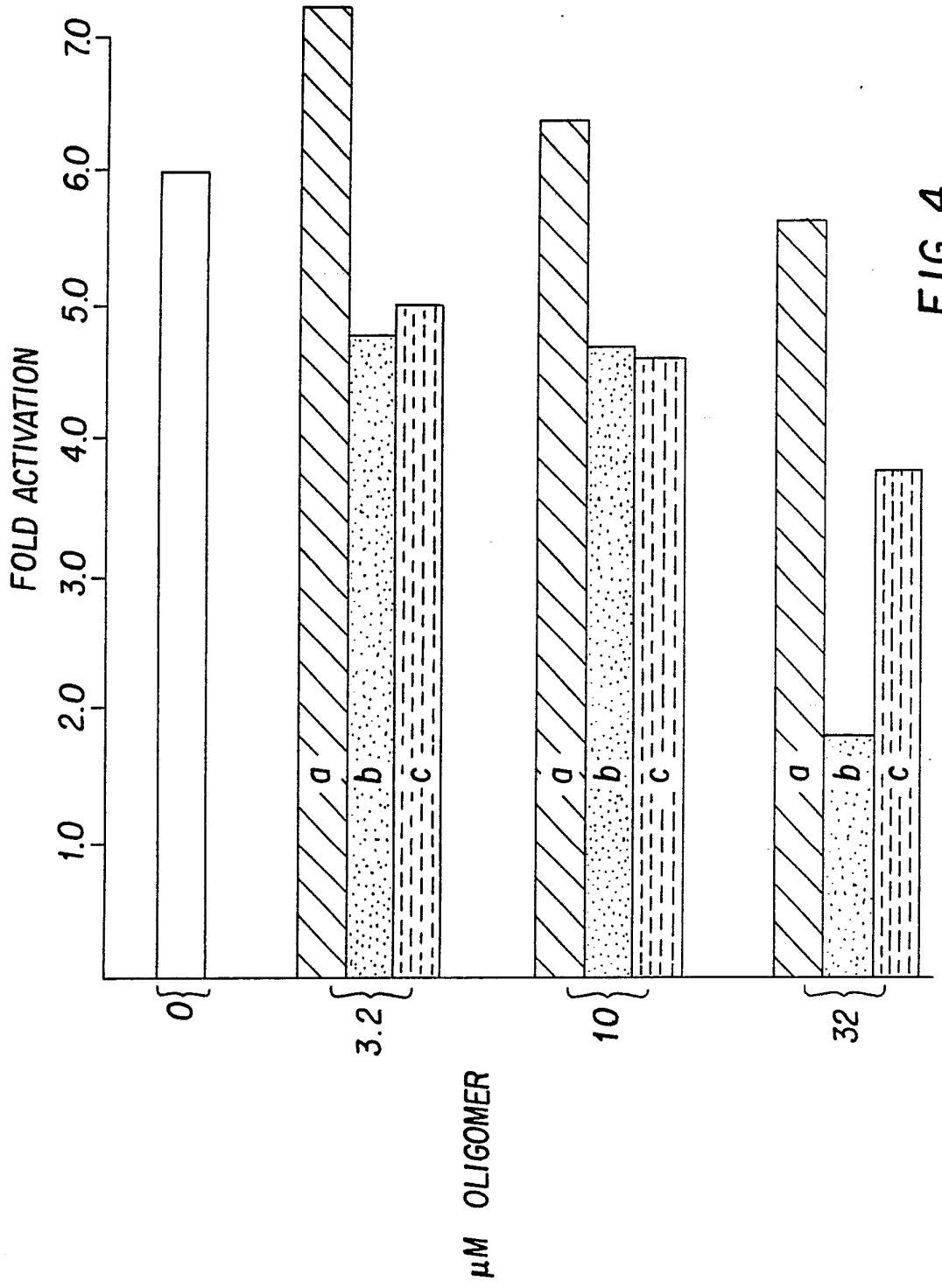

FIG. 4

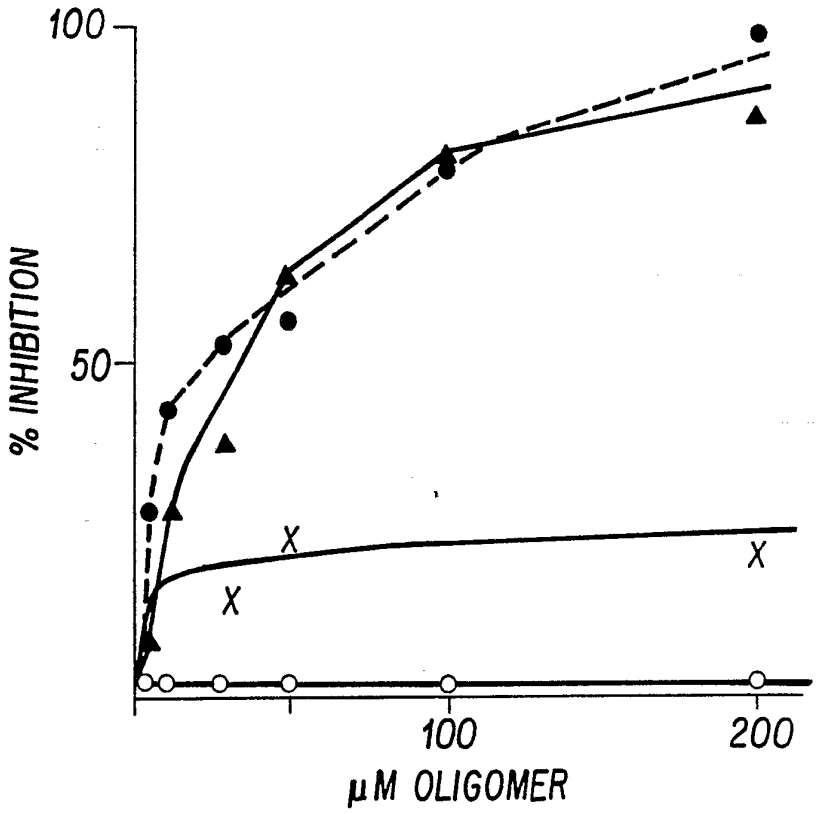

FIG. 5

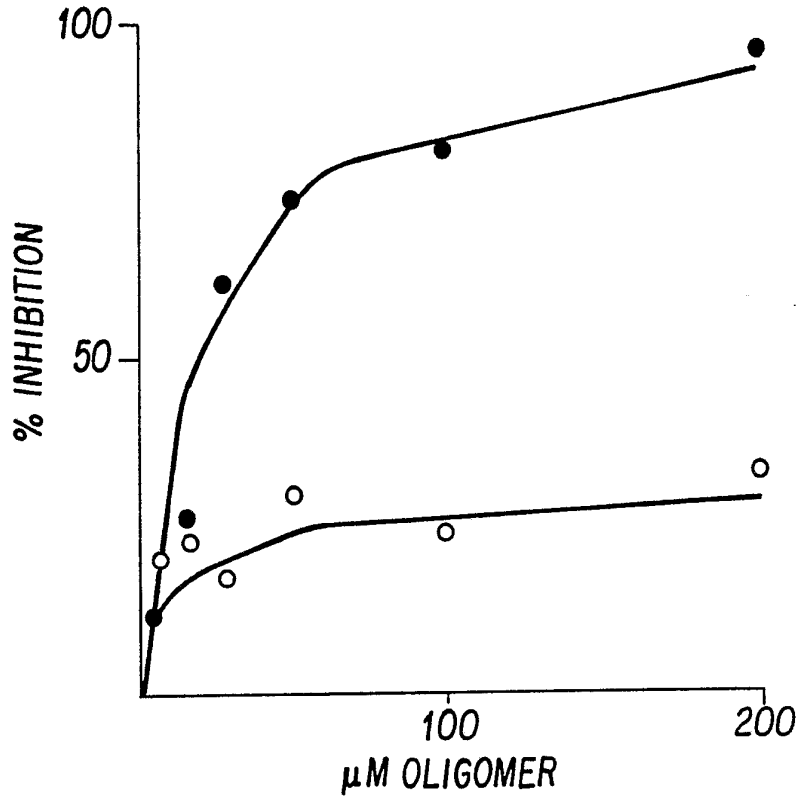

FIG. 6

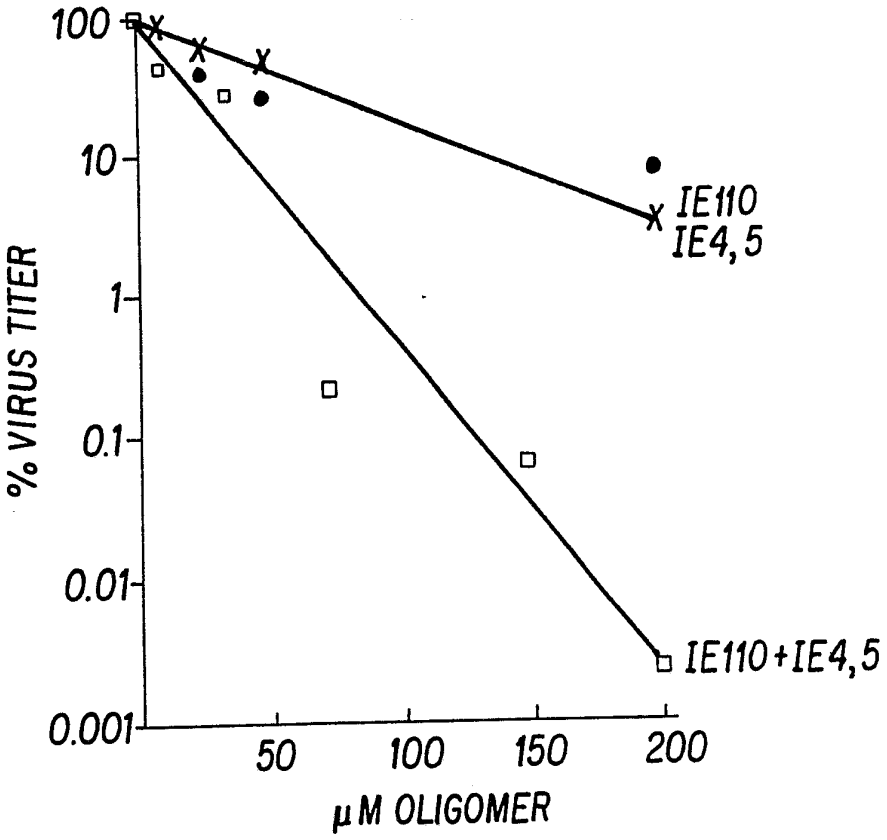
100
10
1
0.1
0.01
0.001
% VIRUS TITER
IE110
IE4,5
IE110+IE4,5
50
100
150
200
μM OLIGOMER

FIG. 7A

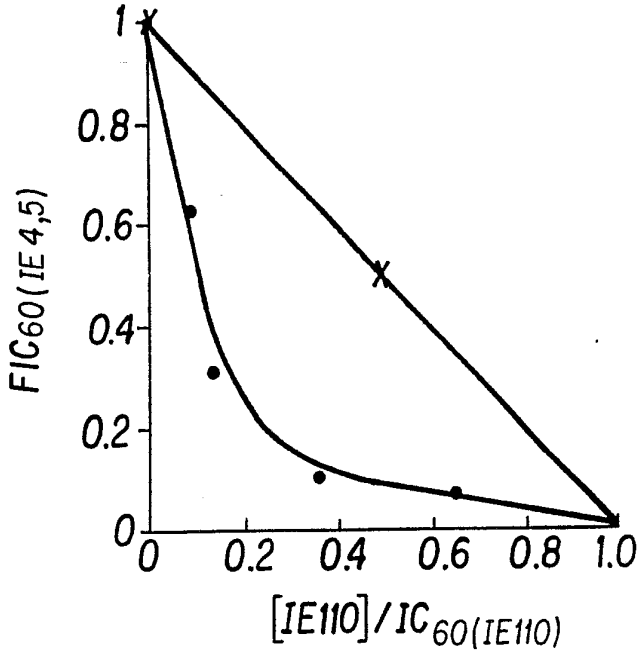
1
0.8
0.6
0.4
0.2
0
FIC60(IE4,5)
0
0.2
0.4
0.6
0.8
1.0
[IE110]/IC60(IE110)

FIG. 7B

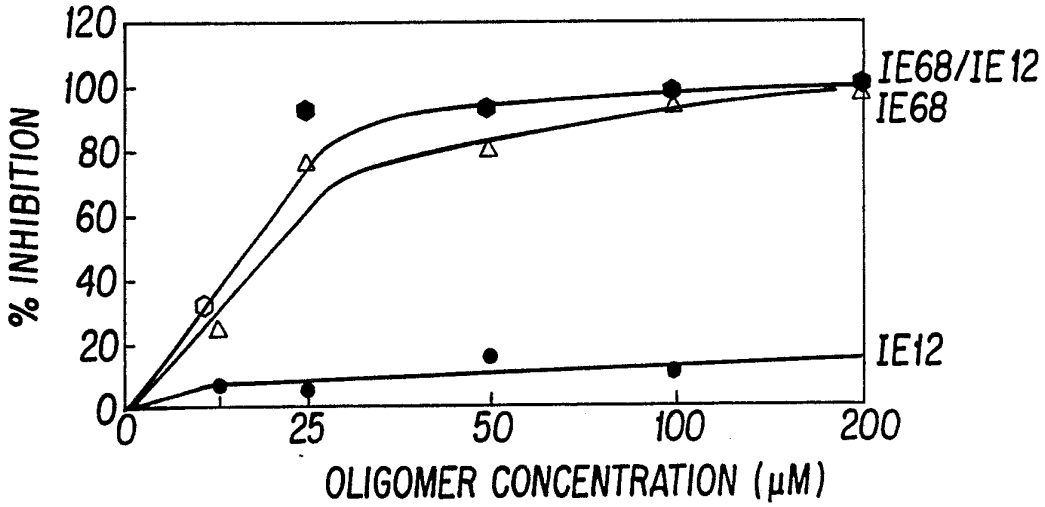
120
100
80
60
40
20
0
% INHIBITION
0
25
50
100
200
OLIGOMER CONCENTRATION (μM)
IE68/IE12
IE68
IE12

FIG. 8

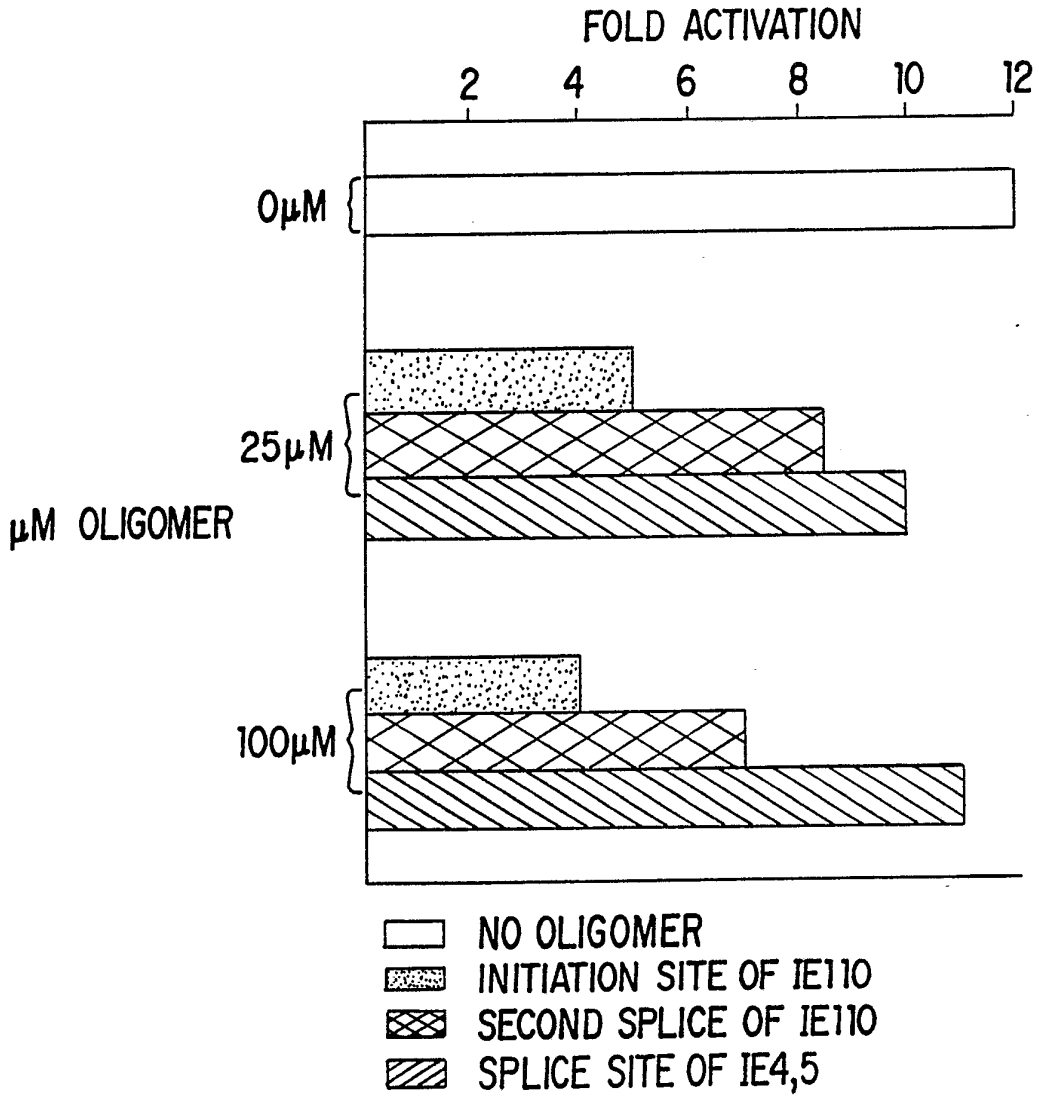
FOLD ACTIVATION
2
4
6
8
10
12
0μM
25μM
100μM
μM OLIGOMER
NO OLIGOMER
INITIATION SITE OF IE110
SECOND SPLICE OF IE110
SPLICE SITE OF IE4,5

FIG. 9

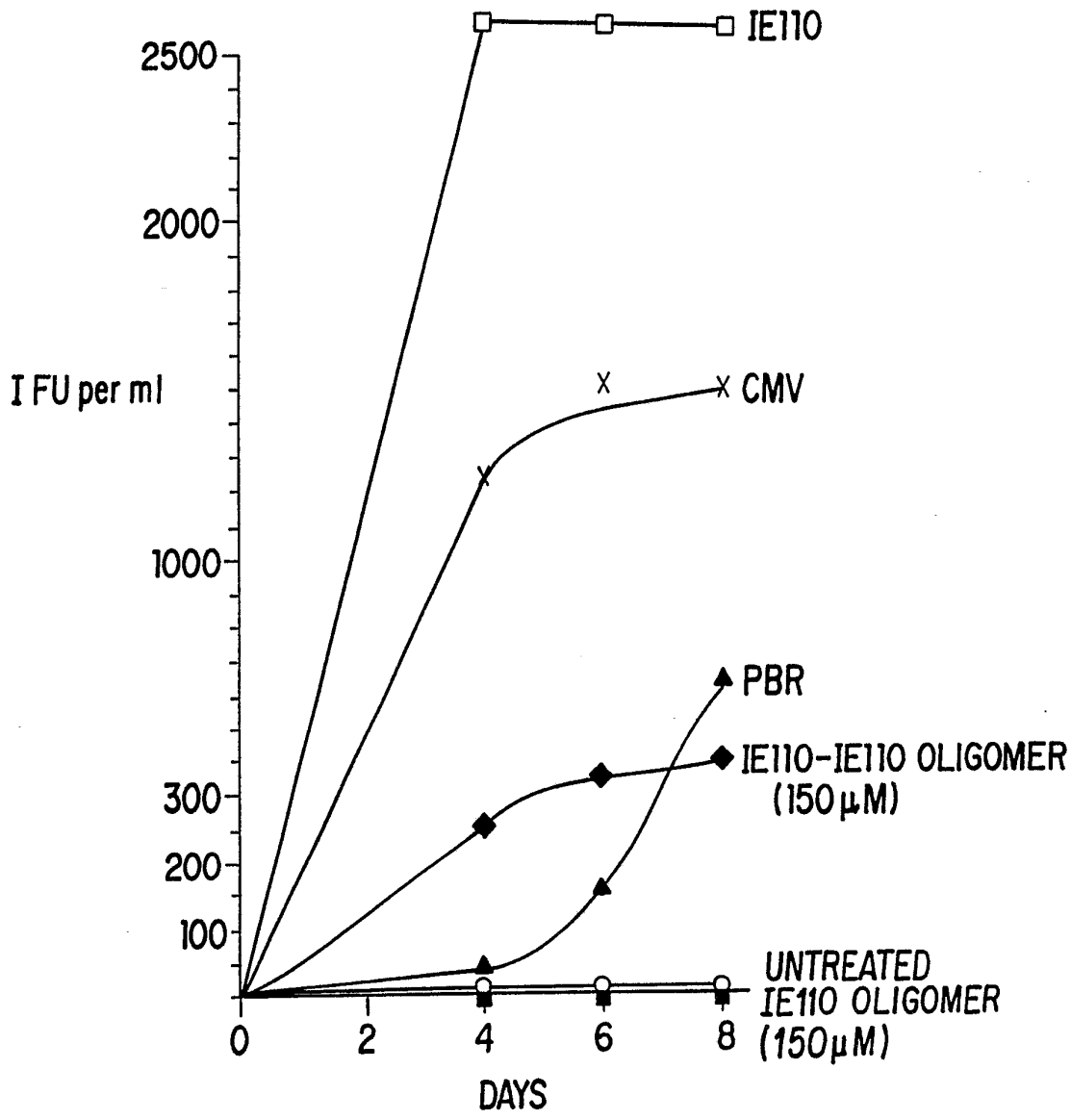
IE110
2500
2000
I FU per ml
CMV
1000
PBR
IE110-IE110 OLIGOMER
(150μM)
300
200
100
UNTREATED
IE110 OLIGOMER
(150μM)
0
2
4
6
8
DAYS

FIG. 10

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/06646

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12Q 1/68, 1/14; C12N 15/00; C07H 21/00; A61K 48/00

U.S. Cl.: 435/6, 36, 320.1; 536/27; 514/44

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/6, 36, 320.1; 536/27; 514/44

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

APS, Dialog; Biosis

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Proceedings National Academy of Science Volume 83. issued May 1986. C.C. Smith et al," Antiviral effect of an alyol nucleoside methylphosphmate) complementary to the splice junction of herpes simplex virus type 1 immediate early pre-m RPAs 4+5," pages 2787-2791. see entire document.	14-16. 21-28.33-39.45-52. 57-60.67-71,81-84. 95-98.109-112,117. 122-125. 131,136-139.150-153
Y	Cancer Research. Volume 48. issued 15 May 1988. A. Stein et al "Oligodeoxynucleotides as Inhibitors of gene Expression: A review". pages 2659-2668. see entire document.	6-8,17-20. 29-32.41-43.53-56. 58-60.71-74,85-88. 99-102. 113-116. 126-129 140-143. 155-157

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
15 January 1991	31 JAN 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Suzanne Ziska ebw

Form PCT/ISA/210 (second sheet) (Rev.11-87)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . . . , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [X] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See Attached

1. [X] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

[X] No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Proceedings Natural Academy of Science. Volume 85. issued August 1988. Goodchild et al. "Inhibition of human immunodeficiency virus replication by antisense alyodeoxynucleotides", pages 5507-5511. see entire document.	1-13.16-25 28-32.35-37.40-49. 52-56.61-67.70-81. 84-95.99-109.112-122. 125-136.139-150.153-158
Y	Biochimie, volume 67. issued 1985. P.S. Miller et al. "Control of ribonucleic acid function by oligonucleoside methyl phosphonates". pages 769-776. see entire document.	14-16. 21-28. 33-39. 45-52. 57-60. 67-71.81-84,95-98. 109-112. 117,122-125.131. 136-139. 150-153
Y	US. A. 4,806,463 (Goodchild et al) 21 February 1989. see entire document.	61-81. 84-95. 98.103-104 57-60
Y,P	US. A. 5,004,810 (Draper et al) 02 April 1991. see entire document.	1.2.57

Group I, consisting of a first product (an oligonucleotide), claims 1-7 8-20, 33-44, 61-74 and 89-102; and a first method of using the product, consisting of a method of inhibiting herpesvirus growth, claims 21-32, 45-56, 75-88 and 103-116;

Group II, consisting of a second method of use of the first product, a method of detecting herpesvirus, claims 57-60;

Group III, consisting of a third method of use of the first product, a method for inhibiting human immunodeficiency virus growth or replication and that of a second virus, claims 117-130;

Group IV, consisting of a second product, a therapeutic composition for inhibiting HIV and a second virus, claims 131-144;

Group V, consisting of first method of use of the second product, a therapeutic method for inhibiting HIV and a second virus, claims 145-158.

Each grouping of claims forms a separate invention not linked to form a single inventive concept.