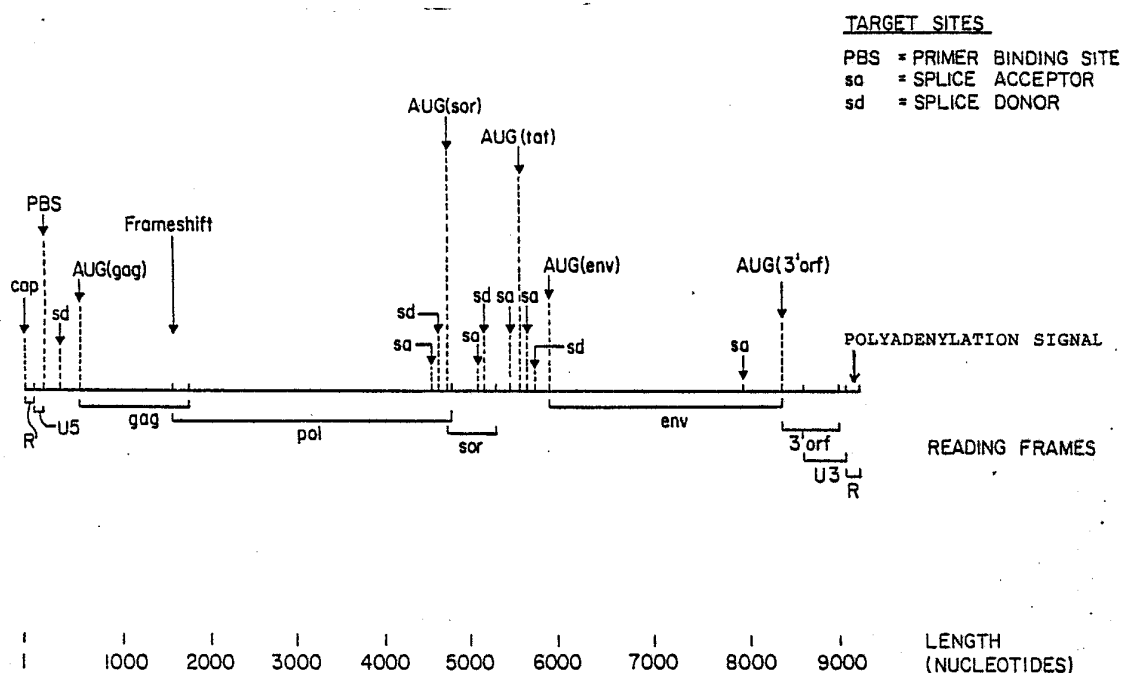




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(54) Title: INHIBITION OF HTLV-III BY EXOGENOUS OLIGONUCLEOTIDES**(57) Abstract**

Inhibition of HTLV-III by administration of an oligonucleotide complementary to highly conserved regions of the HTLV-III genome necessary for HTLV-III replication and/or gene expression is described, as are oligodeoxynucleotide sequences which are complementary to those regions, methods of inhibiting HTLV-III replication and gene expression and methods of determining the presence or absence of HTLV-III virus in samples such as blood, saliva, urine and tears.

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INHIBITION OF HTLV-III BY EXOGENOUS OLIGONUCLEOTIDES

Description

Background

5 The Retroviridae virus family includes viruses
which contain an RNA genome and an RNA-dependent DNA
polymerase activity (reverse transcriptase). During
their growth cycle, retroviridae, or retroviruses as
they are more commonly called, copy their RNA into
10 proviral DNA. Proviral DNA becomes inserted
(integrated) into the chromosomal DNA of the host
where it uses the transcriptional and translational
machinery of the host to express viral RNA and
proteins. Viruses are released from the cell by
15 budding from the cytoplasmic membrane. Most
infections do not kill cells. Rather, infected
cells continue to grow and differentiate while
continuously producing virus.

 Three major classes of human retroviruses have
20 been identified: 1) T-cell lymphotropic viruses; 2)
endogenous genetic elements related to primate
C-type viruses; and 3) foamy viruses.

 Human T-cell leukemia-lymphotropic virus (HTLV)
refers to a family of T cell tropic retroviruses.
25 Such viruses, which have a role in causing certain T
cell neoplasms, are generally divided into three
main types or subgroups: 1) HTLV-type I (HTLV-I),
which appears to cause adult T-cell leukemia-
lymphoma (ATLL); 2) HTLV-type II (HTLV-II), which
30 has been isolated from an individual having a T-cell

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variant of hairy cell leukemia; and 3) HTLV-type III (HTLV-III), which has been identified as the etiologic agent of acquired immune deficiency syndrome (AIDS). HTLV-III is also known as lymphadenopathy-associated virus (LAV), AIDS related virus (ARV) and human immunodeficiency virus (HIV-1). Popovic, M. et al., Science, 224:497-500 (1984); Gallo, R.C. et al., Science, 224:500-503 (1984); Wong-Staal, F. and Gallo, R.C., Nature, 317:395-403 (1985); and Curran, J.W. et al., Science, 229:1352-1357 (1985). More recently, two groups have reported the discovery of what appear to be two different HTLV-type viruses. HTLV-IV has been identified by Kanki and Essex and co-workers in individuals in several Western African countries and HIV-2 by Montagnier in patients who have AIDS but show no signs of HIV-1 infection. Science, 236:390-392 (1987).

AIDS was first recognized in 1981 and since that time, the disease has come to be recognized as a new epidemic. RNA Tumor Viruses (2d edition), Volume 2, pp 437-443, Cold Spring Harbor Laboratory (1985).

Patients with AIDS exhibit clinical manifestations which include severe immunodeficiency which generally involves a depletion of helper T lymphocytes; malignancies; and opportunistic infections. The disease at this time is incurable and the mortality rate among AIDS patients is high.

Because the disease has severe, generally life threatening effects, there is great interest in finding means of protecting the population from it and of treating those who contract it. At the

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present time, much effort is being put into developing methods of detecting the presence of HTLV-III (HIV-1) in body tissues and fluids (e.g., blood, saliva) and into developing vaccines which will
5 protect recipients from HTLV-III. However, there is no known method which is satisfactory, either for preventing the disease or for treating those who become infected with the virus. In fact, current efforts to develop a broad spectrum anti-HTLV-III
10 vaccine may be seriously compromised, in light of the variation in envelope proteins (which are the principal antigenic determinants of the virus) observed among various strains of HTLV-III. Hahn, G.H. et al., Proceedings of the National Academy of
15 Sciences, USA, 82:4813-4817 (1985); Benn, S. et al., Sciences, 230:949-951 (1985). Other methods of blocking the effects of the virus are clearly needed.

Summary of the Invention

20 This invention relates to exogenous oligonucleotides which are complementary to regions of the HTLV-III genome and inhibit HTLV-III replication or gene expression; methods of inhibiting HTLV-III replication and HTLV-III gene expression in cultured
25 human cells; methods of detecting the presence of HTLV-III in biological samples; and methods of administering the oligonucleotides to individuals for the purpose of inhibiting HTLV-III replication or gene expression (hybridization arrest).

30 The oligonucleotides of this invention, which can be oligodeoxyribonucleotides or

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oligoribonucleotides, are complementary to regions on the HTLV-III genome which are highly conserved, and whose function is necessary for normal replication or gene expression by HTLV-III. The
5 oligonucleotides can be used to block HTLV-III replication, gene expression or both and thus can be used as chemotherapeutic agents in inhibiting replication and gene expression by the virus. In addition, they can be used to detect the presence of
10 HTLV-III in samples such as blood, urine and saliva.

Oligonucleotides of the present invention are complementary to target sites which are highly conserved regions of the HTLV-III genome. These include the cap site; the primer binding site;
15 nucleotide sequences vicinal to the primer binding site in the 5' direction; mRNA donor splice and acceptor splice sites; the HTLV-III initiator codons, including those for the gag, the sor, the tat, the env, and the 3'ORF sequences; the art gene
20 or a portion thereof; polyadenylation signal; and the region of the genome responsible for the frameshift known to occur during transcription. These oligodeoxynucleotides can be used to inhibit HTLV-III replication and/or gene expression in the
25 HTLV-III infected cells. They can be administered to individuals to block HTLV-III replication and/or gene expression as a means of chemotherapeutic treatment of acquired immune deficiency syndrome (AIDS) and of AIDS related complex (ARC). The
30 method of the present invention is referred to as hybridization arrest.

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Use of such oligonucleotides has at least two important advantages. First, the antiviral effects observed are very specific. For example, a specific sequence of 20 nucleotides would not be expected to occur at random more often than about one time in 10^{12} . There are about 4×10^9 nucleotide pairs in the human genome and thus, the specificity of a 20-nucleotide sequence chosen from a conserved region of HTLV-III is predicted to be great. Second, the cellular toxicity of the oligonucleotides is also low, in comparison with most nucleoside analogues (e.g., those used in cancer chemotherapy, graft-host immunology and viral inhibition); such analogues are converted into nucleotides, which are subsequently incorporated into cellular DNA.

Oligonucleotides complementary to the same regions of the HTLV-III genome can be used to determine whether HTLV-III is present or absent in a sample such as blood, saliva or urine by determining whether cell death occurs in cells which are normally killed by HTLV-III virus (such as T lymphocytes) when they are cultured with the sample to be tested and whether cell death can be inhibited by the oligonucleotide.

This same approach can also be used to inhibit replication of other retroviruses and other viruses (i.e., viruses whose genomic material is DNA) and, thus, gene expression in host cells. When used to inhibit other retroviruses, the method of the present invention will use oligonucleotides which are complementary to regions of the retroviral genome whose function is necessary for normal

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replication or gene expression and which are highly conserved will be used. In the case of other viruses, oligonucleotides complementary to viral RNA or viral mRNA are used.

5 The method of the present invention can also be used to inhibit the activity of other (non-viral) infectious agents, such as bacteria, fungi, protozoa and worms. In these applications, oligonucleotides complementary to RNA of the infections which is
10 essential to the viability of the infectious agent are used.

Brief Description of the Figure

 The Figure is a schematic representation of the primary nucleotide sequence of the HTLV-III genome
15 and of the location on the genome of oligonucleotide competitive inhibition targets.

Detailed Description of the Invention

INHIBITION OF HTLV-III

 The primary nucleotide sequence of the
20 HTLV-III/LAV genome has been determined by several groups of investigators. Ratner, L. et al., Nature 313:277-284 (1985); Wain-Hobson, S. et al., Cell 40:9-17 (1985); Sanchez-Pescador, R. et al., Science 227:484-492 (1985); Muesing, M.A. et al., Nature
25 313:450-458 (1985).

 The genome of HTLV-III is shown in the Figure. The HTLV-III genome has been shown to be considerably more variable than the genomes of most retroviruses. RNA Tumor Viruses (2d edition) Volume 2, p

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446, Cold Spring Harbor Laboratory (1985). Like other retroviruses, HTLV-III has in its genome three genes which encode viral proteins: 1) the gag gene, which encodes nucleocapsid or internal structural proteins of the virus; 2) the pol gene, which encodes reverse transcriptase (an RNA-directed DNA polymerase responsible for transcribing RNA into DNA); and 3) the env gene, which encodes the envelope glycoproteins of the virion. In addition, two other open reading frames are known; one (sor) overlaps with the 3' end of the pol gene and the other (3'ORF), located at the extreme 3' end of the genome, slightly overlaps the env gene and continues through most of the U₃ region. The genome has also been shown to contain tat-III and art. Tat-III is the trans-activation gene of HTLV-III; it gene encodes for trans-activator protein, which greatly accelerates viral protein synthesis in infected cells. Art (antirepression of the translation-transactivator gene) has only recently been found in the HTLV-III genome and appears to work cooperatively with tat in producing viral core and envelope proteins.

Other regions of the RNA of HTLV-III are a cap nucleotide, which occurs at the extreme 5' end of the genome; a short sequence (R) which is repeated at both ends of the RNA; a short sequence unique to the 5' end (U₅); and a sequence unique to the 3' end (U₃). Each of the last three components is present twice in viral DNA; each forms part of the long terminal repeat (LTR) sequence found at both ends of the unintegrated linear DNA product of reverse

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transcription. The HTLV-III genome also contains a primer binding site (PBS) adjacent to U₅ (at its 3' end); the PBS is complementary to the 3' end of tRNA lysine and functions as primer for synthesis of the minus strand of viral DNA. Donor splice (S.D.) and acceptor splice (S.A.) sites are also located on the viral RNA. Donor splice sites are sequences at which a 5' portion of the viral genome is joined to a portion of the 3' end of viral RNA, forming a spliced, subgenomic messenger RNA. Acceptor splice sites are sequences at which portions of the 3' end of viral RNA join donor splice sites to form subgenomic messenger RNA.

As mentioned above, different HTLV-III strains have been reported to have variations in envelope proteins. These variations may compromise the development of a broad spectrum anti-HTLV-III vaccine. In contrast, the primary nucleotide sequence of the primer area and certain other parts of the HTLV-III genome are highly conserved.

It has been now shown that complementary oligodeoxynucleotides directed toward such highly conserved regions of the HTLV-III genome inhibit virus replication and/or gene expression in cultured HTLV-III-transformed human lymphocytes.

Targeted Regions of the HTLV-III Genome

As mentioned, several regions of the HTLV-III genome are highly conserved; these regions or parts thereof can be targeted for inhibition by complementary oligonucleotide sequences. These regions, referred to as oligonucleotide competitive inhibition targets, include: 1) the cap site; 2) sequences

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of nucleotides 5' to the primer tRNA^{lys} binding site; 3) the primer binding site or a segment thereof; 4) a combination of sequences 5' to the primer tRNA^{lys} binding site and the primer binding site; 5) sequences of the mRNA donor or acceptor splice sites; 6) the initiator codons for the gag, the sor, the tat, the env and the 3'ORF sequences; 7) the art gene or portions thereof; and 8) the region of the genome responsible for the frameshift known to occur during transcription; polyadenylation signal. The location of these regions (except the art gene) is indicated in the Figure; the art gene is located close to the tat gene.

It has been demonstrated that oligodeoxynucleotides complementary to four of the above mentioned highly conserved regions inhibit virus replication or gene expression in cultured HTLV-III-transformed human lymphocytes. That is, oligodeoxynucleotides complementary to 1) sequences 5' to the primary tRNA^{lys} binding site; 2) the primer binding site; 3) sequences of a mRNA donor splice site; or 4) sequences of a mRNA acceptor splice site have been shown to cause inhibition. In general, any highly conserved region of the HTLV-III genome which encodes information necessary for viral replication or gene expression (e.g., protein synthesis) is a potential target for complementary oligodeoxynucleotides.

Complementary Oligonucleotide Sequences

The oligonucleotide sequences complementary to the competitive inhibition targets can be oligoribonucleotide sequences or oligodeoxyribonucleotide

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sequences. Both types are referred to herein as oligonucleotides and, alternatively, as hybridons. As described here, the oligonucleotides were synthesized on an automated DNA synthesizer. It is possible, however, to produce the desired sequences by using genetically engineered organisms, such as bacteria or viruses.

Oligodeoxynucleotide sequences of varying lengths were used to assess their inhibitory effect on viral replication and gene expression. For example, several nucleotide sequences complementary either to nucleotide sequences of the HTLV-III genome which are 5' to the primer tRNA^{lys} binding site or to nucleotide sequences which straddle the primer binding site and the adjacent region (in the 5' direction) were synthesized and their inhibitory effects tested. As described in greater detail in Example 3, a 12-nucleotide sequence (mer), a 20-nucleotide sequence and a 26-nucleotide sequence have been made and their inhibitory effects on viral replication and gene expression measured. The 12-nucleotide and the 20-nucleotide sequences are complementary to portions of the HTLV-III genome close to the primer binding site, in the 5' direction. The 26-nucleotide sequence is complementary to the primer binding site.

In addition, oligodeoxynucleotide sequences complementary to splice donor or splice acceptor sites of HTLV-III mRNA have been made and their inhibitory effects assessed. In particular, a 20-nucleotide sequence complementary to a splice donor site from the 3'-open reading frame region

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(Figure 1) and two 20-nucleotide sequence complementary to the a-1 and a-1' splice acceptor sites, the former necessary for the production of trans-activating factor, have been synthesized and their
5 inhibitory effects measured.

Viral replication was assayed as reverse transcriptase activity level and gene expression as production of viral proteins p15 and p24. Inhibition of viral replication is reflected in reduced
10 reverse transcriptase activity levels; inhibition of viral gene expression is indicated by reduction in viral protein production. As shown in Table 1, HTLV-III replication and protein expression were inhibited in almost every instance. The greatest
15 inhibitory effect was evident when the 20-nucleotide sequence complementary to the splice acceptor site was tested on cultures of HTLV-III infected cells.

Other complementary oligonucleotide sequences which can be used are determined by the competitive
20 inhibition target(s) selected. Oligonucleotide sequences can be complementary to a single competitive inhibition target or can be complementary to more than one such target. For example, sequences can be produced which are complementary to the
25 HTLV-III primer binding site and the region of the genome immediately adjacent to that site in the 5' direction; to two splice donor sites; to two splice acceptor sites; or to any combination of competitive inhibition targets.

30 Other characteristics of the oligonucleotides used to inhibit viral processes include their length; their modification and the location of

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groups used to modify them. For example, the length of the oligonucleotides to be used will be determined by factors such as the desired specificity of inhibition, size necessary to block viral function, and effect on transmembrane passage. For example, the work described herein has made use of complementary oligodeoxynucleotides ranging in length from 14 to 26 nucleotides. However, there is potentially no limit to the length of the oligonucleotides to be used and length must be determined carefully, in light of the fact it plays a role in viral inhibition. Generally, oligonucleotides used to inhibit HTLV-III will be 8-50 nucleotides in length.

Oligonucleotides to be used can be modified at a variety of locations along their length. For example, they can be modified by the addition of groups at the 5' end, the 3' end or both, as well as on the internal phosphate groups or on the bases. Whether oligonucleotides to be used are modified and, if so, the location of the modification(s) will be determined, for example, by the desired effect on viral activity (e.g., inhibition of viral replication, gene expression or both), uptake into infected cells, inhibition of degradation of the oligonucleotides once they are inside cells, and prevention of their use as a primer by reverse transcriptase. For example, if inhibition of reverse transcriptase activity (and thus of viral replication) is desired, it may be necessary to block the 3' end of a sequence complementary to the primer binding site and/or sequences vicinal to the primer binding site in the 5' direction (for example, by a

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2'3' dideoxynucleotide). In this way, the oligonucleotide complementary to either or both of those regions cannot itself serve as a template for transcriptase activity. If the desired effect is increased uptake of the oligonucleotide into infected cells, modification of the oligonucleotide by addition of a lipophilic group at the 5' end would be beneficial. Modification of oligonucleotides can also be carried out by the addition of an intercalating agent (e.g., acridine dye) at 5' or 3' termini, on bases, or on internucleophosphate groups. Modification in this manner may result in stronger binding between the oligonucleotides and the HTLV-III nucleic acids. Asseline, U. et al., C.R. Acad. Sc. Paris, 369-372 (1983). It would be particularly desirable to permanently modify the target site by attaching to the hybridon chemically reactive groups capable of cross linking, cleaving or otherwise modifying the target site. As shown in Table 1, the 12 nucleotide sequences complementary to the region of the HTLV-III genome 5' to the primer binding site were blocked at the 3' end by ddT. Early work on Rous sarcoma virus inhibition indicates that the 3' end blocked hybridon was a more effective inhibitor than an unblocked hybridon. A hybridon is defined as an oligonucleotide complementary to single-stranded DNA or RNA, which modulates the function of the DNA or RNA by competitive hybridization. Zamecnik, P. and M.L. Stephenson, Proceedings of the National Academy of Sciences, USA, 75:280284 (1978).

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Chain terminator(s) to be used in modifying oligonucleotides for use in inhibiting viral replication and gene expression can be, for example, ddT (as described above and in Example 3), the
5 isourea group, the dimethoxytrityl group, or, in fact, any 3' modified function. Selection of the chain terminator is based, for example, on the absence of a 3' OH group (which can act as a substrate for reverse transcriptase); lack of or low
10 cellular toxicity; lipophilicity; and lack of impairment of hydrogen bonding properties of the oligonucleotide.

It has now been demonstrated that 3' or 5' exonucleases, such as phosphodiesterases from snake
15 venom or spleen, can progress past a single methylphosphonate internucleoside linkage. It has also been shown that two such linkages in succession constitute a strong block to the enzyme's activity and can increase the half life of an oligomer in the
20 presence of such an enzyme by 100 fold. Good protection from destruction of an oligodeoxynucleotide by exonucleases can be afforded by using methylphosphonate linkages at the last two positions at each end of the molecule. This
25 enhances the survival of the compound in vivo.

Another modification known to inhibit nuclease digestion is the replacement of internucleoside phosphate by thiophosphate. Matzura, H. and F. Eckstein, European Journal of Biochemistry, 3:448
30 (1968). Thus, some or all of the phosphates in an oligonucleotide sequence can be replaced by thiophosphate to suppress nucleolytic degradation.

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INHIBITION OF HTLV-III-INFECTED CELLS

Using the oligodeoxynucleotide sequences described above and in Example 3, it was possible to inhibit HTLV-III replication and gene expression in HTLV-III-infected cells in tissue culture. The oligodeoxynucleotides described were added to peripheral human blood cells (PB) infected with HTLV-III and to transformed T-lymphocyte (H9) cells infected with HTLV-III. The oligodeoxynucleotide was usually added at time zero only and observation of inhibitory effects was made at 96 hours. In one case, the oligonucleotide was added to fresh culture medium daily for 3 days. Reverse transcriptase activity and viral p15 and p24 protein production were used as indicators of inhibition of HTLV-III replication and gene expression, respectively. As shown in Table 1 and described in detail in Example 3, inhibition was greatest when a 20-nucleotide sequence complementary to a splice acceptor site was added to HTLV-III-infected transformed T-lymphocytes. Inhibition was observed under essentially all experimental conditions (see Table 1).

Important considerations in this context are the concentration at which the complementary oligodeoxynucleotides are applied and the timing (scheduling) of their administration. As shown in Table 1, the oligodeoxynucleotides were added at concentrations ranging from 5ug/ml. to 50ug/ml. culture medium. These concentrations were generally effective in producing an inhibitory effect but this range is by no means to be considered limiting. As described, the oligodeoxynucleotide was usually

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added at one time only; it seems, however, that daily addition (or more frequent addition) is more effective than a single dose.

INHIBITION OF HTLV-III IN HUMANS

5 Based on the information gained from inhibition of HTLV-III-infected cells in tissue culture, it is possible to formulate a strategy for similar inhibition of HTLV-III in AIDS patients, as well as in individuals carrying the AIDS virus but not manifesting symptoms of the disease.

10 The strategy used in treating a particular individual depends on the status of the individual and the objective of the treatment. That is, an individual who has been found to be carrying the HTLV-III virus but shows no symptoms of AIDS might be treated differently, in terms of both the type of oligonucleotide(s) administered and the dose given, than an individual who does, in fact, have AIDS. In addition, treatment might well differ if its objective is to protect uninfected cells or to have an effect on cells which are already infected.

20 For example, an individual known to be harboring the virus but yet manifesting no sign of AIDS could be given a long-term or lifetime maintenance dose of oligonucleotides whose inhibitory effects stop reverse transcription (e.g., oligonucleotides complementary to the primer binding site and/or sequences close to the primer binding site in the 5' direction). In this way, the first step in viral life or replication is inhibited because viral DNA cannot be made and the virus is unable to

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proliferate. However, in an AIDS patient, cells are already infected and treatment must inhibit expression of genes (viral DNA) already present in the infected cells. In this case, oligonucleotides complementary to, for example, initiator codons for genes encoding viral proteins, are required to prevent viral construction. In an AIDS patient, uninfected cells can also be protected by administration of oligonucleotides capable of blocking reverse transcription.

In any treatment situation, however, oligonucleotides must be administered to individuals in a manner capable of getting the oligonucleotides initially into the blood stream and subsequently into cells. As a result, the oligonucleotides can have the desired effects: getting into HTLV-III infected cells to slow down or prevent viral replication and/or into as yet uninfected cells to provide protection.

Oligonucleotides whose presence in cells can stop reverse transcription and oligonucleotides whose presence in cells can inhibit protein synthesis can be administered by intravenous injection, intravenous drip or orally. The dose to be administered varies with such factors as the size and age of the patient, stage of the disease and the type of oligonucleotide to be given.

Detection of the HTLV-III Virus in Samples

The oligonucleotide sequences of the present invention can also be used in determining whether the HTLV-III virus is present or absent in samples such as blood, urine, saliva and tears. An aliquot

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of the sample to be analyzed is added to a culture of cells which are normally killed by the HTLV-III virus (e.g., T lymphocytes); this is the control. A second aliquot is added to a separate culture of T lymphocytes, along with oligonucleotides complementary to one or more of the regions of the HTLV-III genome describe above; this is the test sample. Both cultures are maintained under conditions appropriate for growth and subsequently analyzed (e.g., visually/microscopically) for growth of the T lymphocytes. If the HTLV-III virus is present, the T lymphocytes in the control sample will be killed; if not, the T lymphocytes survive. T lymphocytes in the test sample, however, will continue to be viable because of the protection provided by the complementary oligonucleotides included in the culture. Visual comparison of the two samples makes it possible to determine whether HTLV-III virus is present or absent in each.

20 INHIBITION OF RETROVIRUSES OTHER THAN HTLV-III

As explained above, it is also possible to use oligonucleotides and methods of the present invention to inhibit replication of retroviruses other than HTLV-III. In these applications, oligonucleotide sequences complementary to regions of the retroviral genome whose function is necessary for normal retroviral replication or gene expression and which are highly conserved will be used.

For example, other retroviruses which cause disease in humans are HTLV-I and HTLV-II (which cause leukemia) and HIV-2 (which causes AIDS).

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Oligomers complementary to regions of HTLV-I RNA have been made and shown to inhibit viral replication through the use of assays similar to those described for HTLV-III (Example 3). The

5 sequences used for this work are the following:

1. 5'-AGA AGG CGA AAC AGC AT AGT
2. 5'-GGG CTG ATA ATA AGC ATG GT
3. 5'-GCC GAT AAC GCG TCC ATC GA
4. 5'-GGG GAG TAT TTG CGC ATG GC
- 10 5. 5'-ACT GTG TAC TAA ATT
6. 5'-CCC CAA CTG TGT ACT

Oligonucleotides 1-4 are complementary to putative protein initiation sites in the so called 'X' region near the 3' end of HTLV-I.

- 15 Oligonucleotides 5 and 6 are complementary to the primer binding site region. (See Seiki, M., et al., Proceedings of the National Academy of Sciences, USA, 80:3618 (1983) for HTLV-I sequence).

All of these were shown to be inhibitory to
20 HTLVI. It is also likely that splice sites, the primer binding site, the cap site, the polyadenylation signal, packaging signal and protein sites would be targets for hybridization arrest.

HIV-2 has been shown to be similar in overall
25 structure and biological effect to HIV-1, although there are considerable differences in nucleotide sequence (M. Guyader, et al., Nature, 326:662 (1987)). Analogous target sites for the action of oligonucleotides exist in HIV-2 as occur in HIV-1.
30 However, it appears that only the primer binding site region is sufficiently similar in sequence in

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both HIV-1 and HIV-2 for a single (common) oligonucleotide to be effective in inhibiting replication and gene expression of both viruses.

INHIBITION OF OTHER VIRUSES

5 The oligonucleotides and methods of the present invention can be used to inhibit the activity of any virus pathogenic to humans, animals or plants. As described above for inhibition of retroviruses, oligonucleotides complementary to viral RNA or viral
10 mRNA for both DNA and RNA viruses would be used to inhibit viral replication and gene expression. It is also possible that in some cases, viral DNA will be the target of complementary oligonucleotides. Particularly useful target sites on the RNA are
15 those which serve as binding or recognition sites for proteins (e.g., splice sites, protein initiation sites, polyadenylation signals, capped ends of mRNA, packaging signals, prime binding sites). In those instances in which the virus infects cells with a
20 high ribonuclease H concentration, any sequence which is present within the viral RNA or mRNA and is not found in host RNA would be an acceptable site. Binding of the oligonucleotide to that site would result in enzymatic destruction of the hybridized
25 RNA and hence inactivation of the virus.

Examples of target sites in other viruses are as follows:

1. Foot and Mouth Disease Virus

1. 5'-CGT GAA TTC CTA CTT TCC TG.
- 30 2. 5'-ACC CTA GCG CCC CCT TTC AA.

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The sequence of oligonucleotide 1 is complementary to the only known protein initiation site used by this virus. Robertson, B.H., et al., Journal of Virology, 54:651 (1985). The sequence of oligonucleotide 2 is complementary to the capped end of the virus. Harris, T.J.R., et al., Journal of Virology, 36:659 (1980).

2. Yellow Fever Virus

1. 5'-CGA CCA GAC ATG TTC TGG TC.

2. 5'-ATT AGC ACA CAG GAT TTA CT.

The sequence of oligonucleotide 1 is complementary to the protein initiation site and that of oligonucleotide 2 is complementary to the capped end. Rice, C.M., et al., Science, 229:726 (1985).

3. Varicella-Zoster Virus

Varicella-Zoster virus has seventy genes and is replete with target sites, including splice sites. For example, the sequence 5'-CCT AGG CGT TAC AGG TCC CA, which is complementary to the protein initiation site in the first gene, can be used according to the method of the present invention to inhibit viral function. Davison, A.J. and J.E. Scott, Journal of General Virology, 67:1759 (1986).

4. Herpes Simplex Virus Type 1

Like Varicella-Zoster virus, Herpes Simplex Virus Type 1 is an extremely large

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virus which has many target sites. A sequence complementary to the protein initiation site of the major capsid protein is:

5'-GGA GCG GCC ATG GGG TCG CG. Davison, A.J.
5 and J.E. Scott, Journal of General Virology,
67:2279 (1986).

5. Plant Viruses

10 An example of a plant virus to which the hybridization arrest technique can be applied is cucumber mosaic virus. Its associated
CARNA 5 satellite RNA has been sequenced, and this small RNA molecule has been shown to play a key role in inducing lethal necrosis in the plant. Richards, K.E. et al., Virology, 89:395
15 (1978). Sequence variation of this molecule from one strain to another is very limited. Thus, blockade of the function of this constant feature of the virus by hybridon therapy, administered by aerosol or other means, may
20 provide a new form of plant viral chemotherapy.

6. Viroids

These covalently-closed, circular, single strand RNAs of low molecular weight (MW approximately 100,000) are perhaps the smallest
25 known agents of infectious diseases in plants and animals. Diener, T.O., Viroids and Viroid Diseases, Wiley, New York (1979). Splice junctions of viroids with plant small nuclear RNAs, for example, offer an attractive target
30 site for hybridon inhibition. Diener, T.O.,

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Proceedings of the National Academy of Sciences, U.S.A., 78:5014 (1981). For example, aerosol therapy or addition to the soil in the region of plant roots might be therapeutically useful.

5

INHIBITION OF OTHER INFECTIOUS AGENTS

The hybridization arrest method of the present invention is not limited to viruses. Other infectious agents, including bacteria, fungi, protozoa and worms may be inhibited. In these applications of the method, oligonucleotides which are complementary to RNA of the infectious agent which is essential to the viability of the infectious agent is used. The oligonucleotide is directed against the same type of target sites as described above and should not be complementary to host RNA sequences.

15

Examples of oligonucleotides inhibitory to the trypanosome Trypanosoma brucei gambiense (the protozoa responsible for African sleeping sickness) are:

20

1. 5'-TAC CAA TAT AGT ACA GAA AC
2. 5'-ACT GTT CTA ATA ATA GCG TT

Oligomer 1 is complementary to the splice donor site used to attach the mini-exon found at the 5'-end of all mRNA's in the organism. Oligomer 2 is complementary to the 5'-end of the same mini-exon. Campbell, D.A. et al., Nature, 311:350 (1984).

25

A similar situation is found in Leishmania. In this instance, there is considerable sequence

30

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homology between the protozoa (i.e., T. brucei and Leishmania). The oligomers complementary to Leishmania enriettii corresponding to those against T. brucei given above are:

- 5 1. 5'-TAC CAA TAA AGT ACA GAA AC
2. 5'-ACT GAT ACT TAT ATA GCG TT

An oligonucleotide complementary to the initiation site of a protein common in the female genital complex of the trematode Fasciola hepatica,
10 a liver fluke, is 5'-TGA AAC TTC ATT TTT CAG TG.
Zurita, M. et al., Proceedings of the National Academy of Sciences, USA, 84:2340 (1987).

Other target enzymes are chitin synthetase for anti-fungals and alanine racemase for
15 antibacterials.

USE OF HYBRIDONS FOR THE CONTROL OF NON-INFECTIOUS CONDITIONS

Hybridons may be of benefit in any situation where it is desirable to reduce the level or
20 activity of a given protein or enzyme. This is achieved using oligonucleotides complementary to unique sites on the mRNA for the protein or enzyme and using the same criteria for those sites described previously. Examples where this approach
25 will be beneficial are given below.

Contraceptives

Oligodeoxynucleotides that inhibit the synthesis of structural proteins or enzymes involved largely or exclusively in spermatogenesis, sperm

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mobility, the binding of the sperm to the egg or any other step affecting sperm viability may be used as contraceptives for men. Similarly, contraceptives for women may be oligodeoxynucleotides that inhibit
5 proteins or enzymes involved in ovulation, fertilization, implantation or in the biosynthesis of hormones involved in those processes.

Cardiovascular Disorders

Hypertension can be controlled by oligodeoxy-
10 nucleotides that suppress the synthesis of angiotensin converting enzyme or related enzymes in the renin/angiotensin system; platelet aggregation can be controlled by suppression of the synthesis of enzymes necessary for the synthesis of thromboxane
15 A₂ for use in myocardial and cerebral circulatory disorders, infarcts, arteriosclerosis, embolism and thrombosis; deposition of cholesterol in arterial wall can be inhibited by suppression of the synthesis of fattyacyl co-enzyme A: cholesterol acyl
20 transferase in arteriosclerosis; inhibition of the synthesis of choline phosphotransferase may be useful in hypolipidemia.

Neural Disorders

There are numerous neural disorders in which
25 hybridization arrest can be used to reduce or eliminate adverse effects of the disorder. For example, suppression of the synthesis of monoamine oxidase can be used in Parkinson's disease; suppression of catechol o-methyl transferase can be
30 used to treat depression; and suppression of indole

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N-methyl transferase can be used in treating schizophrenia.

Prostaglandins

- Suppression of selected enzymes in the
- 5 arachidonic acid cascade which leads to prostaglandins and leukotrienes may be useful in the control of platelet aggregation, allergy, inflammation, pain and asthma.

Cancer

- 10 Suppression of the protein expressed by the multidrug resistance (mdr) gene, which is responsible for development of resistance to a variety of anti-cancer drugs and is a major impediment in chemotherapy may prove to be
- 15 beneficial in the treatment of cancer. A sequence complementary to the protein initiation site in the closely-related mouse protein is 5'-CCC AGA ATC ATG CAC AGC TT. Gros, P. et al., Cell, 47:371 (1986). An oligonucleotide having this sequence or a
- 20 sequence complementary to the human mdr gene, once elucidated, can be used to inhibit or suppress protein expression.

- Suppression of proteins encoded by oncogenes, using the hybridization arrest method of the present
- 25 invention and oligonucleotides of the proper sequence, may also prove useful.

Others

There are other, varied disease states in which use of hybridization arrest may prove valuable. For

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example, suppression of the synthesis of aldose reductase may be of benefit in preventing complications in diabetes; suppression of glycolic acid oxidase may be of benefit to prevent

5 crystallization of calcium oxalate (renal lithiation, primary hyperoxalurias, kidney stones); suppression of carbonic anhydrase may be useful in glaucoma, and suppression of hemoglobin synthesis in polycythemia vera might be therapeutically helpful.

10 The present invention will now be further illustrated by the following examples, which are not intended to be limiting in any way.

EXAMPLE 1 Synthesis and Characterization of
Oligodeoxynucleotides

15 Unmodified oligodeoxynucleotides were synthesized on an automated DNA synthesizer (Biosearch SAM I), using either standard triester or phosphoramidite chemistry. Gait, M.J. (Ed.), Oligonucleotide Synthesis, I.R.L. Press (1984). After deblocking,

20 the products were purified first on Merck silica gel 60 thin layer chromatographic plates in *i*-propanol: concentrated ammonia:water (55:35:10) and eluted with ethanol:water (1:3). Where necessary, further purification was performed by high pressure liquid

25 chromatography, using a Waters SAX Radial-PAK cartridge or by polyacrylamide gel electrophoresis (PAGE). The synthetic, preparative and analytical procedures have been described in detail. See Gait, M.J., above. The oligonucleotide with terminal

30 3'-deoxythymidine (ddT) was made by the solution phase triester method. This method is described in

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detail by Narang, S.A. et al., In: Methods in Enzymology, L. Grossman and K. Moldave (Ed.), 65:610-620, Academic Press (1980), the teachings of which are incorporated herein by reference. ddT (Sigma) was used directly in the coupling reaction without protecting groups. The final product was purified first on 2mm thick silica gel plates (Analtech) as above and subsequently by column chromatography on DEAE cellulose in a gradient of 0.02-0.8M triethylammonium bicarbonate.

Oligonucleotides were 5'-end-labeled by T₄ polynucleotide kinase, purified by polyacrylamide gel electrophoresis (PAGE) and sequenced by either the Maxam-Gilbert or wandering spot methods. Maxam, A.M. and W. Gilbert, In: Methods in Enzymology, L. Grossman and K. Moldave (ed.) pp 499-560, Academic Press (1980); Jay, E. et al., Nucleic Acids Research, 1:331-353 (1974). For Maxam-Gilbert sequencing of fragments of this size, it was found necessary to increase reaction times up to 30 minutes at 37°. The presence of ddT at the 3' end of oligodeoxynucleotide did not seem to hinder the action of the exonuclease snake venom phosphodiesterase.

25 EXAMPLE 2 Oligodeoxynucleotide uptake studies

HeLa cells were grown in suspension culture, concentrated by centrifugation at 600 x g for 5 min. and resuspended at a concentration of 5×10^7 to 5×10^8 cells/ml of Dulbecco's modified Eagle's medium (DME) without serum and kept on ice. Synthetic oligodeoxynucleotides to be tested (10-30

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nucleotides in length), were labeled with ^{32}P at the 5'-end by polynucleotide kinase at 2×10^5 cpm/nmol, dissolved in DME without serum, and added to the HeLa cell suspension (40 μl oligodeoxynucleotide solution to 0.7 ml ice cold HeLa cell suspension.). Alternatively, to generate an internally labelled oligonucleotide, two decamers, one of them $5'^{32}\text{P}$ labelled, were joined by T4 DNA ligase in the presence of an oligodeoxynucleotide (12 nucleotides long) part of which was complementary to the 5' end of one of the decamers and part of which was complementary to the 3' end of the other decamer. The concentration of labelled oligodeoxynucleotide in the HeLa cell suspension was usually 1×10^{-5} to 1×10^{-7} M. Cells were incubated under sterile conditions at 37° for up to 20 hours. Samples were cooled at 0° , diluted to 10 ml with DME, and centrifuged lightly to pellet the cells. The supernatant fluid was poured off and saved and the centrifugation tube drained on filter paper. The cell pellets were then washed six times, each time in 9 ml of ice-cold DME. The supernatants were saved and monitored for ^{32}P radioactivity. By the sixth wash, virtually no radioactivity was detected in the wash fluid. The cell pellets were then resuspended on 0.7 ml of ice cold DME and transferred to an electroporation cell. Electroporation was carried out at 0° by a variation of the technique described by Potter and co-workers in Potter et al., Proceedings of the National Academy of Sciences, USA, 81:7161-7165 (1984), the teachings of which are incorporated herein by reference. During

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electroporation, a short high voltage pulse was applied across the electroporation cuvette containing the cell pellets; in this way, the cell membranes were made temporarily leaky or porous, allowing oligonucleotides to pass out of the cells. The electroporation cuvette was kept in an ice bath for 15 min. following electroporation. The contents were transferred to a 1.5 ml Eppendorf microfuge tube, and centrifuged 5 min. at 12,000 x g. The supernatant solution (i.e., oligomer which has entered the cell) was removed, and radioactivity of both the supernatant and the pellet (which contained the nuclear and cell membrane component) was determined by scintillation counting.

Two other variants of this method were also used to determine whether externally added labeled oligodeoxynucleotides enter CEF and HeLa cells. In the case of CEF cells, which had been grown in monolayers in 75cm² Falcon flasks, the DME medium containing serum was removed, the cells were washed once with serum-lacking DME; 2 ml of DME containing ³²P-labeled oligodeoxynucleotide were added; and the resulting combination was incubated at 37°C for 15 minutes. The cells were next washed six times at 37°C (ambient), each time with 10 ml of DME. 2 ml of 1N formic acid was then added, and the cells were kept on ice for 15 min. The same procedure was carried out with CEF or HeLa cells except that instead of 1N formic acid, distilled water was added after incubation to lyse the cells. Results were similar with both procedures; approximately half as much radioactivity was associated with the nuclear

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and cell membrane fraction (sedimented by centrifugation 5 min. at 12,000 x g) as was associated with the non-sedimenting fraction of the cell.

The possibility that treatment of labeled cells with either 1N formic acid or distilled water caused dissociation of radiolabeled oligomer (which had never entered the interior of the cell) from the cell membrane fraction was tested by using the above modified electroporation technique as described.

10 Results using electroporation agree with those where cells were ruptured by hypotonicity or 1N formic acid.

These tests made it possible to assess uptake of ^{32}P -oligonucleotides by the cultured cells described. Inhibition of viral replication by exogenous oligodeoxynucleotides depends upon their uptake in sufficient amounts by the cells; this is not the case when endogenously transcribed or microinjected anti-sense RNAs are used. The permeability of cultured mammalian cells to the oligodeoxynucleotides has been demonstrated by these tests to be as follows:

1) Under the experimental methods described, cellular uptake of 20-nucleotide sequences, labeled with ^{32}P either internally or terminally, increased during the initial few hours of incubation. At an external concentration of $1 \times 10^{-7}\text{M}$, after 4 hours of incubation at 37°C , the internally labeled 20-nucleotide sequence TAGTCTCAAT- ^{32}P -GGGCTGATAA reached a concentration inside the HeLa cell of approximately $2 \times 10^{-9}\text{M}$. In another experiment conducted using the same conditions described, at an

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external concentration of $2 \times 10^{-5} \text{M}$, after 15 minutes of incubation at 37°C , the internally labelled 20-nucleotide sequence TAGTCTCAAT- ^{32}P -GGGCTGATAA reached an apparent concentration inside the CEF
5 cell of about $1.5 \times 10^{-6} \text{M}$.

2) At 15 min. and 4 hour time periods labeled oligodeoxynucleotides released from the cells by electroporation were largely intact, as judged both by migration on thin layer DEAE plates, in Homo V
10 Jay, E. et al., Nucleic Acids Research, 1:331-353 (1974), and by PAGE using oligodeoxynucleotide markers. However, degradation of oligodeoxynucleotides increased with incubation time. By 20 hours, a large fraction of oligodeoxynucleotide was de-
15 graded intracellularly and extracellularly, but undergraded oligomer was still detected, and thus endured long enough to have the desired inhibiting effect.

3) Terminally labeled oligodeoxynucleotides dis-
20 appeared more rapidly than those labeled internally. This indicates that phosphomonoesterase activity is more rapid than endonuclease activity.

EXAMPLE 3 Inhibition of HTLV-III replication by
complementary oligodeoxynucleotides

25 The primary nucleotide sequence of the HTLV-III/LAV genome has been determined during the past year by several groups of investigators, as indicated above. The following regions of the genome were selected as oligonucleotide competitive

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inhibition targets: a) a sequence of nucleotides 5' to the primer tRNA^{lys} binding (association) site; b) a sequence straddling the primer binding site and the adjacent region, in the 5' direction; c) a
5 sequence at the primer binding site and d) sequences from the splice sites (i.e., splice donor site, splice acceptor site) of the pre-mRNA that expresses the 3'-open reading frame regions. Sodroski, J. et al., Journal of Virology, 55:831-835 (1985); Wong-
10 Staal, F. and Gallo, R.C., Nature, 317:395-403 (1985). Their locations on the HTLV-III/LAV genome are indicated in Figure 1.

A) Sequences complementary to the primer binding site and sequences vicinal to the primer
15 binding site in a 5' direction.

Several sequences complementary to regions immediately adjacent, in a 5'-direction, to the tRNA^{lys} primer binding site in HTLV-III, or complementary to the primer binding site were synthesized.
20 These are a 12-nucleotide sequence (5'-CTGCTAGAGATddT) a 20-nucleotide sequence (5'-CTGCTAGAGATTTTCCACAC), and a 26-nucleotide sequence with a 3' terminal non-complementary tail of (pA)₃ (5'-TTCAAGTCCCTGTTCTGGGCGCCAAAA). As shown
25 in Table 1, the 12-nucleotide sequence is complementary to a sequence of nucleotides close to the primer binding site (in the 5' direction); the 20-nucleotide sequence is also complementary to a sequence close to the primer binding site in the 5'
30 direction, and includes the first 11 nucleotides of the 12-nucleotide sequence, as well as nine additional nucleotides. The 26-nucleotide sequence is

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complementary to the primer binding site. These oligodeoxynucleotides were tested on tissue cultures of HTLV-III-infected cells; they were added to the cultures at the concentrations shown in Table 1
5 (column 3). Both reverse transcriptase activity and production of viral-encoded p15 and p24 proteins were measured to determine inhibition of viral replication and inhibition of gene expression, respectively.

Table I: Inhibition of HTLV-III Replication and Protein Expression by Complementary Oligodeoxynucleotides

Sequence	Oligomer Length	Conc. μ g/ml	HTLV-III Binding Site	Cell Line	HTLV-III Added	Percent Inhibition	
						RT p15	p24
0				H9	-	0	0
0				H9	+	0	0
CCCCAACTGTGTACT	15	5	none	H9	+	0	0
"	"	10	none	H9	+	0	0
CTGCTAGAGATGdT	12	5	5'-vicinal to PBS	PB	+	30	17
"	"	10	"	"	+	36	50
"	"	20	"	"	+	40	35
"	"	5	"	H9	+	10	35
"	"	10	"	"	+	17	50
"	"	10	"	H9	+	0	12
"	"	20	"	"	+	0	38
CTGCTAGAGATTTCCACAC	20	50	"	PB	+	50	50
"	"	10x3	"	H9	+	50	75
"	"	50	"	"	+	23	30
TTCAAGTCCTGTGTC- GGCGGCCAAA	26	50	at PBS [†]	H9	+	80	8
GCGTACTCACCAGTCGCCGC	20	50	splice donor site	H9	+	85	60
CTGCTAGAGATTAA	14	50	5'-vicinal to PBS [§]	H9	+	75	11
ACACGCAATTCGAAAATGG	20	50	splice acceptor site	H9	+	67	88

+ 10 μ g/ml. on days 1,2 and 3 from time of infection

† Has 3 non-complementary bases at 3' end

§ Has 2 non-complementary bases at 3' end

A+ PBS indicates directly competing at primer binding site

PB: peripheral human blood cells

H9: transformed immortalized human T cell line

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Reverse transcriptase activity was measured by the method described by Sarin and co-workers, which is a modification of an earlier method described by Baltimore and Smoller. The modified method is
5 described in Sarin, P.S. et al., Biochemica Biophysica Acta, 470:198-206 (1977) and the earlier method in Baltimore, D. and Smoller, D., Proceedings of the National Academy of Sciences, U.S.A., 68: 1507-1511 (1971); the teachings of both references
10 are incorporated herein by reference.

HTLV-III-protein expression was measured by immunofluorescence using monoclonal antibodies to HTLV-III p15 and p24 as described in Sarin et al., Biochemistry and Pharmacology, 34:4075-4078 (1985),
15 the teachings of which are incorporated herein by reference.

In separate experiments, peripheral human blood cells and transformed T-lymphocyte (H9) cells were infected with HTLV-III; the oligodeoxynucleotides
20 were added just once (at time zero), unless otherwise indicated. Assays for inhibition were carried out at 96 hours.

B. Sequences complementary to splice sites of pre-mRNA

25 A 20-nucleotide sequence complementary to a splice donor site from the 3'-open reading frame region, and a 20-nucleotide sequence complementary to a splice acceptor site were produced. These oligodeoxynucleotides were tested as described in
30 part A (above); their effects were also measured through determination of reverse transcriptase activity and production of viral-encoded proteins.

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C. Results of inhibition tests

The results of testing using the oligodeoxy-nucleotides described in A) and B) of this example are shown in Table 1. The greatest inhibition occurred when an oligonucleotide having the sequence ACACCCAATTCTGAAAATGG, which is complementary to the splice acceptor site in H9 cells, was added at 50 ug/ml (9×10^{-6} M). Percent inhibition as shown in the table is based on comparison with control values obtained for HTLV-III-infected cells incubated without oligodeoxynucleotide. As indicated in Table 1 (columns 7-9), reverse transcriptase activity was inhibited by 67%, p15 protein production by 95% and p27 protein production by 88% when this sequence was used. The oligodeoxynucleotide was given just once (at time zero), and inhibitory effects were observed at 96 hours. Marked inhibition was also found with the other oligodeoxynucleotides, as shown in Table 1.

For example, when the 12-mer sequence complementary to the region of the HTLV-III/LAV genome adjacent, in the 5' direction, to the tRNA^{lys} primer binding site was added to HTLV-III-infected cells at the concentrations shown in Table 1, reverse transcriptase activity was inhibited from 10-17% in H9 cells and 30-40% in PB cells. Viral p15 and p24 protein production was inhibited by 15% and by 35-50%, respectively, in H9 cells; in PB cells, inhibition of p15 protein production ranged from 0-35% and of p24 protein production, from 17-50%. When the 20-nucleotide sequence was used, reverse transcriptase activity was inhibited in H9 cells by

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23-50% and viral protein production by 27-75%. Fifty percent inhibition of all three activities was observed in PB cells as a result of addition of the 20-nucleotide sequence. Based on work on inhibition of Rous sarcoma virus in tissue culture, it seems likely that daily addition of competitor oligodeoxynucleotide is more effective than a single dose at time zero. Zamecnik, P.C. and M.L. Stephenson, Proceedings of the National Academy of Sciences, USA, 75: 280-284 (1978). This is also consistent with time-related intracellular and extracellular degradation of added oligodeoxynucleotide, since measurement of efficacy occurs at 96 hours. Although overall variation in assays of other chemotherapeutic agents for HTLV-III is in the vicinity of ± 5 percent, it is considerably higher where oligodeoxynucleotides are being tested (cf. Table I). This may be related to variable nuclease activity, both intracellular and extracellular, in tissue cultures of H9 and PBS cells. Such an effect would be more marked at lower concentration of oligodeoxynucleotides. Oligodeoxynucleotides blocked at the 3' end by ddT, the isourea group, or other chain terminators may prove to be more effective inhibitors than those described above. For example, work on inhibition of Rous sarcoma virus has shown that the 3' end blocked hybridon was a more effective inhibitor than a hybridon having an unblocked 3' end. This is particularly pertinent to prevention of initiation of replication at loci close to the primer binding site.

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Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific
5 embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

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CLAIMS

1. An oligonucleotide consisting essentially of a nucleotide sequence complementary to highly conserved regions of the HTLV-III genome
5 necessary for HTLV-III replication, a nucleotide sequence complementary to highly conserved regions of the HTLV-III genome necessary for HTLV-III gene expression, a nucleotide sequence complementary to highly conserved regions of
10 the HTLV-III genome necessary for HTLV-III replication and to highly conserved regions of the HTLV-III genome necessary for HTLV-III gene expression or equivalents thereof.
2. An oligonucleotide of Claim 1 which is complementary to highly conserved regions of the
15 HTLV-III genome, said regions being selected from the group consisting of:
 - a) the tRNA^{lys} primer binding site;
 - b) regions of the HTLV-III genome vicinal in
20 the 5' direction to the tRNA^{lys} primer binding site;
 - c) the tRNA^{lys} primer binding site and regions of the HTLV-III genome vicinal in
25 the 5' direction to the tRNA^{lys} primer binding site;
 - d) the mRNA donor splice sites;
 - e) the mRNA acceptor splice sites;
 - f) the initiator codon for the gag gene;
 - g) the initiator codon for the env gene;
 - 30 h) the initiator codon for the tat gene;

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- i) the initiator codon for the sor gene;
 - j) the initiator codon for the 3'orf gene;
 - k) the cap nucleotide of the HTLV-III genome;
 - l) the art gene or portions thereof;
 - 5 m) the region of the HTLV-III genome encoding a frameshift; and
 - n) equivalents thereof.
3. An oligodeoxynucleotide of Claim 2 which is at least 8 nucleotides long.
- 10 4. An oligodeoxynucleotide of Claim 2, said oligodeoxynucleotide being a synthetic oligodeoxynucleotide selected from the group consisting of:
- a) CTGCTAGAGATddT;
 - 15 b) CTGCTAGAGATTTTCCACAC;
 - c) TTCAAGTCCCTGTTCGGGCGCCAAA;
 - d) GCGTACTCACCAGTCGCCGC;
 - e) CTGCTAGAGATTAA;
 - f) ACACCCAATTCTGAAAATGG; and
 - 20 g) equivalents thereof.
5. A method of inhibiting HTLV-III replication, HTLV-III gene expression or both in cells containing HTLV-III comprising introducing into the cells oligodeoxynucleotides which are
- 25 complementary to highly conserved regions of the HTLV-III genome necessary for HTLV-III replication, HTLV-III gene expression, or both, said oligodeoxynucleotides being introduced into the cells in sufficient quantities to

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cause inhibition of HTLV-III replication,
HTLV-III gene expression or both.

6. A method of inhibiting HTLV-III replication,
HTLV-III gene expression or both in cells
5 containing HTLV-III, comprising contacting the
cells with an oligodeoxynucleotide comple-
mentary to one or more regions of the HTLV-III
genome, the regions being selected from the
group consisting of:
- 10 a) the tRNA^{lys} primer binding site;
b) regions of the HTLV-III genome vicinal in
the 5' direction to the tRNA^{lys} primer
binding site;
c) the tRNA^{lys} primer binding site of the
15 HTLV-III genome and regions of the
HTLV-III genome vicinal in the 5' direc-
tion to the tRNA^{lys} primer binding site;
d) the mRNA donor splice sites of the
HTLV-III genome;
20 e) the mRNA acceptor splice sites;
f) the initiator codon for the gag gene;
g) the initiator codon for the env gene;
h) the initiator codon for the tat gene;
i) the initiator codon for the sor gene;
25 j) the initiator codon for the 3'orf gene;
k) the cap nucleotide of the HTLV-III genome;
l) the art gene or portions thereof;
m) the region of the HTLV-III genome encoding
a frameshift; and
30 n) equivalents thereof,

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under conditions appropriate for passage of said oligodeoxynucleotides into the cells in sufficient quantity to inhibit HTLV-III replication, HTLV-III gene expression or both.

- 5 7. A method of Claim 6, in which the oligodeoxy-
nucleotides are selected from the group con-
sisting of:
- a) CTGCTAGAGATddT;
 - b) CTGCTAGAGATTTTCCACAC;
 - 10 c) TTCAAGTCCCTGTTCTGGGCGCCAAA;
 - d) GCGTACTCACCAGTCGCCGC; and
 - e) CTGCTAGAGATTAA;
 - f) ACACCCAATTCTGAAAATGG; and
 - g) equivalents thereof.
- 15 8. A chemotherapeutic method of inhibiting
HTLV-III replication, HTLV-III gene expression
or both in an individual, comprising
administering to said individual an
oligonucleotide complementary to highly
20 conserved regions of the HTLV-III genome
necessary for HTLV-III replication or gene
expression in sufficient concentrations to
inhibit HTLV-III replication, HTLV-III gene
expression, or both.
- 25 9. A chemotherapeutic method of inhibiting
HTLV-III replication, HTLV-III gene expression
or both in an individual, comprising the oral
or intravenous administration of an oligodeoxy-
nucleotide complementary to one or more regions

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of the HTLV-III genome, said regions being selected from the group consisting of:

- a) the tRNA^{lys} primer binding site;
- b) regions of the HTLV-III genome vicinal in the 5' direction to the tRNA^{lys} primer binding site;
- c) the tRNA^{lys} primer binding site and regions of the HTLV-III genome vicinal in the 5' direction to the tRNA^{lys} primer binding site;
- d) the mRNA donor splice sites;
- e) the mRNA acceptor splice sites;
- f) the initiator codon for the gag gene;
- g) the initiator codon for the env gene;
- h) the initiator codon for the tat gene;
- i) the initiator codon for the sor gene;
- j) the initiator codon for the 3'orf gene;
- k) the cap nucleotide;
- l) the art gene or portions thereof;
- m) the region of the HTLV-III genome encoding a frameshift; and
- n) equivalents thereof.

10. A method of Claim 9 in which the oligodeoxy-nucleotide is a synthetic oligonucleotide and is selected from the group consisting of:

- a) CTGCTAGAGATddT;
- b) CTGCTAGAGATTTTCCACAC;
- c) TTCAAGTCCCTGTTCTGGGCGCCAAA;
- d) GCGTACTCACCAGTCGCCGC; and
- e) CTGCTAGAGATTAA;
- f) ACACCCAATTCTGAAAATGG;
- g) equivalents thereof.

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11. A method of detecting the presence of HTLV-III virus in a sample by demonstrating inhibition of replication of said virus in cells which are normally killed by the HTLV-III virus after the
5 cells have been combined with said sample and an oligonucleotide complementary to at least one highly conserved region of the HTLV-III genome necessary for HTLV-III replication and maintained under conditions appropriate for
10 cell growth.
12. A method of detecting the presence of HTLV-III virus in a liquid sample, comprising the steps of:
- a. combining an aliquot of the sample with
15 cells which are normally killed by HTLV-III virus, to produce a control combination;
- b. combining a second aliquot of the sample with cells which are normally killed by
20 the HTLV-III virus and an oligodeoxy-nucleotide complementary to at least one highly conserved region of the HTLV-III genome necessary for HTLV-III replication, to produce a test combination;
- c. maintaining said control combination and said test combination under conditions appropriate for cell growth; and
- 25 d. comparing the growth of cells in said control combination with the growth of
30 cells in said test combination.

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13. A method of Claim 12 in which the cells which are normally killed by HTLV-III virus are T lymphocytes and the oligodeoxynucleotide complementary to at least one highly conserved region of the HTLV-III genome is complementary to at least one region of the HTLV-III genome selected from the group consisting of:
- a) the tRNA^{lys} primer binding site;
 - b) regions of the HTLV-III genome vicinal in the 5' direction to the tRNA^{lys} primer binding site;
 - c) the tRNA^{lys} primer binding site of the HTLV-III genome and regions of the HTLV-III genome vicinal in the 5' direction to the tRNA^{lys} primer binding site;
 - d) the mRNA donor splice sites of the HTLV-III genome;
 - e) the mRNA acceptor splice sites;
 - f) the initiator codon for the gag gene;
 - g) the initiator codon for the env gene;
 - h) the initiator codon for the tat gene;
 - i) the initiator codon for the sor gene;
 - j) the initiator codon for the 3'orf gene;
 - k) the cap nucleotide of the HTLV-III genome;
 - l) the art gene or portions thereof;
 - m) the region of the HTLV-III genome encoding a frameshift; and
 - n) equivalents thereof.

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14. A method of detecting the presence of HTLV-III virus in a liquid sample, comprising the steps of:
- 5 a. combining a first aliquot of the sample with transformed T lymphocyte cells, under conditions appropriate for cell growth;
 - b. combining a second aliquot of the sample with transformed T lymphocyte cells and
10 oligodeoxynucleotides complementary to highly conserved regions of the HTLV-III genome necessary for HTLV-III replication, under conditions appropriate for cell growth;
 - 15 c. maintaining the combination formed in (a) and the combination formed in (b) under conditions appropriate for cell growth; and
 - 20 d. comparing visually the growth of cells in the combination formed in (a) with the growth of cells in the combination formed in (b).
15. A synthetic oligonucleotide for use in inhibiting activity of a virus in a cell, consisting essentially of a nucleotide sequence
25 complementary to a region of viral RNA or viral mRNA necessary for viral replication; a nucleotide sequence complementary to a region of viral RNA or viral mRNA necessary for viral gene expression; a nucleotide sequence
30 complementary to viral RNA or mRNA necessary for viral replication and to viral RNA or mRNA

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necessary for viral gene expression, or equivalents thereof.

16. A synthetic oligonucleotide for use in
inhibiting activity of an infectious agent in a
5 cell, consisting essentially of a nucleotide
sequence complementary to a region of RNA or
mRNA of the infectious agent necessary for
replication of the infectious agent; a
nucleotide sequence complementary to a region
10 of RNA or mRNA of the infectious agent
necessary for expression of genes of the
infectious agent; a nucleotide sequence
complementary to a region of RNA or mRNA of the
infectious agent necessary for replication of
15 the infectious agent and to RNA or mRNA of the
infectious agent necessary for expression of
genes of the infectious agent, or equivalents
thereof.

TARGET SITES

PBS = PRIMER BINDING SITE
sa = SPLICE ACCEPTOR
sd = SPLICE DONOR

