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Review

GENE THERAPY FOR HIV-1 INFECTION: ARE LETHAL GENES A VALUABLE TOOL?

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Abstract - At present, virus replication is quite efficiently blocked by highly active antiretroviral therapy (HAART). However current HAART therapies still have important limitations that compromise in the long term the health of the patient. This failure is due to the existence of viral sanctuaries of replication-competent HIV-1, which can persists for the whole life. Two main strategies of HIV-1 gene therapy have been tried. One is based in the rationale of killing the infected cells before the virus can produce infective particles and the other strategy is to protect the cell from the virus. The first strategy uses lethal genes that are expressed in response to the expression of a HIV-1 provirus. Although technical limitations and safety concerns have limited the use of this approach in the last years, recent development of efficient lentiviral vectors and the need of the elimination of the latently infected cells are reasons to reconsider and come back to use this approach. Elimination of latently infected cells would also represent a perfect complement of the HAART therapy and may represent a hope in the long term for the development of a real cure of this infection.

Key words: Gene therapy, HIV-1, lethal genes, lentiviral vectors

INTRODUCTION

In the last 8 years it has been developed new drug combinations that represent important improvements in the treatment of the infection by HIV-1. In developed countries, and for those patients that can receive the anti-retroviral therapy, this mortal disease has become into a chronic disease. At present, virus replication is in most patients quite efficiently blocked by highly active antiretroviral therapy (HAART). However, we are still far from a real cure of the disease, and the current HAART therapies still have important limitations that compromise in the long term the health of the patient. One limitation of the therapies is originated because the virus has such a high rate of mutation, that even in combined triple or quadruple drug therapeutic regimens, drug resistant strains emerge in the patients in prolonged HAART therapies (17,98). At the same time, the serious side effects of HAART make it intolerable for some patients, and in general, it compromises the health of the patient in the long term. The side effects also produce, in many cases, failures in the drug adherence, which in turns accelerates the emergence of drug resistant strains. However, the most severe limitation of current therapies is the failure to eradicate the virus and as a consequence, the patient is chronically infected. This failure is due to the existence of viral sanctuaries of replication-competent HIV-1, which can persists in the patient probably for its whole life (25,42,111). For one hand, drugs cannot reach some tissues as it is the central nervous system (CNS) where the virus can infect macrophages and microglia cells (86), or as the retina or testis (88,117). But the most important sanctuary is constituted by the latently infected resting $CD4^+$ T cells because they can persist for the whole life of the patient (41,92,93,99). These cells contain an integrated provirus that is silent, but that can become productive at any moment if the cell is activated by the presence of an antigen. Current estimations indicate that there are about 0.1 to 1 per 10^6 $CD4^+$ T lymphocytes infected with replication competent viruses resting in patients receiving efficient suppressive HAART regimens (41,42,56,92,118). The complexity of the treatment of the HIV-1 infection and relevance of the disease justifies that many efforts have been made aimed to develop efficient anti-HIV-1 gene therapy approaches. In this review it is made a revision of the main strategies used in HIV-1 therapy and the limitations and possibilities of future development.

Abbreviations: **CNS**: central nervous system; **HAART**: highly active antiretroviral therapy

BASIC HIV-1 GENETICS

HIV-1 is a member of the retrovirus family and it belongs to the lentivirus genus, which shows remarkable differences with other retroviruses as the oncoviruses. The HIV-1 genome contains three genes that are common to all retroviruses (*gag*, *pol* and *env*), and six genes that are specific of HIV-1 and related lentiviruses (*tat, rev, nef, vif, vpr* and *vpu*) (Fig. 1). Viral particles contain two genomic single-stranded RNAs that are reverse transcribed shortly after infection of susceptible cells. The double-stranded cDNA once inside the nucleus of the cell is integrated into chromosomal DNA. At this stage it becomes a provirus, which is expressed in response to host factors and normally leads to the production of new viral particles and to the cell death.

The *gag* and *env* genes encode for the structural proteins and the *pol* gene encodes for the protease, reverse transcriptase and integrase enzymes. The *pol* gene products are the targets for the HAART therapies, especially the reverse transcriptase and the protease. The *gag-pol* and the *env* genes are traduced as long proproteins that are processed and activated by the protease activity. The *vif, vpr, vpu* and *nef* genes encode for proteins that have been termed "accessory" or "auxiliary" to reflect the fact that they are needed for virus replication in some cellular types. However, these genes are essential to make an effective infection in the patient.

The viral genes are expressed in response to cellular transcription factors (48), and by the activity of the Tat and Rev proteins (107). Integrated provirus is expressed in a full length RNA that contains several splicing donors and acceptor sites (100). Fully spliced RNA encodes three proteins that accumulate in the cell nucleus, Tat, Rev and Nef. Tat is a potent transactivator that together to host factors binds a 5' stem-loop of the nascent RNA (TAR) that stabilise the RNA polymerase II complex by hyperphosphorylation (10,57). Rev protein is inactive until a threshold is reached, then multiple Rev proteins bind a sequence present inside the *env* gene (RRE) and at the

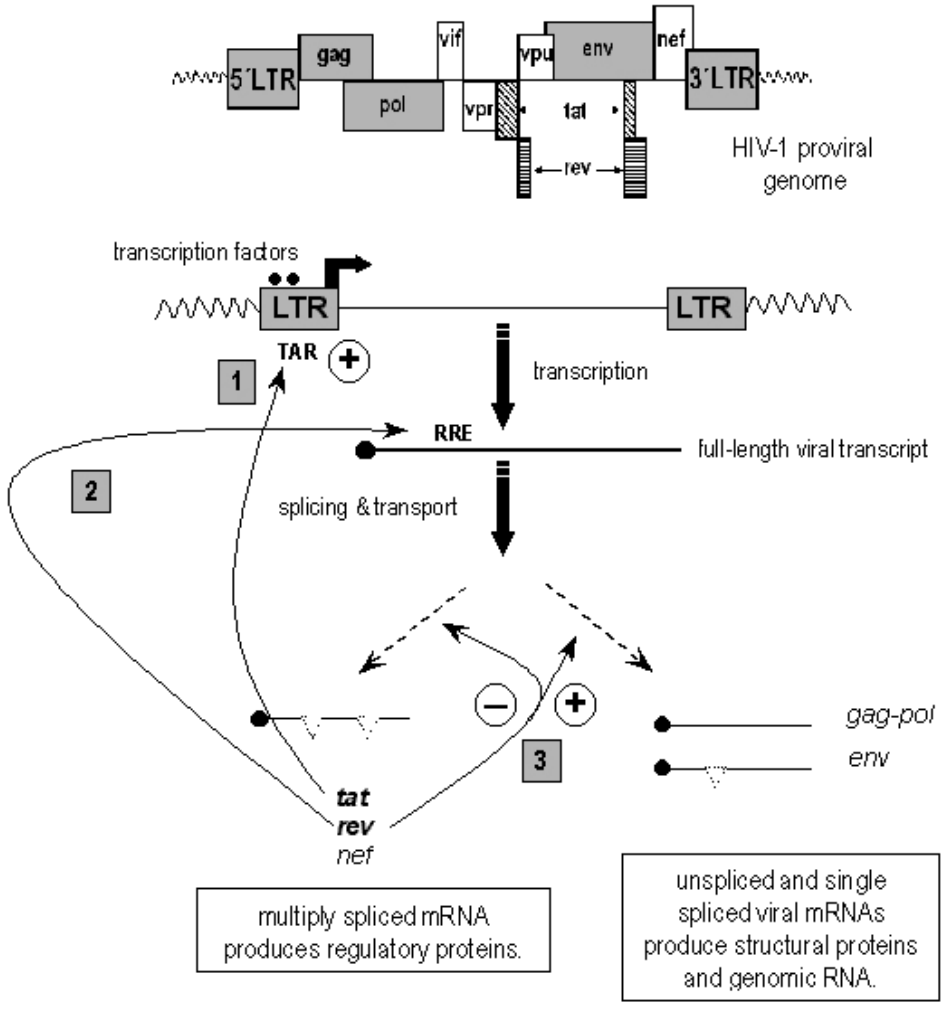

Fig. 1 HIV-1 genome and the expression of their genes

same time associates with the host nuclear export protein CRM1 (43,55,82). Rev protein is therefore involved in nuclear export of unspliced or single spliced transcripts, and allows the expression of the genes (*gag, pol, env, vif, vpu* and *vpr*) (38,51,72) (Fig. 1).

MAIN STRATEGIES USED IN THE GENE THERAPY OF HIV-1 INFECTION

Gene therapy of HIV-1 has been performed by many different experimental approaches. However, two main strategies have been tried. One is based in the rationale of killing the infected cells before the virus can produce infective particles, with the aim of producing a kind of sink where the virus can not replicate and get the clearance of the virus from the blood and infected tissues. Another strategy is to protect the cell from the virus, avoiding its infection or if infected, producing an inhibition of the HIV-1 replication.

Lethal genes used to eliminate infected cells

The first strategy uses toxic genes to induce the cell death if the cell is infected. The vector expressing the lethal gene must eliminate the infected cell faster than the virus produce its progeny. The idea of eliminate the infected cells is quite reasonable because it is a rapid way of eliminating the virus, and it is preferable to get rid of the infected cells than maintain them in the patient for an undetermined period of time. However, this strategy is receiving less attention in the last years, probably because the population of transduced cells is expected to be diminishing and the probable outcome is the elimination of them (74,76). Therefore, this strategy utility seems to be limited by the need of the development of a very efficient *in vivo* transduction or repeated events of transduction. Another concern regarding this approach is that the introduction of toxic or lethal genes into healthy cells may compromise the survival and / or the functionality of the cell. Therefore, any gene therapy strategy that uses this kind of approach should contain enough safety mechanisms to avoid any harm to the cell or any interference with the normal function.

The strategy of eliminating the infected cells has the limitation of the long-term lost of the transduced cells, which make that the efficiency decay with the time. However, if a huge number of HIV-1 target cells were genetically modified, this kind of approach would be most efficient to get in the total elimination of the virus or at least producing a long-term suppression of the virus spread. Any therapy that can get the patient rid of the severe adverse side effects of the HAART therapies would be of great benefits for the patients. For that reason, and although it is true that there are still technical difficulties to get such an efficient transduction, it is up to some extend surprising that in the last years it has been put least effort in this strategy than in the last decade. This type of strategy is performed using suicide vectors that express a toxic gene in response to viral regulatory proteins as Tat or Rev. *in vitro* virus spread inhibition and selective killing of infected cells was obtained with the *herpes simplex* virus type 1 thymidine kinase gene, which induce acyclovir and ganciclovir sensitivity (16,22), diphteria toxin A chain gene (30,32,53,54,91), the interferon $\alpha 2$ gene (91) and the cytosine deaminase gene (91). Induction of apoptosis by the over expression of P53 in response to the presence of HIV-1 provirus, produce a very efficient and specific destruction of infected cells (R. Oya, L Saniger and F. Luque, unpublished data).

Anti-HIV-1 genes used to inhibit viral replication

The second strategy produces inhibition of the normal replication virus cycle by interfering viral or cellular function required for the viral cycle. This strategy has the disadvantage that it does not eliminate infected cells and therefore the virus is not eliminated. On the contrary, the aim of this approach is to get a population of cells resistant to the virus that might reconstitute the immune function. An important difference of this strategy with the use of toxic genes is that in this case the transduced cells have a selective advantage over the untransduced, and therefore it will produce a tendency of getting augmented its proportion over the untransduced sensitive cells (76). Therefore, a subpopulation of virus resistant cells will be established in the patient. However, it remains unclear if this subpopulation of transduced cells may be sufficient to restore an efficient immune function. An interesting approach is to make the genetic modification of haematopoietic stem cells, which might be accompanied by myeloablation to increase the fraction of final cells resistant to the virus infection. Although, there are still technical difficulties to may use this approach, as it is a reduced lymphopoietic capacity observed in HIV-1-infected patients (2), and an observed epigenetic silencing of the inserted genes during differentiation of the haematopoietic stem cells (60,81,83,85,116), recent studies using lentiviral derived vectors reveals promising advances for the use of this approach (31,52).

To obtain a subpopulation of cells resistant to the viral infection it has been carried out genetic interventions to block any step of the viral life cycle. The first step in the viral life cycle is the entry in the cell. Preventing the virus entry in the cell it is an ideal target to inhibit the replication of the virus. The virus receptor is the CD4 transmembrane receptor. However, this protein is essential for the normal function of the $CD4^+$ lymphocyte, and therefore it is not a possible target to be blocked. CCR5 is a coreceptor needed for normal entry of the virus in the initial infection and subsequent dissemination (24,94). The absence of this membrane protein does not have any known effect, and it

is then an ideal target to inhibit the viral entry. Another coreceptor, CXCR4 (11,39), it's used for some viral strains that appear along the infection and represents another target to inhibit virus entry. Its silencing using small interfering RNAs (siRNA) does not produce serious side effects (73). Thus, the expression of both receptors has been blocked using the expression of ribozymes, intracellular single chain antibodies, intrakines and siRNA (1,5,7,15,20,40,64). Recently, peptides derived from the Env protein gp41 have been used to inhibit the HIV-1 entry in the cell at the level of membrane fusion (35). A second target used, is the conversion of the viral RNA into an integrated provirus, thus reverse transcriptase and integrase have been blocked by the use of monoclonal antibodies (14,66,97). A third step of the life cycle is the provirus gene expression. This step has been the focus of a number of different experimental approaches to avoid the effective expression of the inserted provirus. The preferred target has been the inhibition of the Tat induced transactivation of gene expression. In this way, it is inhibited the transcription of the provirus, which can avoid the expression of viral proteins that interfere with cellular functions, and very important, it is suppressed the multiple adverse effects of the Tat protein (37,46,47,58,68,84,96,114,115,120). Tat activity has been blocked by transdominant negative Tat mutants (44,95), anti-Tat intracellular antibodies (89,90), anti-hCyclinT1 intrabodies that prevent the Tat interaction with the positive transcription elongation factor b (6), or by the suppression of its interaction with the TAR element by RNA decoys (18,45,69,70), TAR antisense (23,109) or ribozymes-anti-TAR (105). Transcription has been also blocked with ribozymes directed against the LTR U5 region to get degraded the viral transcripts (34,49,63,113). Another target to avoid the viral gene expression is to block the Rev activity, and in this way to prevent the nuclear export of unspliced or partially spliced transcripts. The expression of the genes that are processed in the multiple spliced mRNA is in this way avoided. Many of the toxic effects of the viral proteins can be avoided, but not those produced by the Tat protein. Transdominant negative Rev mutants (4,9,13,21,28,75,77,87,109), RRE decoys RNAs (21), and siRNA (3,8,9,65) have been used to efficiently block the Rev function. Finally, structural genes can be blocked to prevent from virion formation. Transdominant Gag mutants (106,108) and intrabodies anti-*env* (89) have been used succesfully. Also the α1 antitrypsin gene inhibits cellular serine proteases as well as the viral protease and produced a potent inhibition of HIV-1 replication in transduced lymphocytes (29). However, the cytotoxic effects of the other viral proteins are not prevented with this strategy. The emergence of resistant mutant strains is probably the main problem of these strategies where the target chosen to inhibit the viral replication is a viral gene, however the use of RNA decoys are probably a very good approach to limit this problem. Another strategy to solve the problem of emergence of resistant strains is to develop vectors that contain two antiviral genes, thus a combination of a transdominant negative Rev and an antisense *env* can inhibit quite efficiently the HIV-1 replication (77).

Most studies of gene therapy of HIV-1 can be classified into these two main strategies, however, an interesting different approach has been proposed. This approach is aimed not to eradicate the virus, nor to reconstitute a immune function with genetically modified cells, but to introduce a conditionally replicating HIV-1 (crHIV-1) that interfere with the virus replication in such a way that the wild virus spread is sufficiently diminished to delay or avoid the onset of AIDS. Therefore, in this strategy the patient would receive a crHIV-1 that behaves as a parasite of the wild HIV-1. The crHIV-1 should be innocuous for the cells, and its introduction in the patient should make that the infection would be tolerated (33,34,110). The main difficulty for the feasibility of this approach is the high mutation rate of the wild HIV-1 that could allow the emergence of viruses resistant to the parasitic activity of the crHIV-1. Another concern is that recombinant forms between the wild virus and the crHIV-1 could appear and compromise the efficiency of the strategy.

VECTORS USED IN HIV-1 GENE THERAPY

Many different vectors have been used to deliver anti-viral genes into target cells in the HIV-1 gene therapy. Those vectors were based on viruses such as Moloney murine leukemia virus (MoMLV), adeno-associated virus (AAV), simian virus 40 (SV40) or lentiviral viruses as HIV-1, HIV-2, simian inmunodeficiency virus (SIV) and feline immunodeficiency virus (FIV). In the last few years it's being developed and used lentiviral derived vectors because these viruses can infect both dividing and quiescent cells, while oncoretroviruses can only infect dividing cells (67). Extensive review of lentiviruses used for a HIV-1 gene therapy can be found in references (19,74,76). Lentiviral vectors must be packaged into virions by a system of helper cell line where the vector is introduced to produce the packaged vector RNA (Fig. 2). HIV-1 or HIV-2 derived vectors have the advantage of been able to be directed very specifically towards the same target cells than the wild type virus. An efficient vector delivery and at the same time, a restricted delivery of the vector to the HIV-1 target cells are desirable characteristics that should have an anti-HIV-1 vector. Another advantage of HIV-1-based vectors is that they are less sensitive to gene silencing during cell differentiation (71), and for this reason may be ideal for hematopoietic cells transduction. Although, packaging of lentiviral derived vectors is still less efficient than the obtained with other viral systems and needs to be improved to may proceed with an *in vivo* gene therapy, relevant

improvements have been made recently, and an efficient large-scale production and concentration of HIV-1 derived lentiviral vectors has been got (26). Lentiviral vectors directed against HIV-1 target cells should be packaged into HIV-1 wild type virions that contain the viral Env proteins. Some HIV-1 strains show a tropism towards CNS cells. The vesicular stomatitis virus G-protein (VSV-G) broadens the spectrum of target cells including the CNS cells and also yields higher vector titers, as well as, it gives greater stability to the packaged vector viral particles (112). Lentiviral vectors pseudotyped with VSV-G have been successfully used for the delivery of lentiviral vectors into the CNS (12,59,61,62,80,119). SV40-based vectors can be very efficiently packaged and transduce non-dividing cells, including CNS cells (101,103,104). An efficient protection of the neurons from the HIV-1 has been obtained with anti-HIV-1 transgenes delivered by SV40 vectors (28). The efficiency of the production of highly titer of SV40 derived vector makes an interesting delivery system to carry out and *in vivo* gene therapy of HIV-1 infection (27-29,50,102), but on the other hand, it still remains the fact that these vectors can transduce and modify genetically many cells that are not HIV-1 targets, which it would be preferable to be avoided.

FUTURE PERSPECTIVES

As it has just been described, none of the strategies lacks of limitations, but many of them are merely technical and therefore should be solved in the near future. Of special interest is the *ex vivo* modification of hematopoietic cells that can engraft and produce multiple cell types (36,78,79), and that may exhibit resistance to the virus replication (8). This type of approach may represent a hope for the near future for patients that do not tolerate the HAART therapy and later on for most patients. However, and although this type of approaches aimed to make less aggressive the infection may give good results in a shorter term, it should be considered that the real solution for this disease would come with the complete eradication of the virus from the patient. Recent improvements in delivery systems using lentiviral vectors make conceivable that in the future it will be possible to get an efficient *in vivo* vector delivery to HIV-1 target cells, which represents a hope for the virus eradication from the patient. In the long-term, this should be the horizon for the treatment of the HIV-1 infection, because it will represent a real cure for this infection, and also it will prevent new infections of people that might

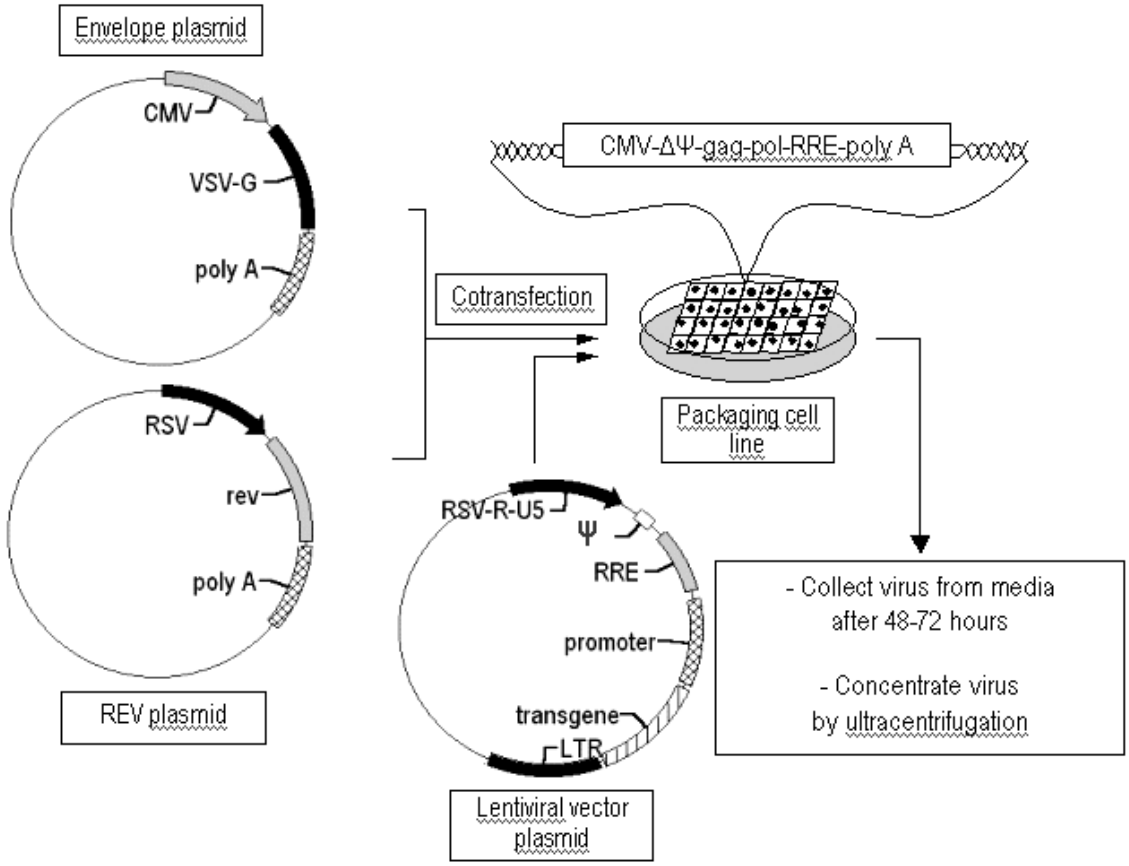

Fig. 2 Packaging system to produce virions with the vector RNA

have unprotected sexual contact with the patient. However, to get a complete eradication of the virus it should be developed an experimental strategy directed to eliminate the reservoir of latently infected resting $CD4^+$ T lymphocytes. As mentioned previously, this reservoir can last for the whole life of the patient and it represents and constant peril of reactivation of the virus. The elimination of infected cells using lethal genes is a strategy that in the last years has received very little attention, but that it is worth to be reconsidered in order to eliminate the HIV-1 latent reservoir. This would be an important task to advance in the viral eradication and would represent a complement of the current HAART therapy. The development of vectors that express the lethal gene in a very tight regulation system it is needed to avoid any harm to healthy transduced cells. The use of physiologic cellular genes as p53 to induce apoptosis may be an alternative to toxic genes to preserve the normal function of the uninfected transduced cells. This approach would take advantage of the success of the antiretroviral therapy and instead of being an alternative to HAART, it might produce a synergistic effect to it. Thus, as a conclusion, we believe that the development of efficient gene delivery by lentiviral vectors and the objective of eliminating the latent reservoir make that the use of lethal genes in HIV-1 gene therapy, unattended in the last years, should be positively reconsidered.

REFERENCES

1. Anderson, J., Banerjea, A., Planelles, V. and Akkina, R., Potent suppression of HIV type 1 infection by a short hairpin anti-CXCR4 siRNA. *AIDS Res. Hum. Retroviruses* 2003, **19**: 699-706.
2. Bahner, I., Kearns, K., Coutinho, S., Leonard, E.H. and Kohn, D.B., Infection of human marrow stroma by human immunodeficiency virus-1 (HIV-1) is both required and sufficient for HIV-1-induced hematopoietic suppression *in vitro*: demonstration by gene modification of primary human stroma. *Blood* 1997, **90**: 1787-1798.
3. Bahner, I., Kearns, K., Hao, Q.L., Smogorzewska, E.M. and Kohn, D.B., Transduction of human $CD34^+$ hematopoietic progenitor cells by a retroviral vector expressing an RRE decoy inhibits human immunodeficiency virus type 1 replication in myelomonocytic cells produced in long-term culture. *J. Virol.* 1996, **70**: 4352-4360.
4. Bahner, I., Zhou, C., Yu, X.J., Hao, Q.L., Guatelli, J.C. and Kohn, D.B., Comparison of trans-dominant inhibitory mutant human immunodeficiency virus type 1 genes expressed by retroviral vectors in human T lymphocytes. *J. Virol.* 1993, **67**: 3199-3207.
5. Bai, J., Gorantla, S., Banda, N., Cagnon, L., Rossi, J. and Akkina, R., Characterization of anti-CCR5 ribozyme-transduced $CD34^+$ hematopoietic progenitor cells *in vitro* and in a SCID-hu mouse model *in vivo*. *Mol. Ther.* 2000, **1**: 244-254.
6. Bai, J., Sui, J., Zhu, R.Y., Tallarico, A.S., Gennari, F., Zhang, D. and Marasco, W.A., Inhibition of Tat-mediated transactivation and HIV-1 replication by human anti-hCyclinT1 intrabodies. *J. Biol. Chem.* 2003, **278**: 1433-1442.
7. Bai, X., Chen, J.D., Yang, A.G., Torti, F. and Chen, S.Y., Genetic co-inactivation of macrophage- and T-tropic HIV-1 chemokine coreceptors CCR-5 and CXCR-4 by intrakines. *Gene Ther*. 1998, **5**: 984-994.
8. Banerjea, A., Li, M.J., Bauer, G., Remling, L., Lee, N.S., Rossi, J. and Akkina, R., Inhibition of HIV-1 by lentiviral vector-transduced siRNAs in T lymphocytes differentiated in SCID-hu mice and $CD34^+$ progenitor cell-derived macrophages. *Mol. Ther.* 2003, **8**: 62-71.
9. Bauer, G., Valdez, P., Kearns, K., Bahner, I., Wen, S.F., Zaia, J.A. and Kohn, D.B., 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**: 2259-2267.
10. Berkhout, B., Silverman, R.H. and Jeang, K.T., Tat trans-activates the human immunodeficiency virus through a nascent RNA target. *Cell* 1989, **59**: 273-282.
11. Bleul, C.C., Farzan, M., Choe, H., Parolin, C., Clark-Lewis, I., Sodroski, J. and Springer, T.A., The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* 1996, **382**: 829-833.
12. Blomer, U., Naldini, L., Kafri, T., Trono, D., Verma, I.M. and Gage, F.H., Highly efficient and sustained gene transfer in adult neurons with a lentivirus vector. *J. Virol*. 1997, **71**: 6641-6649.
13. Bonyhadi, M.L., Moss, K., Voytovich, A., Auten, J., Kalfoglou, C., Plavec, I., Forestell, S., Su, L., Bohnlein, E. and Kaneshima, H., RevM10-expressing T cells derived *in vivo* from transduced human hematopoietic stem-progenitor cells inhibit human immunodeficiency virus replication. *J. Virol.* 1997, **71**: 4707-4716.
14. BouHamdan, M., Duan, L.X., Pomerantz, R.J. and Strayer, D.S., Inhibition of HIV-1 by an anti-integrase single-chain variable fragment (SFv): delivery by SV40 provides durable protection against HIV-1 and does not require selection. *Gene Ther.* 1999, **6**: 660-666.
15. BouHamdan, M., Strayer, D.S., Wei, D., Mukhtar, M., Duan, L.X., Hoxie, J. and Pomerantz, R.J., Inhibition of HIV-1 infection by down-regulation of the CXCR4 co-receptor using an intracellular single chain variable fragment against CXCR4. *Gene Ther.* 2001, **8**: 408-418.
16. Brady, H.J., Miles, C.G., Pennington, D.J. and Dzierzak, E.A., Specific ablation of human immunodeficiency virus Tat-expressing cells by conditionally toxic retroviruses. *Proc. Natl. Acad. Sci. USA* 1994, **91**: 365-369.
17. Brenner, B., Wainberg, M.A., Salomon, H., Rouleau, D., Dascal, A., Spira, B., Sekaly, R.P., Conway, B. and Routy, J.P., Resistance to antiretroviral drugs in patients with primary HIV-1 infection. Investigators of the Quebec Primary Infection Study. *Int. J. Antimicrob. Agents* 2000, **16**: 429-434.
18. Browning, C.M., Cagnon, L., Good, P.D., Rossi, J., Engelke, D.R. and Markovitz, D.M., Potent inhibition of human immunodeficiency virus type 1 (HIV-1) gene expression and virus production by an HIV-2 tat activation-response RNA decoy. *J. Virol.* 1999, **73**: 5191-5195.

Buchschacher, G.L. Jr. and Wong-Staal, F., Development of lentiviral vectors for gene therapy for human diseases. *Blood* 2000, **95**: 2499-2504.

20. Cagnon, L. and Rossi, J.J., Downregulation of the CCR5 beta-chemokine receptor and inhibition of HIV-1 infection by stable VA1-ribozyme chimeric transcripts. *Antisense Nucl. Acid Drug Dev*. 2000, **10**: 251-261.
21. Caputo, A., Rossi, C., Bozzini, R., Betti, M., Grossi, M.P., Barbanti-Brodano, G. and Balboni, P.G., Studies on the effect of the combined expression of anti-tat and anti-rev genes on HIV-1 replication. *Gene Ther.* 1997, **4**: 288-295.
22. Caruso, M. and Klatzmann, D., Selective killing of $CD4^+$ cells harboring a human immunodeficiency virus-inducible suicide gene prevents viral spread in an infected cell population. *Proc. Natl. Acad. Sci. USA* 1992, **89**: 182-186.

23. Chadwick, D.R. and Lever, A.M., Antisense RNA sequences targeting the 5' leader packaging signal region of human immunodeficiency virus type-1 inhibits viral replication at post-transcriptional stages of the life cycle. *Gene Ther.* 2000, **7**: 1362-1368.
24. Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P.D., Wu, L., Mackay, C.R., LaRosa, G., Newman, W., Gerard, N., Gerard, C. and Sodroski, J., The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 1996, **85**: 1135-1148.
25. Chun, T.W., Stuyver, L., Mizell, S.B., Ehler, L.A., Mican, J.A., Baseler, M., Lloyd, A.L., Nowak, M.A. and Fauci, A.S., Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. USA* 1997, **94**: 13193-13197.
26. Coleman, J.E., Huentelman, M.J., Kasparov, S., Metcalfe, B.L., Paton, J.F., Katovich, M.J., Semple-Rowland, S.L. and Raizada, M.K., Efficient large-scale production and concentration of HIV-1-based lentiviral vectors for use *in vivo*. *Physiol. Genomics* 2003, **12**: 221-228.
27. Cordelier, P. and Strayer, D.S., Conditional expression of alpha(1)-antitrypsin delivered by recombinant SV40 vectors protects lymphocytes against HIV. *Gene Ther.* 2003, **10**: 2153-2156.
28. Cordelier, P., Van Bockstaele, E., Calarota, S.A. and Strayer, D.S., Inhibiting AIDS in the central nervous system: gene delivery to protect neurons from HIV. *Mol. Ther.* 2003, **7**: 801-810.
29. Cordelier, P., Zern, M.A. and Strayer, D.S., HIV-1 proprotein processing as a target for gene therapy. *Gene Ther*. 2003, **10**: 467-477.
30. Curiel, T.J., Cook, D.R., Wang, Y., Hahn, B.H., Ghosh, S.K. and Harrison, G.S., Long-term inhibition of clinical and laboratory human immunodeficiency virus strains in human T cell lines containing an HIV-regulated diphtheria toxin A chain gene. *Hum. Gene Ther.* 1993, **4**: 741-747.
31. Davis, B.M., Humeau, L. and Dropulic, B., *In vivo* selection for human and murine hematopoietic cells transduced with a therapeutic MGMT lentiviral vector that inhibits HIV replication. *Mol. Ther*. 2004, **9**: 160-172.
32. Dinges, M.M., Cook, D.R., King, J., Curiel, T.J., Zhang, X.Q. and Harrison, G.S., HIV-regulated diphtheria toxin A chain gene confers long-term protection against HIV type 1 infection in the human promonocytic cell line U937. *Hum. Gene Ther.* 1995, **6**: 1437-1445.
33. Dropulic, B. Lentivirus in the clinic. *Mol. Ther*. 2001, **4**: 511-512.
34. Dropulic, B., Hermankova, M. and Pitha, P.M., A conditionally replicating HIV-1 vector interferes with wild-type HIV-1 replication and spread. *Proc. Natl. Acad. Sci. USA* 1996, **93**: 11103-11108.
35. Egelhofer, M., Brandenburg, G., Martinius, H., Schult-Dietrich, P., Melikyan, G., Kunert, R., Baum, C., Choi, I., Alexandrov, A. and von Laer, D., Inhibition of human immunodeficiency virus type 1 entry in cells expressing gp41-derived peptides. *J. Virol.* 2004, **78**: 568-575.
36. Engel, B.C. and Kohn, D.B., Stem cell directed gene therapy. *Front. Biosci.* 1999, **4**: e26-33.
37. Ensoli, B., Barillari, G., Salahuddin, S.Z., Gallo, R.C. and Wong-Staal, F., Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. *Nature* 1990, **345**: 84-86.
38. Felber, B.K., Hadzopoulou-Cladaras, M., Cladaras, C., Copeland, T. and Pavlakis, G.N., rev protein of human immunodeficiency virus type 1 affects the stability and transport of the viral mRNA. *Proc. Natl. Acad. Sci. USA* 1989, **86**: 1495-1499.
39. Feng, Y., Broder, C.C., Kennedy, P.E. and Berger, E.A., HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G-protein-coupled receptor. *Science* 1996, **272**: 872-877.
40. Feng, Y., Leavitt, M., Tritz, R., Duarte, E., Kang, D., Mamounas, M., Gilles, P., Wong-Staal, F., Kennedy, S., Merson, J., Yu, M. and Barber, J.R., Inhibition of CCR5-dependent HIV-1 infection by hairpin ribozyme gene therapy against CC-chemokine receptor 5. *Virology* 2000, **276**: 271-278.
41. Finzi, D., Blankson, J., Siliciano, J.D., Margolick, J.B., Chadwick, K., Pierson, T., Smith, K., Lisziewicz, J., Lori, F., Flexner, C., Quinn, T.C., Chaisson, R.E., Rosenberg, E., Walker, B., Gange, S., Gallant, J. and Siliciano, R.F., Latent infection of $CD4^+$ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat. Med.* 1999, **5**: 512-517.
42. Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R.E., Quinn, T.C., Chadwick, K., Margolick, J., Brookmeyer, R., Gallant, J., Markowitz, M., Ho, D.D., Richman, D.D. and Siliciano, R.F., Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997, **278**: 1295-1300.
43. Fornerod, M., Ohno, M., Yoshida, M. and Mattaj, I.W., CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell* 1997, **90**: 1051-1060.
44. Fraisier, C., Abraham, D.A., van Oijen, M., Cunliffe, V., Irvine, A., Craig, R, and Dzierzak, E.A., Inhibition of Tat-mediated transactivation and HIV replication with Tat mutant and repressor domain fusion proteins. *Gene Ther.*1998, **5**: 946-954.
45. Fraisier, C., Irvine, A., Wrighton, C., Craig, R. and Dzierzak, E., High level inhibition of HIV replication with combination RNA decoys expressed from an HIV-Tat inducible vector. *Gene Ther*. 1998, **5**: 1665-1676.
46. Frankel, A.D. and Pabo, C.O., Cellular uptake of the tat protein from human immunodeficiency virus. *Cell* 1988, **55**: 1189-1193.
47. Gallo, R.C., Tat as one key to HIV-induced immune pathogenesis and Tat (correction of Pat) toxoid as an important component of a vaccine. *Proc. Natl. Acad. Sci. USA* 1999, **96**: 8324-8326.
48. Garcia, J.A., Wu, F.K., Mitsuyasu, R. and Gaynor, R.B., Interactions of cellular proteins involved in the transcriptional regulation of the human immunodeficiency virus. *EMBO J.* 1987, **6**: 3761-3770.
49. Gervaix, A., Li, X., Kraus, G. and Wong-Staal, F., Multigene antiviral vectors inhibit diverse human immunodeficiency virus type 1 clades. *J. Virol.* 1997, **71**: 3048-3053.
50. Goldstein, H., Pettoello-Mantovani, M., Anderson, C.M., Cordelier, P., Pomerantz, R.J. and Strayer, D.S., Gene therapy using a simian virus 40-derived vector inhibits the development of *in vivo* human immunodeficiency virus type 1 infection of severe combined immunodeficiency mice implanted with human fetal thymic and liver tissue. *J. Infect. Dis.* 2002, **185**: 1425-1430.
51. Hammarskjold, M.L., Heimer, J., Hammarskjold, B., Sangwan, I., Albert, L. and Rekosh, D., Regulation of human immunodeficiency virus env expression by the rev gene product. *J. Virol*. 1989, **63**: 1959-1966.
52. Hanawa, H., Hematti, P., Keyvanfar, K., Metzger, M.E., Krouse, A., Donahue, R.E., Kepes, S., Gray, J., Dunbar, C.E., Persons, D.A. and Nienhuis, A.W., Efficient gene transfer into rhesus repopulating hematopoietic stem cells using a simian immunodeficiency virus-based lentiviral vector system. *Blood* 2004.
53. Harrison, G.S., Long, C.J., Curiel, T.J., Maxwell, F. and Maxwell, I.H., Inhibition of human immunodeficiency virus-1 production resulting from transduction with a retrovirus containing an HIV-regulated diphtheria toxin A chain gene. *Hum. Gene Ther.* 1992, **3**: 461-469.
54. Harrison, G.S., Long, C.J., Maxwell, F., Glode, L.M. and Maxwell, I.H., Inhibition of HIV production in cells containing an integrated, HIV-regulated diphtheria toxin A chain gene AIDS. *Res. Hum. Retroviruses* 1992, **8**: 39-45.
55. Henderson, B.R. and Percipalle, P., Interactions between HIV Rev and nuclear import and export factors: the Rev nuclear localisation signal mediates specific binding to human importin-beta. *J. Mol. Biol*. 1997, **274**: 693-707.
56. Hermankova, M., Siliciano, J.D., Zhou, Y., Monie, D., Chadwick, K.,

Margolick, J.B., Quinn, T.C. and Siliciano, R.F., Analysis of human immunodeficiency virus type 1 gene expression in latently infected resting $CD4^+$ T lymphocytes *in vivo*. *J. Virol.* 2003, **77**: 7383-7392.
57. Herrmann, C.H. and Rice, A.P. Lentivirus Tat proteins specifically associate with a cellular protein kinase, TAK, that hyperphosphorylates the carboxyl-terminal domain of the large subunit of RNA polymerase II: candidate for a Tat cofactor. *J. Virol.* 1995, **69**: 1612-1620.
58. Huang, L., Bosch, I., Hofmann, W., Sodroski, J. and Pardee, A.B., Tat protein induces human immunodeficiency virus type 1 (HIV-1) coreceptors and promotes infection with both macrophage-tropic and T-lymphotropic HIV-1 strains. *J. Virol*. 1998, **72**: 8952-8960.
59. Jakobsson, J., Ericson, C., Jansson, M., Bjork, E. and Lundberg, C., Targeted transgene expression in rat brain using lentiviral vectors. *J. Neurosci. Res.* 2003, **73**: 876-885.
60. Klug, C.A., Cheshier, S. and Weissman, I.L., Inactivation of a GFP retrovirus occurs at multiple levels in long-term repopulating stem cells and their differentiated progeny. *Blood* 2000, **96**: 894-901.
61. Kordower, J.H., Emborg, M.E., Bloch, J., Ma, S.Y., Chu, Y., Leventhal, L., McBride, J., Chen, E.Y., Palfi, S., Roitberg, B.Z., Brown, W.D., Holden, J.E., Pyzalski, R., Taylor, M.D., Carvey, P., Ling, Z., Trono, D., Hantraye, P., Deglon, N. and Aebischer, P., Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science* 2000, **290**: 767-773.
62. Kyrkanides, S., Miller, J.H. and Federoff, H.J., Systemic FIV vector administration: transduction of CNS immune cells and Purkinje neurons. *Mol. Brain Res.* 2003, **119**: 1-9.
63. Leavitt, M.C., Yu, M., Yamada, O., Kraus, G., Looney, D., Poeschla, E. and Wong-Staal, F., Transfer of an anti-HIV-1 ribozyme gene into primary human lymphocytes. *Hum. Gene Ther*. 1994, **5**: 1115-1120.
64. Lee, M.T., Coburn, G.A., McClure, M.O. and Cullen, B.R., Inhibition of human immunodeficiency virus type 1 replication in primary macrophages by using Tat- or CCR5-specific small interfering RNAs expressed from a lentivirus vector. *J. Virol.* 2003, **77**: 11964-11972.
65. Lee, N.S., Dohjima, T., Bauer, G., Li, H., Li, M.J., Ehsani, A., Salvaterra, P., Rossi, J., Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. *Nat. Biotechnol*. 2002, **20**: 500-505.
66. Levy-Mintz, P., Duan, L., Zhang, H., Hu, B., Dornadula, G., Zhu, M., Kulkosky, J., Bizub-Bender, D., Skalka, A.M. and Pomerantz, R.J., Intracellular expression of single-chain variable fragments to inhibit early stages of the viral life cycle by targeting human immunodeficiency virus type 1 integrase. *J. Virol*. 1996, **70**: 8821-8832.
67. Lewis, P.F. and Emerman, M., Passage through mitosis is required for oncoretroviruses but not for the human immunodeficiency virus. *J. Virol.* 1994, **68**: 510-516.
68. Li, C.J., Friedman, D.J., Wang, C., Metelev, V. and Pardee, A.B., Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. *Science* 1995, **268**: 429-431.
69. Lisziewicz, J., Rappaport, J. and Dhar, R., Tat-regulated production of multimerized TAR RNA inhibits HIV-1 gene expression. *New Biol.* 1991, **3**: 82-89.
70. Lisziewicz, J., Sun, D., Smythe, J., Lusso, P., Lori, F., Louie, A., Markham, P., Rossi, J., Reitz, M. and Gallo, R.C., Inhibition of human immunodeficiency virus type 1 replication by regulated expression of a polymeric Tat activation response RNA decoy as a strategy for gene therapy in AIDS. *Proc. Natl. Acad. Sci USA* 1993, **90**: 8000-8004.
71. Lois, C., Hong, E.J., Pease, S., Brown, E.J. and Baltimore, D., Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors. *Science* 2002, **295**: 868-872.
72. Malim, M.H., Hauber, J., Le, S.Y., Maizel, J.V. and Cullen, B.R., The HIV-1 rev trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. *Nature* 1989, **338**: 254-257.
73. Martinez, M.A., Gutierrez, A., Armand-Ugon, M., Blanco, J., Parera, M., Gomez, J., Clotet, B. and Este, J.A., Suppression of chemokine receptor expression by RNA interference allows for inhibition of HIV-1 replication. *AIDS* 2002, **16**: 2385-2390.
74. Mautino, M.R., Lentiviral vectors for gene therapy of HIV-1 infection. *Curr. Gene Ther.* 2002, **2**: 23-43.
75. Mautino, M.R., Keiser, N. and Morgan, R.A., Inhibition of human immunodeficiency virus type 1 (HIV-1) replication by HIV-1-based lentivirus vectors expressing transdominant Rev. *J. Virol*. 2001, **75**: 3590-3599.
76. Mautino, M.R. and Morgan, R.A., Gene therapy of HIV-1 infection using lentiviral vectors expressing anti-HIV-1 genes. *AIDS Patient Care STDS* 2002, **16**: 11-26.
77. Mautino, M.R. and Morgan, R.A., Inhibition of HIV-1 replication by novel lentiviral vectors expressing transdominant Rev and HIV-1 env antisense. *Gene Ther.* 2002, **9**: 421-431.
78. Miyoshi, H., Gene delivery to hematopoietic stem cells using lentiviral vectors. *Meth. Mol. Biol*. 2004, **246**: 429-438.
79. Miyoshi, H., Smith, K.A., Mosier, D.E., Verma, I.M. and Torbett, B.E., Transduction of human $CD34^+$ cells that mediate long-term engraftment of NOD/SCID mice by HIV vectors. *Science* 1999, **283**: 682-686.
80. Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F.H., Verma, I.M. and Trono, D., *In vivo* gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* 1996, **272**: 263-267.
81. Neff, T., Shotkoski, F. and Stamatoyannopoulos, G., Stem cell gene therapy, position effects and chromatin insulators. *Stem Cells* 1997, **15 (1)**, 265-271.
82. Neville, M., Stutz, F., Lee, L., Davis, L.I. and Rosbash, M., The importin-beta family member Crm1p bridges the interaction between Rev and the nuclear pore complex during nuclear export. *Curr. Biol*. 1997, **7**: 767-775.
83. Osborne, C.S., Pasceri, P., Singal, R., Sukonnik, T., Ginder, G.D. and Ellis, J., Amelioration of retroviral vector silencing in locus control region beta-globin-transgenic mice and transduced F9 embryonic cells. *J. Virol*. 1999, **73**: 5490-5496.
84. Ott, M., Emiliani, S., Van Lint, C., Herbein, G., Lovett, J., Chirmule, N., McCloskey, T., Pahwa, S. and Verdin, E., Immune hyperactivation of HIV-1-infected T cells mediated by Tat and the CD28 pathway. *Science* 1997, **275**: 1481-1485.
85. Pannell, D., Osborne, C.S., Yao, S., Sukonnik, T., Pasceri, P., Karaiskakis, A., Okano, M., Li, E., Lipshitz, H.D. and Ellis, J., Retrovirus vector silencing is *de novo* methylase independent and marked by a repressive histone code. *EMBO J.* 2000, **19**: 5884-5894.
86. Pierson, T., McArthur, J. and Siliciano, R.F., Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. *Annu. Rev. Immunol* .2000, **18**: 665-708.
87. Plavec, I., Agarwal, M., Ho, K.E., Pineda, M., Auten, J., Baker, J., Matsuzaki, H., Escaich, S., Bonyhadi, M. and Bohnlein, E., High transdominant RevM10 protein levels are required to inhibit HIV-1 replication in cell lines and primary T cells: implication for gene therapy of AIDS. *Gene Ther*. 1997, **4**: 128-139.
88. Pomerantz, R.J., Kuritzkes, D.R., de la Monte, S.M., Rota, T.R., Baker, A.S., Albert, D., Bor, D.H., Feldman, E.L., Schooley, R.T. and Hirsch, M.S., Infection of the retina by human immunodeficiency virus type I. *N. Engl. J. Med*. 1987, **317**: 1643-1647.
89. Poznansky, M.C., Foxall, R., Mhashilkar, A., Coker, R., Jones, S., Ramstedt, U. and Marasco, W., 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**: 487-496.
90. Poznansky, M.C., La Vecchio, J., Silva-Arietta, S., Porter-Brooks, J., Brody, K., Olszak, I.T., Adams, G.B., Ramstedt, U., Marasco, W.A. and Scadden, D.T., 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**: 2505-2514.
91. Ragheb, J.A., Couture, L., Mullen, C., Ridgway, A. and Morgan, R.A., Inhibition of human immunodeficiency virus type 1 by Tat/Rev-regulated expression of cytosine deaminase, interferon alpha2, or diphtheria toxin compared with inhibition by transdominant *Rev. Hum Gene Ther* .1999, **10**: 103-112.
92. Ramratnam, B., Mittler, J.E., Zhang, L., Boden, D., Hurley, A., Fang, F., Macken, C.A., Perelson, A.S., Markowitz, M. and Ho, D.D., The decay of the latent reservoir of replication-competent HIV-1 is inversely correlated with the extent of residual viral replication during prolonged anti-retroviral therapy. *Nat. Med*. 2000, **6**: 82-85.
93. Ramratnam, B., Ribeiro, R., He, T., Chung, C., Simon, V., Vanderhoeven, J., Hurley, A., Zhang, L., Perelson, A.S., Ho, D.D. and Markowitz, M., Intensification of antiretroviral therapy accelerates the decay of the HIV-1 latent reservoir and decreases, but does not eliminate, ongoing virus replication. *J. Acquir. Immun. Defic. Synd.* 2004, **35**: 33-37.
94. Ross, T.M., Bieniasz, P.D. and Cullen, B.R., Role of chemokine receptors in HIV-1 infection and pathogenesis. *Adv. Virus Res*. 1999, **52**: 233-267.
95. Rossi, C., Balboni, P.G., Betti, M., Marconi, P.C., Bozzini, R., Grossi, M.P., Barbanti-Brodano, G. and Caputo, A., 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**: 1261-1269.
96. Secchiero, P., Zella, D., Capitani, S., Gallo, R.C. and 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**: 2427-2431.
97. Shaheen, F., Duan, L., Zhu, M., Bagasra, O. and Pomerantz, R.J., Targeting human immunodeficiency virus type 1 reverse transcriptase by intracellular expression of single-chain variable fragments to inhibit early stages of the viral life cycle. *J. Virol.* 1996, **70**: 3392-3400.
98. Si-Mohamed, A., Kazatchkine, M.D., Heard, I., Goujon, C., Prazuck, T., Aymard, G., Cessot, G., Kuo, Y.H., Bernard, M.C., Diquet, B., Malkin, J.E., Gutmann, L. and Belec, L., Selection of drug-resistant variants in the female genital tract of human immunodeficiency virus type 1-infected women receiving antiretroviral therapy. *J. Infect. Dis.* 2000, **182**: 112-122.
99. Siliciano, J.D., Kajdas, J., Finzi, D., Quinn, T.C., Chadwick, K., Margolick, J.B., Kovacs, C., Gange, S.J. and Siliciano, R.F., Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting $CD4^+$ T cells. *Nat. Med*. 2003, **9**: 727-728.
100. Stevenson, M., Stanwick, T.L., Dempsey, M.P. and Lamonica, C.A., HIV-1 replication is controlled at the level of T cell activation and proviral integration. *EMBO J.* 1990, **9**: 1551-1560.
101. Strayer, D.S., SV40 as an effective gene transfer vector *in vivo*. *J. Biol. Chem.* 1996, **271**: 24741-24746.
102. Strayer, D.S., Branco, F., Landre, J., BouHamdan, M., Shaheen, F. and Pomerantz, R.J., Combination genetic therapy to inhibit HIV-1. *Mol. Ther.* 2002, **5**: 33-41.
103. Strayer, D.S., Kondo, R., Milano, J. and Duan, L.X., Use of SV40-based vectors to transduce foreign genes to normal human peripheral blood mononuclear cells. *Gene Ther.* 1997, **4**: 219-225.
104. Strayer, D.S. and Milano, J., SV40 mediates stable gene transfer *in vivo*. *Gene Ther.* 1996, **3**: 581-587.
105. Sun, L.Q., Ely, J.A., Gerlach, W. and Symonds, G., Anti-HIV ribozymes. *Mol. Biotechnol.* 1997, **7**: 241-251.
106. Tewari, D., Notkins, A.L. and Zhou, P., Inhibition of HIV-1 replication in primary human T cells transduced with an intracellular anti-HIV-1 p17 antibody gene. *J. Gene Med.* 2003, **5**: 182-189.
107. Tong-Starksen, S.E., Luciw, P.A. and Peterlin, B.M., Human immunodeficiency virus long terminal repeat responds to T cell activation signals. *Proc. Natl. Acad. Sci. USA* 1987, **84**: 6845-6849.
108. Trono, D., Feinberg, M.B., and Baltimore, D., HIV-1 Gag mutants can dominantly interfere with the replication of the wild-type virus. *Cell* 1989, **59**: 113-120.
109. Vandendriessche, T., Chuah, M.K., Chiang, L., Chang, H.K., Ensoli, B. and Morgan, R.A., Inhibition of clinical human immunodeficiency virus (HIV) type 1 isolates in primary $CD4^+$ T lymphocytes by retroviral vectors expressing anti-HIV genes. *J. Virol*. 1995, **69**: 4045-4052.
110. Weinberger, L.S., Schaffer, D.V. and Arkin, A.P., Theoretical design of a gene therapy to prevent AIDS but not human immunodeficiency virus type 1 infection. *J. Virol*. 2003, **77**: 10028-10036.
111. Wong, J.K., Hezareh, M., Gunthard, H.F., Havlir, D.V., Ignacio, C.C., Spina, C.A. and Richman, D.D., Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 1997, **278**: 1291-1295.
112. Yee, J.K., Miyanohara, A., LaPorte, P., Bouic, K., Burns, J.C. and Friedmann, T., A general method for the generation of high-titer, pantropic retroviral vectors: highly efficient infection of primary hepatocytes. *Proc. Natl. Acad. Sci. USA* 1994, **91**: 9564-9568.
113. Yu, M., Leavitt, M.C., Maruyama, M., Yamada, O., Young, D., Ho, A.D. and Wong-Staal, F., Intracellular immunization of human fetal cord blood stem/progenitor cells with a ribozyme against human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* 1995, **92**: 699-703.
114. Zagury, D., Lachgar, A., Chams, V., Fall, L.S., Bernard, J., Zagury, J.F., Bizzini, B., Gringeri, A., Santagostino, E., Rappaport, J., Feldman, M., Burny, A. and Gallo, R.C., Interferon alpha and Tat involvement in the immunosuppression of uninfected T cells and C-C chemokine decline in AIDS. *Proc. Natl. Acad. Sci. USA* 1998, **95**: 3851-3856.
115. Zauli, G., La Placa, M., Vignoli, M., Re, M.C., Gibellini, D., Furlini, G., Milani, D., Marchisio, M., Mazzoni, M. and Capitani, S., An autocrine loop of HIV type-1 Tat protein responsible for the improved survival/proliferation capacity of permanently Tat-transfected cells and required for optimal HIV-1 LTR transactivating activity. *J. Acquir. Immun. Defic. Syndr. Hum. Retrovirol.* 1995, **10**: 306-316.
116. Zentilin, L., Qin, G., Tafuro, S., Dinauer, M.C., Baum, C. and Giacca, M., Variegation of retroviral vector gene expression in myeloid cells. *Gene Ther*. 2000, **7**: 153-166.
117. Zhang, H., Dornadula, G., Beumont, M., Livornese, L. Jr., Van Uitert, B., Henning, K. and Pomerantz, R.J., Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. *N. Engl. J. Med*. 1998, **339**: 1803-1809.
118. Zhang, L., Ramratnam, B., Tenner-Racz, K., He, Y., Vesanen, M., Lewin, S., Talal, A., Racz, P., Perelson, A.S., Korber, B.T., Markowitz, M. and Ho, D.D., Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. *N. Engl. J. Med*. 1999, **340**: 1605-1613.
119. Zhao, C., Strappe, P.M., Lever, A.M. and Franklin, R.J., Lentiviral vectors for gene delivery to normal and demyelinated white matter. *Glia* 2003, **42**: 59-67.
120. Zocchi, M.R., Rubartelli, A., Morgavi, P. and Poggi, A., HIV-1 Tat inhibits human natural killer cell function by blocking L-type calcium channels. *J. Immunol.* 1998, **161**: 2938-2943.