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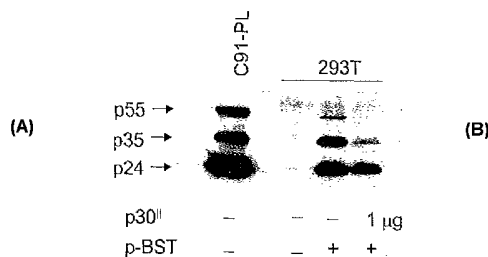
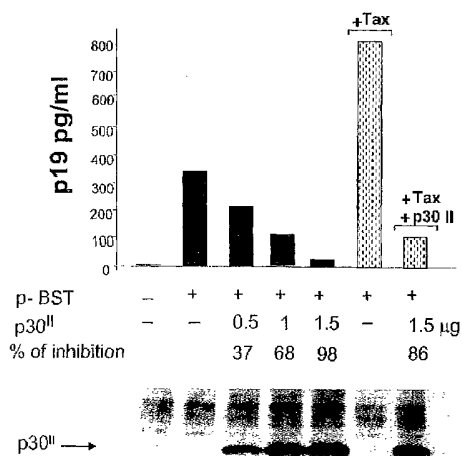
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(54) Title: HTLV-I p30^{II} AND p12^I PROTEINS AS THERAPEUTIC TARGETS IN HTLV-I INFECTED INDIVIDUALS



(57) Abstract: The present invention provides methods for identifying compounds for breaking viral latency in subjects having a retroviral infection (e.g., HTLV-1 or HIV). The present invention also provides methods for treating such patients with retroviral infections.

HTLV-I p30^{II} AND p12^I PROTEINS AS THERAPEUTIC TARGETS IN HTLV-I INFECTED INDIVIDUALS

5 **CROSS-REFERENCE TO RELATED APPLICATION**

This application claims priority to U.S. Provisional Application No. 60/404,580, filed August 19, 2002, which is herein incorporated by reference in its entirety.

ACKNOWLEDGMENTS

10 This invention was made with intramural support from the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

 The present invention generally relates to methods for identifying compounds
15 that can be used to break viral latency in subjects having a retroviral infection (e.g., HTLV-1 or HIV), and methods for treating such patients.

BACKGROUND OF THE INVENTION

 Human T-cell leukemia/lymphoma virus type 1 (HTLV-1) infects and
20 transforms mature human CD4⁺ T-cells *in vitro* and causes adult T-cell leukemia/lymphoma (ATLL) in approximately 2% of infected individuals in a lifetime. The time lag between infection and leukemia development has led to the hypothesis that long-term proliferation of HTLV-1-infected T-cells causes the accumulation of genetic mutations that culminates in overt ATLL. At present, all available ATLL
25 treatments fail to induce long-term remission.

 The ability of HTLV-1 to seize control of T-cell proliferation likely depends on antigen stimulation and cycling of T-cells, since cell division is required for viral-DNA integration. HTLV-1-infected cycling memory T-cells may revert to a resting state and, until the next antigen exposure, the provirus remains dormant. Thus, chronic antigen
30 stimulation may create a vicious cycle that results in transient viral expression and accumulation of genetic mutations in infected T-cells. Indeed, chronic immune-system

stimulation by pathogens has been associated with a higher risk of leukemia development.

The fate of the infected T-cells depends on their ability to evade the host immune response, as well as on control of proliferative and anti-apoptotic signals mediated by viral and cellular proteins. T-cell growth and survival results from
5 balanced integration of the interleukin-2 (IL-2) and the IL-2 receptor (IL-2R) pathways, cell cycle, and anti-apoptotic pathways. Indeed, HTLV-1 has evolved several strategies to arrogate all these pathways.

The stoichiometry and catalytic activity of Tax, the viral transactivator,
10 determines T-cell progression through the G1 phase of the cell cycle and confers proliferative advantage to infected T-cells. The Rex protein, a positive regulator of viral expression, promotes viral production by regulating the transport of genomic and envelope viral mRNAs to the cytoplasm and influences the expression of other cellular genes. Another viral protein, p12¹, appears to function as an adaptor that integrates T-
15 cell activation and proliferative signals while affecting major histocompatibility complex class I (MHC I) trafficking to the cell surface. Thus, expression of viral genes, and particularly of Tax, can result in temporal control of T-cell growth/survival and allow the expansion of a pool of T-cells carrying the provirus.

Because Tax is highly immunogenic in infected cells, HTLV-1 appears to have
20 evolved a dedicated genetic function to reduce the expression of viral proteins (including Tax) and become latent in order to evade host immune surveillance, a strategy commonly used by DNA viruses. In support of this hypothesis, studies in a nonhuman primate model of HTLV-1 infection suggest that viral latency occurs within a few weeks from infection. Furthermore, a discrepancy between the number of
25 provirus-carrying T-cells and the number of viral-RNA-expressing T-cells has been described.

In addition to ATLL, HTLV-1 can also cause inflammatory diseases, such as tropical spastic paraparesis/HTLV-1-associated myelopathy; (TSP/HAM; a progressive demyelinating disease), isolated peripheral polyneuropathy, myopathy, arthropathy, and
30 uveitis, in HTLV-1-infected individuals.

Because viral latency allows HTLV-1-infected cells to evade the immune system, which results in persistence of HTLV-1 infection, and because such infection

can lead to ATLL and other diseases, there is a need in the art for therapeutic strategies that can be used to unmask infected cells to the host immune system and facilitate viral clearance.

5

SUMMARY OF THE INVENTION

Long-term persistence of HTLV-1 infection can lead to leukemia/lymphoma (ATLL) and other diseases. The present invention is based upon the inventors' discovery, as described herein, that p30^{II}, a protein encoded by open reading frame (ORF) II within the 3' end of the viral genome, binds to and retains in the nucleus the mRNA that encodes positive regulators of viral expression (i.e., the Tax and Rex proteins), thereby inhibiting HTLV-1 expression and contributing to viral latency *in vivo*. In addition, p12^I, an ORF I-encoded protein, appears to be up-regulated by p30^{II}. Because p12^I is able to down-regulate major histocompatibility complex class I (MHC I) A, B, and C heavy chains, its expression in concert with the reduction of the expression of Tax, Gag, Pol, and Env induced by p30^{II} likely contributes to escape of the infected cells from immune recognition.

In a first aspect, the invention features a method of identifying a compound for breaking retroviral latency in a subject with a retroviral infection, including: a) contacting a sample with the compound, wherein the sample includes: i. an mRNA comprising an HTLV-1 middle exon sequence between HTLV-1 nucleotides 4641-4831, and ii. p30^{II}; and b) detecting the amount of binding between the mRNA and p30^{II}, whereby a decrease in the amount of binding between the mRNA and p30^{II} in the sample, compared to the amount of binding between the mRNA and p30^{II} in a sample not contacted by the compound, identifies a compound for breaking retroviral latency in a subject with a retroviral infection.

In a second aspect, the invention features a method of identifying a compound for breaking retroviral latency in a subject with a retroviral infection, including: a) contacting a sample with the compound, wherein the sample includes p30^{II}; and b) detecting p30^{II} activity, whereby a decrease in p30^{II} activity in the sample, relative to p30^{II} activity in a sample not contacted with the compound, identifies a compound for breaking retroviral latency in a subject with a retroviral infection.

In one example of the first and second aspects of the invention, the compound can be for breaking HTLV-1 latency.

In another example of the first and second aspects of the invention, the compound can be for breaking HIV latency.

5 In yet another example of the first and second aspects of the invention, the compound can decrease the level or activity of p12^I.

In a third aspect, the invention features a method of treating a patient with a retroviral infection, including administering, to the patient, a compound identified by the method of claim 1 or 2, thereby treating the patient with a retroviral infection.

10 The method of the third aspect of the invention can further include, e.g., administering a reverse transcriptase inhibitor or a retroviral protease inhibitor to the patient.

In a fourth aspect, the invention features a method of treating a patient with a retroviral infection, including: a) vaccinating the patient with a retroviral protein; b) 15 administering a compound that inhibits the activity of a p30^{II}-analogous latency protein to the patient; and c) administering a reverse transcriptase inhibitor or a retroviral protease inhibitor to the patient, thereby treating a patient with retroviral infection.

In various examples of the fourth aspect of the invention, the retroviral structural protein can be, e.g., Gag, Env, Pol, or Tax.

20 In other examples of the fourth aspect of the invention, the retrovirus can be, e.g., HTLV-1 or HIV.

In still other examples of the fourth aspect of the invention, p30^{II}-analogous latency protein can be, e.g., p30^{II} or Vpr.

In yet other examples of the fourth aspect of the invention, the compound is 25 identified by a method as set forth in the first or second aspects of the invention.

Additional advantages of the invention will be set forth in part in the description which follows, and will be apparent from the description, or can be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended 30 claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A-1B: Inhibition of HTLV-1 viral production by p30^{II}.

5 Fig. 1A. The HTLV-1 molecular clone p-BST (5 µg) was cotransfected in 293T cells with increasing amounts of p30^{II}-HA1 (0.5, 1, and 1.5 µg), as demonstrated by Western blot using the αHA1 antibody. Tax (0.25 µg) was also added in the presence or absence of 1.5 µg of p30^{II}. The amount of p19 Gag in the supernatant was measured by ELISA forty-eight hours after transfection. Results are representative of three
10 independent experiments.

 Fig. 1B. Western blot analysis of cell lysates from 293T cells cotransfected with 5 µg of p-BST with or without 1 µg of pMHP30^{II}-HA1.

Fig. 2A-2C: p30^{II} does not affect transcription from the viral LTR. HTLV
15 LTR-*Luc* reporter construct (1 µg) was transfected along with increasing amounts of pMHP30^{II} (0.25, 0.5, 1 µg). Results are average values from two independent experiments and were normalized for transfection efficiencies using values from the thymidine kinase *RL-TK* cotransfection construct (0.02 µg). *Luc* activity was assayed thirty-six hours post-transfection using the dual reporter assay.

20 Fig. 1A. Without Tax.

 Fig. 1B. With Tax (0.5 µg). Tax and p30^{II} expression was confirmed by Western blot.

 Fig. 1C. HTLV LTR-*Luc* vector (1 µg) was coexpressed with the HTLV molecular clone p-BST (5 µg) and increasing amounts of pMHP30^{II} (0.25, 0.5, 1 µg).
25 Normalized results on the *RL-TK* are representative of two independent experiments. p30^{II} expression was demonstrated by Western blot using the αHA1 antibody.

Fig. 3A-3D: p30^{II} is a post-transcriptional regulator of viral expression and affects nucleo-cytoplasmic export of specific viral mRNAs. 293T cells were
30 transfected with p-BST (5 µg) and increasing amounts of pMHP30^{II} (0.5, 1, and 1.5 µg). Tax (0.2 µg) was added in trans to increase sensitivity. However, similar results were obtained in the absence of exogenous Tax.

Fig. 3A. Schematic representation of part of the HTLV-1 transcription and specific primer pairs (LTR2, Gag1/Gag2, Ik1, Rpx4) used in RT-PCR. Gag1: 5'-GCTCCTCCCTCGTGGC-3' (SEQ ID NO: 5). Gag2: 5'-GCCACGAGGGAGGAGC-3' (SEQ ID NO: 6). LTR2: 5'-CCTACCTGAGGCCGCCATCCACGCGGTTG-3' (SEQ ID NO: 7). Ik1 and Rpx4 have been previously reported (Koralnik, I. et al. "Protein isoforms encoded by the pX region of the human T-cell leukemia/lymphotropic virus type I." *Proc. Natl. Acad. Sci. USA* **89**, 8813-8817 (1992)).

Southern blot analysis of splice junctions and GAPDH control amplified from total RNA (Fig. 3A), cytoplasmic RNA (Fig. 3C), and nuclear RNA (Fig. 3D) using the primers LTR2/Rpx4. Identity of amplification products was further confirmed by sequencing.

Fig. 4A-4D: p30^{II} specifically interacts with the *tax/rex* splice junction.

Fig. 4A. PCR products from the splice junctions of the *tax/rex* and *p21^{rex}* RNAs were cloned at the 3' end of the *RL-TK* vector between the stop codon and poly(A) site.

Fig. 4B. 293T cells were transfected with *RL-TK-p21^{rex}* (1 µg) or *RL-TK-tax/rex* (1 µg) in the presence of increasing amounts of pMHP30^{II} (0.25 and 0.5 µg). Results were normalized for transfection efficiencies by coexpression of a CMV-*Luc* vector. *RL* activity was assessed using the dual reporter assay and results are representative of three independent experiments.

Fig. 4C. Biotinylated *tax/rex* and *p21^{rex}* RNA splice junctions were transcribed *in vitro*. The *tax/rex* (lanes 6-8) or *p21^{rex}* mRNA (lanes 3-5) bound to beads was mixed with nuclear extracts of CMT3 cells (lanes 3 and 6) or transfected with pMHP30^{II} (lanes 4-5 and 7-8). Purified complexes were immunoprecipitated and identified by Western blot using anti-HA antibody. Competition with non-biotinylated RNA was performed for specificity (lanes 5 and 8). One-fifth of the input used in binding reactions is shown in lane 2.

Fig. 4D. Top panels: Heterokaryon of CMT3 cells transfected with p30^{II}-GFP or DsRed1 expression vectors. Bottom panels: Heterokaryon of CMT3 cells transfected with Rev-GFP or DsRed1.

Fig. 5A-5C: p30^{II} inhibits viral production from chronic virus-producer human T-cell lines. p30^{II} was cloned into the HRCMV lentiviral vector at BamH1-Xho1 sites (Naldini, L. et al. "In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector." *Science* **272**, 263-267 (1996)). VSV G pseudotype viruses were produced in 293T cells and concentrated by ultracentrifugation, as previously described (Nicot, C., et al. "Bcl-XL is upregulated by HTLV type I and type II in vitro and in ex vivo ATLL samples." *Blood* **96**, 275-281 (2000)). In these conditions, more than 70% of cells are transduced by GFP pseudotypes (Fig. 5C). HTLV-1 producer cell lines HUT102 (Fig. 5A) and MT2 and C91PL (Fig. 5B) were infected with saturating amounts of virus for twelve hours, washed, and cultured in complete media. Viral production was assayed from duplicate experiments thirty-six hours post-infection by ELISA. Results are representative of two independent experiments. Expression of p30^{II} decreased the level of the Tax protein in cell extracts (Fig. 5C) of the HUT102 cell line.

DETAILED DESCRIPTION OF THE INVENTION

HTLV-1 infections persist despite a vigorous virus-specific host immune response. HTLV-1 promotes T-cell growth and can cause ATLL, inflammatory diseases (such as TSP/HAM, isolated peripheral polyneuropathy, myopathy, arthropathy, and uveitis), and other manifestations, in a fraction of HTLV-1-infected individuals. Virion production is positively regulated by the viral proteins Tax, which transcriptionally activates the viral promoter, and Rex, which facilitates the nuclear export of mRNAs for the structural (Gag and Env) and enzymatic (Pol) proteins.

As described herein, the present invention is based upon our discovery that two viral proteins, p30^{II} and p12^I, likely work in concert in allowing the replication of HTLV-1-infected T-cells while avoiding immune recognition in the host. In accordance with the present invention, each of these proteins can be employed as therapeutic targets to "break" HTLV-1 latency and to enhance detection/disposal of HTLV-1-infected cells by the immune system, which in turn will decrease an HTLV-1-infected patient's chance of developing ATLL and/or other manifestations associated with HTLV-1 infection.

As described herein, HTLV-1 has evolved a genetic function to restrict its own expression by a novel post-transcriptional mechanism. Specifically, HTLV-1-encoded p30^{II} is the first viral protein shown to specifically block the transport of viral mRNA to the cytoplasm. p30^{II} is a nuclear-resident protein that binds to and retains in the nucleus the doubly spliced mRNA encoding the Tax and Rex proteins, thereby reducing Tax and Rex protein levels and inhibiting virus expression. Since diseases such as leukemia are associated with long-term HTLV-1 infection, inhibition of p30^{II}-induced latency can be used as a therapeutic strategy to unmask infected cells to the host immune cells and facilitate viral clearance.

Here we demonstrate that the viral protein p30^{II} is a nuclear-resident protein that directly or indirectly binds to and retains the *tax/rex* mRNA in the nucleus. In turn, p30^{II} decreases Tax-mediated viral transcription, by decreasing the level of Tax, and transport of genomic and singly spliced mRNAs, by decreasing the level of Rex.

Virus-encoded proteins able to regulate viral-RNA transport have been described for other viruses (Cullen, B. R. Regulation of HIV-1 gene expression. *FASEB J.* **5**, 2361-2368 (1991)). These viral proteins, including HTLV-1 Rex and HIV-1 Rev, share common nuclear/nucleolar cellular distribution and augment viral expression by increasing viral-mRNA nuclear export. The functional activity of the HTLV-1 p30^{II} protein, which also localizes to the nuclear/nucleolar compartment, is novel in that it negatively regulates viral replication by blocking export of a specific viral mRNA.

Importantly, our discovery defines a novel viral target for the eradication of persistent viruses, such as HTLV-1, that hide so well in the human genome. The present discovery of the interaction of p30^{II} and *tax/rex* mRNA allows the design of inhibitors that can be used to "break" latency *in vivo*. In this scenario, infected T-cells can be revealed more efficiently to and eliminated by the host immune cells. *De novo* infection by the resulting virions can be blocked by available reverse-transcriptase inhibitors. Since the magnitude of clonal expansion of HTLV-1-infected T-cells correlates with disease development, strategies aimed at curtailing the number of infected cells are likely to delay or even eliminate disease development in HTLV-1-infected individuals.

Test Compounds

In general, compounds that are useful for breaking HTLV-1 latency and/or unmasking viral-infected cells to the immune system in accordance with the present invention can be identified from large libraries of natural products or synthetic (or
5 semi-synthetic) extracts or chemical libraries according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such
10 extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-,
15 peptide-, and nucleic acid-based compounds (e.g., but not limited to, antibodies, peptides, and aptamers). Synthetic compound libraries are commercially available, e.g., from Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI).

Alternatively, libraries of natural compounds in the form of bacterial, fungal,
20 plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceanographics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are generated, if desired, according to methods known in the art, e.g., by standard extraction and fractionation methods.
25 Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art of drug discovery and development readily understand that methods for dereplication (e.g., taxonomic dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the
30 elimination of replicates or repeats of materials already known for their effect on the particular target enzyme being studied should be employed whenever possible.

When a crude extract is found to have a desired activity, further fractionation of the positive lead extract is necessary to isolate chemical constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude
5 extract having an activity that breaks HTLV-1 latency and/or unmasks viral-infected cells to the immune system. The same assays described herein for the detection of activities in mixtures of compounds can be used to purify the active component and to test derivatives thereof. Methods of fractionation and purification of such heterogeneous
10 extracts are known in the art. If desired, compounds shown to be useful agents for treatment are chemically modified according to methods known in the art. Compounds identified as being of therapeutic value may be subsequently analyzed using animal models for diseases or conditions in which it is desirable to regulate activity of the target enzyme.

15 Methods of administration

The compounds identified using the screening methods disclosed herein may be administered to subjects with a pharmaceutically acceptable diluent, carrier, or excipient, in unit dosage form. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered
20 to an individual along with a compound of the invention without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the pharmaceutical composition in which it is contained. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such compositions to subjects. Any appropriate route of
25 administration may be employed, for example, but not limited to, intravenous, parenteral, transcutaneous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, intrarectal, intravaginal, aerosol, or oral administration. Therapeutic
30 formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; for intranasal formulations, in the form of powders, nasal drops, or aerosols; for intravaginal

formulations, vaginal creams, suppositories, or pessaries; for transdermal formulations, in the form of creams or distributed onto patches to be applied to the skin.

Methods well known in the art for making formulations are found in, for example, *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for molecules of the invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

Dosage

The compounds of the invention may be administered to a subject in an amount sufficient to break viral latency and/or to enhance detection/disposal of HTLV-1-infected cells by the immune system in a subject in need thereof, i.e., a subject infected with HTLV-1, who may or may not be suffering from a disease or condition associated with HTLV-1 infection (e.g., but not limited to, ATLL, TSP/HAM, isolated peripheral polyneuropathy, myopathy, arthropathy, and uveitis). One of ordinary skill in the art will understand that optimal dosages used will vary according to the individual being treated, the particular compound being used, and the chosen route of administration. The optimal dosage will also vary among individuals on the basis of age, size, weight, gender, and physical condition. Methods for determining optimum dosages are described, for example, in *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995.

The amount of active ingredient that may be combined with a carrier material to produce a single dosage form will vary depending upon the viral load, the individual

being treated, and the particular mode of administration. A therapeutically effective amount may be determined by routine experimentation as will be appreciated by one of ordinary skill in the art. For example, a unit dose of a compound of the invention may preferably contain between 0.001 milligram (mg) and 1 gram of the active ingredient.

5 Typically, for treatment of humans, a compound of the invention would be administered in an amount ranging from approximately 0.001 to 10 mg/kg of body weight, and preferably, from 0.1 to 3 mg/kg would be administered. The compounds may be administered daily one to four times per day, preferably once or twice a day. Therapeutic compounds may also be administered weekly, monthly, or sporadically

10 (for example, after coitus), as is well known in the art.

The following examples are intended to be purely exemplary of the invention and are not intended to limit the scope of the invention, since numerous modifications and variations thereto will be readily apparent to those of ordinary skill in the art.

15

EXAMPLE I

The experiments described herein provide insight into the underlying mechanisms by which HTLV-1 allows cells to evade the immune system, and have identified two proteins encoded by HTLV-1 that play a key role in rendering cells

20 transparent to immune recognition while they undergo cell division with consequent generation of one additional infected cell (carrying the provirus) for each division.

Although the mRNA for the HTLV-1 p12^I and p30^{II} proteins, encoded by the ORF I and ORF II, respectively (Koralnik IJ, et al. Protein isoforms encoded by the pX region of human T-cell leukemia/lymphotropic virus type I. *Proc. Natl. Acad. Sci. USA*,

25 89:8813-8817, 1992), has been found in infected cells, the proteins have not been identified in infected cells, raising skepticism in the field about their existence. We nevertheless have confirmed the existence of these proteins and have found that indeed the p12^I protein is produced by the virus only under conditions of very restricted production of other viral proteins such as Gag, Env, and Tax. In fact, p12^I can be

30 detected only when p30^{II} is expressed. In turn, p30^{II} blocks viral expression through a post-transcriptional mechanism that involves retention in the nucleus of the genomic

RNA as well as some doubly or singly spliced mRNAs (Env, Tat, and Gag) but not the singly spliced mRNA for p12^I, which in turn is also increased by p30^{II}.

Because p12^I is able to down-regulate major histocompatibility complex class I (MHC I) A, B, and C heavy chains, its expression in concert with the reduction of the expression of Tax, Gag, Pol, and Env induced by p30^{II} likely contributes to escape of the infected cells from immune recognition.

The p30^{II} protein is a nucleolar protein (Koralnik et al. The p12^I, p13^{II}, and p30^{II} proteins encoded by human T-cell leukemia/lymphotropic virus type I open reading frames I and II are localized in three different cellular compartments. *J. Virol.* 67:2360-2366, 1993) that has been reported to affect transcription initiated on the HTLV-1 long-terminal repeat (LTR). However, analysis performed in our laboratory did not confirm these findings. In fact, even with an increase of p30^{II}, no change in the amount of luciferase expressed under the control of the HTLV-1 LTR could be observed. Similarly, Tax increased trans-activation of the HTLV-1 LTR-luc construct and was not inhibited by increasing the amount of p30^{II}.

Surprisingly, however, when we assessed the effect of p30^{II} on the Tax protein expressed within the context of a biologically competent (for replication) proviral clone, p30^{II} exerted a significant suppressive effect on the activity of the Tax produced by the provirus on the HTLV-1 LTR-luc reporter gene. This result was tantalizing inasmuch as it suggests that the suppressive effect of p30^{II} was likely to be post-transcriptional. Increasing the amount of p30^{II} resulted in a decrease in viral production (measured as virus-specific-antigen release in the supernatants) of cells transfected with the HTLV-1 molecular clone pBST. This decrease was observed both in the presence and absence of additional Tax.

These finding were further confirmed using two different proviral molecular clones (PCSHTLV). The viral p24 Gag protein is detected in Western blot in the supernatant or in the total cellular lysate. Thus, the suppressive effect of p30^{II} resulted not only in decreased Tax activity but also in suppression of viral replication.

To further prove that the effect observed on viral replication was post-transcriptional and to address the underlying mechanism(s), the alternatively spliced mRNAs encoding viral proteins were studied in detail. Indeed, analysis of total or cytoplasmic RNA revealed that following transfection of the pBST clone with

increasing amounts of p30^{II} no changes in the total amount of RNA for Gag, Env, and pX Tax/Rex occurred. However all these mRNAs were decreased in the cytoplasmic RNA.

In contrast, the p21 Rex mRNA did not change and unexpectedly the pX ORF I mRNA was significantly augmented by the co-expression of p30^{II} in the total RNA. Hybridization and DNA sequencing of the splice junctions confirmed the specificity of the bands. These data suggest that p30^{II} regulates the export of selected mRNA to the cytoplasm and that some may be retained in the nucleus.

A common feature of these mRNAs is the presence of the middle exon located between nucleotides 4641-4831 (i.e. this region is included in the mRNAs for the Gag, Polymerase Envelope, Tax and Rex proteins). Thus, to further investigate the mechanism underlying the p30^{II} biological effect, DNA fragments including the splice junctions of the Env, Tax/Rex and p21Rex (RLTK-Env, RLTK-R4 and RLTK-R2 respectively) were cloned downstream of the Renilla gene expressed from the TK promoter. Co-expression of p30^{II} resulted in a marked decrease of both constructs carrying the exon4631-4831 (RLTK-Env and RLTK4) while no significant reduction was observed with RLTKR2.

These data suggest that likely the p30^{II} binds to RNAs carrying this sequence and interferes with their transport to the cytoplasm. This is supported by the observation that p30^{II} does not shuttle from the nucleolus to the cytoplasm. In fact we have extended this observation to the p30^{II} protein even when it is expressed in the context of the virus. In other proteins that shuttle in and out of the nucleus a consensus sequence has been described that carries four leucines (L) with a precise spacing among them. Indeed mutation of leucine to alanine in the c-Abl proto-oncogene disrupts its shuttling ability. Interestingly, the p30^{II} protein also carries a mutation of that leucine suggesting that this leucine-rich domain is important for p30^{II} function, which is supported by experiments with truncation mutants of p30^{II}.

Lastly, we proved that the increase in the pXORF1 mRNA induced by p30^{II} encoding the p12 protein indeed was associated with an increase in p12 expression

The pBST-HA1 molecular clone in which the p12 open reading frame was modified to express the HA1 tag and this clone was transfected in the absence or the presence of p30^{II}. As a positive control a plasmid encoding only p12 was transfected in

the same cells. Expression of p12 was observed only in the presence of p30^{II}. Because p12 is able to induce T-cell proliferation and at the same time to downmodulate the Major Histocompatibility Complex Class I (MHC I) likely it is in the interest of the virus to decrease viral expression through p30^{II} and increase p12 expression to further
5 avoid immune recognition, by interfering with MHCI antigen presentation.

Collectively these data demonstrate for the first time the expression of p12 from a molecular clone but more importantly they demonstrate that both p12 and p30^{II} are expressed in condition of a decreased expression of the structural genes (Gag, Pol, and Env) and the regulatory Tax and Rex proteins. These data indicate that both p12 and
10 p30^{II} are "latency proteins" that are likely essential for the survival of infected cells in vivo, and provide key information on the mechanism used by the virus to become "latent." As such, rational design of drugs able to interfere with the interaction of p30^{II} with RNA should be able to break latency and allow the immune system to contain the expansion of viral infected cells and prevent the pathogenic effect of the virus.

15 The RNA sequence or RNA structure recognized by HTLV-1 p30^{II} is likely to be similar in other retroviruses, such as Human Immunodeficiency Virus (HIV). Moreover, each such retrovirus is likely to encode a protein that functions analogously to HTLV-1 p30^{II} (a "p30^{II}-analogous latency protein"), i.e., by binding viral RNAs and preventing their export into the cytoplasm, thereby maintaining viral latency. For
20 example, the HIV-encoded protein Vpr is likely to function in HIV latency in a manner analogous to p30^{II} in HTLV-1 latency (i.e., Vpr is a "p30^{II}-analogous latency protein"). Accordingly, compounds that inhibit Vpr activity (e.g., Vpr binding to HIV mRNA) can be used to break viral latency in HIV-infected individuals. It is likely that other oncogenic human viruses also employ such strategies.

25

EXAMPLE II**A) METHODS**

Construction of DNA plasmids. HTLV-1 molecular clones p-BST, pCSHTLV, and p-ACH and the pCMV4 Tax construct have been previously described (Nicot, C. et al.

- 5 “Establishment of HTLV-I-infected cell lines from French, Guianese and West Indian patients and isolation of a proviral clone producing viral particles.” *Virus Research* **30**, 317-334 (1993); Kimata, J. T., ET AL. “Construction and characterization of infectious human T-cell leukemia virus type 1 molecular clones.” *Virology* **204**, 656-664 (1994); Derse, D., et al. “Examining the molecular genetics of HTLV-I with an infectious
10 molecular clone of the virus and permissive cell culture systems.” *J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol.* **12**, 1-5 (1996); Smith, M. R. & Greene, W. C. “Identification of HTLV-I tax trans-activator mutants exhibiting novel transcriptional phenotypes.” *Genes Dev.* **4**, 1875-1885 (1990)).

- p30^{II}* cDNA was PCR-amplified using the primers *p30^{II}-Hind 5’-*
15 *CCCCAAGCTTCCATGGCACTATGCTGTTTCGCC-3’* (SEQ ID NO: 1) and *p30^{II}-Eco 5’-CCGAATTCAGGTTCTCTGGGTGGGGAAGG-3’* (SEQ ID NO: 2) from the pME Tax ORF II plasmid (Koralnik, I. J., et al. “The p12^I, p13^{II}, and p30^{II} proteins encoded by human T-cell leukemia/lymphotropic virus type I open reading frames I and II are localized in three different cellular compartments.” *J. Virol.* **67**, 2360-2366
20 (1993)), digested with *HindIII* and *EcoRI* and cloned at the same sites of the pMH vector (Roche Molecular, Indianapolis, Indiana) that provided the HA epitope in frame at the carboxy terminus of p30^{II}. p30^{II}-GFP (GFP fused at the amino terminus) was constructed by inserting the *HindIII-EcoRI p30^{II}* cDNA fragment into the EGP3N3 vector (Promega, Madison, Wisconsin). Rev-GFP was previously described (Stauber,
25 R., et al. “Analysis of trafficking of Rev and transdominant Rev proteins in living cells using green fluorescent protein fusions: transdominant Rev blocks the export of Rev from the nucleus to the cytoplasm.” *Virology* **213**, 439-449 (1995)).

- DsRed1 vector was purchased from BD Biosciences Clontech, Palo Alto, California. *RL-TK-tax/rex* and *RL-TK-p21^{rex}* reporter constructs were made by insertion
30 of the corresponding cDNA fragment between the Xba1 and Not1 restriction sites in the *RL-TK* vector. cDNAs for *tax/rex* and *p21^{rex}* were PCR-amplified using RNA extracted from 293T cells transfected with p-BST using the primers LTR-Xba, 5’-

AATCTAGACCTACCTGAGGCCGCCATCCACGCGGTTG-3' (SEQ ID NO: 3), and
Rpx-Not, 5'-AAGCGGCCGCAACACGTAGACTGGGTATCC-3' (SEQ ID NO: 4).
HIV lentiviral p30^{II}- or GFP-expressing vectors were generated by cloning p30^{II} or
GFP between BamH1 and Xho1 in the pCMVHR vector (Naldini, L. et al. "In vivo
5 gene delivery and stable transduction of nondividing cells by a lentiviral vector."
Science **272**, 263-267 (1996)). Production of high-titer pseudotype virus stocks were
generated as previously described (Nicot, C. et al. "HTLV-1 p12(I) protein enhances
STAT5 activation and decreases the interleukin-2 requirement for proliferation of
primary human peripheral blood mononuclear cells." *Blood* **98**, 823-829 (2001)).
10 HTLV-1 LTR-*Luc* and *RL-TK* vectors have been previously reported (Nicot, C. et al.
"HTLV-I Tax transrepresses the human c-Myb promoter independently of its
interaction with CBP or p300." *Oncogene* **19**, 2155-2164 (2000)).

Transfections, ELISA, and *Luc* assays. Transfections were performed using
Effectene (Qiagen, Valencia, California) and the DNA amounts are specified in each
15 experiment. Transfection efficiencies were normalized using either *RL-TK* or
cytomegalovirus (CMV)-*Luc*. Viral production was assayed in duplicate thirty-six
hours post-transfection or post-infection by ELISA using the RETRO-TEK HTLV p19
antigen ELISA kit according to manufacturer's instructions (Zeptometrix, Buffalo,
New York). Electroporation of CMT3 cells was performed as previously reported
20 (Dundr, M. et al. "A kinetic framework for a mammalian RNA polymerase in vivo."
Science **298**, 1623-1626 (2002)).

Protein detection. Western blot analysis for p30^{II} or Tax expression was
performed using 50 µg of total protein lysate, anti-HA (12C5), or anti-Tax monoclonal
antibodies (kindly provided by John Brady, National Cancer Institute, Bethesda,
25 Maryland), and an anti-mouse HRP-conjugated secondary antibody. GFP-fused
proteins were detected by confocal microscopy using Zeiss equipment (Thornwood,
New York). Metabolic labeling of HTLV-1-transformed HUT102 cells was performed
using DMEM media lacking methionine and cysteine supplemented with Express S³⁵
Met protein label mix (PerkinElmer, Boston, Massachusetts) for forty hours. Cell
30 extracts were resuspended in RIPA buffer and immunoprecipitated.

Heterocaryon assays. Heterocaryon assay was performed to evaluate p30^{II}
shuttling abilities. CMT3 cells were transfected with either p30^{II}-GFP or red fluorescent

protein (RFP) expression vectors. Transfected cells were mixed. The following day, thirty minutes prior to fusion with polyethylene glycol (PEG), cells were incubated with cyclohexamide (25 µg/ml) to prevent *de novo* protein synthesis and, after fusion, cells were maintained with cyclohexamide for an additional hour, fixed, and observed
5 by confocal microscopy. HIV Rev-GFP was used as positive control. Similar results were obtained in HeLa and NIH3T3 cells from one to two hours.

RNA binding assays. *tax/rex* and *p21^{rex}* cDNAs were cloned in pMH vector. Biotinylated RNA was transcribed *in vitro* using the RiboMAXTM production system (Promega) and biotin-14-CTP (Invitrogen, Carlsbad, California) according to
10 manufacturer instructions, purified, and immobilized on streptavidin magnetic particles (Roche Molecular). RNA on beads was mixed with nuclear extracts of CMT3 cells or transfected with pMHp30^{II} in 10 mM Hepes, 50 mM KCl, 0.1 mM EDTA, 0.5 mM DTT, and proteases and RNase inhibitors. Complexes were purified with a magnet after several washes in binding buffer. Bound proteins were eluted in RIPA,
15 immunoprecipitated, and identified by Western blot using anti-HA antibody.

B) RESULTS

Decreased viral production by ectopic expression of p30^{II}. The replication-competent HTLV-1 molecular clone p-BST (Nicot, C. et al. "Establishment of HTLV-
20 I-infected cell lines from French, Guianese and West Indian patients and isolation of a proviral clone producing viral particles." *Virus Research* **30**, 317-334 (1993)) was co-expressed with increasing amounts of the p30^{II} protein, and viral production was assessed by measuring p19 Gag in the supernatant of transfected cells (Fig. 1A). p30^{II} decreased viral production in a dose-dependent manner and this suppressive effect
25 remained evident even when Tax was expressed *in trans* (Fig. 1A). The decreased level of Gag protein in the virions was not due to an inhibition of the viral protease since the p55 Gag precursor was not accumulated and processed normally even in the presence of exogenous p30^{II} (Fig. 1B). The difference in viral production observed in the presence of p30^{II} was not due to a difference in transfection efficiency, which was
30 controlled by coexpressing a *Renilla (RL)-TK* gene construct in all assays.

Importantly, the negative effect of p30^{II} on viral replication was not restricted to the p-BST clone since similar results were obtained when two additional HTLV-1

molecular clones, p-ACH (Kimata, J. T., et al. "Construction and characterization of infectious human T-cell leukemia virus type 1 molecular clones." *Virology* **204**, 656-664 (1994)) and p-CSH (Derse, D., et al. "Examining the molecular genetics of HTLV-I with an infectious molecular clone of the virus and permissive cell culture systems." *J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol.* **12**, 1-5 (1996)), were tested.

p30^{II} inhibits Tax at a post-transcriptional level. We hypothesized at first that the reduction of p30^{II}-induced viral expression may be related to the inhibition of Tax transcriptional activity on the long terminal repeat (LTR). However, in our experimental system, co-expression of p30^{II} with a full-length HTLV-1 LTR fused to a *luciferase (Luc)* reporter gene did not significantly affect basal or Tax-mediated transcription from the viral LTR (Figs. 2A-2B) when transfection efficiency was internally controlled by using the *RL-TK* construct. These data demonstrate that p30^{II} likely did not target Tax as a protein.

Interestingly, p30^{II} suppressed Tax activity on the HTLV-1 LTR-*Luc* reporter gene only when Tax was produced from the doubly spliced mRNA transcript within the HTLV-1 proviral clone (Fig. 2C), suggesting that p30^{II} may target the *tax* mRNA.

p30^{II} sequesters the doubly spliced *tax/rex* mRNA in the nucleus. To investigate further whether p30^{II} affected the level of viral transcripts, semi-quantitative reverse-transcription PCR (RT-PCR) of total, nuclear, and cytoplasmic RNAs from cells cotransfected with the p-BST provirus and p30^{II} was performed using primers able to amplify the genomic, *env*, *tax/rex*, and *p21^{rex}* mRNAs (Koralnik, I. et al. Protein isoforms encoded by the pX region of the human T-cell leukemia/lymphotropic virus type I. *Proc. Natl. Acad. Sci. USA* **89**, 8813-8817 (1992)) (Fig. 3A). While p30^{II} did not significantly affect the levels of any of these viral mRNAs in the total cellular RNA (Fig. 3B, lanes 2-3), in the cytoplasmic RNA, a significant decrease of the doubly spliced *tax/rex* mRNA (Fig. 3C, lanes 5-6) but not the *gag*, *env*, and *p21^{rex}* mRNAs was observed. In contrast, only the *tax/rex* mRNA accumulated in the nucleus in the presence of p30^{II} (Fig. 3D, lanes 8-9).

p30^{II} specifically binds to the *tax/rex* mRNA splice junction. The *tax/rex* mRNA is generated by double splicing of the genomic RNA (Seiki, M., et al. "Expression of the pX gene of HTLV-I: general splicing mechanism in the HTLV family." *Science* **228**, 1532-1534 (1985); Aldovini, A., et al. "Molecular analysis of a

deletion mutant provirus of type I human T-cell lymphotropic virus: evidence for a doubly spliced x-lor mRNA." *Proc. Natl. Acad. Sci. U.S.A* **83**, 38-42 (1986); and Wachsmann, W. et al. "HTLV x-gene product: requirement for the env methionine initiation codon." *Science* **228**, 1534-1537 (1985)) from the splice-donor site within the
 5 R region of the viral LTR (nucleotide 119) to an acceptor site within the *pol* gene (nucleotide 4641) and from a donor site at nucleotide 4831 to an acceptor site, at nucleotide 6950. The *p21^{rex}* mRNA is completely spliced from the same splice-donor site within the LTR region to the most distant acceptor site at nucleotide 6950 (Kiyokawa, T. et al. "p27x-III and p21x-III, proteins encoded by the pX sequence of
 10 human T-cell leukemia virus type I." *Proc. Natl. Acad. Sci. USA* **82**, 8359-8363 (1985)) (Fig. 3A).

The ability of p30^{II} to decrease the cytoplasmic levels of *tax/rex* mRNA but not of *p21^{rex}* mRNA suggested a specific interaction of p30^{II} with the mRNA that encodes both Tax and Rex positive regulators. To prove this hypothesis in a heterologous
 15 system, the cDNAs for both *tax/rex* and *p21^{rex}* were inserted at the 3' end of a construct expressing *RL (RL-TK)* (Fig. 4A). Coexpression of p30^{II} resulted in a decreased *RL* expression from the *RL-TK-tax/rex* construct but not from the construct that contained the *p21^{rex}* cDNA (Fig. 4B), confirming the specificity of p30^{II} for the *tax/rex* mRNA splice junction.

20 These results suggested that p30^{II} may form complexes with the *tax/rex* but not the *p21^{rex}* mRNA. To investigate this, the *tax/rex* and *p21^{rex}* RNA splice junctions (Fig. 4A) were synthesized *in vitro*, biotinylated, and immobilized on streptavidin magnetic beads and used in pull-down assays following incubation with cell extracts from control or p30^{II}-expressing CMT3 cells. In agreement with results shown in Fig. 4B, p30^{II}
 25 specifically interacted with the *tax/rex* mRNA splice junction (the HTLV-1 middle exon sequence) but not that of the *p21^{rex}* mRNA (Fig. 4C, lanes 4 and 7). The specificity of this interaction was further demonstrated by binding competition using the non-biotinylated *tax/rex* RNA splice junction (Fig. 4C, lanes 5 and 8). These results indicate that a region of RNA between nt 4641 and 4831 (the middle exon
 30 sequence) of the *tax/rex* RNA is involved in p30^{II}-mediated latency of HTLV-1.

p30^{II} is a nuclear-resident protein. The notion that HTLV-1 p30^{II} is a nuclear/nucleolar protein coupled with the finding that p30^{II} binds to and retains the

tax/rex mRNA in the nucleus suggested that p30^{II} may not be able to shuttle between the nucleus and the cytoplasm. To test this hypothesis, green fluorescent protein (GFP) was fused to the amino terminus of p30^{II} and the shuttling ability of this chimeric protein was tested by heterokaryon assay (Fig. 4D). Cells were transfected independently with the DsRed1 fluorescent protein or p30^{II}-GFP and subsequently fused. In parallel, a Rev-GFP construct was used as a positive control since the human immunodeficiency virus type 1 (HIV-1) Rev protein shuttles into and out of the nucleus. As expected, Rev-GFP was found in DsRed1-positive fused acceptor cells, indicative of its ability to shuttle. In contrast, p30^{II} remained localized to the nucleus of p30^{II}-transfected cells and did not shuttle to acceptor nuclei of the DsRed1 fused cells (Fig. 4D).

p30^{II} reduces viral production in HTLV-1-infected T-cells. Collectively, the above data demonstrate that p30^{II} decreases viral replication by forming a protein-RNA complex(es) that is retained in the nucleus. We next investigated whether this would also be the case in the context of human HTLV-1-infected T-cells. A recombinant lentivirus vector carrying the *p30^{II}* gene was pseudotyped with vesicular stomatitis virus (VSV) Env protein and used to transduce p30^{II} in the chronically infected human T-cell lines HUT102, C91PL, and MT2. Expression of p30^{II} indeed resulted in both a decrease in the level of the Tax protein in cell extracts (Fig. 5C) and a decrease in p19 Gag production in the supernatant of the HUT102 cell line (Fig. 5A). Transduction of p30^{II} also reduced viral expression in MT2 and C91PL cell lines (Fig. 5B). Thus, p30^{II} also suppresses viral replication in human T-cells, the natural target of HTLV-1 infection.

Incorporation by Reference

Throughout this application, various publications, patents, and/or patent applications are referenced in order to more fully describe the state of the art to which this invention pertains. The disclosures of these publications, patents, and/or patent applications are herein incorporated by reference in their entireties, and for the subject matter for which they are specifically referenced in the same or a prior sentence, to the same extent as if each independent publication, patent, and/or patent application was specifically and individually indicated to be incorporated by reference.

Other Embodiments

It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

What is claimed is:

1. A method of identifying a compound for breaking retroviral latency in a subject with a retroviral infection, comprising:
 - a) contacting a sample with the compound, wherein the sample comprises:
 - i. an mRNA comprising an HTLV-1 middle exon sequence between HTLV-1 nucleotides 4641-4831, and
 - ii. p30^{II}; and
 - b) detecting the amount of binding between the mRNA and p30^{II}, whereby a decrease in the amount of binding between the mRNA and p30^{II} in the sample, compared to the amount of binding between the mRNA and p30^{II} in a sample not contacted by the compound, identifies a compound for breaking retroviral latency in a subject with a retroviral infection.
2. A method of identifying a compound for breaking retroviral latency in a subject with a retroviral infection, comprising:
 - a) contacting a sample with the compound, wherein the sample comprises p30^I; and
 - b) detecting p30^{II} activity, whereby a decrease in p30^{II} activity in the sample, relative to p30^{II} activity in a sample not contacted with the compound, identifies a compound for breaking retroviral latency in a subject with a retroviral infection.
3. The method of claim 1 or 2, wherein the compound is for breaking HTLV-1 latency.
4. The method of claim 1 or 2, wherein the compound is for breaking HIV latency.
5. The method of claim 1 or 2, wherein the compound decreases the level or activity of p12^I.

6. A method of treating a patient with a retroviral infection, comprising administering, to the patient, a compound identified by the method of claim 1 or 2, thereby treating the patient with a retroviral infection.
7. The method of claim 6, further comprising administering a reverse transcriptase inhibitor or a retroviral protease inhibitor to the patient.
8. A method of treating a patient with a retroviral infection, comprising:
 - a) vaccinating the patient with a retroviral protein;
 - b) administering a compound that inhibits the activity of a p30^{II}-analogous latency protein to the patient; and
 - c) administering a reverse transcriptase inhibitor or a retroviral protease inhibitor to the patient, thereby treating a patient with retroviral infection.
9. The method of claim 8, wherein the retroviral structural protein is Gag, Env, Pol, or Tax.
10. The method of claim 8, wherein the retrovirus is HTLV-1.
11. The method of claim 8, wherein the retrovirus is HIV.
12. The method of claim 8, wherein the p30^{II}-analogous latency protein is p30^{II}.
13. The method of claim 8, wherein the p30^{II}-analogous latency protein is Vpr.
14. The method of claim 8, wherein the compound is a compound identified by the method of claim 1 or 2.

Figure 1A

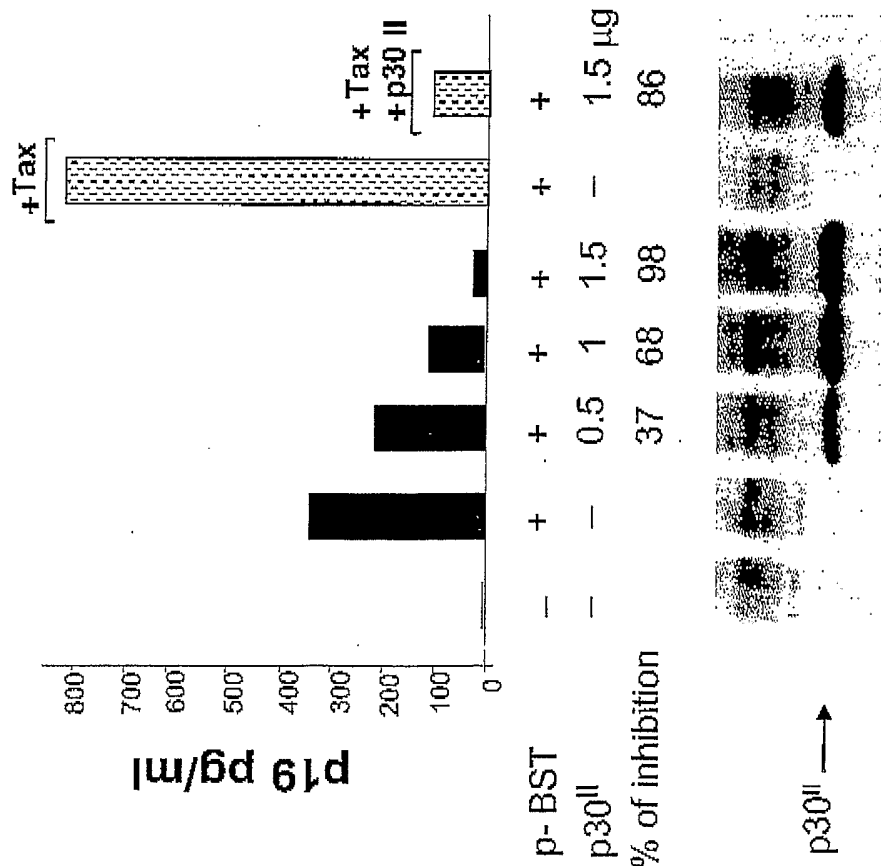


Figure 1B

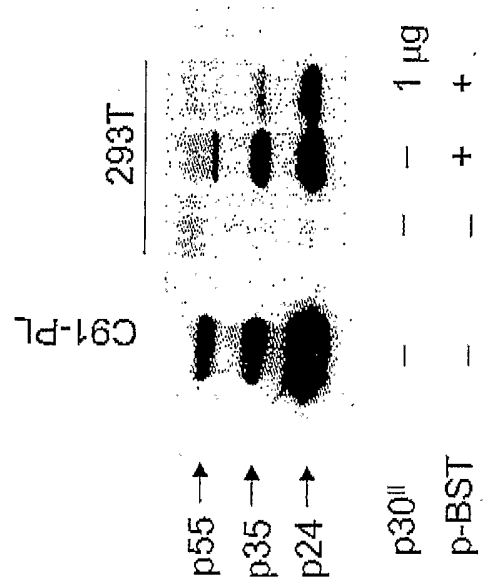


Figure 2A

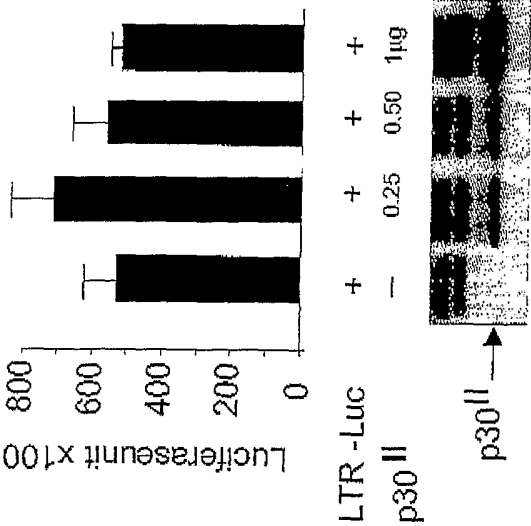


Figure 2B

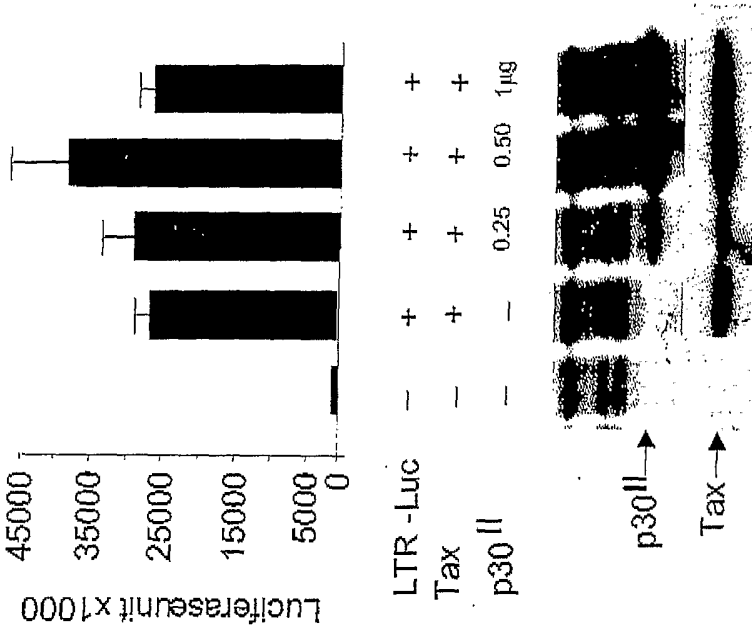


Figure 2C

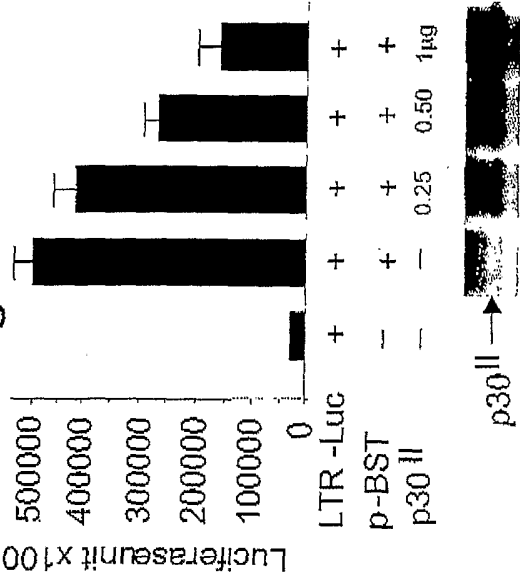


Figure 4A

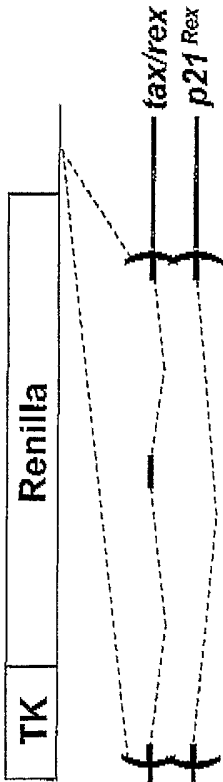


Figure 4C

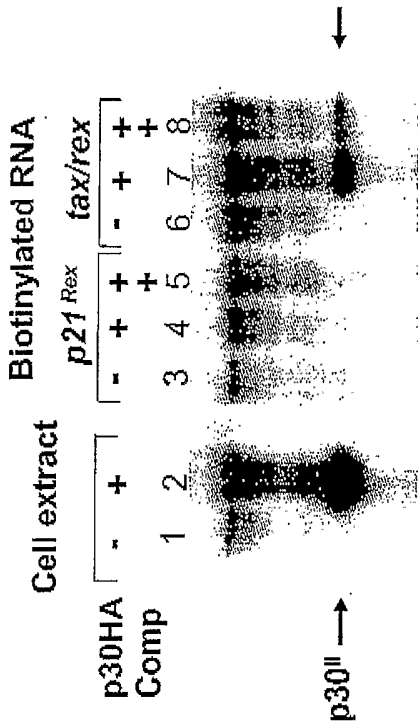


Figure 4B

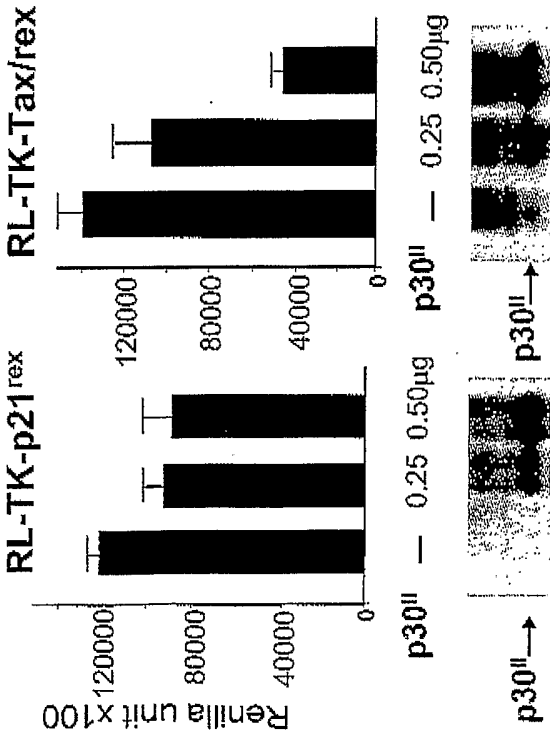


Figure 4D

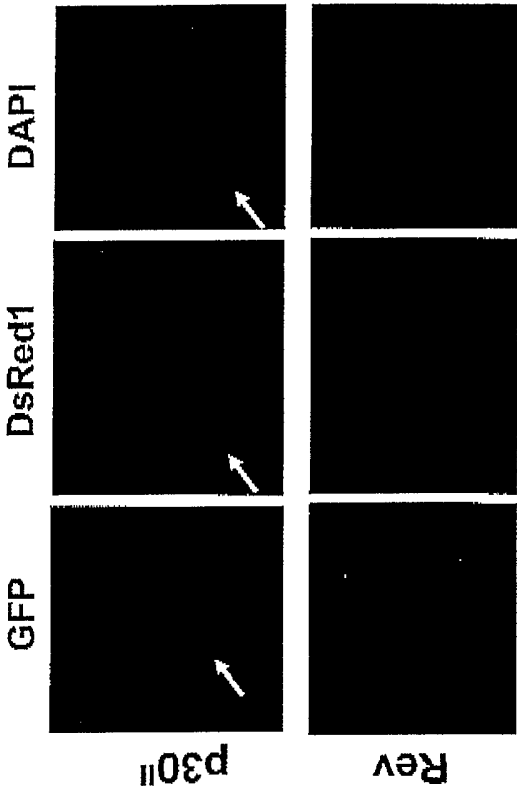


Figure 5A

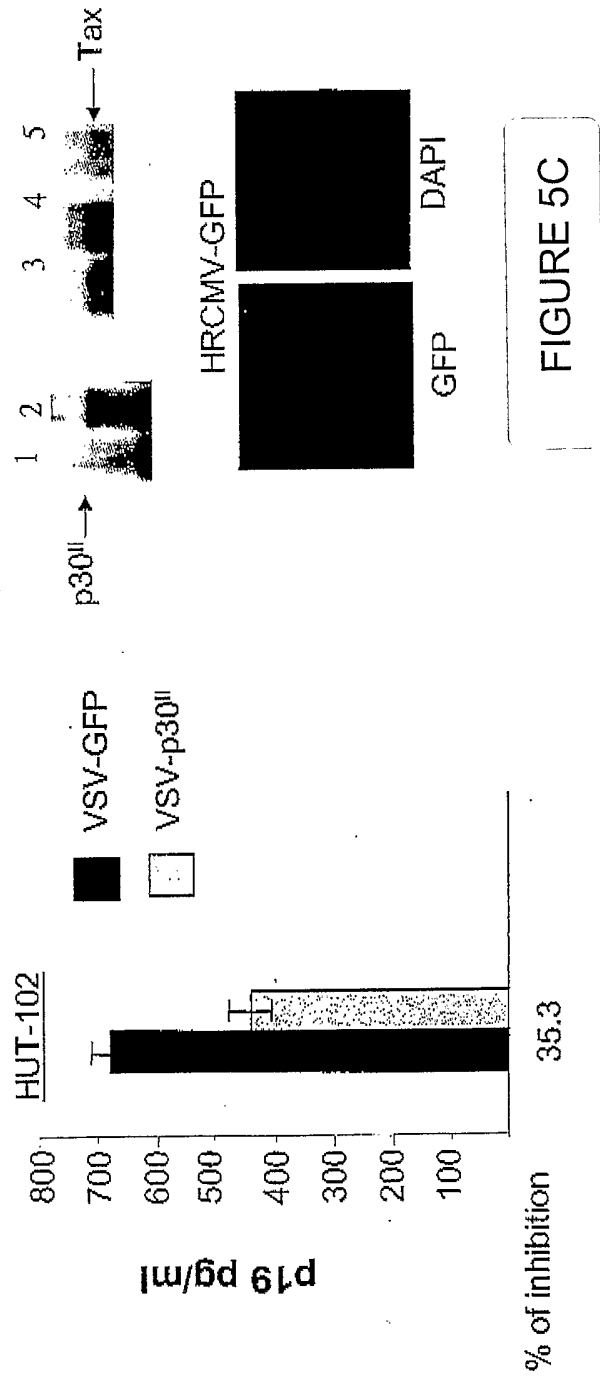
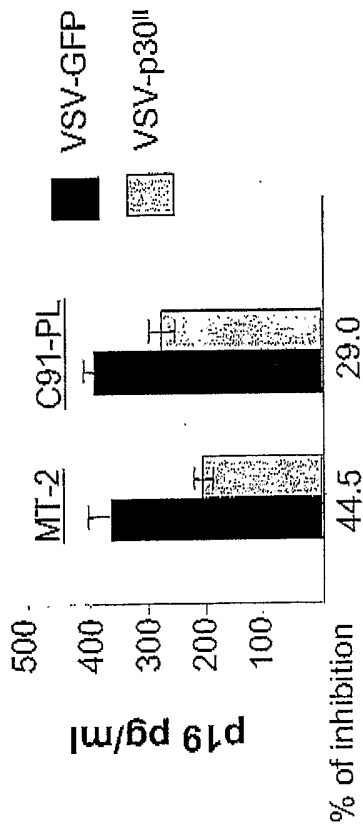


Figure 5B



SEQUENCE LISTING

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as represented by the Secretary, Department of
Health and Human Services

Genoveffa Franchini
Christophe Nicot

<120> HTLV-1 p30II AND p12I PROTEINS AS
THERAPEUTIC TARGETS IN HTLV-1 INFECTED INDIVIDUALS

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