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Hematopoietic Stem Cell-based Gene Therapy Against HIV Infection: Promises and Caveats

Johan van Griensven, Erik De Clercq and Zeger Debyser
Rega Institute for Medical Research, K.U. Leuven and Kulak, Leuven, Belgium

Abstract

Stem cell-based gene therapy of HIV infection aims at inhibiting HIV replication and the progression to AIDS by the introduction of antiviral genes in primitive hematopoietic stem cells (HSC). Ideally, after differentiation into mature blood cells, these antiviral genes should create a host-cell population that is resistant to HIV infection. Although the current gene therapy clinical trials established the safety and provided proof-of-principle for gene therapy of HIV-1 infection, the overall results have been disappointing, and many issues still remain to be resolved before this approach can be efficiently used against HIV infection. Since a significant percentage of the stem cells in the patient have to be transduced to obtain a significant impact on HIV replication, the first prerequisite for successful gene therapy of HIV-1 infection consists of increasing the amount of transduced HSCs. Further improvements in gene transfer and gene therapy strategies will probably lead to future clinical successes. On the other hand, HIV-1 infection is a very complex disease, affecting various organs in addition to the T-cells, with an impact on T-cell homeostasis that is currently not fully understood. Even at low viral loads and before the advent of clinical symptoms, a high turnover of CD4+ cells exists in HIV-infected patients with functional implications for the homeostasis of the thymus, bone marrow and T-cell homeostasis which may hamper the CD34$^+$ HSC approach. Hence, the extent to which these alterations hamper a gene therapy approach, or can be reversed upon HAART, will determine the feasibility of future gene therapy against AIDS. **(AIDS Reviews 2005;7:44-55)**

Key words

HIV. Gene therapy. Hematopoietic stem cells. Retroviral vectors.

Introduction

Four classes of antiretroviral drugs are currently available to treat HIV-1 infection: nucleoside and nucleotide analogs, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors[1]. The fusion inhibitor T-20 (enfuvirtide) was launched in 2003 as the prototype of a fifth class of HIV-1 inhibitors[2]. Research is also focusing on immunomodulatory approaches with cytokines or vaccination; however, until now this has not shown any clear clinical benefit[3]. Highly active antiretroviral therapy (HAART), generally utilizing an HIV protease inhibitor and two out of several available reverse transcriptase inhibitors, can effectively reduce the viral load to undetectable levels in the peripheral blood, with a subsequent increase in CD4+ T-cell numbers and improved clinical status[4,5]. However, a number of problems with the current therapies limit their usefulness. First, the cost of the drugs constitutes a significant burden to individuals and governments worldwide, and virtually precludes their general availability in developing countries. The vanished hope of eradicating the virus, the serious side effects, and the inconvenient and complicated medication schedules all have a negative affect on patient compliance[6]. Furthermore, because the existing drugs are only able to suppress viral replication, but do not eliminate the virus from the body, they must be administered indefinitely; however, the long-term side effects of current drug therapies and long-term consequences of subclinical

Correspondence to:
Zeger Debyser
Molecular Virology and Gene Therapy KULAK and KULeuven
Kapucijnenvoer, 33
B-3000 Leuven, Flanders, Belgium
E-mail: zeger.debyser@med.kuleuven.ac.be

HIV infection remain largely unknown. Most importantly, because of the high mutation rate of the virus, drug-resistant strains continuously emerge in the infected patients with subsequent failure of virologic control[7]. Treatment failures due to drug resistance are currently a major problem for the management of HIV-1 infection. For all of these reasons, alternative or adjuvant treatment strategies for HIV infection, including gene therapy, are being investigated.

Gene therapy of HIV infection aims at inhibiting HIV replication and the progression to AIDS by the introduction of antiviral genes in the host cells of the virus. Ideally, these antiviral genes create a host-cell population that is resistant to HIV infection. This approach was originally referred to as "intracellular immunization". Alternatively, gene therapy may be used to selectively eliminate HIV-infected cells by using targeted toxins or by augmenting the immune response to HIV. An example of this strategy is DNA vaccination[8].

Gene therapy can target the virus directly by inserting anti-HIV genes in the genome of the cell types infected by HIV. Furthermore, because the cell itself produces the therapeutic agent, no systemic toxicity is associated with expression of the transgene. In addition, because the anti-HIV gene is integrated into the genome, theoretically few repeat doses will be required, avoiding compliance issues. Finally, the capability to target conserved viral structures (at the protein, RNA, or DNA level) that are difficult to target by antiviral compounds, and the ease of combining several antiviral genes targeting different steps in the HIV replication cycle, may confound the emergence of viral resistance. This review focuses on hematopoietic stem cell (HSC)-based gene therapeutic approaches against HIV-1 infection. After a general overview, we will discuss the current progress in the HSC-based gene therapy field and address the challenges for and feasibility of this approach as a therapeutic strategy against HIV-1 infection.

Target cells

The major host cells for HIV replication are of lymphoid (CD4+ lymphocyte) or myeloid (macrophage, monocyte, microglia, dendritic cell) origin. Since pluripotent HSCs generate cells of both lymphoid and myeloid origin, all HIV host cells could be made resistant to replication if antiviral genes could be efficiently transferred into the stem cells. Due to the self-renewal capacity of these cells, the resistant population should persist for a lifetime. It has been shown that CD34+ cells, a population enriched in HSCs, can be isolated from the peripheral blood from HIV patients after mobilization with growth factors without affecting the viral load[9]. Since stem cells are apparently not infected by HIV[10], autologous HSCs of the patient could be isolated, transduced, and reinfused. The impressive clinical results with HSC transplantation for X-linked severe combined immunodeficiency (SCID-X1) provide further support for this approach[11]. Both animal and patient data indicate that, upon institution of HAART, renewed hematopoiesis and thymopoiesis take place[12-14]. Current problems associated with this approach are low efficiency of *in vivo* gene marking.

An alternative target for gene therapy of AIDS is the CD4+ lymphocyte. CD4+ cells can be easily isolated, transduced, expanded and selected in culture, and reinfused. For this reason, initial clinical trials for the gene therapy of AIDS have been based on transfusion of CD4+ cells. If the cells have a survival advantage (e.g. resistance to HIV), they could be selected for in the patient. The obvious disadvantage is the temporal benefit of this strategy due to the limited lifespan and growth potential of CD4+ cells *in vivo*. Moreover, antiviral resistance is restricted to CD4+ T-cells. Various phase I clinical trials using transduced CD4+ cells have been performed. Although only low levels of transduced cells were observed, the general observation of a preferential survival of cells containing an antiviral gene provides proof-of-principle for the gene therapy for AIDS.

Since HSCs and lymphocytes can be easily collected and cultured, transduction is done *in vitro*. Subsequently, transduced cells can be reinfused intravenously. *In vitro* transduction allows for controlled exposure of the target cells and is not confounded by immunological reactions.

Gene-transfer systems

Oncoretroviral vectors

Successful gene therapy with HSCs requires efficient gene delivery to a high percentage of long-term, reconstituting HSCs, stable persistence of the gene as the HSCs undergo extensive proliferation, and an appropriate level of expression of the gene in the relevant cell types. Although recent advances have been made, adenoviral and adeno-associated viral vectors currently suffer from several disadvantages for successful gene transfer into HSCs. Since the transgene has to be transmitted from the HSC to the differentiated progeny, and since prolonged antiviral resistance against HIV infection is required, integration of the antiviral gene in the host chromosome of the dividing target cells of HIV is necessary and, as a consequence, retroviral vectors have been most commonly used. Since the use of murine oncoretroviral vectors is limited to dividing cells, prior stimulation of HSCs with cytokines is required. Virtually all the clinical trials for gene therapy using HSCs have used murine oncoretroviral –derived from Moloney murine leukemia virus (MoMLV)–

vectors. The disappointing results of the first clinical trials employing retroviral vectors were thought to be partly related to the loss of pluripotency and the induction of differentiation associated with stimulation of HSCs[15,16].

Recent data from gene therapy trials for SCID-X1 and ADA-SCID demonstrate the feasibility of efficient gene therapy into HSCs using oncoretroviral vectors, at least for these specific diseases and in pediatric patients[11]. On the other hand, in these trials the theoretical risk for insertional mutagenesis proved to be a real risk, since two children developed a malignant lymphoproliferation. Ongoing research in retroviral vectorology is aimed at reducing this risk by using self-inactivating promoters and insulators[17].

Lentiviral vectors

Since lentiviral vectors, derived from lentiviruses such as HIV, retain the property to transduce nondividing cells, the use of lentiviral vectors should enable transduction of primitive, quiescent HSCs, and thus enable avoidance of the engraftment defect seen with HSCs manipulated for uptake of oncoretroviral vectors. Although pre-stimulation and cell-cycling induction can be avoided for lentiviral transduction, controversy persists on whether lentiviral vectors can transduce truly quiescent HSCs[18]. Indeed, most data suggest that HSCs in G_0 phase are relatively resistant to lentiviral vector transduction, suggesting that some form of cellular activation of HSCs is necessary to render them susceptible to lentiviral vector transduction[19,20]. Further progression within the cell cycle is not required for successful lentiviral transduction, but is associated with an increased transduction efficiency[20]. Therefore, transduction of primitive hematopoietic cells has, in general, been improved by a brief culture in the presence of hematopoietic cytokines. The vast majority of studies reported so far with lentiviral vectors have been aimed at developing gene transfer with minimal *in vitro* culture to reduce negative effects on the survival and perhaps the long-term *in vivo* repopulation ability of stem cells[18]. HIV-derived vectors were able to transduce HSC, and both long-term engraftment and multilineage reconstitution have been shown in non-obese diabetic (NOD)-SCID mice and rhesus monkeys without the need of *in vitro* cell-cycle induction[21-23]. Several additional advantages exist. The preintegration complex of lentiviruses is relatively stable, increasing the time window for integration[24]. Lentiviral vectors ensure better expression of the transgene; current data suggest that lentiviral vectors express the transgene at higher levels[25] and expression of lentiviral vectors might be less prone to gene silencing[18,24,26,27]. Finally, complex regulatory gene regions can be more easily incorporated[28,29].

Antiviral constructs

The gene therapy strategies for HIV infection can be divided into two basic approaches:

Selective elimination: removal of infected cells, either directly using HIV-1 inducible genes encoding toxins, or indirectly with genes encoding antigens resulting in stimulation of immune responses. Gene therapy approaches that attempt to elicit a cytotoxic T-lymphocyte (CTL) response directly in infected patients or in cell culture followed by reinfusion of "primed" anti-HIV CTLs –adoptive immune transfer– have been investigated. Two phase I and one phase II clinical trials have been completed that investigated the potential antiviral effects of gene-modified HIV-specific T-cell therapy[32]. Although no clear clinical benefit was observed, measures of viral load suggested a possible antiviral effect[33]. An interesting approach is vector-mediated introduction of viral genes into dendritic cells where the expression of viral antigens can elicit an anti-HIV CTL response[34,35]. Using a dendritic cell-based approach, impressive results in chimpanzees have recently been reported[36]. Therapeutic DNA vaccination is based on the same principle[37]. Although several clinical trials of immunotherapy have been performed, proof-of-principle that a clinical benefit can be achieved is currently lacking[3,38,39].

Intracellular immunization: the introduction of *antiviral genes* into target cells (or their progenitors) of HIV-1 that encode nucleic acid sequences or proteins that interfere specifically with HIV replication. The gene product that mediates the antiviral effect is either a protein or RNA. Both have advantages and disadvantages. Proteins can be immunogenic, while RNAs are not. Apart from viral structures, cellular proteins involved in HIV-1 pathogenesis could be targeted as well. Indeed, due to the high mutation rate, viral escape mutants occur relatively easily. By strategically targeting cellular proteins, it is hoped that the development of resistance would be more difficult. Transfer of these genes into T-cells has been proven to inhibit HIV-1 replication *in vitro*. In addition, introduction of these constructs into HSCs has been shown to confer antiviral resistance to the progeny in culture. This review will focus on the latter approach.

Protein-based approach

Dominant-negative mutants of viral proteins are nonfunctional mutants that inhibit the function of the wild-type counterpart when co-expressed in cells. Dominant-negative mutants of both structural and regulatory proteins have been used as antiviral agents[8,40]. By far the most intensively studied is a Rev mutant termed RevM10[41,42]. The RevM10 mutant can still bind the

Rev-response element (RRE) and multimerize with wild-type Rev, but is nonfunctional. Transdominant negative mutants of cellular proteins involved in HIV replication have been targeted as well[43].

Single-chain antibodies are intracellularly expressed antibodies, referred to as "intrabodies". Apart from multiple viral targets, several cellular proteins have been targeted like CCR5 and CXCR4[44-46].

Nucleic acid-based approach

Genetically engineered *antisense RNA* molecules have the potential, by binding to complementary viral sequences, to interfere both with early and late stages of the viral replication cycle. The RNA duplex is degraded by cellular RNase or the translation is blocked. Use of antisense DNA oligonucleotides has been hampered by poor cellular uptake, intracellular degradation, or nonspecific effects. More promising is viral vector-mediated delivery of antisense genes; transcription will lead to production of antisense mRNA. Multiple viral structures have been targeted with this approach. Antisense RNAs can tolerate a high degree of nucleotide sequence divergence with the target RNA, making the emergence of antisense-resistant variants less likely. Long-term and high levels of antisense are required to effectively inhibit viral replication, and the antisense has to be present at least at an equimolar ratio as the sense transcript. Hence, the promoter driving antisense gene expression has to be strong enough.

An interesting development is the use of the HIV long terminal repeat (LTR) as promoter of the antisense transcript; since this promoter is Tat-dependent, expression of antisense can be made dependent upon infection of the target cell by HIV[47]. Currently, a phase I clinical trial in HIV-1 infected patients, based on vector-mediated *env*-antisense delivery into CD4+ T-cells, is taking place[48]. In cell culture, this antisense construct achieved 3-log suppression of HIV-1 replication at low multiplicities of virus infection (MOI). Mobilization of the vector by rescue with HIV is expected to spread the antiviral gene further[49]. This is the first clinical trial using HIV vectors. The trial plans to enroll a total of five patients who are infected with HIV and have failed two regimens of HAART. As at November 2004, three patients were being treated. For additional information, we refer to the company's website (http://www.virxsys.com).

Ribozymes are small antisense RNA molecules capable of cleaving specific target RNA sequences in a catalytic reaction. In contrast to antisense RNA, a single ribozyme can inactivate many target RNA molecules. Since ribozymes are encoded by small transcriptional units, multiple ribozymes targeting different genomic regions can be incorporated into one vector. Both incoming genomic RNA and newly transcribed RNA from the integrated virus can be targeted[50,51]. Ribozymes are also more specific than antisense RNA. A disadvantage for HIV gene therapy is the high mutation rate of HIV; a single mutation in the binding or cleavage sequence may render the ribozyme inactive. Cellular gene transcripts involved in HIV replication, like the CCR5 molecule, have been targeted in particular, since it is known that people who naturally lack functional CCR5 are less susceptible to HIV infection and disease progression[52]. CD34+ cells have been transduced with a vector encoding an anti-CCR5 ribozyme. These cells were able to differentiate in cells resistant to HIV infection *in vitro* and in animal models[53,54].

RNA decoys function by sequestration of essential viral or cellular proteins. Both TAR and RRE sequences have been used. A RRE decoy, used in a study with pediatric patients infected with HIV, was only of limited success[55].

RNA interference is a general mechanism for silencing the mRNA transcript of an active gene[56]. This process of post-transcriptional gene silencing is initiated by small interfering RNA (siRNA), a 21 to 23 bp long double-stranded form of RNA that is highly specific for the nucleotide sequence of its target mRNA. It was found that transfection of differentiated mammalian cells with synthetic siRNA results in highly sequence-specific RNA interference[57]. To avoid the degradation of siRNA by cellular enzymes, DNA plasmids that encode siRNAs in the form of stem-loop hairpins are being used. Recently, siRNA constructs have been successfully incorporated in various viral vector systems, including lentiviral vectors[58-60]. This opens the way for clinical applications. RNA interference quickly became a weapon for multifrontal attacks on HIV[61]. Both viral and cellular sequences have been targeted and both preintegration and post-integration mRNA could be degraded[62]. Simultaneous attacks by siRNA targeting strategically important viral and cellular functions will most likely act synergistically.

Genetic intervention at different points of the HIV-1 lifecycle

There is an order of preference regarding which viral gene products are the most ideal targets for genetic intervention[65]. Preferably, the very early steps of the viral lifecycle should be blocked, thereby abolishing the possibility of viral replication and the transcription of toxic gene products at a very early step in the viral lifecycle. This could be accomplished by preventing virus entry –by acting on CXCR4 and/or CCR5– or the formation and integration of proviral DNA. If proviral DNA integration cannot be prevented, then the next step to be targeted is the expression of the early viral RNAs, e.g. by ribozymes targeting the U5 region of the mRNA. The following target is the stimulation of tran-

Table 1. Gene therapy trials for HIV-1 infection

Cells	Vector	Construct	N	Patients	AR	VL	Results	Ref.
CD4	Gold	RevM10	3		N?	NR	Detection up to 8 weeks, preferential survival	[74]
	RV	RevM10	3		M	L	Detection for 6 months, preferential survival	[71]
	RV	RevM10-Tat-AS	NR	Syngeneic	NR	NR	Not reported in literature	[75]
	RV	Tat-ribozyme	4	Syngeneic	Y/N	NR	Marking up to 4 years	[76]
	RV	LTR-ribozyme			NR	NR	Low-level marking, preferential survival?	[8,72]
	LV	Env-AS	3	Failed HAART	N?	H?	Currently being performed	[48]
CD34								
BM	RV	RRE-decoy	4	Pediatric patients	Y	H	Very short and low level of marking	[55]
PB	RV	TdRev	1	Non-myeloablative conditioning	Y	U	Low level of marking no preferential survival	[77,78]
PB	RV	Ribozyme	10		Y/N	Mo	Gene marking up to 3 years, no preferential survival	[79]
PB	RV	Tat/Rev-ribozyme	5	Myeloablative conditioning (HIV-1 lymphoma)	Y	L	Short-lived cell marking; 10- to 50-fold increase due To myeloablation	[80,81]

VL: viral load; H: high; L: low; Mo: moderate; U: undetectable; AR: antiretroviral therapy; Y: yes (HAART); N: no; M: monotherapy; RV: oncoretroviral; LV: lentiviral; BM: bone marrow; PB: peripheral blood; Td: transdominant; AS: antisense; NR: not reported.

scription by Tat, e.g. by TAR-decoy, ribozymes, or Tat-antisense. If transcription cannot be stopped, the nuclear export might be. Here, the transdominant negative mutant RevM10 has been very effective. Finally, blocking virion formation and maturation has been achieved by transdominant-negative mutants of Gag and cellular proteins. Of note, blocking the function of the late gene products will reduce virion production, but will not prevent the expression of cytotoxic viral gene products[65].

Early gene therapy trials for HIV-1 infection

In the first clinical trials of gene therapy against HIV infection, CD4+ T-cells were transduced, since oncoretroviral transduction of HSCs was rather unsuccessful at that time (Table 1). A small phase I clinical trial was performed using an oncoretroviral vector to introduce RevM10 into peripheral CD4+ T-cells of HV-1 infected patients[71]. The procedure appeared safe, and cells containing RevM10 could be detected for an average of about six months and they preferentially survived over cells transduced by the control vector. No anti-RevM10 antibodies were detected. Clinical trials involving the use of anti-HIV ribozymes have been undertaken as well. The ribozyme-containing T-cells demonstrated a preferential survival[72]. However, only low levels of transduced cells were detected. In contrast, in a ADA-SCID trial using similar protocols, persistence of gene-marked cells has been demonstrated for 12 years[73]. The pioneering clinical trials supported the safety and feasibility of this approach and provided proofs-of-principle. There was hope that, by targeting HSCs, a more sustained impact could be obtained.

In pediatric HIV-infected patients, CD34+ cells isolated from bone marrow were transduced with a murine retroviral vector encoding an RRE decoy sequence, and transduced cells were reinfused into the patients[55]. Only extremely low levels of gene-containing leukocytes could be detected in the patients after infusion of RRE decoy-containing transduced cells, and only on the day after cell infusion. No survival advantage could be evidenced. The disappointing data stand in contrast to the results using similar methods of gene transfer into CD34+ cells derived from the cord blood of infants with ADA-SCID, where higher levels of gene marking were obtained for prolonged time periods. A new phase I clinical trial using optimized transduction conditions is currently being conducted in pediatric HIV-patients[70]. In another trial, CD34+ HSCs were mobilized with granulocyte colony stimulating factor (G-CSF), isolated by apheresis and transduced with a ribozyme-encoding oncoretroviral vector before being reinfused. Ten patients were enrolled in the study; each tolerated the procedure well and the feasibility of the protocol was established with gene-marked cells persisting for at least three years[79].

The experience demonstrating the feasibility of stem-cell mobilization and administration of myeloablative

chemotherapy in HIV-positive individuals formed the basis of trials exploring the use of gene transfer into stem cells in an attempt to obtain long-term control of HIV infection. In a phase I/II study, five patients with AIDS lymphoma underwent autologous (peripheral blood) stem-cell transplantation and received, in addition to the un-manipulated peripheral blood stem cells, selected CD34+ cells transduced with oncoretroviral vector-encoding ribozymes targeted to *tat* and *rev*[80]. Engraftment times were similar to the HIV-negative setting and no increase in regimen-related toxicity was seen. All the patients were maintained on HAART during the transplant period. No significant changes in viral load were observed, and the CD4 counts rose above baseline by 10 to 12 months posttransplant. Thus, the gene manipulation appeared to be safe and did not have deleterious long-term effects on the underlying HIV infection. In terms of gene marking, the five subjects with AIDS lymphoma undergoing conditioning before stem-cell transplantation showed a 10- to 50-fold increase in marked cells posttransplant as compared to prior, healthy, HIV-infected volunteers who received transduced cells without myeloablative chemotherapy[81]. However, the durability of this engraftment was short-lived. There was an observable marking in multiple cell lineages during the first six months posttransplant, but this declined to minimum levels of detection over the next six months.

Hence, the use of chemotherapy and stem-cell transplantation is safe in HIV-infected patients on HAART. The technologies used in clinical trails until now, however, were able to produce only low levels of gene marking in adult patients. Due to the morbidity and mortality associated with myeloablation, non-myeloablative conditioning would be a more interesting option. In a study of HIV-infected patients with hematologic malignancies, non-myeloablative conditioning in one patient was combined with transplantation of allogeneic HSCs, transduced with an oncoretroviral vector encoding for transdominant Rev[77]. Although gene marking was observed during the three years after transplantation, only 0.01% of cells were marked, with similar levels for myeloid and lymphoid cells. Relative to control vector-transduced cells, no survival or proliferative advantage of cells carrying the therapeutic gene could be observed, although the absence of detectable HIV replication might have removed any selective pressure[77].

Recent progress in the field of HSC-based gene therapy

Although the current gene therapeutic clinical trials have established the safety and provided proof-of-principle for gene therapy of HIV-1 infection, the overall results have been disappointing, with only low-level and/or short-term gene marking and no unambiguous clinical benefit[70]. These disappointing clinical results mirror the findings of other gene-marking trials, all using non-optimized gene transfer conditions. In addition, immune clearance of transduced cells has been documented in HIV-1 and other gene therapy trials, possibly explaining the short-term marking in some studies[70]. However, further improvements in gene transfer and gene therapy strategies will probably lead to future clinical successes. Since a significant percentage of the stem cells in the patient have to be transduced to obtain a significant impact on HIV replication, the first requisite for successful gene therapy of HIV-1 infection will consist of increasing the amount of gene-marked HSCs.

Optimization of oncoretrovirus-mediated transduction efficiency

After the disappointing results of the early clinical trials, researchers returned to the bench to work on the optimization of oncoretroviral vectors for gene transfer into human HSCs[18] (Table 2). In addition, the importance of preclinical testing of gene transfer approaches *in vivo* in adequate animal models, such as NOD-SCID mice and nonhuman primate animal models, was emphasized[82]. A major observation was that not cell cycling per se, but rather the specific cytokine environment, critically determines the engraftment deficit. Optimization of the cytokine cocktail and/or adhesion to fibronectin may favor HSC cycling with preservation of stem-cell properties including self-renewal and *in vivo* engraftment capacity[83-85]. Using these cocktails, successful oncoretroviral transduction both in NOD/SCID and in nonhuman primate animal models has been obtained[86,87]. The progress in oncoretroviral vector-based transduction has been translated into successful gene therapy. Indeed, recent data from gene therapy trials for SCID-X1 and ADA-SCID demonstrate the feasibility of efficient gene therapy into HSCs using oncoretroviral vectors, at least for these specific diseases and in pediatric patients[11,30].

Lentiviral vectors

Although lentiviral vectors have several theoretical advantages compared to oncoretroviral vectors, evidence of the superiority of lentiviral vectors for clinical applications is still lacking[65]. The first clinical trial using lentiviral vectors (in HIV-infected patients) has been started recently (see above)[48]. Given the safety concerns associated with lentiviral vectors for human use, it is clear that they must be shown to have significant technological advantages over standard retroviral vectors. Any difference in the risk of insertional mutagen-

Table 2. Major technological advances in (onco)retroviral HSC gene transfer

- Transduction modalities: fibronectin
- Different viral envelopes: VSV-G, GALV, RD-114
- Improved gene expression: MSCV
- Improved cytokines to maintain repopulating capacity
  - Thrombopoietin (TPO)
  - Stem cell factor (SCF)
  - FLT-3 ligand
- Alternative vector systems: lentiviral vectors

esis might be a crucial feature. Indeed, since different integration-site profiles were reported, the risk factors for the use of MLV- or HIV-1-based vectors for gene therapy may not be identical[31]. Since insertional mutagenesis can increase the risk of activation of cellular genes, hope has been raised that lentiviral vectors might be less prone to activate cellular genes[31].

An additional advantage of the use of HIV-1-based vectors for gene therapy for HIV-1 infection is the inhibition of HIV-1 replication by HIV-1-derived vector constructs. A first mechanism of inhibition is the sequestration of Tat and Rev by the TAR and RRE sequences present in the vector backbone[63], although other authors have minimized this effect[64]. Moreover, mobilization of vector genomic RNA by infectious HIV-1 virus results in a competition for RNA packaging and an interference with reverse transcription in heterodimeric virions. Both mechanisms need an intact LTR to allow the transcription of genomic RNA, stimulated upon Tat production during HIV-1 infection. Another consequence of vector mobilization is the potential spread of the vector genome to non-transduced cells. Although this mechanism can increase the level of gene-marked cells, this has also raised biosafety concerns[65]. The use of self-inactivating (SIN) vectors, lacking a functional LTR promoter, can thus increase the biosafety of lentiviral vectors, but as a consequence the inhibition of HIV-1 replication and the vector mobilization will be greatly lost. Some people argue that the use of SIN vectors is preferable for other gene therapy applications to minimize the risk of recombination and insertional mutagenesis, whereas in the context of HIV-1 infection, a vector containing an intact LTR should be used[65].

For HIV-1 gene therapy, the use of conditionally replicating HIV-1 vectors has even been proposed to induce a pseudo-latent state in virus-producing cells, potentially reducing the HIV-1 set point [66]. In essence, the aim of this approach is to create a parasite of HIV. Based on a mathematical model of HIV-1 *in vivo* dynamics, it was calculated that the use of these vectors could reduce HIV-1 set point to levels comparable to HAART, provided the vector is more efficiently packaged than HIV-1. Although this certainly raises concerns on biosafety, it remains a challenging idea[66].

Post-transduction selection or expansion strategies

In vivo *selection*

During competitive repopulation, gene-modified cells are diluted, resulting in a low percentage of transduced cells. However, if a strong selective advantage is conferred to the transduced cells, extensive selection and accumulation of the modified cells can occur *in vivo*[88]. This was clearly demonstrated in the gene therapy trial for patients with SCID-X1, where impressive clinical results were obtained without prior bone marrow chemo-ablation[11]. In this trial, transduction of CD34+ HSCs with a retroviral vector expressing the common γ-chain confers an *in vivo* growth advantage for certain lymphoid progenitor cells, resulting in full repopulation of the T-cell compartment despite relatively low transduction efficiency and very low levels of marking in the myeloid compartment. In this study, the marking of myeloid cells was only 0.01 to 0.1%, demonstrating both the difficulty of gene marking and engraftment of HSC, without marrow-conditioning regimens when no strong selection pressure exists. To detect a clinically important survival and/or growth advantage, selection at the progenitor level is thought to be required[88]. If no extensive selection of transduced cells can be expected *in vivo*, additional strategies will be necessary to increase the level of gene-marked cells in the peripheral blood. A recent study of gene therapy for ADA-SCID, employing non-myeloablative bone marrow conditioning with busulfan, demonstrated that marrow conditioning may enhance engraftment and therefore gene therapy marking, even where no or only low selective advantage is expected (the myeloid compartment) and at the same time it may enhance a selective advantage where one is expected (T-lymphocytes)[30]. A more elegant approach is the transfer of genes that confer resistance to drugs that are capable of efficiently killing stem cells, enabling *in vivo* selection of transduced HSCs. Although many different drug-selection genes have been investigated, expression of the multidrug resistance gene 1, conferring resistance to a variety of chemotherapeutic agents,

has been studied in most detail over the past ten years[89]. More recent clinical data from HSC transplantation in cancer patients support the feasibility of this approach, although still only modest increases in genetically modified cells occurred following cytotoxic drug treatment[90]. Interestingly, using the methylguanine methyltransferase resistance gene, highly efficient *in vivo* selection in a non-myeablative setting in dogs was recently reported. A phase I clinical trial using this approach is currently being performed[91].

In vivo *expansion*

Instead of relying on *in vivo* selection on genes that confer resistance to cytotoxic drugs, it is also possible to use a cell-growth switch, allowing a minor population of transduced cells to be amplified *in vivo*. Although promising results were obtained in mice, using a chimeric gene composed of the granulocyte colony-stimulating factor receptor gene and the estrogen receptor gene hormone-binding domain, the only study in non-human primates was less successful[92]. Recently, a similar system in which the erythropoietin receptor is used has been described[93]. Another approach to amplify genetically modified cells is to confer a competitive advantage to genetically corrected cells through the use of molecules that promote HSC regeneration and self-renewal, such as the HOXB4 transcription factor. A selective *in vivo* growth advantage by HOXB4-overexpression was confirmed in human stem cells. However, high HOXB4 expression substantially impaired the myeloerythroid differentiation program and the B-cell development[94].

In vitro *expansion*

Recently, several studies have been published reporting on different cytokine cocktails for extensive expansion of primitive cells from cord blood without loss of the stem-cell properties[84,95]. These data indicate that *in vitro* manipulation and expansion of human primitive HSCs does not necessarily lead to loss of self-renewal and multilineage differentiation capacity. Rather, gene transfer into primitive HSCs, followed by *in vitro* expansion, could provide a means to facilitate and accelerate the engraftment of genetically modified stem cells.

In conclusion, earlier clinical trials were hampered by inefficient gene transfer into true multipotent HSCs, poor competition of cells manipulated *in vitro* with endogenous HSCs, and poor *in vivo* transgene expression. Recent clinical successes were not only based on improvements in HSC gene-transfer techniques, but also dependent on a strong growth selective advantage of gene-corrected precursor cells[88]. Further development of post-transduction selection, and/or expansion strategies either *in vitro* or *in vivo* will most likely be necessary for clinical applications in disorders without a significant inherent growth advantage of corrected cells such as HIV-1 infection.

General remarks on the feasibility of gene therapy against HIV-1 infection

It has to be realized that HIV-1 infection is a very complex disease, affecting various organs in addition to the T-cells, with an impact on T-cell homeostasis that is currently not fully understood[96]. The extent to which these alterations hamper a gene therapy approach, or can be reversed upon HAART, will determine the feasibility of future gene therapy against AIDS.

Altered hematopoiesis and thymopoiesis during HIV-1 infection

A first potential difficulty arises from the prolonged, extensive, HIV replication in the patients, resulting in significant functional and morphologic changes in various organs. It is known that HIV infection can affect hematopoiesis and interferes with thymopoiesis[13,14]. Hence, functional defects at both levels could hamper the clinical benefit from HSC transfusion. Although most data demonstrate that antiretroviral therapy can reverse the functional defects of HIV-1 infection on thymopoiesis and hematopoiesis[13], minor but clinically important alterations are not excluded. Hence, the extent and the rate of production of gene-transduced naive T-cells will critically affect the clinical benefit of this approach.

The currently successful gene therapeutic trials have been performed in pediatric patients, where active thymopoiesis takes place. In addition, some data suggest that significant differences exist between adult and neonatal stem cells[97-99]. Hence, successful gene therapeutic approaches in pediatric patients cannot be automatically expected to be applicable to the adult population. T-cell production involves two pathways: thymus-dependent development of new T-lymphocytes and thymus-independent proliferation of mature cells in the peripheral blood and lymphoid tissues. Although recent data suggest a persistent role of the thymus for T-cell production in adults[100], the issue is still controversial. The key question remains unanswered: how much thymic contribution to the peripheral blood pool occurs in HIV-1 infection, both before and after HAART. Indeed, whether the increase in naive T-cells upon institution of HAART is caused by renewed thymopoiesis is not universally acknowledged [101]. However, the extent of production of naive T-cells by the thymus will critically determine the feasibility of stem cell-based gene therapy for HIV-1 infection[102,103]. In a recently reported trial using an HSC-based approach against HIV-1 infection, naive

T-cells expressing the antiviral gene were detected in some patients, albeit at low levels[79].

Impact of generalized immune activation and apoptosis?

Although the limited clinical data currently available suggest that some form of selection might take place at the T-cell level, the extent of the selection will clearly be less pronounced than at the progenitor level, as observed in the SCID-trials[88]. Whether selection would also occur in the advent of efficient HAART-induced suppression of viral replication, will have to be determined. Similar observations were made in the ADA-SCID gene therapeutic trials where, in general, patients continued to receive regular injections with ADA-PEG. Only recently, it was evidenced that withdrawal of ADA-PEG did result in a clear survival advantage of transduced cells, resulting in an increase in gene marking from between 1 and 3% up to 100%[104]. To what extent selection and/or protection of transduced cells will still occur in the event of high-level viral replication (in the absence of HAART) is not known. HIV infection is characterized by altered T-cell homeostasis and distribution, increased apoptosis, and a generalized activation of the immune system[96]. This results in a shortened survival of both infected and uninfected T-cells[105]. Even under treatment with HAART, some degree of over-activation of the immune system persists[106]. Hence, whether the *in vitro* resistance of the transduced cells will result in increased T-cell survival, *in vivo* selection, and reduction of HIV replication are questions that can only be answered in animal models and patient studies.

Induction of HIV-specific immune responses?

Whether the presence of resistant T-cells will also increase the HIV-specific immune responses is an open question[14]. Whereas efficient suppression of viral replication with HAART leads to the restoration of an effective immunity to viral pathogens, HIV-specific immunity is not restored to the extent of controlling HIV-1 replication upon removal of HAART. Several factors might contribute. The current view is that HIV-specific T-cells are preferentially infected[107]. Indeed, residual HIV-1 replication, even with efficient HAART, is thought to occur and this could eliminate activated/functional HIV-1 specific cells. However, HIV-specific immune cells can be detected, even in the chronic phase of the infection, pointing to a problem at the functional level[14]. Alternatively, it could be that HAART removes the antigenic trigger necessary for priming of the immune response[106]. This constitutes the rationale basis for immunotherapeutic approaches such as therapeutic immunization[37]. Only in the first scenario could the presence of the antiviral gene possibly contribute to mounting an efficient antiviral immune response after chronic infection has taken place. Even then, the resistant HIV-1 specific cells will have to be functional.

Combination of various antiviral constructs?

It is generally accepted that, for any therapy to be effective against HIV infection, different antiviral targets have to be targeted at once. Classical drug therapy today combines inhibitors of reverse transcriptase and protease; in the future, entry and integrase inhibitors will likely be added to these combination regimens. The same is true for gene therapy strategies. In cell-culture experiments, a single antiviral gene typically reduces viral load by 1 to 2 log; only at lower MOI can virus breakthrough be prevented[65]. In contrast, antiviral drugs can completely block HIV replication at much higher MOI. Therefore, it will be necessary to combine different antiviral genes in one vector, and this is technologically easier to achieve than designing effective drug combinations[6]. Several studies demonstrate HIV inhibition at higher MOI when several antiviral genes are combined, or when high expression levels of the antiviral gene can be obtained[67-69]. Cell-culture experiments generally utilize selected cell lines, where virtually every cell expresses the antiviral gene. If essentially all of the patient's target cells could be rendered resistant, viral load is likely to decrease. Theoretically, inhibition of HIV-1 replication in 99.9% of target cells would be necessary to obtain a 3-log reduction in viral load, as can be obtained with HAART[70]. However, since the transduced cells will be diluted upon administration, only a fraction of the cells in HIV patients will be protected. Combination of drug therapy and gene therapy (as in ongoing clinical trials for anti-HIV gene therapy) is desirable. Potent drug cocktails block replication of HIV; antiviral genes create a reservoir of immune cells that are resistant to HIV infection. If the protected cells acquire a selective survival advantage, the limited number of protected cells should gradually increase, resulting in some preservation of immune function.

Adequate animal models

Unfortunately, conclusions based on *in vitro* assays are of limited value since they do not represent a true scenario of an *in vivo* HIV-1 infection, where the interplay between different cell types involved in HIV-1 infection, and the interactions with the host organism, can be evaluated altogether. Since gene therapy is a clinical discipline, progress requires –next to basic research– studies in animal models and in patients. A major drawback is the lack of clinically relevant data

from animal models[65]. Infection of Rhesus monkeys with simian immunodeficiency virus (SIV) provides a suitable model of HIV-1 infection in humans[108]. Using this model and oncoretroviral delivery of an antisense construct in CD4+ T-cells, a significant attenuation of subsequent SIV infection was observed[109]. The feasibility of transplantation of CD34+ monkey HSCs transduced with lentivirus vectors expressing marker genes has already been demonstrated[110]. Various other nonhuman primate models exist[111,112]. Interestingly, a recent report describes rats transgenic for HIV-1 receptors as an animal model[113]. The ultimate goal at the preclinical level would be to assess gene therapeutic approaches in an adequate animal model. Despite the high costs and difficulties associated with experiments in nonhuman primates, these models are the only ones that allow testing of the real efficacy of these gene therapy approaches and provide the likelihood of success before its application in human clinical trials[82]. Many relevant questions could be (partially) answered by animal studies.

Perspectives

Initially, high hopes were raised for the gene therapy of AIDS. The initial gene therapy trials were hampered by insufficient gene transfer. Recent progress in this field has resulted in the first successful gene therapy trial for X-SCID. However, one has to admit that gene therapy for AIDS does not have the potential to cure AIDS. Still, in conjunction with HAART and other adjuvant therapies, it could be highly valuable. In this view, there are some similarities with chemotherapeutic agents for the treatment of cancer, which can be used in different stages of disease: a drug can be used for induction (before the application of other therapies), as consolidation (to increase the effect of a certain therapy), or it can be used as a last resort when other therapies have failed. One potential clinical application would be to use gene therapy in patients that have failed HAART due to the development of multidrug resistance. The current gene therapy trial using lentiviral vectors is being performed in this setting[48]. Although the presence of resistant cells could potentially reduce the viral load, the persistence of a sufficient level of CD4 T-cells, leading to some preservation of immune function, would be the major benefit. The severe side effects, and the potential development of virus-drug resistance after several years of successful HAART therapy, constitute two of the major drawbacks of the current therapy.

Current therapeutic guidelines recommend that the initiation of HAART is delayed[114]. Therefore, gene therapy could be applied prior to initiation of HAART. By affecting the rate of viral replication and the impact on the CD4 count, the use of HAART could be delayed with several years. Finally, the concurrent application of HAART and gene therapy might be preferred. Potentially, this could increase or prolong the suppression of viral replication, or could counteract the gradual decrease in CD4 count over the years. Clearly, many issues still remain to be resolved before this approach can be efficiently used against HIV infection. However, recent progress in the field of HSC-based gene therapy makes it a promising strategy in the fight against AIDS.

References

1. De Clercq E. New anti-HIV agents and targets. Med Res Rev 2002;22:531-65.
2. Jenny-Avital E. Enfuvirtide, an HIV-1 fusion inhibitor. N Engl J Med 2003;349:1770-1.
3. Letvin N, Walker B. Immunopathogenesis and immunotherapy in AIDS virus infections. Nat Med 2003;9:861-6.
4. Autran B, Carcelain G, Li T, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 1997;277:112-6.
5. Palella F, Delaney K, Moorman A, et al. Declining morbidity and mortality among patients with advanced HIV infection. N Engl J Med 1998;338:853-60.
6. Powderly W. Limitations of current HIV therapies: opportunities for improvement. J Acquir Immune Defic Syndr 2003;33 (suppl 1):7-12.
7. Shafer R, Winters M, Palmer S, et al. Multiple concurrent reverse transcriptase and protease mutations and multidrug resistance of HIV-1 isolates from heavily treated patients. Ann Intern Med 1998;128:906-11.
8. Buchschacher G, Wong-Staal F. Approaches to gene therapy for HIV infection. Hum Gene Ther 2001;12:1013-9.
9. Nielsen S, Afzelius P, Dam-Larsen S, et al. Effect of granulocyte colony-stimulating factor (G-CSF) in HIV-infected patients: increase in numbers of naive CD4 cells and CD34 cells makes G-CSF a candidate for use in gene therapy or to support antiretroviral therapy. J Infect Dis 1998;177:1733-6.
10. Shen H, Cheng T, Preffer F, et al. Intrinsic HIV type 1 resistance of hematopoietic stem cells despite coreceptor expression. J Virol 1999;73:728-37.
11. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, et al. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 2000;288:669-72.
12. Douek D, McFarland R, Keiser P, et al. Changes in thymic function with age and during the treatment of HIV infection. Nature 1998;396:690-5.
13. Amado R, Mitsuyasu R, Zack J. Gene therapy for the treatment of AIDS: animal models and human clinical experience. Front Biosci 1999;4:468-75.
14. Scadden D. Stem cells and immune reconstitution in AIDS. Blood Rev 2003;17:227-31.
15. Peters S, Kittler E, Ramshaw H, et al. Murine marrow cells expanded in culture with IL-3, IL-6, IL-11, and SCF acquire an engraftment defect in normal hosts. Exp Hematol 1995;23:461-9.
16. Habibian H, Peters S, Hsieh C, et al. The fluctuating phenotype of the lymphohematopoietic stem cell with cell cycle transit. J Exp Med 1998;188:393-8.
17. Barquinero J, Eixarch H, Perez-Melgosa M. Retroviral vectors: new applications for an old tool. Gene Therapy 2004;11 (suppl):3-9.
18. Brenner S, Malech H. Current developments in the design of oncoretrovirus and lentivirus vector systems for hematopoietic cell gene therapy. Biochim Biophys Acta 2003;1640:1-24.
19. Piacibello W, Bruno S, Sanavio F, et al. Lentiviral gene transfer and *ex vivo* expansion of human primitive stem cells capable of primary, secondary, and tertiary multilineage repopulation in NOD/SCID mice. Non-obese diabetic/severe combined immunodeficient. Blood 2002;100:4391-400.

20. Ailles L, Schmidt M, Santoni de Sio F, et al. Molecular evidence of lentiviral vector-mediated gene transfer into human self-renewing, multipotent, long-term NOD/SCID repopulating hematopoietic cells. Mol Ther 2002;6:615-26.
21. Miyoshi H, Smith K, Mosier D, et al. Transduction of human CD34+ cells that mediate long-term engraftment of NOD/SCID mice by HIV vectors. Science 1999;283:682-6.
22. An D, Kung S, Bonifacino A, et al. Lentivirus vector-mediated hematopoietic stem cell gene transfer of common gamma-chain cytokine receptor in rhesus macaques. J Virol 2001;75:3547-55.
23. Zielske S, Gerson S. Cytokines, including stem cell factor alone, enhance lentiviral transduction in nondividing human LTCIC and NOD/SCID repopulating cells. Mol Ther 2003;7:325-33.
24. Chang L, Gay E. The molecular genetics of lentiviral vectors-current and future perspectives. Curr Gene Ther 2001;1:237-51.
25. Zaiss A, Son S, Chang L. RNA 3' readthrough of oncoretrovirus and lentivirus: implications for vector safety and efficacy. J Virol 2002; 76:7209-19.
26. Gropp M, Itsykson P, Singer O, et al. Stable genetic modification of human embryonic stem cells by lentiviral vectors. Mol Ther 2003;7:281-7.
27. Bai Y, Soda Y, Izawa K, et al. Effective transduction and stable transgene expression in human blood cells by a third-generation lentiviral vector. Gene Ther 2003;10:1446-57.
28. May C, Rivella S, Callegari J, et al. Therapeutic hemoglobin synthesis in beta-thalassaemic mice expressing lentivirus-encoded human beta-globin. Nature 2000;406:82-6.
29. Pawliuk R, Westerman K, Fabry M, et al. Correction of sickle cell disease in transgenic mouse models by gene therapy. Science 2001;294:2368-71.
30. Aiuti A, Slavin S, Aker M, et al. Correction of ADA-SCID by stem cell gene therapy combined with non-myeloablative conditioning. Science 2002;296:2410-3.
31. Trono D. Virology. Picking the right spot. Science 2003;300:1670-1.
32. Fanning, G, Amado R, Symonds G. Gene therapy for HIV/AIDS: the potential for a new therapeutic regimen. J Gene Med 2003;5:645-53.
33. Deeks S, Wagner B, Anton P, et al. A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy. Mol Ther 2002;5:788-97.
34. Gruber A, Chen I, Kuhen K, et al. Generation of dendritic cells from lentiviral vector-transduced CD34+ cells from HIV+ donors. J Med Virol 2003;70:183-6.
35. Gruber A, Kan-Mitchell J, Kuhen K, et al. Dendritic cells transduced by multiply deleted HIV-1 vectors exhibit normal phenotypes and functions and elicit an HIV-specific cytotoxic T-lymphocyte response *in vitro*. Blood 2000;96:1327-33.
36. Lu W, Wu X, Lu Y, Guo W, Andrieu JM. Therapeutic dendritic-cell vaccine for simian AIDS. Nat Med 2003;9:27-32.
37. Autran B, Debre P, Walker B, et al. Therapeutic vaccines against HIV need international partnerships. Nat Rev Immunol 2003;3:503-8.
38. Hardy G, Imami N, Gotch F. Improving HIV-specific immune responses in HIV-infected patients. J HIV Ther 2002;7:40-5.
39. Lisziewicz J, Bakare N, Lori F. Therapeutic vaccination for future management of HIV/AIDS. Vaccine 2003;21:620-3.
40. Feinberg M, Trono D. Intracellular immunization: trans-dominant mutants of HIV gene products as tools for the study and interruption of viral replication. AIDS Res Hum Retroviruses 1992;8:1013-22.
41. Malim M, Bohnlein S, Hauber J, et al. Functional dissection of the HIV-1 Rev trans-activator - derivation of a trans-dominant repressor of Rev function. Cell 1989;58:205-14.
42. Mautino M, Keiser N, Morgan R. Inhibition of HIV-1 replication by HIV-1-based lentivirus vectors expressing transdominant Rev. J Virol 2001;75:3590-9.
43. Yung E, Sorin M, Pal A, et al. Inhibition of HIV-1 virion production by a transdominant mutant of integrase interactor 1. Nat Med 2001;7:920-6.
44. Steinberger P, Andris-Widhopf J, Buhler B, et al. Functional deletion of the CCR5 receptor by intracellular immunization produces cells that are refractory to CCR5-dependent HIV-1 infection and cell fusion. Proc Natl Acad Sci USA 2000;97:805-10.
45. BouHamdan M, Strayer D, Wei D, et al. Inhibition of HIV-1 infection by down-regulation of the CXCR4 coreceptor using an intracellular single chain variable fragment against CXCR4. Gene Ther 2001; 8:408-18.
46. Bai J, Sui J, Zhu R, et al. Inhibition of Tat-mediated transactivation and HIV-1 replication by human anti-hCyclinT1 intrabodies. J Biol Chem 2003;278:1433-42.
47. Dropulic B, Hermankova M, Pitha P. A conditionally replicating HIV-1 vector interferes with wild-type HIV-1 replication and spread. Proc Natl Acad Sci USA 1996;93:11103-8.
48. MacGregor R. Clinical protocol. A phase 1 open-label clinical trial of the safety and tolerability of single escalating doses of autologous CD4 T cells transduced with VRX496 in HIV-positive subjects. Hum Gene Ther 2001;12:2028-9.
49. Podsakoff G. Lentiviral vectors approach the clinic but fall back: National Institutes of Health Recombinant DNA Advisory Committee review of a first clinical protocol for use of a lentiviral vector. Mol Ther 2001;4:282-3.
50. Yamada O, Yu M, Yee J, et al. Intracellular immunization of human T cells with a hairpin ribozyme against HIV type 1. Gene Ther 1994;1:38-45.
51. Ramezani A, Ding S, Joshi S. Inhibition of HIV-1 replication by retroviral vectors expressing monomeric and multimeric hammerhead ribozymes. Gene Ther 1997;4:861-7.
52. Liu R, Paxton W, Choe S, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply exposed individuals to HIV-1 infection. Cell 1996;86:367-77.
53. Bai J, Gorantla S, Banda N, et al. Characterization of anti-CCR5 ribozyme-transduced CD34+ hematopoietic progenitor cells *in vitro* and in a SCID-hu mouse model *in vivo*. Mol Ther 2000;1:244-54.
54. Bai J, Banda N, Lee N, et al. RNA-based anti-HIV-1 gene therapeutic constructs in SCID-hu mouse model. Mol Ther 2002;6:770-82.
55. Kohn D, Bauer G, Rice C, et al. A clinical trial of retroviral-mediated transfer of a rev-responsive element decoy gene into CD34(+) cells from the bone marrow of HIV-1-infected children. Blood 1999;94:368-71.
56. Hannon G. RNA interference. Nature 2002;418:244-51.
57. Kitabwalla M, Ruprecht R. RNA interference – a new weapon against HIV and beyond. N Engl J Med 2002;347:1364-7.
58. Shen C, Buck A, Liu X, et al. Gene silencing by adenovirus-delivered siRNA. FEBS Lett 2003;539:111-4.
59. Abbas-Terki T, Blanco-Bose W, Deglon N, et al. Lentiviral-mediated RNA interference. Hum Gene Ther 2002;13:2197-201.
60. Barton G, Medzhitov R. Retroviral delivery of small interfering RNA into primary cells. Proc Natl Acad Sci USA 2002;99:14943-5.
61. Pomerantz R. RNA interference meets HIV-1: Will silence be golden? Nat Med 2002;8:659-60.
62. Surabhi R, Gaynor R. RNA interference directed against viral and cellular targets inhibits HIV type 1 replication. J Virol 2002;76:12963-73.
63. Corbeau P, Wong-Staal F. Anti-HIV effects of HIV vectors. Virology 1998;243:268-74.
64. An D, Morizono K, Li Q, et al. An inducible HIV-1 vector which effectively suppresses HIV-1 replication. J Virol 1999;73:7671-7.
65. Mautino M, Morgan R. Gene therapy of HIV-1 infection using lentiviral vectors expressing anti-HIV-1 genes. AIDS Patient Care STDS 2002;16:11-26.
66. Weinberger L, Schaffer D, Arkin A. Theoretical design of a gene therapy to prevent AIDS but not HIV type 1 infection. J Virol 2003;77:10028-36.
67. Strayer D, Branco F, Landre J, et al. Combination genetic therapy to inhibit HIV-1. Mol Ther 2002;5:33-41.
68. Mautino M, Morgan R. Inhibition of HIV-1 replication by novel lentiviral vectors expressing transdominant Rev and HIV-1 env antisense. Gene Ther 2002;9:421-31.
69. Ding S, Lombardi R, Nazari R, et al. A combination anti-HIV-1 gene therapy approach using a single transcription unit that expresses antisense, decoy, and sense RNAs, and trans-dominant negative mutant Gag and Env proteins. Front Biosci 2002;7:15-28.
70. Engel B, Kohn D. Gene therapy for inborn and acquired immune deficiency disorders. Acta Haematol 2003;110:60-70.
71. Ranga U, Woffendin C, Verma S, et al. Enhanced T cell engraftment after retroviral delivery of an antiviral gene in HIV-infected individuals. Proc Natl Acad Sci USA 1998;95:1201-6.

72. Wong-Staal F, Poeschla E, Looney D. A controlled, phase I clinical trial to evaluate the safety and effects in HIV-1 infected humans of autologous lymphocytes transduced with a ribozyme that cleaves HIV-1 RNA. Hum Gene Ther 1998;9:2407-25.
73. Muul L, Tuschong L, Soenen S, et al. Persistence and expression of the adenosine deaminase gene for 12 years and immune reaction to gene transfer components: long-term results of the first clinical gene therapy trial. Blood 2003;101:2563-9.
74. Woffendin C, Ranga U, Yang Z, et al. Expression of a protective gene prolongs survival of T cells in HIV-infected patients. Proc Natl Acad Sci USA 1996;93:2889-94.
75. Morgan R, Walker R. Gene therapy for AIDS using retroviral mediated gene transfer to deliver HIV-1 antisense TAR and transdominant Rev protein genes to syngeneic lymphocytes in HIV-1 infected identical twins. Hum Gene Ther 1996;7:1281-306.
76. Cooper D, Penny R, Symonds G, et al. A marker study of therapeutically transduced CD4+ peripheral blood lymphocytes in HIV discordant identical twins. Preclinical characterization of an anti-tat ribozyme for therapeutic application. Hum Gene Ther 1999; 10:1401-21.
77. Kang E, de Witte M, Malech H, et al. Non-myeloablative conditioning followed by transplantation of genetically modified HLA-matched peripheral blood progenitor cells for hematologic malignancies in patients with acquired immunodeficiency syndrome. Blood 2002; 99:698-701.
78. Kang E, De Witte M, Malech H. Gene therapy-based treatment for HIV-positive patients with malignancies. J Hematother Stem Cell Res 2002;11:809-16.
79. Amado R, Mitsuyasu R, 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. Junker U, Moon J, Kalfoglou C, et al. Hematopoietic potential and retroviral transduction of CD34+ Thy-1+ peripheral blood stem cells from asymptomatic HIV type-1-infected individuals mobilized with granulocyte colony-stimulating factor. Blood 1997;89: 4299-306.
81. Krishnan A, Zaia J, Molina A. Stem cell transplantation and gene therapy for HIV-related lymphomas. J Hematother Stem Cell Res 2002;11:765-75.
82. Donahue R, Dunbar C. Update on the use of nonhuman primate models for preclinical testing of gene therapy approaches targeting hematopoietic cells. The use of nonhuman primate models to improve gene transfer into hematopoietic stem cells. Hum Gene Ther 2001;12:607-17.
83. Astori G, Malangone W, Adami V, et al. A novel protocol that allows short-term stem cell expansion of both committed and pluripotent hematopoietic progenitor cells suitable for clinical use. Blood Cells Mol Dis 2001;27:715-24.
84. Gammaitoni L, Bruno S, Sanavio F, et al. *Ex vivo* expansion of human adult stem cells capable of primary and secondary hemopoietic reconstitution. Exp Hematol 2003;31:261-70.
85. Heike T, Nakahata T. *Ex vivo* expansion of hematopoietic stem cells by cytokines. Biochim Biophys Acta 2002;1592:313-21.
86. Kiem H, Andrews R, Morris J, et al. Improved gene transfer into baboon marrow repopulating cells using recombinant human fibronectin fragment CH-296 in combination with interleukin-6, stem cell factor, FLT-3 ligand, and megakaryocyte growth and development factor. Blood 1998;92:1878-86.
87. Hematti P, Sellers S, Agricola B, et al. Retroviral transduction efficiency of G-CSF+SCF-mobilized peripheral blood CD34+ cells is superior to G-CSF or G-CSF+Flt3-L-mobilized cells in nonhuman primates. Blood 2003;101:2199-205.
88. Fischer A, Hacein-Bey S, Cavazzana-Calvo M. Gene therapy of severe combined immunodeficiencies. Nat Rev Immunol 2002;2:615-21.
89. Persons D, Allay E, Sawai N, et al. Successful treatment of murine {beta}-thalassemia using *in vivo* selection of genetically-modified, drug-resistant hematopoietic stem cells. Blood 2003;102:506-13.
90. Abonour R, Williams D, Einhorn L, et al. Efficient retrovirus-mediated transfer of the multidrug resistance 1 gene into autologous human long-term repopulating hematopoietic stem cells. Nat Med 2000;6:652-8.
91. Bank A. Hematopoietic stem cell gene therapy: selecting only the best. J Clin Invest 2003;112:1478-80.
92. Hanazono Y, Nagashima T, Takatoku M, et al. *In vivo* selective expansion of gene-modified hematopoietic cells in a nonhuman primate model. Gene Ther 2002;9:1055-64.
93. Nagashima T, Ueda Y, Hanazono Y, et al. *In vivo* expansion of gene-modified hematopoietic cells by a novel selective amplifier gene utilizing the erythropoietin receptor as a molecular switch. J Gene Med 2004;6:22-31.
94. Schiedlmeier B, Klump H, Will E, et al. High-level ectopic HOXB4 expression confers a profound *in vivo* competitive growth advantage on human cord blood CD34+ cells, but impairs lymphomyeloid differentiation. Blood 2003;101:1759-68.
95. Denning-Kendall P, Evely R, Singha S, et al. *In vitro* expansion of cord blood does not prevent engraftment of severe combined immunodeficient repopulating cells. Br J Haematol 2002;116: 218-28.
96. Combadiere B, Carcelain G, Debre P, et al. Plasticity of lymphoid compartments during HIV infection and treatments: hopes and limits. Microbes Infect 2002;4:575-80.
97. Wilpshaar J, Falkenburg J, Tong X, et al. Similar repopulating capacity of mitotically active and resting umbilical cord blood CD34(+) cells in NOD/SCID mice. Blood 2000;96:2100-7.
98. Traycoff C, Abboud M, Laver J, et al. Human umbilical cord blood hematopoietic progenitor cells: are they the same as their adult bone marrow counterparts? Blood Cells 1994;20:382-90.
99. Zandstra P, Conneally E, Piret J, et al. Ontogeny-associated changes in the cytokine responses of primitive human hemopoietic cells. Br J Haematol 1998;101:770-8.
100. Rodewald H. The thymus in the age of retirement. Nature 1998; 396:630-1.
101. Hazenberg M, Otto S, Wit F, et al. Discordant responses during antiretroviral therapy: role of immune activation and T cell redistribution rather than true CD4 T cell loss. AIDS 2002;16:1287-9.
102. Mackall C, Fleisher T, Brown M, et al. Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. N Engl J Med 1995;332:143-9.
103. Mackall C, Gress R. Pathways of T-cell regeneration in mice and humans: implications for bone marrow transplantation and immunotherapy. Immunol Rev 1997;157:61-72.
104. Aiuti A, Vai S, Mortellaro A, et al. Immune reconstitution in ADA-SCID after PBL gene therapy and discontinuation of enzyme replacement. Nat Med 2002;8:423-5.
105. Hazenberg M. T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock. Nat Immunol 2000;1:285-9.
106. Cohen Stuart J, Hazebergh M, Hamann D, et al. The dominant source of CD4+ and CD8+ T-cell activation in HIV infection is antigenic stimulation. J Acquir Immune Defic Syndr 2000;25:203-11.
107. Douek D, Brenchley J, Betts M, et al. HIV preferentially infects HIV-specific CD4+ T cells. Nature 2002;417:95-8.
108. Gardner M. Simian AIDS: an historical perspective. J Med Primatol 2003;32:180-6.
109. Donahue R, Bunnell B, Zink M, et al. Reduction in SIV replication in rhesus macaques infused with autologous lymphocytes engineered with antiviral genes. Nat Med 1998;4:181-6.
110. An D, Wersto R, Agricola B, et al. Marking and gene expression by a lentivirus vector in transplanted human and nonhuman primate CD34(+) cells. J Virol 2000;74:1286-95.
111. Nath B, Schumann K and Boyer J. The chimpanzee and other non-human-primate models in HIV-1 vaccine research. Trends Microbiol 2000;8:426-31.
112. Locher C, Witt S, Herndier B, et al. Baboons as an animal model for HIV pathogenesis and vaccine development. Immunol Rev 2001; 183:127-40.
113. Keppler O, Welte F, Ngo T, et al. Progress toward a human CD4/CCR5 transgenic rat model for de novo infection by HIV type 1. J Exp Med 2002;195:719-36.
114. Yeni P, Hammer S, Carpenter C, et al. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. JAMA 2002;288:222-35.