Drugs of the Future 2002, 27(5): 481-493

CCC: 0377-8282/2002

Review Article

HIV-1 Tat protein: a target for the development of anti-AIDS therapies

Marco Rusnati* and Marco Presta

Unit of General Pathology and Immunology, Department of Biomedical Sciences and Biotechnology, University of Brescia, Via Valsabbina 19, 25123 Brescia, Italy.
**Correspondence*

CONTENTS

Introduction

The clinical features that characterize AIDS cannot be ascribed to the simple infection of $CD4^+$ cells by HIV, especially when considering that only a small percentage of T cells are infected by the virus throughout the progression of the disease (1). Accordingly, classical anti-antiviral monotherapies have demonstrated very limited benefits. Also, AIDS-associated pathologies such as dementia, Kaposi's sarcoma (KS) and the increased incidence of tumors cannot be explained merely by the death of HIV-infected cells. The complex pathogenesis of AIDS involves additional extracellular factors which are able to act on HIV uninfected cells. Several cytokines aberrantly produced during HIV infection may explain, at least in part, some of the pathological effects observed in AIDS patients (2). In addition, viral proteins may act as extracellular "toxins" significantly contributing to some of the pathological events associated with AIDS (3). Among these, the transactivating factor (Tat) of HIV-1 has been shown to be crucial for HIV replication and to be involved in the pathogenesis of AIDS-associated diseases. Thus, Tat is considered as a target for the development of novel anti-AIDS therapies.

Tat biology has been the subject of several reviews (4-7). The aim of the present paper is to review recent studies examining how interference with HIV-Tat biology may be a key therapeutic strategy to cure AIDS.

The HIV-1 Tat gene encodes for a 86-101 amino acid polypeptide that acts as the main transactivating factor of HIV (6). Tat protein can be actively released by HIV-infected cells (7) and is detected in the serum of HIV-infected individuals (8). Extracellular Tat is able to enter latently HIV-infected cells and activate the transcription of the viral genome. In AIDS patients, the reactivation of HIV by extracellular Tat may explain the burst of replication associated with early phases of HIV infection, where synchronized virion replication takes place (9). Moreover, extracellular Tat promotes HIV-1 coreceptors expression (10), thus inducing a self-perpetuating permissiveness for HIV-1 infection.

Extracellular Tat also acts on different types of uninfected cells by interacting with several receptors including integrins $\alpha_5\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$; the vascular endothelial growth factor (VEGF) receptors VEGFR1/Flt-1 and VEGFR2/KDR; and the chemokine receptors CCR2 and CCR3 and CD26 (also known as dipeptidyl peptidase IV) (7). More recently, Tat has been shown to also bind the chemokine receptor CXCR-4 (11), the low density lipoprotein receptor-related protein (LPR) (12) and heparan sulfate proteoglycans (HSPGs) (13).

Given the variety of receptors bound by extracellular Tat, it can be expected that a complex network of signal transduction pathways must be activated by the transactivating factor in target cells (7). Also, LPR and HSPG receptors mediate the internalization of Tat inside the cell. As already mentioned, internalized Tat retains the capacity to transactivate viral and/or cellular genes.

Extracellular Tat exerts a variety of biological activities some of which have been tentatively associated with distinct AIDS-associated pathologies (Fig. 1). For example, Tat is considered a neurotoxin implicated in the pathogenesis of AIDS dementia (14) and also acts on different cells of immunity contributing to the immune suppression in AIDS patients (4, 7). Tat appears to be responsible, at

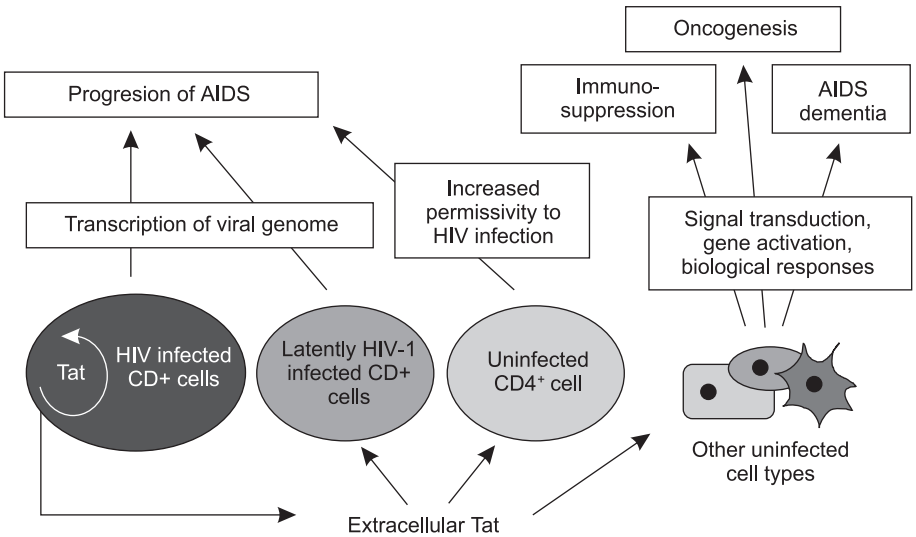

Fig. 1. Intra- and extracellular Tat in AIDS progression and AIDS-associated pathologies.

least in part, for the observed increased incidence of tumors in AIDS patients (4), as well. Also relevant to this point, extracellular Tat induces neovascularization (7) that is required for tumor growth and metastasis. Therefore, extracellular Tat has been implicated in the development of hypervascularized KS (7). Furthermore, extracellular Tat transactivates the genome of human herpes virus 8 (15), hepatitis virus C (16) and human cytomegalovirus (17), thus favoring secondary infections. And finally, extracellular Tat induces upregulation of a wide spectrum of cytokines, chemokines, growth factors and their receptors (4). This suggests that a complex interplay may occur in which Tat-driven upregulation of cytokines amplifies the spectrum of the biological effects associated with the action of the transactivating factor.

Tat as a potential target for the treatment of AIDS

Many features make HIV peculiar among human viruses and HIV infection difficult to eradicate. The high levels of viremia found in the initial acute HIV infection quickly decline as the immune response develops, but latent infection persists throughout the long period of latency. Under the pressure of the immune response, the initial homogeneity of the virus is replaced by the rapid appearance of different viral strains. This represents the main obstacle to classical vaccine approaches to AIDS treatment. Indeed, antibodies directed toward HIV structural proteins cannot take into account the great epitope diversity existing among and within infected individuals. Thus, anti-AIDS therapies have been based mainly on inhibitors of HIV replication (highly active antiretroviral therapy [HAART]) which have significantly decreased AIDS mortality in the U.S. However, because the virus latently resides in resting memory $CD4^+$ cells and cannot be completely eradicated, HAART must be administered chronically, increasing its cost and leading to the development of highly drug resistant HIV strains. Moreover, HAART is frequently discontinued by patients as a drug "holiday" or because of drug intolerance thus allowing for the reactivation of HIV-1 and progression of AIDS. Therefore, novel anti-AIDS therapies should control AIDS progression and cure associated pathologies rather than eradicate HIV itself. Moreover, the cost of anti-AIDS drugs must minimized due to the dramatic increase of AIDS infection in the third world (18, 19).

Tat plays a pivotal role in HIV replication and in several AIDS-associated pathologies and is also implicated in the massive initial viral output that correlates with unfavorable prognosis and to the development of HIV mutants that overwhelm the immune system and/or vaccination (20). Also, Tat is secreted and is characterized by a high amino acid conservation (20). Interestingly, a stretch of repeated Arg and Lys residues (basic domain) occurs in the Tat protein and is implicated in several aspects of Tat biology. Indeed, it drives nuclear and nucleolar delivery of Tat, mediates the interaction of Tat with some cell surface receptors and nucleic acids and is required for some of the biological activities of extracellular Tat (21). The basic domain is a highly immunoreactive region and is well conserved among Tat proteins isolated from different HIV-1 strains (20, 22). These characteristics make Tat a good candidate for the development of an anti-AIDS vaccine and/or drugs (20).

When considering Tat as a target for drug/vaccine approaches, it should be keep in mind that it acts both as an intracellular transactivating factor and as an extracellular, pleiotropic cytokine. However, it is worth noting that extracellular Tat can be internalized by target cells where it retains its intracellular transactivating potential. These features help to identify different possible anti-Tat

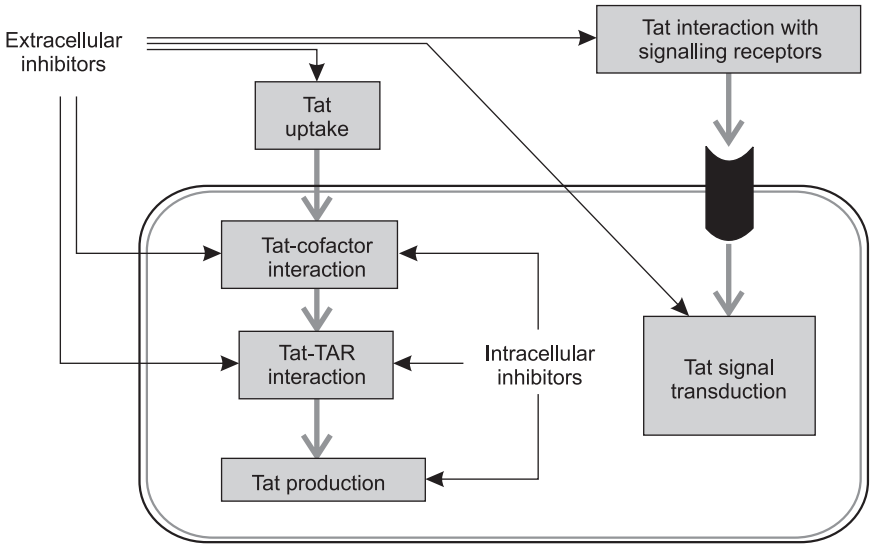

Fig. 2. Anti-Tat strategies for the development of anti-AIDS therapies. Intracellular and extracellular Tat inhibitors can act on multiple targets.

strategies, such as the inhibition of extracellular Tat interaction with target cells and/or its intracellular uptake, the intracellular blockade of Tat production and release by HIV-infected cells and the disruption of Tat interaction with its intracellular cofactors or with TAR RNA (Fig. 2). On this basis, extracellular and intracellular Tat antagonists will be discussed separately.

Extracellular Tat antagonists

Theoretically, the biological activity of extracellular Tat can be neutralized by anti-Tat antibodies, Tat-binding compounds, Tat receptor antagonists or inhibitors of Tat activated signal transduction pathways (Fig. 2).

Tat as a vaccine

Vaccination against HIV is a major goal for the treatment of AIDS because it would provide both prophylactic and therapeutic benefits at costs significantly lower than those of HAART therapies.

More than 60 phase I/II trials of up to 30 candidate AIDS vaccines have been conducted thus far (23). Accordingly, up to 600 articles have been published about AIDS vaccines, erroneously suggesting that this kind of cure is at hand. Unfortunately, this may not be true. Indeed, the most promising AIDS vaccine, one directed against the gp120 protein, was recently demonstrated to be ineffective and harmful to humans (24). For many years Tat was not considered a candidate target for an AIDS vaccine because of its intracellular localization. However, the discovery of an extracellular role for Tat would enable use of a strategy for AIDS vaccination that is radically different from previous ones. This new vaccine would be similar to toxoid vaccines for diphtheria or tetanus in that it could be directed against a viral product implicated in the pathogenesis of the disease rather than against the virion itself.

Several considerations point to Tat as a good candidate for an anti-AIDS vaccine. First, HIV infection does lead to an immune response. Among the viral proteins, reaction against Tat is the quickest both in primates (25) and humans (26, 27). High levels of humoral and cell-mediated immune response against Tat correlate with better prognosis (3, 26-30). Second, Tat increases the permissiveness for HIV-1 infection (31), thus suggesting that neutralization of Tat may lead to protection from HIV infection. Third, immunization with a Tat vaccine may prevent or control those AIDS-associated pathologies in which extracellular Tat plays a pathogenic role. Fourth, Tat is the most conserved protein of HIV-1, making the development of viral clones resistant to Tat vaccination unlikely (32).

Cafaro *et al*. (33) vaccinated cynomolgus monkeys with a native Tat protein and obtained complete protection against the highly pathogenic simian-human immunodeficiency virus (SHIV)-89.6P strain in 5 of 7 animals. The use of native Tat protein as a vaccine is strongly debated (34). Whereas Cafaro *et al*. reported that the animals inoculated with native Tat protein or with tat cDNA showed no signs of toxicity (33, 35), Cohen *et al*. demonstrated that native Tat is highly immunosuppressive *in vivo* (36). The rationale for the use of native Tat is based on the possibility that the structural modifications introduced in a toxoid would destroy the main immunogenic epitopes of the protein. On the other hand, the side effects caused by immunization with gp120 in humans (24) suggest caution when using native, bioactive HIV proteins.

Le Buanec *et al*. (37) developed a Tat toxoid that was demonstrated to be safe in mice, seronegative humans and immunocompromised HIV-1 infected patients (38). Subsequent studies with macaques immunized with the Tat toxoid and challenged with SHIV-89.6P demonstrated an attenuation of SHIV replication and disease but no protection against infection (37).

Novel approaches to the preparation of a Tat vaccine can be forseen. Immunogenic Tat sequences that overlap functional domains of Tat have been identified which can be exploited to design safe and efficient Tat vaccines (40, 41). These proteins elicit specific cellular or humoral responses in animals (42-44) and also minimize chronic plasma viremia in rhesus macaques infected with SHIV (45). Alternatively, immunization with cDNA encoding for Tat mutants has been shown to induce a humoral and cellular response against wild-type Tat (35, 46).

Despite its potential therapeutical implications, a Tat vaccine might not be sufficient to cure AIDS. More likely, Tat should be considered as a constituent of a composite vaccine (34). Indeed, vaccination with cDNAs encoding for Tat and other HIV proteins are under study (46-50).

Extracellular Tat-binding antagonists

The demonstration that heparin binds Tat (51) and that HSPGs act as Tat receptors (13) suggests that polyanionic heparin-like compounds may be used to sequester extracellular Tat, thus inhibiting cell interaction and biological effects. Indeed, the polysulfonated suramin acts as an extracellular Tat antagonist in different experimental models (21, 51, 52). However, its toxicity and lack of specificity represent serious limitations for the use of suramin as an anti-Tat drug. To overcome these limitations, a series of polysulfonated distamycine derivatives mimicking suramin were tested for Tat antagonist activity. These compounds compete with heparin/HSPGs for the binding to Tat (21). They inhibit the mitogenic and HIV-LTR-transactivating activity of Tat *in vitro* (21, 53) and its angiogenic, tumorigenic and metastatic potential *in vivo* (53, 54). Interestingly, distamycine derivatives retain their inhibitory activity when delivered to target cells by lipofection (53).

Two of the distamycin derivatives tested (PNU-151779 and PNU-157666) inhibited the LTR-transactivating activity and mitogenic capacity of extracellular Tat with a potency significantly higher than that of suramin and comparable to that of heparin (21). Interestingly, it is possible to dissociate the Tat antagonist activity of distamycin derivatives from their FGF2-antagonist activity by modification of their chemical structure (M. Rusnati, unpublished observations). These findings suggest that appropriate modifications in the backbone structure or in the distribution of the sulfonated groups of distamycine derivatives can lead to compounds with increased Tat antagonist potency and/or specificity.

Dextrin-2-sulphate (D2S) represents another polyanionic compound that has been demonstrated to bind extracellular Tat and to inhibit its transactivating activity (55).

Pentosan polysulfate (PPS) interacts with Tat protein with a 10-fold higher affinity than that of heparin. Accordingly, PPS is highly effective in preventing the interaction of Tat with target cells and in inhibiting the LTR-transactivating and mitogenic activity of Tat *in vitro*. Also, PPS inhibits Tat-induced neovascularization *in vivo* (56).

Recently, it was shown that it is possible to produce "biotechnological heparin-like molecules" by controlled sulfation of the *Escherichia coli* K5 polysaccharide (57). These products have been demonstrated to interact with FGF2 and to inhibit its biological activity *in vitro* and *in vivo* (58). Experiments performed in our laboratory have shown that an appropriate sulfation confers Tat binding capacity and specific Tat antagonist activity to K5 polysaccharide (Urbinati *et al*., manuscript in preparation).

Inhibitors of extracellular Tat receptors

Because Tat can interact with different cell surface receptors, they might be considered targets for Tat antagonists under selected conditions when the cytokine-like action of Tat plays a pathogenic role.

Anti-KDR antibodies inhibit Tat-driven chemotactic migration of endothelial cells *in vitro* and Tat-induced neovascularization *in vivo* (59). Accordingly, anti-KDR antibodies inhibit the mitogenic activity of extracellular Tat in epithelial cells (Urbinati C., unpublished observations).

Arg-Gly-Asp (RGD)-containing peptides are well known antagonists of integrin receptors (60). Accordingly, we have observed that linear RGD-containing peptides inhibit integrin-mediated cell adhesion to immobilized Tat. Interestingly, cyclization of RGD-containing peptides increases their Tat inhibitory potency (Urbinati *et al*., manuscript in preparation). However, RGD-containing peptides may exhibit aspecific inhibition that can interfere with the function of platelet-associated integrin $alpha_{IIb}beta_3$ that is required for a correct coagulation process. Based on this, an RGD-peptidomimetic compound (SCH-221153) has been developed that selectively binds to $\alpha_v\beta_3$ and $\alpha_v\beta_5$ but not to $\alpha_{IIb}\beta_3$ (60). Preliminary experiments have shown that SCH-221153 exerts significant Tat antagonist activity *in vitro* (Urbinati *et al*., manuscript in preparation).

Inhibitors of Tat-activated intracellular second messengers

The interaction of extracellular Tat with cell membrane receptors activates a variety of intracellular signaling pathways and some second messengers appear to be activated by intracellular Tat.

We have observed that the mitogenic activity exerted by extracellular Tat in epithelial cells is inhibited by the tyrosine phosphorylation inhibitor tyrphostin, the protein

kinase C (PKC) inhibitor GF-109203X and the mitogen activated kinase (MAPK) p38 inhibitor SB-203580, but not by the phosphatidyl-inositol 3 kinase inhibitor LY-294002 (Rusnati *et al.*, unpublished data). Accordingly, the PKC inhibitors curcumin (61) and staurosporine (62) inhibit the transactivating activity of Tat.

The MAPK $ERK_{1/2}$ is activated by extracellular Tat in many different cell types and may be located at the convergence of the signal transduction pathways activated by the interaction of Tat with KDR and integrins (63). In microglia, 17β-estradiol suppresses Tat-induced MAPK activation and consequent phagocytosis and superoxide/TNF-α release (64). In addition, the specific $ERK_{1/2}$ inhibitors PD-098059 and U-0126 effectively inhibit $ERK_{1/2}$ phosphorylation in endothelial (63) and epithelial cells (Rusnati *et al.*, unpublished observations). They also inhibit the mitogenic activity of Tat in cultured epithelial cells (Rusnati *et al.*, unpublished observations) but have no effect on Tat-induced neovascularization *in vivo* (63).

Arachidonic acid metabolism can be activated by Tat and quinacrine and chloroquine inhibit the LTR-transactivating activity of Tat (62). Tat also induces the production of phosphatidic acids. The specific inhibitor CT-2576 prevents the transactivating activity exerted by Tat in epithelial cells and HIV replication in promonocytic cells (65).

Diazepam has been shown to inhibit Tat-induced migration of human microglia by blocking Tat-induced Ca^{2+} mobilization (66).

Tat activation of the intracellular second messenger NF-κB is required for the transcription of the viral genome. Transdominant mutants of IκB α (an inhibitor of NF-κB) inhibit the transactivating activity of Tat and HIV replication when transfected in target cells (67). Also, natural molecules such as interleukin 16 (68) and nitric oxide (69) inhibit Tat-induced NF-κB activation.

In conclusion, specific inhibitors of several second messengers can be used to inhibit different biological activities of Tat. In addition to the possibility of identifying effective Tat antagonists, these studies may help to elucidate the signal transduction pathways responsible for the biological effects of extracellular Tat in target cells.

Intracellular Tat antagonists

Long before Tat was demonstrated to be secreted by HIV-infected cells, intracellular Tat was considered a target for anti-HIV therapies. Accordingly, several drugs and gene therapies aimed at blocking the biological activity of intracellular Tat have been described (Fig. 3).

Anti-tat antisense

Gene therapies based on tat antisense cDNA have been suggested to be capable of blocking the translation of tat mRNA in HIV-infected cells (70). Transfection with tat antisense cDNA efficiently inhibits HIV infection in T lymphocytes and monocytes (71, 72). This inhibition can also be obtained in the presence of high multiplicity infections and inflammatory cytokines (73), an experimental condition that more closely resembles HIV infection in human patients. Accordingly, rhesus macaques infused with autologous lymphocytes engineered with antisense anti-tat gene and challenged with SIV showed a reduction in viral load, a sustained number of CD4+ cells and a decrease in lymph node disruption (74).

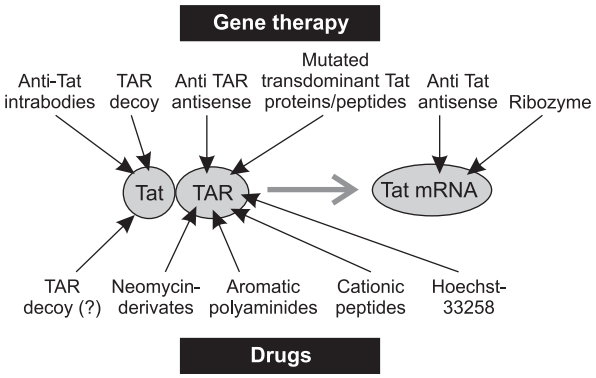

Fig. 3. Intracellular anti-Tat strategies aimed at blocking Tat-TAR interaction and Tat mRNA translation.

Ribozymes are metalloenzymes that retain the properties of an antisense RNA with the additional capacity of catalytic cleavage of specific RNA sequences (75). Ribozymes directed against tat mRNA and expressed in target cells by plasmid transfection or retroviral infection effectively inhibit HIV replication (76-80). Ribozymes were also shown to exert their Tat inhibitory effect on primary drug-resistant HIV strains with no mutations or loss of sensitivity to the ribozyme observed in a series of HIV passages in ribozyme-expressing cells (81).

Finally, novel chimeric ribozymes have been developed that contain the target hybridizing arms in the form of DNA instead of RNA. DNA enzymes may be endowed with higher stability and a longer half-life in plasma with respect to the classical RNA-based ribozyme (82, 83).

Also relevant to anti-tat gene therapy is the fact that hematopoietic stem cells, as opposed to circulating cells, can be transfected with anti-HIV genes without altering their differentiation (79, 84-86). Theoretically, this may lead to the reconstitution of immune function in AIDS patients. Also, anti-HIV genes can be engineered under the control of Tat-dependent HIV-LTR promoter to drive their expression only in HIV-infected cells (73).

Anti-TAR antisense, decoy and antagonists

Tat stimulates HIV-1 transcription elongation by interacting with a stem-loop RNA element (TAR) formed at the extreme 5' end of all viral transcripts (6). Thus, the Tat-TAR complex is considered an important target for anti-HIV strategies. Specific antisense oligonucleotides

complementary to the TAR apical stem-loop block Tat binding *in vitro* (87, 88). Accordingly, infection of target cells with retroviral vectors carrying antisense-TAR inhibits the transactivating activity of Tat (73, 89). Inhibition of Tat-TAR interaction can also be achieved by means of a TAR RNA decoy that blocks the binding of Tat to the authentic TAR region. Indeed, when expressed in target cells, the TAR decoy significantly reduces HIV infection (89-91). It has been proposed that, after proper structural modifications, TAR decoys may be administered exogenously to target cells (92), also with the aim of targeting extracellular Tat.

Tat-TAR interaction can also be prevented by means of transdominant Tat proteins or Tat peptides. Overexpression of suitable Tat mutants inhibits the transactivating activity of Tat and HIV production in various cell types (93-96). Accordingly, expression of Tat peptides or their electroporation into the cell inhibits LTR transactivation, HIV replication and virus transmission in U1 cells (97). Using a combinatorial library, Hamy *et al*. (98) selected the peptide-based compound CGP-642222 that mimics the basic peptide of Tat and binds to TAR, thus preventing Tat transactivating activity and HIV replication in human lymphocytes. Similar results were obtained by Choudhury *et al*. (99) with the basic *N*-acetyl-Arg-Lys-Lys-Arg-Gln-Arg-Arg-Arg-Cys(biotin)-NH_2 peptide.

TAR can also be targeted by chemical compounds such as the bisenzimidazole Hoechst-33258 (100), aromatic polyamidines (101) and tetrahydropyrimidine derivatives (102).

Anti-Tat intracellular intrabodies

Intracellular antibodies (intrabodies) can bind and inactivate intracellular antigens when expressed intracellularly in transfected cells. Marasco *et al*. (103) demonstrated that the expression of anti-Tat intrabodies in HIV-infected cells exhibits different HIV antagonist effects and that the structure of anti-Tat intrabodies can be genetically manipulated to increase their Tat antagonist activity (86, 103-105).

Inhibitors of intracellular Tat cofactors

Several intracellular cofactors are required for the nuclear delivery of Tat protein and for its interaction with TAR-RNA (5, 6). Friedler *et al*. (106) developed a cyclic peptidomimetic compound that functionally mimics the basic domain of Tat. This compound blocks the interaction of intracellular Tat with importin-β and its nuclear accumulation. The effect is specific since the peptidomimetic does not influence the nuclear delivery of other nuclear proteins.

P-TEFb is an intracellular complex composed of cyclin-dependent kinase (Cdk) 9 and cyclin T1. Tat-P-TEFb interaction is required for the transactivation of the HIV promoter (5). Flavopiridol, a Cdk inhibitor (107), and mC2p, a pseudo-substrate for Cdk7 (108), effectively block the transactivating activity of Tat and HIV replication *in vitro*.

Another intracellular Tat cofactor is the transcription factor Sp1 (5, 6). Sp3, an homolog of Sp1, represses Tat-mediated transactivation when overexpressed in target cells. Sp3 acts by competing with Sp1 for interaction with the specific DNA-binding sequence (109). Accordingly, the plant lignan 3'-*O*-methyl nordihydroguaiaretic acid inhibits Tat-dependent transactivation *in vitro* by binding to Sp1 sites (110).

Intracellular Tat antagonists with unknown mechanisms of action

Other synthetic compounds have been shown to act as Tat antagonists by inhibiting HIV replication even though their mechanism of action has not been fully elucidated. Among them, the benzodiazepine derivatives Ro 5-3335 and Ro 24-7429 were found to exert an inhibitory activity on several HIV strains in different HIV-infected cell types without causing emergence of drug-resistant HIV strains (111-114). Unfortunately, a randomized trial failed to show significant antiviral activity of Ro 24-7429 in AIDS patients (115). This lack of effect may be due to the fact that benzodiazepines are ineffective on chronically infected monocytes (116).

In the search for effective HIV-1 transcription inhibitors, Baba *et al*. identified a series of Tat antagonists including the 2-glycineamide-5-chlorophenyl 2-pyrryl ketone (117), the fluoroquinoline derivatives K-37 (118) and K-38 (119) and a novel substance produced by *Streptomyces* named EM-2487 (120) that inhibits HIV replication in acutely or chronically HIV-infected cells.

In addition, glucocorticoids (121), D-penicillamine and its derivatives (122, 123), the topoisomerase I inhibitor camptothecin (124), the TNF-α release antagonist calventol (125) and keto/enol epoxy steroids (126) have been shown to inhibit the transactivating activity of Tat. Finally, β-interferon plus 13-*cis*-retinoic acid inhibit neovascularization induced by Tat *in vivo*, suggesting that this combination may have possible therapeutic implications in epidemic KS (127).

Tat antagonists: the multitarget hypothesis

Up to 300 papers have been published to date addressing the possibility of blocking extracellular/intracellular Tat. Most of these studies, however, are limited to *in vitro* experiments. Only a few Tat antagonists were also effective *in vivo* and only one compound was tested in a clinical trial with poor results (115). This lack of success may be due to the diverse features and mechanisms of action of Tat. Indeed, the blocking of Tat-TAR interaction may inhibit HIV replication but be ineffective against the pathological effects of extracellular Tat. In turn, extracellular Tat antagonists may not be able to access the

transactivating factor inside HIV-infected cells. Finally, drugs that efficiently block extracellular and/or intracellular Tat may not reach the central nervous system, thus being ineffective on microglia-released Tat and related AIDS dementia. An efficient anti-Tat therapy must incorporate all these aspects and, at least in theory, overcome the abovementioned obstacles. For instance, cell permeable cyclic peptides endowed with Tat antagonist activity have been described (106). Tat-binding polyanionic compounds have been shown to block intracellular Tat once encapsulated in liposomes (53). Finally, the Tat antagonists diazepam and Ro 5-4864, readily cross the blood-brain barrier (66).

In some AIDS-associated diseases Tat acts via or synergistically with other growth factors/cytokines, thus making it difficult to inhibit these pathological processes with specific Tat inhibitors. Tat mimics heparin-binding angiogenic growth factors like VEGF and FGF2. They share structural features, receptors and/or signal transduction pathways. On the other hand, VEGF and FGF2 cooperate with Tat during the pathogenesis of AIDS-associated KS (128, 129). It is thus conceivable that drugs capable of "multitargeting" Tat and other angiogenic growth factors may be useful for the topical treatment of KS lesions. For instance, PPS, recently demonstrated to act as a Tat antagonist, binds to and neutralizes FGF-2 (56). Inhibitors of KDR or integrins may simultaneously block the effects of Tat, FGF2 and VEGF. Finally, $ERK_{1/2}$ is located downstream to the signal transduction pathways activated by Tat, VEGF and FGF2 (63), suggesting that its inhibitors can exert multitargeted effects.

Multitarget activity can also be directed to Tat and other HIV proteins. Indeed, some of the polyanionic Tat antagonists described in this review have been shown to also interact with different HIV proteins interfering with various aspects of the viral cycle (Fig. 4).

Heparin disrupts the CD4-gp120 complex by binding to both proteins (130, 131). PPS prevents HIV-1 replication (132) and sincitium formation (133) by inhibiting reverse transcriptase (134) and by binding to gp120 but not to CD4 (131). In contrast, D2S inhibits HIV adsorption

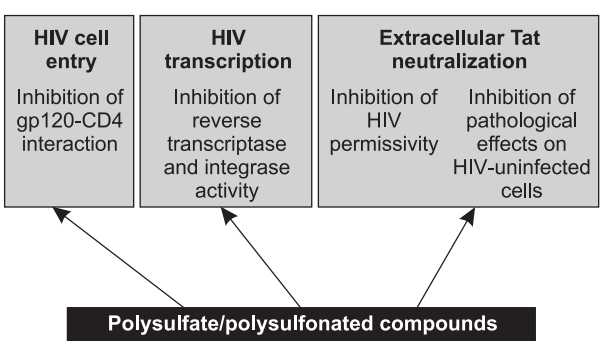

Fig. 4. Multitarget activity of polysulfated/polysulfonated compounds in anti-AIDS therapy. See text for further details.

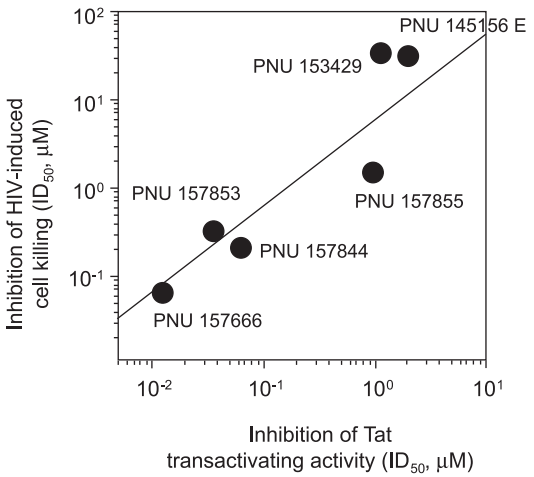

Fig. 5. Multitarget activity of suramin-related distamycin derivatives. Different polysulfonated distamycin derivatives were assayed for their capacity to inhibit HIV-1-induced cell killing (135) and the LTR-transactivating activity of extracellular Tat (20). The potency (ID_{50}) shown by each compound in the two assays was plotted and linear regression was calculated ($t = 0.92$, $p < 0.01$).

to cell surface (55) by binding to CD4 but not to gp120 (135). Distamycine derivatives prevent HIV-1-induced cell killing (136) by inhibiting HIV-1 reverse transcriptase (137) and integrase (138). They also downregulate chemokine receptors that act as HIV-1 coreceptors (139) and may also alter CD4 expression (140).

Given these characteristics, these compounds should be considered only prototypes of multitargeting HIV antagonists since further development requires knowledge of the molecular basis of their interactions with HIV-related proteins. Interestingly, both Tat and gp120 contain a stretch of positively charged amino acids that mediates their interaction with polyanionic molecules (21, 141). On the other hand, specific patterns of sulfated/sulfonated groups within polyanionic compounds determine the specificity of the binding to the different proteins (142). It is conceivable that the identification of the structural features of polyanionic compounds required for Tat and/or gp120 binding will bring new insights to the design of efficient multitarget anti-HIV drugs. In this regard, a positive correlation exists between Tat antagonists and anti-HIV-1 activities of distamycin derivatives (Fig. 5). Thus, it is possible that the multitarget effects of polyanionic compounds can be effectively investigated and possibly enhanced by modification of chemical structures.

Other compounds have been found to interfere with different aspects of HIV biology. The cell permeable peptidomimetic compound described by Friedler *et al.* (106) inhibits nuclear import of Tat and rev-RNA interaction simultaneously. The peptidoid CGP-64222 inhibits both Tat-TAR and HIV-CXC-4 interactions (143). Similarly, the oligocationic peptide ALX40-4C inhibits both Tat-TAR interaction and HIV adsorption to target cells (144).

Compounds such as the diphenylfuran derivatives prevent Tat-TAR interaction and exert antibiotic activity against *Pneumocystis carinii*, one the major causes of mortality in AIDS patients (145). The antibiotics neamin, neomycin and their derivatives also block TAR-Tat interaction and may simultaneously prevent secondary infections in HIV-infected individuals (144, 146).

Purine nucleoside analogs such as 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (147) and carbocyclic adenosine analogs (148, 149) inhibit the transactivating activity of Tat and are expected to also elicit antiviral activity.

It has been hypothesized that an anti-HIV multitargeted effect can be achieved with combinations of drugs. Hsu *et al*. (114) suggested using the Tat antagonist Ro 24-7429 in combination with classic reverse transcriptase and/or proteases inhibitors to reduce new infections and viral chronicity. In fact, the anti-HIV effects of the compound were increased when it was combined with the NF-κB specific inhibitor, pentoxifylline (150).

A multitarget approach to block intracellular Tat has also been developed that involves anti-tat antisenses coupled to TAR antisense which results in increased therapeutic efficacy (89, 73, 151, 152). Transdominant Tat and Rev proteins transfected simultaneously into target cells exert an anti-HIV effect that is greater than that obtained with the transdominant proteins used alone (93). An anti-CCR5 ribozyme confers resistance to viral infection, but its efficiency is increased when used in combination with an anti-tat-rev ribozyme (153). A double hammerhead ribozyme targeted to cleave both tat and rev transcripts has been cominbed with a rev decoy and a rev transdominant mutant resulting in better inhibition of HIV replication (85). Anti-Tat intrabodies have been successfully used in combination with anti-gp120 intrabodies (154) or with NF-κB antagonists (155).

Conclusions

Tat can be considered a target for the development of anti-AIDS therapies. Because it was believed to be the main HIV transactivator with an intracellular mechanism of action, initial efforts focused on the development and optimization of anti-tat gene therapies.

In the early 1990s, the discovery that Tat was present in the serum of HIV-infected individuals triggered a series of studies aimed at preparation of a Tat vaccine. The preliminary results are controversial and previous experience with vaccination against structural proteins of HIV suggest that caution should be used. In addition to vaccination, some recent papers discussed the possibility of designing and producing synthetic compounds that inhibit either the interaction of extracellular Tat with target uninfected cells or extracellular Tat-generated signal transduction. These recent developments have increased the therapeutic options available for the treatment of AIDS and its related pathologies.

AIDS has long been treated as a classic infectious disease with therapies aimed at eradicating its etiological agent. It is now clear that this strategy is not successful and that an effective anti-AIDS therapy will require a multitargeted approach in which classic antiviral drugs and protease inhibitors are combined with novel extracellular Tat antagonists. This approach should prevent the development of drug-resistant HIV strains, decrease the dosage and related toxicity of each single drug and lead to a cure for AIDS-associated pathologies.

Acknowledgements

This work was supported in part by grants from European Community ("Biologically Active Novel Glycosaminoglycans" contract no. QLK3-CT-1999-00536), Istituto Superiore di Sanità (AIDS Project), Associazione Italiana per la Ricerca sul Cancro, National Research Council (Target Project on Biotechnology) to M.P., from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (Cofin 2000; Project "Meccanismi molecolari di communicazione intracellulare: le molecole solubili" to M.P., Cofin 2000: Project "Trasduzione proteica attraverso le membrane biologiche: un approccio integrato di biologia molecolare e fisica biomolecolare" to M.R., and "ex 60%" to M.R. and M.P.).

References

1. Zagury, D., Bernard, J., Leonard, R. et al. *Long-term cultures of HTLV-III-infected T cells: A model of cytopathology of T-cell depletion in AIDS.* Science 1986, 231: 850-3.

2. Ambrus, J.L., Poiesz, B.J., Lillie, M.A., Stadler, I., Di Berardino, L.A., Chadha, K.C. *Interferon and interferon inhibitor levels in patients infected with varicella-zoster virus, acquired immunodeficiency syndrome, acquired immunodeficiency syndrome-related complex, or Kaposi's sarcoma, and in normal individuals.* Am J Med 1989, 87: 405-7.

3. Zagury, D., Lachgar, A., Chams, V. et al. *Interferon α and Tat involvement in the immunosuppression of uninfected T cells and C-C chemokine decline in AIDS.* Proc Natl Acad Sci USA 1998, 95: 3851-6.

4. Caputo, A., Betti, M., Boarini, C., Mantovani, I., Corallini, A., Barbanti-Brodano, G.B. *Multiple functions of human immunodeficiency virus type 1 Tat protein in the pathogenesis of AIDS.* Recent Res Dev Virol 1999 I, 753-71.

5. Jeang, K.T., Xiao, H., Rich, E.A. *Multifaceted activities of the HIV-1 transactivator of transcription, Tat.* J Biol Chem 1999, 274: 28837-40.

6. Gatignol, A., Jeang, K.T. *Tat as a transcriptional activator and a potential therapeutic target for HIV-1.* Adv Pharmacol 2000, 48: 209-27.

7. Noonan, D., Albini, A. *From the outside in: Extracellular activities of HIV Tat.* Adv Pharmacol 2000, 48: 229-50.

8. Westendorp, M.O., Frank, R., Ochsenbauer, C. et al. *Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120.* Nature 1995, 375: 497-500.

9. Peterlin, B.M., Adams, M., Alonso, A. et al. In: Human Retroviruses. Cullen, B. R. (Ed.). Oxford University Press: Oxford 1993, 75-100.

10. Secchiero, P., Zella, D., Capitani, S., Gallo, R.C., Zauli, G. *Extracellular HIV-1 Tat protein up-regulates the expression of surface CXC-chemokine receptor 4 in resting CD4⁺ T cells.* J Immunol 1999, 162: 2427-31.

11. Ghezzi, S., Noonan, D.M., Aluigi, M.G. et al. *Inhibition of CXCR4-dependent HIV-1 infection by extracellular HIV-1 Tat.* Biochem Biophys Res Commun 2000, 270: 992-6.

12. Liu, Y., Jones, M., Hingtgen, C.M. et al. *Uptake of HIV-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands.* Nat Med 2000, 6: 1380-7.

13. Tyagi, M., Rusnati, M., Presto, M., Giacca, M. *Internalization of HIV-1 Tat requires cell surface heparan sulfate proteoglycan.* J Biol Chem 2001, 276: 3254-61.

14. Dewhurst, S., Gelbard, H.A., Fine, S.M. *Neuropathogenesis of AIDS.* Mol Med Today 1996, January: 16-23.

15. Huang, L.M., Chao, M.F., Chen, M.Y. et al. *Reciprocal regulatory interaction between human herpesvirus 8 and human immunodeficiency virus type 1.* J Biol Chem 2001, 276: 13427-32.

16. Ferbeyre, G., Bourdeau, V., Cedergren, R. *Does HIV tat protein also regulate genes of other viruses present in HIV infection?* Trends Biochem Sci 1997, 22: 115-6.

17. Toth, F.D., Mosborg-Petersen, P., Kiss, J. et al. *Interactions between human immunodeficiency virus type 1 and human cytomegalovirus in human term syncytiotrophoblast cells coinfected with both viruses.* J Virol 1995, 69: 2223-32.

18. Bridges, S.H., Sarver, N. *Gene therapy and immune restoration for HIV disease.* Lancet 1995 345: 427-32.

19. Pomerantz, R.J., Trono, D. *Genetic therapies for HIV infections: Promise for the future.* AIDS 1995, 9: 985-93.

20. Goldstein, G. *HIV-1 Tat protein as a potential AIDS vaccine.* Nat Med 1996, 2: 960-4.

21. Rusnati, M., Tulipano, G., Urbinati, C. et al. *The basic domain in HIV-1 Tat protein as a target for polysulfated heparin-mimicking extracellular Tat antagonists.* J Biol Chem 1998, 273: 16027-37.

22. Gregoire, C.J., Loret, E.P. *Conformational heterogeneity in two regions of TAT results in structural variations of this protein as a function of HIV-1 isolates.* J Biol Chem 1996, 271: 22641-6.

23. Mulligan, M.J., Weber, J. *Human trials of HIV-1 vaccines.* AIDS 1999, 13 (Suppl A): S105-12.

24. Veljkovic, V., Metlas, R., Kohler, H. et al. *AIDS epidemic at the beginning of the third millennium: Time for a new AIDS vaccine strategy.* Vaccine 2001, 19: 1855-62.

25. Stevceva, L., Tryniszewska, E., Hel, Z. et al. *Differences in time of virus appearance in the blood and virus-specific immune responses in intravenous and intrarectal primary SIVmac251 infection of rhesus macaques: A pilot study.* BMC Infect Dis 2001, 1: 9.

26. Froebel, K.S., Aldhous, M.C., Mok, J.Y., Hayley, J., Arnott, M., Peutherer, J.F. *Cytotoxic T lymphocyte activity in children infected with HIV.* AIDS Res Hum Retroviruses 1994, 10(Suppl. 2): S83-8.

27. Addo, M.M., Altfeld, M., Rosenberg, E.S. et al. *The HIV-1 regulatory proteins Tat and Rev are frequently targeted by cytotoxic T lymphocytes derived from HIV-1-infected individuals.* Proc Natl Acad Sci USA 2001, 98: 1781-6.

28. Re, M.C., Furbini, G., Vignoli, M., Ramazzotti, E., Zauli, G., La Placa, M. *Antibody against human immunodeficiency virus type 1 (HIV-1) Tat protein may have influenced the progression of AIDS in HIV-1-infected hemophiliac patients.* Clin Diagn Lab Immunol 1996, 3: 230-2.

29. Re, M.C., Gibellini, D., Furbini, G. et al. *Relationship between the presence of anti-Tat antibody, DNA and RNA viral load.* New Microbiol 2001, 24: 207-15.

30. Van Baalen, C.A., Pontesilli, O., Huisman, R.C. et al. *Human immunodeficiency virus type 1 Rev- and Tat-specific cytotoxic T lymphocyte frequencies inversely correlate with rapid progression to AIDS.* J Gen Virol 1997, 78: 1913-8.

31. Li, C.J., Ueda, Y., Shi, B. et al. *Tat protein induces self-perpetuating permissivity for productive HIV-1 infection.* Proc Natl Acad Sci USA 1997, 94: 8116-20.

32. Korber, B. et al. (Eds.). Human Retroviruses and AIDS 1995. A Compilation and Analysis of Nucleic Acids and Amino Acid Sequences. II-A-55, 56 (Theoretical Biology and Biophysic) Los Alamos National Laboratory, Los Alamos, New Mexico 1995.

33. Cafaro, A., Caputo, A., Fracasso, C. et al. *Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine.* Nat Med 1999, 5: 643-50.

34. Gallo, R.C. *Tat as one key to HIV-induced immune pathogenesis and Tat toxoid as an important component of a vaccine.* Proc Natl Acad Sci U S A 1999, 96: 8324-6.

35. Cafaro, A., Titti, F., Fracasso, C. et al. *Vaccination with DNA containing tat coding sequences and unmethylated CpG motifs protects cynomolgus monkeys upon infection with simian/human immunodeficiency virus (SHIV89.6P).* Vaccine 2001, 19: 2862-77.

36. Cohen, S.S., Li, C., Ding, L. et al. *Pronounced acute immunosuppression in vivo mediated by HIV Tat challenge.* Proc Natl Acad Sci USA 1999, 96: 10842-7.

37. Le Buanec, H., Lachgar, A., Bizzini, B. et al. *A prophylactic and therapeutic AIDS vaccine containing as a component the innocuous Tat toxoid.* Biomed Pharmacother 1998, 52: 431-5.

38. Gringeri, A., Santagostino, E., Muca-Perja, M. et al. *Safety and immunogenicity of HIV-1 Tat toxoid in immunocompromised HIV-1-infected patients.* J Hum Virol 1998, 1: 293-8.

39. Pauza, C.D., Trivedi, P., Wallace, M. et al. *Vaccination with Tat toxoid attenuates disease in simian/HIV-challenged macaques.* Proc Natl Acad Sci USA 2000, 97: 3515-9.

40. Demirhan, I., Chandra, A., Mueller, F. et al. *Antibody spectrum against the viral transactivator protein in patients with human immunodeficiency virus type 1 infection and Kaposi's sarcoma.* J Hum Virol 2000, 3: 137-43.

41. Husain, M., Hahn, T., Yedavalli, V.R., Ahmad, N. *Characterization of HIV type 1 tat sequences associated with perinatal transmission.* AIDS Res Hum Retroviruses 2001, 17: 765-73.

42. Boykins, R.A., Ardans, J.A., Wahl, L.M., Lal, R.B., Yamada, K.M., Dhawan, S. *Immunization with a novel HIV-1-Tat multiple-peptide conjugate induces effective immune response in mice.* Peptides 2000, 21: 1839-47.

43. Morris, C.B., Thanawastien, A., Sullivan, D.E., Clements, J.D. *Identification of a peptide capable of inducing an HIV-1 Tat-specific CTL response.* Vaccine 2002, 20: 12-5.

44. Betti, M., Voltan, R., Marchisio, M. et al. *Characterization of HIV-1 Tat proteins mutated in the transactivation domain for prophylactic and therapeutic application.* Vaccine 2001, 19: 3408-19.

45. Goldstein, G., Manson, K., Tribbick, G., Smith, R. *Minimization of chronic plasma viremia in rhesus macaques immunized with synthetic HIV-1 Tat peptides and infected with a chimeric simian/human immunodeficiency virus (SHIV33).* Vaccine 2000, 18: 2789-95.

46. Caselli, E., Betti, M., Grossi, M.P. et al. *DNA immunization with HIV-1 Tat mutated in the trans activation domain induces humoral and cellular immune responses against wild-type Tat.* J Immunol 1999, 162: 5631-8.

47. Hinkula, J., Lundholm, P., Wahren, B. *Nucleic acid vaccination with HIV regulatory genes: A combination of HIV-1 genes in separate plasmids induces strong immune responses.* Vaccine 1997, 15: 874-8.

48. Chenciner, N., Randrianarison-Jewtoukoff, V., Delpeyroux, F. et al. *Enhancement of humoral immunity to SIVenv following simultaneous inoculation of mice by three recombinant adenoviruses encoding SIVenv/poliovirus chimeras, Tat and Rev.* AIDS Res Hum Retroviruses 1997, 13: 801-6.

49. Osterhaus, A.D., van Baalen, C.A., Gruters, R.A. et al. *Vaccination with Rev and Tat against AIDS.* Vaccine 1999, 17: 2713-4.

50. Kjerrstrom, A., Hinkula, J., Engstrom, G. et al. *Interactions of single and combined human immunodeficiency virus type 1 (HIV-1) DNA vaccines.* Virology 2001, 284: 46-61.

51. Rusnati, M., Coltrini, D., Oreste, P. et al. *Interaction of HIV-1 Tat protein with heparin: Role of the backbone structure, sulfation, and size.* J Biol Chem 1997, 272: 11313-20.

52. Rusnati, M., Tulipano, G., Spillmann, D. et al. *Multiple interaction of HIV-1 Tat protein with size-defined heparin oligosaccharides.* J Biol Chem 1999, 274: 28198-205.

53. Corallini, A., Betti, M., Rusnati, M. et al. *Characterization of the effects of two polysulfonated distamycin A derivatives, PNU145156E and PNU153429, on HIV type 1 Tat protein.* AIDS Res Hum Retroviruses 1998, 14: 1561-71.

54. Possati, L., Campioni, D., Sola, F. et al. *Antiangiogenic, antitumoural and antimetastatic effects of two distamycin A derivatives with anti-HIV-1 Tat activity in a Kaposi's sarcoma-like murine model.* Clin Exp Metastasis 1999, 17: 575-582.

55. Watson, K., Gooderham, N.J., Davies, D.S., Edwards, R.J. *Interaction of the transactivating protein HIV-1 Tat with sulphated polysaccharides.* Biochem Pharmacol 1999, 57: 775-83.

56. Rusnati, M., Urbinati, C., Caputo, A. et al. *Pentosan polysulfate as inhibitor of extracellular HIV-1 Tat.* J Biol Chem 2001, 276: 22420-5.

57. Naggi, A., Torri, G., Casu, B. et al. *Toward a biotechnological heparin through combined chemical and enzymatic modification of the Escherichia coli K5 polysaccharide.* Semin Thromb Hemost 2001, 27: 437-43.

58. Leali, D., Belleri, M., Urbinati, C. et al. *Fibroblast growth factor-2 antagonist activity and angiostatic capacity of sulfated Escherichia coli K5 polysaccharide derivatives.* J Biol Chem 2001, 276: 37900-8.

59. Albini, A., Soldi, R., Giunciuglio, D. et al. *The angiogenesis induced by HIV-1 Tat protein is mediated by the Flk-1/KDR receptor on vascular endothelial cells.* Nat Med 1996, 2: 1371-5.

60. Kumar, C.C., Malkowski, M., Yin, Z. et al. *Inhibition of angiogenesis and tumor growth by SCH221153, a dual $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin receptor antagonist.* Cancer Res 2001, 61: 2232-8.

61. Barthelemy, S., Vergnes, L., Moynier, M., Guyot, D., Labidalle, S., Bahraoui, E. *Curcumin and curcumin derivatives inhibit Tat-mediated transactivation of type 1 human immunodeficiency virus long terminal repeat.* Res Virol 1998, 149: 43-52.

62. Jiang, M.C., Lin, J.K., Chen, S.S.L. *Inhibition of HIV-1 Tat-mediated transactivation by quinacrine and chloroquine.* Biochem Biophys Res Commun 1996, 226: 1-7.

63. Rusnati, M., Urbinati, C., Musulin, B. et al. *Activation of endothelial cell mitogen activated protein kinase $ERK_{(1/2)}$ by extracellular HIV-1 Tat protein.* Endothelium 2001, 8: 65-74.

64. Bruce-Keller, A.J., Barger, S.W., Moss, N.I., Pham, J.T., Keller, J.N., Nath, A. *Pro-inflammatory and pro-oxidant properties of the HIV protein Tat in a microglial cell line: Attenuation by 17β-estradiol.* J Neurochem 2001, 78: 1315-24.

65. Leung, D.W., Peterson, P.K., Weeks, R. et al. *CT-2576, an inhibitor of phospholipid signaling, suppresses constitutive and induced expression of human immunodeficiency virus.* Proc Natl Acad Sci USA 1995, 92: 4813-7.

66. Lokensgard, J.R., Hu, S., Hegg, C.C., Thayer, S.A., Gekker, G., Peterson, P.K. *Diazepam inhibits HIV-1 Tat-induced migration of human microglia.* J Neurovirol 2001, 7: 481-6.

67. Beauparlant, P., Kwon, H., Clarke, M. et al. *Transdominant mutants of I κ B α block Tat-tumor necrosis factor synergistic activation of human immunodeficiency virus type 1 gene expression and virus multiplication.* J Virol 1996, 70: 5777-85.

68. Maciaszek, J.W., Parada, N.A., Cruikshank, W.W., Center, D.M., Kornfeld, H., Viglianti, G.A. *IL-16 represses HIV-1 promoter activity.* J Immunol 1997, 158: 5-8.

69. Chen, F., Lu, Y., Castranova, V. et al. *Nitric oxide inhibits HIV Tat-induced NF-κB activation.* Am J Pathol 1999, 155: 275-84.

70. Lisziewicz, J., Sun, D., Lisziewicz, A., Gallo, R.C. *Antitat gene therapy: A candidate for late-stage AIDS patients.* Gene Ther 1995, 2: 218-22.

71. Manca, F., Fenoglio, D., Franchin, E. et al. *Anti-HIV genetic treatment of antigen-specific human CD4 lymphocytes for adoptive immunotherapy of opportunistic infections in AIDS.* Gene Ther 1997, 4: 1216-24.

72. Li, Y., Starr, S.E., Lisziewicz, J., Ho, W.Z. *Inhibition of HIV-1 replication in chronically infected cell lines and peripheral blood mononuclear cells by retrovirus-mediated antitat gene transfer.* Gene Ther 2000, 7: 321-8.

73. Lisziewicz, J., Sun, D., Gallo, R.C., Ensoli, B., Lori, F. *Efficacy of antitat gene therapy in the presence of high multiplicity infection and inflammatory cytokines.* Hum Gene Ther 1996, 7: 2209-16.

74. Donahue, R.E., Bunnell, B.A., Zink, M.C. et al. *Reduction in SIV replication in rhesus macaques infused with autologous lymphocytes engineered with antiviral genes.* Nat Med 1998, 4: 181-6.

75. Zhou, D.-M., Taira, K. *The hydrolysis of RNA: From theoretical calculations to the hammerhead ribozyme-mediated cleavage of RNA.* Chem Rev 1998, 98: 991-1026.

76. Ojwang, J.O., Hampel, A., Looney, D.J., Wong-Staal, F., Rappaport, J. *Inhibition of human immunodeficiency virus type 1 expression by a hairpin ribozyme.* Proc Natl Acad Sci USA 1992, 89: 10802-6.

77. Zhou, C., Bahner, I.C., Larson, G.P., Zaia, J.A., Rossi, J.J., Kohn, E.B. *Inhibition of HIV-1 in human T-lymphocytes by retrovirally transduced anti-tat and rev hammerhead ribozymes.* Gene 1994, 149: 33-9.

78. Sun, L.Q., Wang, L., Gerlach, W.L., Symonds, G. *Target sequence-specific inhibition of HIV-1 replication by ribozymes directed to tat RNA.* Nucleic Acids Res 1995, 23: 2909-13.

79. Amado, R.G., Mitsuyasu, R.T., Symonds, G. et al. *A phase I trial of autologous CD34⁺ hematopoietic progenitor cells transduced with an anti-HIV ribozyme.* Hum Gene Ther 1999, 10: 2255-70.

80. Kuwabara, T., Warashina, M., Nakayama, A., Ohkawa, J., Taira, K. *tRNAVal-heterodimeric maxizymes with high potential as gene inactivating agents: Simultaneous cleavage at two sites in HIV-1 Tat mRNA in cultured cells.* Proc Natl Acad Sci USA 1999, 96: 1886-91.

81. Wang, L., Witherington, C., King, A. et al. *Preclinical characterization of an anti-tat ribozyme for therapeutic application.* Hum Gene Ther 1998, 9: 1283-91.

82. Unwalla, H., Banerjea, A.C. *Novel mono- and di-DNA-enzymes targeted to cleave TAT or TAT-REV RNA inhibit HIV-1 gene expression.* Antiviral Res 2001, 51: 127-39.

83. Unwalla, H., Banerjea, A.C. *Inhibition of HIV-1 gene expression by novel macrophage-tropic DNA enzymes targeted to cleave HIV-1 TAT/Rev RNA.* Biochem J 2001, 357: 147-55.

84. Rosenzweig, M., Marks, D.F., Hempel, D., Lisziewicz, J., Johnson, R.P. *Transduction of CD34⁺ hematopoietic progenitor cells with an antitat gene protects T-cell and macrophage progeny from AIDS virus infection.* J Virol 1997, 71: 2740-6.

85. Bauer, G., Valdez, P., Kearns, K. et al. *Inhibition of human immunodeficiency virus-1 (HIV-1) replication after transduction of granulocyte colony-stimulating factor-mobilized CD34⁺ cells from HIV-1-infected donors using retroviral vectors containing anti-HIV-1 genes.* Blood 1997, 89: 2259-67.

86. Poznansky, M.C., La Vecchio, J., Silva-Arietta, S. et al. *Inhibition of human immunodeficiency virus replication and growth advantage of CD4⁺ T cells and monocytes derived from CD34⁺ cells transduced with an intracellular antibody directed against human immunodeficiency virus type 1 Tat.* Hum Gene Ther 1999, 10: 2505-14.

87. Arzumanov, A., Walsh, AP., Liu, X., Rajwanshi, V.K., Wengel, J., Gait, M.J. *Oligonucleotide analogue interference with the HIV-1 Tat protein-TAR RNA interaction.* Nucleosides Nucleotides Nucleic Acids 2001, 20: 471-80.

88. Arzumanov, A., Walsh, A.P., Rajwanshi, V.K., Kumar, R., Wengel, J., Gait, M. J. *Inhibition of HIV-1 Tat-dependent trans activation by steric block chimeric 2'-O-methyl/LNA oligoribonucleotides.* Biochemistry 2001, 40: 14645-54.

89, Chang, H.K., Gendelman, R., Lisziewicz, J., Gallo, R.C., Ensoli, B. *Block of HIV-1 infection by a combination of antisense tat RNA and TAR decoys: A strategy for control of HIV-1.* Gene Ther 1994, 1: 208-16.

90. Lee, S.W., Gallardo, H.F., Gaspar, O., Smith, C., Gilboa, E. *Inhibition of HIV-1 in CEM cells by a potent TAR decoy.* Gene Ther 1995, 2: 377-84.

91. Jayan, G.C., Cordelier, P., Patel, C. et al. *SV40-derived vectors provide effective transgene expression and inhibition of HIV-1 using constitutive, conditional, and pol III promoters.* Gene Ther 2001, 8: 1033-42.

92. Bohjanen, P.R., Colvin, R.A., Puttaraju, M., Been, M.D., Garcia-Blanco, M.A. *A small circular TAR RNA decoy specifically inhibits Tat-activated HIV-1 transcription.* Nucleic Acids Res 1996, 24: 3733-8.

93. Ulich, C., Harrich, D., Estes, P., Gaynor, R.B. *Inhibition of human immunodeficiency virus type 1 replication is enhanced by a combination of transdominant Tat and Rev proteins.* J Virol 1996, 70: 4871-6.

94. Orsini, M.J., Debouck, C.M. *Inhibition of human immunodeficiency virus type 1 and type 2 Tat function by transdominant Tat protein localized to both the nucleus and cytoplasm.* J Virol 1996, 70: 8055-63.

95. Rossi, C., Balboni, P.G., Betti, M. et al. *Inhibition of HIV-1 replication by a Tat transdominant negative mutant in human peripheral blood lymphocytes from healthy donors and HIV-1-infected patients.* Gene Ther 1997, 4: 1261-9.

96. Fraisier, C., Abraham, D.A., van Oijen, M. et al. *Inhibition of Tat-mediated transactivation and HIV replication with Tat mutant and repressor domain fusion proteins.* Gene Ther 1998, 5: 946-54.

97. Kashanchi, F., Sadaie, M.R., Brady, J.N. *Inhibition of HIV-1 transcription and virus replication using soluble Tat peptide analogs.* Virology 1997, 227: 431-8.

98. Hamy, F., Felder, E.R., Heizmann, G. et al. *An inhibitor of the Tat/TAR RNA interaction that effectively suppresses HIV-1 replication.* Proc Natl Acad Sci USA 1997, 94: 3548-53.

99. Choudhury, I., Wang, J., Rabson, A.B. et al. *Inhibition of HIV-1 replication by a Tat RNA-binding domain peptide analog.* J Acquir Immune Defic Syndr Hum Retrovirol 1998, 17: 104-11.

100. Dassonneville, L., Hamy, F., Colson, P., Houssier, C., Bailly, C. *Binding of Hoechst 33258 to the TAR RNA of HIV-1. Recognition of a pyrimidine bulge-dependent structure.* Nucleic Acids Res 1997, 25: 4487-92.

101. Mischiati, C., Jeang, K.T., Feriotto, G. et al. *Aromatic polyamidines inhibiting the Tat-induced HIV-1 transcription recognize structuredTAR-RNA.* Antisense Nucleic Acid Drug Dev 2001, 11: 209-17.

102. Lapidot, A., Ben-Asher, E., Eisenstein, M. *Tetrahydropyrimidine derivatives inhibit binding of a Tat-like, arginine-containing peptide, to HIV TAR RNA in vitro.* FEBS Lett 1995, 367: 33-8.

103. Marasco, W.A., La Vecchio, J., Winkler, A. *Human anti-HIV-1 Tat sFv intrabodies for gene therapy of advanced HIV-1-infection and AIDS.* J Immunol Methods 1999, 231: 223-38.

104. Mhashilkar, A,M., La Vecchio, J., Eberhardt, B. et al. *Inhibition of human immunodeficiency virus type 1 replication in vitro in acutely and persistently infected human CD4⁺ mononuclear cells expressing murine and humanized anti-human immunodeficiency virus type 1 Tat single-chain variable fragment intrabodies.* Hum Gene Ther 1999, 10: 1453-67.

105. Mhashilkar, A.M., Bagley, J., Chen, S.Y., Szilvay, A.M., Helland, D.G., Marasco, W.A. *Inhibition of HIV-1 Tat-mediated*

LTR transactivation and HIV-1 infection by anti-Tat single chain intrabodies. EMBO J 1995, 14: 1542-51.

106. Friedler, A., Friedler, D., Luedtke, N.W., Tor, Y., Loyter, A., Gilon, C. *Development of a functional backbone cyclic mimetic of the HIV-1 Tat arginine-rich motif.* J Biol Chem 2000, 275: 23783-9.

107. Chao, S.H., Fujinaga, K., Marion, J.E. et al. *Flavopiridol inhibits P-TEFb and blocks HIV-1 replication.* J Biol Chem 2000, 275: 28345-8.

108. Okamoto, H., Cujec, T.P., Peterlin, B.M., Okamoto, T. *HIV-1 replication is inhibited by a pseudo-substrate peptide that blocks Tat transactivation.* Virology 2000, 270: 337-44.

109. Majello, B., De Luca, P., Hagen, G., Suske, G., Lania, L. *Different members of the Sp1 multigene family exert opposite transcriptional regulation of the long terminal repeat of HIV-1.* Nucleic Acids Res 1994, 22: 4914-21.

110. Gnabre, J.N., Brady, J.N., Clanton, D.J., Ito, Y., Dittmer, J., Bates, R.B., Huang, R.C. *Inhibition of human immunodeficiency virus type 1 transcription and replication by DNA sequence-selective plant lignans.* Proc Natl Acad Sci USA 1995, 92: 11239-43.

111. Hsu, M.C., Schutt, A.D., Holly, M. et al. *Inhibition of HIV replication in acute and chronic infections in vitro by a Tat antagonist.* Science 1991, 254: 1799-802.

112. Shahabuddin, M., Volsky, B., Hsu, M.C., Volsky, D.J. *Restoration of cell surface CD4 expression in human immunodeficiency virus type 1-infected cells by treatment with a Tat antagonist.* J Virol 1992, 66: 6802-5.

113. Hsu, M.C., Schutt, A.D., Holly, M. et al. *Discovery and characterization of an HIV-1 Tat antagonist.* Biochem Soc Trans 1992, 20: 525-31.

114. Hsu, M.C., Dhingra, U., Earley, J.V. et al. *Inhibition of type 1 human immunodeficiency virus replication by a Tat antagonist to which the virus remains sensitive after prolonged exposure in vitro.* Proc Natl Acad Sci USA 1993, 90: 6395-9.

115. Haubrich, R.H., Flexner, C., Lederman, M.M. et al. *A randomized trial of the activity and safety of Ro 24-7429 (Tat antagonist) versus nucleoside for human immunodeficiency virus infection. The AIDS Clinical Trials Group 213 Team.* J Infect Dis 1995, 172: 1246-52.

116. Dunne, A.L., Siregar, H., Mills, J., Crowe, S.M. *HIV replication in chronically infected macrophages is not inhibited by the Tat inhibitors Ro-5-3335 and Ro-24-7429.* J Leukoc Biol 1994, 56: 369-73.

117. Kira, T., Hashimoto, K., Baba, M., Okamoto, T., Shigeta, S. *2-Glycineamide-5-chlorophenyl 2-pyrryl ketone, a non-benzodiazepin Tat antagonist, is effective against acute and chronic HIV-1 infections in vitro.* Antiviral Res 1996, 32: 55-62.

118. Okamoto, H., Cujec, T.P., Okamoto, M., Peterlin, B.M., Baba, M., Okamoto, T. *Inhibition of the RNA-dependent transactivation and replication of human immunodeficiency virus type 1 by a fluoroquinoline derivative K-37.* Virology 2000, 272: 402-8.

119. Baba, M., Okamoto, M., Kawamura, M. et al. *Inhibition of human immunodeficiency virus type 1 replication and cytokine production by fluoroquinoline derivatives.* Mol Pharmacol 1998, 53: 1097-103.

120. Baba, M., Okamoto, M., Takeuchi, H. *Inhibition of human immunodeficiency virus type 1 replication in acutely and chronically infected cells by EM2487, a novel substance produced by a Streptomyces species.* Antimicrob Agents Chemother 1999, 43: 2350-5.

121. Mitra, D., Sikder, S., Laurence, J. *Inhibition of tat-activated, HIV-1 long terminal repeat-mediated gene expression by glucocorticoids.* AIDS Res Hum Retroviruses 1993, 9: 1055-6.

122. Chandra, A., Demirhan, I., Arya, S.K., Chandra, P. *D-Penicillamine inhibits transactivation of human immunodeficiency virus type-1 (HIV-1) LTR by transactivator protein.* FEBS Lett 1988, 236: 282-6.

123. Demirhan, I., Chandra, A., Sarin, P.S., Hasselmayer, O., Hofmann, D., Chandra, P. *Inhibition of Tat-mediated HIV-1-LTR transactivation and virus replication by sulfhydryl compounds with chelating properties.* Anticancer Res 2000, 20: 2513-7.

124. Li, C.J., Wang, C., Pardee, A.B. *Camptothecin inhibits Tat-mediated transactivation of type 1 human immunodeficiency virus.* J Biol Chem 1994, 269: 7051-4.

125. Biswas, D.K., Tius, M.A., Zhuo, J., Pardee, A.B. *Canventol inhibits HIV-1 replication by Tat-induced Tar-independent mechanism.* J Acquir Immune Defic Syndr Hum Retrovirol 1996, 12: 120-7.

126. Michne, W.F., Schroeder, J.D., Bailey, T.R. et al. *Keto/enol epoxy steroids as HIV-1 Tat inhibitors: Structure-activity relationships and pharmacophore localization.* J Med Chem 1995, 38: 3197-206.

127. Iurlaro, M., Benelli, R., Masiello, L., Rosso, M., Santi, L., Albini, A. *β Interferon inhibits HIV-1 Tat-induced angiogenesis: Synergism with 13-cis retinoic acid.* Eur J Cancer 1998, 34: 570-6.

128. Barillari, G., Sgadari, C., Palladino, C. et al. *Inflammatory cytokines synergize with the HIV-1 Tat protein to promote angiogenesis and Kaposi's sarcoma via induction of basic fibroblast growth factor and the $\alpha_v\beta_3$ integrin.* J Immunol 1999, 163: 1929-35.

129. Sodhi, A., Montaner, S., Patel, V. et al. *The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1α.* Cancer Res 2000, 60: 4873-80.

130. Lederman, S., Gulick, R., Chess, L. *Dextran sulfate and heparin interact with CD4 molecules to inhibit the binding of coat protein (gp120) of HIV.* J Immunol 1989, 143: 1149-54.

131. Schols, D., Pauwels, R., Desmyter, J., De Clercq, E. *Dextran sulfate and other polyanionic anti-HIV compounds specifically interact with the viral gp120 glycoprotein expressed by T-cells persistently infected with HIV-1.* Virology 1990, 175: 556-61.

132. Anand, R., Nayyar, S., Galvin, T.A., Merril, C.R., Bigelow, L.B. *Sodium pentosan polysulfate (PPS), an anti-HIV agent also exhibits synergism with AZT, lymphoproliferative activity, and virus enhancement.* AIDS Res Hum Retroviruses 1990, 6: 679-89.

133. Baba, M., Schols, D., Pauwels, R., Nakashima, H., De Clercq, E. *Sulfated polysaccharides as potent inhibitors of HIV-induced syncytium formation: A new strategy towards AIDS chemotherapy.* J Acquir Immune Defic Syndr 1990, 3: 493-9.

134. Sydow, G., Klocking, H.P. *Effect of pentosan polysulfate (SP 54) on the reverse transcriptase activity of several retroviruses.* Biomed Biochim Acta 1987, 46: 527-30.

135. Weber, J., Nunn, A., O'Connor, T. et al. *'Chemical condoms' for the prevention of HIV infection: Evaluation of novel agents against SHIV(89.6PD) in vitro and in vivo.* AIDS 2001, 15: 1563-8.

136. Clanton, D.J., Buckheit, R.W. Jr., Terpening, S.J. et al. *Novel sulfonated and phosphonated analogs of distamycin which inhibit the replication of HIV.* Antiviral Res 1995, 27: 335-54.

137. Ryabinin, V.A., Zakharova, O.D., Yurchenko, E.Y. et al. *Synthesis and evaluation of oligo-1,3-thiazolecarboxamide derivatives as HIV-1 reverse transcriptase inhibitors.* Bioorg Med Chem 2000, 8: 985-93.

138. Neamati, N., Mazumder, A., Sunder, S. et al. *Highly potent synthetic polyamides, bisdistamycins, and lexitropsins as inhibitors of human immunodeficiency virus type 1 integrase.* Mol Pharmacol 1998, 54: 280-90.

139. Howard, O.M., Korte, T., Tarasova, N.I. et al. *Small molecule inhibitor of HIV-1 cell fusion blocks chemokine receptor-mediated function.* J Leukoc Biol 1998, 64: 6-13.

140. Manetti, F., Corelli, F., Monelli, N., Borgia, A.L., Botta, M. *Research on anti-HIV-1 agents. Investigation on the CD4-Suradista binding mode through docking experiments.* J Comput Aided Mol Des 2000, 14: 355-68.

141. Batinic, D., Robey, F.A. *The V3 region of the envelope glycoprotein of human immunodeficiency virus type 1 binds sulfated polysaccharides and CD4-derived synthetic peptides.* J Biol Chem 1992, 267: 6664-71.

142. Rusnati, M., Presta, M. *Interaction of angiogenic basic fibroblast growth factor with endothelial cell heparan sulfate proteoglycans: Biological implications in neo-vascularization.* Int J Clin Lab Res 1996, 26: 15-23.

143. Daelemans, D., Schols, D., Witvrouw, M. et al. *A second target for the peptoid Tat/transactivation response element inhibitor CGP64222: Inhibition of human immunodeficiency virus replication by blocking CXC-chemokine receptor 4-mediated virus entry.* Mol Pharmacol 2000, 57: 116-24.

144. O'Brien, W.A., Sumner-Smith, M., Mao, S.H., Sadeghi, S., Zhao, J.Q., Chen, I.S. *Anti-human immunodeficiency virus type 1 activity of an oligocationic compound mediated via gp120 V3 interactions.* J Virol 1996, 70: 2825-31.

145. Gelus, N., Bailly, C., Hamy, F., Klimkait, T., Wilson, W.D., Boykin, D.W. *Inhibition of HIV-1 Tat-TAR interaction by diphenylfuran derivatives: Effects of the terminal basic side chains.* Bioorg Med Chem 1999, 7: 1089-96.

146. Hamasaki, K., Ueno, A. *Aminoglycoside antibiotics, neamine and its derivatives as potent inhibitors for the RNA-protein interactions derived from HIV-1 activators.* Bioorg Med Chem Lett 2001, 11: 591-4.

147. Marciniak, R.A., Sharp, P.A. *HIV-1 Tat protein promotes formation of more-processive elongation complexes.* EMBO J 1991, 10: 4189-96.

148. Daelemans, D., Este, J.A., Witvrouw, M. et al. *S-adenosylhomocysteine hydrolase inhibitors interfere with the replication of human immunodeficiency virus type 1 through inhibition of the LTR transactivation.* Mol Pharmacol 1997, 52: 1157-63.

149. De Clercq, E. *Carbocyclic adenosine analogues as S-adenosylhomocysteine hydrolase inhibitors and antiviral agents: Recent advances.* Nucleosides Nucleotides 1998, 17: 625-34.

150. Biswas, D.K., Ahlers, C.M., Dezube, B.J., Pardee, A.B. *Cooperative inhibition of NF-κ B and Tat-induced superactivation of human immunodeficiency virus type 1 long terminal repeat.* Proc Natl Acad Sci USA 1993, 90: 11044-8.

151. Chatterjee, S., Johnson, P.R., Wong, K.K. Jr. *Dual-target inhibition of HIV-1 in vitro by means of an adeno-associated virus antisense vector.* Science 1992, 258: 1485-8.

152. Liu, D., Donegan, J., Nuovo, G., Mitra, D., Laurence, J. *Stable human immunodeficiency virus type 1 (HIV-1) resistance in transformed CD4$^+$ monocytic cells treated with multitargeting HIV-1 antisense sequences incorporated into U1 snRNA.* J Virol 1997, 71: 4079-85.

153. Bai, J., Rossi, J., Akkina, R. *Multivalent anti-CCR ribozymes for stem cell-based HIV type 1 gene therapy.* AIDS Res Hum Retroviruses 2001, 17: 385-99.

154. Poznansky, M.C., Foxall, R., Mhashilkar, A. et al. *Inhibition of human immunodeficiency virus replication and growth advantage of CD4$^+$ T cells from HIV-infected individuals that express intracellular antibodies against HIV-1 gp120 or Tat.* Hum Gene Ther 1998, 9: 487-96.

155. Mhashilkar, A.M., Biswas, D.K., LaVecchio, J., Pardee, A.B., Marasco, W.A. *Inhibition of human immunodeficiency virus type 1 replication in vitro by a novel combination of anti-Tat single-chain intrabodies and NF-κ B antagonists.* J Virol 1997, 71: 6486-94.