

Review

Gene-Based Immunotherapy for Human Immunodeficiency Virus Infection and Acquired Immunodeficiency Syndrome

BORO DROPULIC¹ and CARL H. JUNE²

ABSTRACT

More than 40 million people are infected with human immunodeficiency virus (HIV), and a successful vaccine is at least a decade away. Although highly active antiretroviral therapy prolongs life, the maintenance of viral latency requires life-long treatment and results in cumulative toxicities and viral escape mutants. Gene therapy offers the promise to cure or prevent progressive HIV infection by interfering with HIV replication and CD4⁺ cell decline long term in the absence of chronic chemotherapy, and approximately 2 million HIV-infected individuals live in settings where there is sufficient infrastructure to support its application with current technology. Although the development of HIV/AIDS gene therapy has been slow, progress in a number of areas is evident, so that studies to date have significantly advanced the field of gene-based immunotherapy. Advances have helped to define a series of ongoing and planned trials that may shed light on potential mechanisms for the successful clinical gene therapy of HIV.

INTRODUCTION

THE RATIONALE for continued investigation into novel treatment and prevention strategies for human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) remains strong. Although the introduction of highly active antiretroviral therapy (HAART) has provided an effective means to suppress viral replication, HAART is not curative because the virus hides in latent reservoirs, ready to reinstate the infection even after prolonged therapy (Siliciano *et al.*, 2003). Emergence of drug-resistant or multidrug-resistant strains of HIV has been on the rise, as exemplified by increases in transmission of these viruses (Wensing and Boucher, 2003; Wensing *et al.*, 2005). These caveats with HAART support the rationale to develop novel approaches to treat the disease, including gene therapy.

AIDS is a disorder of the immune system that is caused by collapse of immunity driven primarily by depletion of CD4⁺ T cells. Therefore, protection of the T cell compartment via genetic modification of T cells or stem cells is an attractive hypothesis for prevention of AIDS onset. This has been examined

since the 1990s in a series of phase I and phase II clinical trials, and continues to be enthusiastically investigated. Despite the work that has been done, it remains theoretical as to what the *in vivo* mechanism of a successful HIV gene therapeutic would be. Although the antiviral efficacy of genetic payloads can easily be tested, the complex dynamics between HIV replication, T cell homeostasis, and anti-HIV immunity in the body make our understanding of an effective clinical approach challenging at best. This review is intended to provide an overview of the payloads, vectors, and cellular targets that have been investigated to date in clinical trials. We summarize lessons that have been learned and describe where this field of research is going, and we illustrate some of the challenges and opportunities for development of a successful treatment for HIV/AIDS.

ANTI-HIV PAYLOADS

Several classes of anti-HIV genetic payloads have been shown to be effective in inhibition of HIV replication *in vitro*. These include dominant negative mutant proteins, intracellular

¹Lentigen, Baltimore, MD 21227.

²Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA 19104.

antibodies, antisense, RNA decoys, ribozymes, interfering RNA, and HIV-activated T cell receptors, among some additional novel approaches. There are ample choices for anti-HIV payloads, as is shown below. When choosing an approach it is important to balance efficacy with durability regarding rapid virus evolution, low immunogenicity (because an immune response would lead to rejection of genetically modified cells), and targeting of conserved regions of HIV whenever possible to allow for panclade efficacy.

Dominant negative proteins

Dominant negative mutant proteins interfere with the HIV life cycle. Several have been tested that target both the virus and cellular proteins that are involved in the life cycle of the virus. The most extensively studied dominant negative mutant protein is Rev M10, which interferes with efficient export of unspliced genomic HIV RNA from the nucleus into progeny viral particles. Rev M10 has a mutation in a highly conserved domain known to interact with cellular factors, and has been shown to efficiently inhibit HIV replication *in vitro* (Malim *et al.*, 1989, 1992). Cells expressing this protein have shown a survival advantage over non-Rev M10-expressing cells in clinical trials in which HIV-infected individuals were infused with cells genetically modified to express the mutant protein (Ranga *et al.*, 1998; Morgan *et al.*, 2005).

Other viral targets include the Gag protein and other proteins expressed during the late stages of the HIV life cycle. A *trans*-dominant mutant Gag has been shown to effectively inhibit HIV *in vitro* (Trono *et al.*, 1989; Cara *et al.*, 1998). Another target is the Vif protein, which is essential for the production of infectious HIV particles in lymphocytes and macrophages. Its role is to overcome the deaminating effects of APOBEC3G protein on the viral genome, thereby facilitating efficient infection of cells by progeny virions. *Trans*-dominant mutant peptides of Vif have been developed that disrupt Vif function by interfering with its oligomerization (Yang *et al.*, 2001, 2003). Experiments with these mutant Vif peptides have shown significant inhibition of HIV replication, demonstrating their potential utility. Finally, peptides derived from HIV reverse transcriptase have been shown to inhibit the ability of the virus to integrate, thus providing an early block to HIV replication in the cell (Oz Gleenberg *et al.*, 2005).

Other studies have attempted to develop *trans*-dominant mutant proteins that interfere with cellular targets. Attractive cellular targets are the CCR5 receptor, which is a coreceptor for infection of CD4⁺ cells with CCR5 (R5) strains of HIV, and restriction factors such as the tripartite motif protein TRIM5 α (Stremlau *et al.*, 2004). *Trans*-dominant mutant variants of CCR5 have been shown to interfere with HIV infection of CD4⁺ T cells (Luis Abad *et al.*, 2003). Further support for this approach comes from the fact that individuals homozygous for a 32-bp deletion mutant of the CCR5 receptor (Δ 32-CCR5) are more resistant to R5 strains of HIV than are individuals who express the wild-type receptor. Another cellular target is the protein that interacts with HIV integrase (IN), INI1. A fragment of this protein acts in a dominant negative fashion to inhibit interaction of IN with the functional cellular protein (Yung *et al.*, 2001). Finally, peripheral blood lymphocytes transduced with a simian virus 40 (SV40) vector expressing the native α ₁-an-

titrypsin gene were shown to decrease the processing of p55^{gag} and gp160^{env} proteins, significantly decreasing HIV replication (Cordelier *et al.*, 2003b).

Intrabodies

Intracellularly expressed recombinant antibodies, termed intrabodies, have gained increased popularity. Intrabodies that bind to both viral and cellular targets have been developed. An intrabody targeted to HIV p17^{gag} showed strong inhibition of several strains of HIV (Deepanker Tewari, 2003). Several single-chain antibodies targeted to the reverse transcriptase molecule of HIV have been identified (Herschhorn *et al.*, 2003), but more studies are required to confirm their general utility for HIV gene therapy. More analysis has been performed for an anti-Vif intrabody that significantly affects its function, presumably by interfering with oligomerization. Peripheral blood lymphocytes transduced with a lentiviral vector expressing an anti-Vif intrabody was shown to significantly inhibit both laboratory and primary strains of HIV (Goncalves *et al.*, 2002).

Cellular targets for intrabody gene therapy may be more attractive than viral targets because of virus escape mutations. Single-chain antibodies targeted to CXCR4 and cyclin T1 have been developed and were shown to inhibit the replication of various HIV strains (BouHamdan *et al.*, 2001; Bai *et al.*, 2003). However, further testing is required to show that targeting such cellular factors in the primary lymphoid cells will not result in toxicity. Intrabodies to the CCR5 receptor are also in development (Cordelier *et al.*, 2004). A significant challenge for all protein-based therapeutics is to avoid immunogenicity in order to circumvent immune-mediated rejection of vector-transduced cells.

Anti-HIV RNAs

Numerous RNA-based strategies against HIV have been evaluated. These include ribozymes, RNA decoys, antisense, and, most recently, RNA interference (RNAi). There are distinct advantages to an RNA approach: (1) heterologous RNA molecules expressed in cells are not immunogenic; (2) there are several types of RNA inhibitory molecule to choose from including antisense, ribozymes, decoys, and, most recently, RNAi; (3) multiple sequences on the HIV genome can be targeted simultaneously, addressing the issue of HIV resistance; and (4) both viral and cellular sequences can be targeted simultaneously, offering the possibility of attacking HIV replication at multiple steps of the life cycle and further against cellular targets that are not prone to HIV resistance.

Numerous antisense targets have been tested, targeting both coding and noncoding regions of the HIV genome, and most have been shown to effectively inhibit HIV replication (Goodchild *et al.*, 1988; Sczakiel *et al.*, 1992; Chuah *et al.*, 1994; Sun *et al.*, 1995; Vandendriessche *et al.*, 1995; Veres *et al.*, 1996, 1998; Humeau *et al.*, 2004; Lu *et al.*, 2004b). The size of antisense plays an important role in antiviral efficacy, with shorter sequences showing lesser or no inhibition, and sequences greater than 1000 bases having the best efficacy (Goodchild *et al.*, 1988; Veres *et al.*, 1996). An advantage of using long antisense is that it is difficult for HIV to develop resistance against it, because such extensive mutation would render the virus replication incompetent or severely debilitated. This concept was

demonstrated in the laboratory by Lu and colleagues (2004b), where a lentiviral vector expressing long antisense targeted to the envelope region of HIV was evaluated in an *in vitro* system of serial selection in order to isolate HIV resistance mutants. Although several mutants were isolated, none had developed resistance to the antisense and only one mutant retained the ability to replicate autonomously. Ultimately, follow-up studies in the clinic will be needed to fully establish the lack of HIV antisense resistance mutations.

Ribozymes are similar to antisense but have the added feature of cleaving their target catalytically. Several studies have shown inhibition of HIV replication, using ribozymes (Rossi, 1999; Morris and Rossi, 2004). More recent studies have shown that many types of ribozymes require modified structures for true catalytic activity, suggesting that the anti-HIV effects of earlier studies in some cases may be the result of the innate antisense effects, rather than their purported catalytic properties (Khvorova *et al.*, 2003). A ribozyme approach is likely to result in HIV escape mutants if it is not used in a multipayload setting, or against a cellular target.

In contrast to antisense and ribozymes, decoys do not attack target RNAs directly; rather, they mimic RNA structures involved in the viral life cycle and decoy viral or cellular factors away from propagation of the virus. HIV has several *cis*-acting nucleic acid sequences, and two in particular specifically interact with the HIV proteins Tat and Rev and have been tested as antivirals (Sullenger *et al.*, 1990, 1991; Lee *et al.*, 1994; Bahner *et al.*, 1996; Bukovsky *et al.*, 1999; Li *et al.*, 2005). HIV Tat binds to the *trans*-acting response element (TAR) sequence to facilitate transcriptional elongation for production of full-length HIV genomes. Rev binds to the Rev response element (RRE) to facilitate the export of unspliced genomic RNA from the nucleus. Both of these interactions are critically involved in the replication cycle of the virus. The RRE has been stably expressed in cells via murine leukemia virus (MLV) retroviral vectors and shown to interfere with HIV replication (Bahner *et al.*, 1996). TAR decoys expressed by lentiviral vectors are presently anticipated for clinical testing in a triple payload vector construct (Li *et al.*, 2005). Of note, HIV-based lentiviral vectors are generally configured to contain TAR and RRE elements, so even without additional anti-HIV sequences they express *cis*-acting elements that can decoy factors away from replication of infectious HIV.

More recently, RNAi has become a powerful tool for gene silencing, and the first report regarding its utility against HIV was made in 2002 (Das *et al.*, 2004). Since that time, improved methods for expression of RNAi by gene therapy vectors, most notably short hairpin RNAs (shRNAs) driven by Pol III promoters such as the U6 promoter, have enabled the practical application of this technology (An *et al.*, 2003). As with antisense and ribozymes, RNAi has been used to degrade various viral and host cell targets involved in the life cycle of HIV-1 (Coburn and Cullen, 2002; Novina *et al.*, 2002). However, targeting HIV RNA with shRNA, although effective in the short term, is problematic for long-term inhibition of HIV replication as even a single point mutation on the target RNA can dramatically affect the efficacy of the shRNA molecule, and by now many investigators have reported RNAi escape (Boden *et al.*, 2003; Das *et al.*, 2004). Given the diversity of HIV strains and the ease with which HIV mutates, single-modal shRNAs that target HIV are not viable for use in HIV gene therapy.

To address this issue, Rossi and colleagues developed multitargeted RNA interference-based lentiviral vectors that target both HIV and cellular proteins involved in the life cycle of HIV (Li *et al.*, 2005). In this study, the combination of a Pol III U6 promoter-driven shRNA targeting the *rev* and *tat* mRNAs of HIV-1, a U6-transcribed nucleolar-localizing TAR RNA decoy, and a VA1-derived Pol III cassette that expresses an anti-CCR5 ribozyme was engineered into a self-inactivating lentiviral vector. The study demonstrated that the combination vector was effective in controlling the replication of HIV and appeared to prevent the production of escape mutants. A clinical trial to test this approach is in preparation (www4.od.nih.gov/oba/rac/minutes/RAC_minutes_09-05.pdf).

Other approaches

A potential mechanism for success in HIV gene therapy involves the boosting of HIV immunity to enable the body to control virus replication and spread. Typically this is attempted via therapeutic vaccination, but these approaches have historically been unsuccessful. Studies initiated in the early 1990s examined the potential of engineering HIV-specific cytotoxic T lymphocytes (CTLs), using the CD4 extracellular domain or a gp41-specific antibody coupled to the ζ signaling chain of the CD3 T cell receptor (Roberts *et al.*, 1994; Yang *et al.*, 1997). These preclinical studies demonstrated the proof of principle that the redirected CD8⁺ T cells were capable of responding by interleukin (IL)-2 secretion on binding to HIV, and exhibited robust HIV CTL activity that equaled or superseded historical CTL controls. The CD4-CD3 ζ approach was then translated to clinical application (Mitsuyasu *et al.*, 2000; Walker *et al.*, 2000; Deeks *et al.*, 2002).

It has been proposed that blocking of an early step in the HIV life cycle will be important to confer a selective advantage to vector-modified cells in the body, and hence allow outgrowth of HIV-resistant cells in the patient (von Laer *et al.*, 2006). There are few antiviral genes available that block HIV preintegration. The new antiretroviral drug enfuvirtide, commonly known as T20, has been released to the market (Lalezari *et al.*, 2003). T20 blocks HIV entry by inhibiting the conformational changes needed for fusion of the viral envelope with the cellular membrane (Eckert and Kim, 2001). For a genetic approach, the T20 peptide was modified with an anchor protein for cell surface expression, and further optimized for reduced immunogenicity and improved expression and stability, and this optimized peptide was called M87o (Egelhofer *et al.*, 2004). An issue is that, although resistance has not yet been documented in the gene therapy setting, the fact that resistance is characterized after T20 treatment generates some concern over the approach if it is used without a multitargeted payload (Poveda *et al.*, 2004; Sista *et al.*, 2004). Therefore it may be important to use anti-HIV surface peptides in combination with other surface inhibitors or other modalities to interfere with the virus replication cycle.

There are several additional modalities that have shown preclinical promise, using unconventional approaches for halting HIV replication or spread. One interesting approach places the herpes simplex type 1 thymidine kinase (TK) gene under the control of the HIV long terminal repeat (LTR). After HIV infection and in the presence of acyclovir, the HIV-infected cells

died before the virus could spread in the culture, offering a unique approach for deletion of HIV-containing cells in the patient (Caruso and Klatzmann, 1992). A second approach also used the HIV LTR to trigger conditional gene expression of interferon- α , which interferes with the subsequent round of infection and hence spread of HIV (Sanhadji *et al.*, 1997; Cordelier *et al.*, 2003a). Another group designed an HIV LTR-specific translational inhibitor (Segal *et al.*, 2004).

Studies have shown that the expression of simian TRIM5 α , which binds to the HIV capsid and interferes with the uncoating process, strongly protects human cells from productive HIV-1 infection (Stremlau *et al.*, 2004). The human version of TRIM5 α is not efficient at blocking HIV, presumably because the capsid protein has evolved to reduce the interaction. This is also observed with decreased binding of the simian immunodeficiency virus (SIV) capsid to the simian TRIM5 α when compared with HIV capsid binding to the simian protein. Because the simian protein has a high level of homology to the human protein, it is anticipated that gene therapy using this gene may not be immunogenic.

Worthy of consideration in light of heightened awareness of risks associated with integrating vectors, zinc finger-binding proteins fused to a nuclease domain (zinc finger nucleases) have been developed and proof of principle was established for the treatment of immunodeficiency due to common γ -chain gene mutations (Urnov *et al.*, 2005). This is a "hit and run" approach because only genetic deletion occurs, thus obviating safety concerns about potential adverse effects of vector integration. Zinc finger nucleases targeting the CCR5 locus are now being evaluated in preclinical development for ultimate clinical application for patients with HAART-resistant strains of R5-tropic HIV-1.

CLINICAL VECTORS FOR HIV GENE THERAPY

Similar to HAART, with the exception of gene therapy-based vaccines, any successful gene therapy for HIV requires durability because of the latent stage of the virus. Accordingly, only integrating viral vectors have been used to date in gene therapy trials for HIV/AIDS: murine leukemia virus (MLV)-based retroviral and HIV-based lentiviral vectors. Although both vector types are derived from the Retroviridae family of viruses, a convention has evolved to call vectors derived from MLV "retroviral vectors" whereas vectors derived from lentivirus are called "lentiviral vectors." Until recently, all the HIV gene therapy trials have employed MLV vectors (Table 1).

HIV clinical trials that have employed MLV vectors established that the vectors are well tolerated and reported no vector-related adverse events. Although these vectors have been suitable, a disadvantage has been that quiescent cells cannot be readily transduced because the MLV preintegration complex requires cell division for access to the cellular genome for integration (Roe *et al.*, 1993). Also, MLV vectors integrate preferentially near transcriptional start sites (Mitchell *et al.*, 2004), increasing the possibility for insertional mutagenesis through activation of putative oncogenes.

We predict that lentiviral vectors will be the preferred choice in the future for reasons of safety and efficacy. Lentiviral vec-

tors not only can transduce nondividing cells, but also can transduce stimulated cells with efficiencies exceeding 90%, abrogating the need for selection of transduced cells and improving the potential effectiveness of each cellular dose (Humeau *et al.*, 2004). Like retroviral vectors, lentiviral vectors can be pseudotyped with envelope proteins that broaden their tropism and stabilize the vector during its manufacture (Yee *et al.*, 1994; Burns *et al.*, 1993).

Lentiviral vectors can be derived from the feline immunodeficiency virus (FIV), equine infectious anemia virus (EIAV), bovine immunodeficiency virus (BIV), simian immunodeficiency virus (SIV), and the human immunodeficiency virus. Only HIV-derived lentiviral vectors have been used in a clinical setting, although lentiviral vectors based on non-HIV species have been developed (e.g., SIV, BIV, EIAV, and FIV). One perceived advantage of non-HIV-based lentiviral vectors is that they are not known to cause disease in humans, and therefore could be safer. However, because the consequence of a recombination between an animal lentiviral vector and wild-type HIV in the event of infection of transduced cells is unknown, there remains concern that this could lead to the creation of new human pathogens. At least in the case of HIV, recombination between the same types of viruses has occurred and the outcome is well understood.

In addition to improved efficacy of gene transfer, it is anticipated that lentiviral vectors have an improved safety profile in terms of insertional oncogenesis. Preclinical data have shown that lentiviral vectors do not have the enhancer activity in their promoter region that retroviral vectors have, which could contribute to distal aberrant gene expression in the cell (Lu *et al.*, 2004a). In addition, the insertion profile of lentiviral vectors tends to occur more within the gene coding region, where gene disruption instead of activation would happen, instead of upstream in the 5' noncoding region, such as retroviruses are known to do (Mitchell *et al.*, 2004). Finally, MLV has been historically known to have oncogenic potential that is related to its insertion (Tsichlis, 1987). In stark contrast, natural HIV infection is not associated with T cell leukemogenesis. However, more clinical data with long-term follow-up are needed to establish the safety of lentiviral vectors with confidence.

Clinical experience with HIV-based lentiviral gene therapy

Because of the perceived risks described above, only in 2003 was the first lentiviral vector trial initiated. This vector expressed a long antisense against the HIV envelope gene in autologous CD4⁺ T cells. This trial has been concluded, and it showed that the patients tolerated the lentiviral vector-modified T cells well and that there were no adverse events associated with the vector (MacGregor *et al.*, 2005; Manilla *et al.*, 2005). Three of five subjects had prolonged engraftment with lentivirally modified T cells for at least 1 year after infusion, and although no statistically significant anti-HIV effects can be observed in a pilot trial, it is notable that one patient developed a sustained decrease of >1.5 log in viral load. A second phase I/II trial has just been initiated to evaluate the therapy in the context of structured treatment interruption (STI) and a follow-up phase II repeat dosing exploratory trial is in progress. Two additional unrelated lentivirus vector-based trials are anti-

TABLE 1. EXAMPLES OF COMPLETED AND ONGOING GENE THERAPY TRIALS FOR HIV/AIDS

<i>Name^a or description</i>	<i>Phase</i>	<i>Payload</i>	<i>Cellular vehicle</i>	<i>Transfer vector</i>	<i>Reference(s)</i>
Completed Trials					
T cells					
“Ribozyme Gene Therapy of HIV-1 Infection”	I	Anti-HIV-1 R _z to the U5 leader sequence	Autologous CD4 ⁺ cells; single dose	Murine retrovirus	Wong-Staal, 1998
A clinical trial evaluating the safety and tolerability of a single infusion of autologous CD4 ⁺ T cells modified with a dominant negative anti-HIV gene	I/II	Rev M10	Autologous CD4 ⁺ cells; single dose	Gold particles/murine retrovirus	Ranga, 1998; Woffendin 1996
“A Marker Study of Therapeutically Transduced CD4 ⁺ Peripheral Blood Lymphocytes in HIV Discordant Identical Twins”	I	Anti-HIV-1 <i>tat</i> ribozyme (Rz2)	Syngeneic CD4 ⁺ cells (twin study); single dose	Murine retrovirus	Macpherson, 2005; Cooper, 1999
A clinical trial evaluating the safety and tolerability of multiple infusions of syngeneic CD4 ⁺ lymphocytes modified with anti-HIV genes	I	<i>Trans</i> -dominant rev and/or <i>trans</i> -dominant rev with TAR antisense	Syngeneic CD4 ⁺ cells (twin study); 2 doses	Murine retrovirus	Morgan, 2005
A clinical trial evaluating the safety, tolerability, and persistence of escalating and repeat doses of genetically modified syngeneic CD8 ⁺ or CD4 ⁺ and CD8 ⁺ cells	I/II	CD4 receptor coupled with the CD3 signaling chain	Syngeneic CD8 ⁺ or CD4 ⁺ and CD8 ⁺ cells; single or multiple doses	Murine retrovirus	Walker, 2000
A clinical trial evaluating the safety, tolerability, and tissue trafficking of a single dose of genetically modified autologous CD4 ⁺ and CD8 ⁺ cells	I/II	CD4 receptor coupled with the CD3 signaling chain	Autologous CD4 ⁺ and CD8 ⁺ cells; single dose	Murine retrovirus	Mitsuyasu, 2000
“A Phase II Randomized Study of HIV-Specific T-Cell Gene Therapy in Subjects with Undetectable Plasma Viremia on Combination Antiretroviral Therapy”	II	CD4 receptor coupled with the CD3 signaling chain	Autologous CD4 ⁺ and CD8 ⁺ T cells; repeat doses (3)	Murine retrovirus	Deeks, 2002
A phase I/II study evaluating the safety, tolerability, and antiviral effects of autologous CD4 ⁺ T cells expressing the HIV fusion inhibitor M87	I	gp41 fusion peptide inhibitor	Autologous CD4 ⁺ T cells	Murine retrovirus	von Laer, 2005 (http://www.asgt.org/am05/executive-summary_friday.pdf)
“A Phase I Clinical Trial of the Safety and Tolerability of a Single Dose of Autologous T Cells Transduced with VRX496 in HIV Positive Patient-Subjects”	I/II	Anti-HIV-1 antisense against the envelope gene	Autologous CD4 ⁺ T cells; single dose	HIV-derived lentivirus, conditionally replicating	MacGregor, 2001; Levine <i>et al.</i> , submitted

(continued)

TABLE 1. EXAMPLES OF COMPLETED AND ONGOING GENE THERAPY TRIALS FOR HIV/AIDS (CONTINUED)

Name ^a or description	Phase	Payload	Cellular vehicle	Transfer vector	Reference(s)
Stem cells					
“Nonmyeloablative Conditioning Followed by Transplantation of Genetically Modified HLA-Matched Peripheral Blood Progenitor Cells for Hematologic Malignancies in Patients with Acquired Immunodeficiency Syndrome”	I	<i>Trans</i> -dominant Rev	Autologous CD34 ⁺ cells isolated from mobilized peripheral blood	Murine retrovirus	Kang, 2002
“A Clinical Trial of Retroviral-Mediated Transfer of a <i>rev</i> -Responsive Element Decoy Gene into CD34 ⁺ Cells from the Bone Marrow of Human Immunodeficiency Virus-1-Infected Children”	I	RRE decoy	Autologous CD34 ⁺ bone marrow cells	Murine retrovirus	Kohn, 1999
A clinical trial evaluating the safety, tolerability, and persistence of transplantation with autologous bone marrow transduced with a retroviral vector expressing dominant negative <i>rev</i> or a control gene	I	<i>Trans</i> -dominant Rev	Autologous CD34 ⁺ bone marrow cells	Murine retrovirus	Podsakoff, 2005
“A Phase I Trial of Autologous CD34 ⁺ Hematopoietic Progenitor Cells Transduced with an Anti-HIV Ribozyme”	I	Anti-HIV-1 <i>tat</i> ribozyme (Rz2)	Autologous CD34 ⁺ cells isolated from mobilized peripheral blood	Murine retrovirus	Armado, 1999, 2004
Ongoing Trials					
“A Randomized Phase II, Double-Blind, Controlled Trial to Evaluate the Safety and Efficacy of Autologous CD34 ⁺ Hematopoietic Progenitor Cells Transduced with Placebo or an Anti-HIV-1 Ribozyme (OZ1) in Patients with HIV-1 Infection”	II	Anti-HIV ribozyme OZ-1	Autologous CD34 ⁺ cells isolated from mobilized peripheral blood	Murine retrovirus	http://clinicaltrials.gov/show/NCT00074997
“A Phase I/II, Open-Label, Single-Center Study to Evaluate the Tolerability, Trafficking, and Therapeutic Effects of Repeated Doses of Autologous T Cells Transduced with VRX496 in HIV-Infected Subjects”	I/II	Anti-HIV-1 antisense against the envelope gene	Autologous CD4 ⁺ T cells; repeat doses (6)	HIV-derived lentivirus, conditionally	http://www.clinicaltrials.gov/ct/show/NCT00295477?order=4
“A Phase II, Open-Label, Multicenter Study to Evaluate the Safety, Tolerability, and Biological Activity of Repeated Doses of Autologous T Cells Transduced with VRX496 in HIV-Positive Subjects”	II	Anti-HIV-1 antisense against the envelope gene	Autologous CD4 ⁺ T cells; repeat doses (4 or 8)	HIV-derived lentivirus conditionally replicating	http://www.clinicaltrials.gov/ct/show/NCT00131560?order=1

Abbreviations: RRE, Rev response element; Rz, ribozyme; TAR, *trans*-acting response element.

^aOfficial titles are in quotation marks.

pated to begin at the City of Hope National Medical Center (Duarte, CA) in the near future.

CELLULAR TARGETS

For HIV, all gene therapy trials have used *ex vivo* gene transfer in part because there does not yet exist efficient *in vivo* targeting for gene therapy. In addition, *ex vivo* gene transfer permits optimized gene transfer efficiency; reduces the risk of gene transfer to nontarget cells, which is a particular safety issue for integrating vectors; and typically reduces or avoids development of immunity against the vector because it is washed out by the time the patient receives the product. *Ex vivo* cellular targets for HIV gene therapy have historically been either T lymphocytes or stem cells (Table 1). The advantage to transducing stem cells is that then all blood cell progeny will express the antiviral gene, thus conferring resistance to all HIV-susceptible cells including T cells, monocytes, macrophages, dendritic cells, and microglial cells. Using T cells as the gene therapy vehicle offers some advantages including the potential to augment anti-HIV immunity, and the ability to expand the cells and increase each dose size to improve engraftment and, it is hoped, the therapeutic effect.

T cells

Significant advances have been made in the manipulation and growth of T cells *ex vivo*. In particular, the discovery that the anergy induced by stimulation of T cells with CD3 alone could be overcome through costimulation of the CD3 and CD28 receptors enabled large-scale amplification of T cells (Boise *et al.*, 1995; Levine *et al.*, 1995, 1997; Noel *et al.*, 1996; Radvanyi *et al.*, 1996). Furthermore, CD28 costimulation induces a state of resistance to HIV infection in CD4⁺ cells (Carroll *et al.*, 1997). The feasibility of T cell processing for production of sufficient doses of cellular product to conceivably have a clinical impact has been demonstrated (Levine *et al.*, 1995, 1996, 2002). The T cell-based HIV gene therapy trials to date have reported no or modest effects on viral load, but have established an encouraging body of data supporting safety, a selective advantage of gene-modified HIV-resistant T cells in the body, and also the ability of the cells to persist long term.

CD4⁺ T lymphocytes were first genetically modified to express the *trans*-dominant negative protein Rev M10 in a clinical trial in which autologous CD4⁺ T cells were modified with gold microparticles or by an MLV vector expressing the modified Rev M10. This trial was novel in that patient cells were transduced with either a vector expressing the antiviral gene, or a marking vector. A mixture of both cell populations was given to the patient. This trial demonstrated a selective advantage in the transduced cells containing the antiviral gene, but not the control transduced cells, in patients chronically infected with HIV. Rev M10-expressing cells were detectable for an average of 6 months compared with 3 weeks with control cells (Ranga *et al.*, 1998).

In subsequent trials, the CD4–CD3 ζ construct was used to modify CD4⁺ and CD8⁺ T cells in patients via MLV vector delivery. One phase I trial evaluated syngeneic cell transfer of one to six infusions of gene-modified cells, and the second trial

evaluated a single larger infusion of autologous product (Mitsuyasu *et al.*, 2000; Walker *et al.*, 2000). This second study established lymphoid tissue trafficking and *in vivo* persistence of modified cells through the analysis of rectal mucosal biopsies. Stable engraftment was demonstrated, as the CD4–CD3 ζ gene was detected in 1–3% of blood mononuclear cells at 8 weeks and 0.1% at 1 year postinfusion (Mitsuyasu *et al.*, 2000). A randomized phase II study of the CD4–CD3 ζ vector was then conducted in a total of 40 patients (20 treated and 20 control patients), and it confirmed that the T cell infusions resulted in elevated CD4⁺ T cell counts and the stable persistence of vector-modified cells. Furthermore, it demonstrated a modest antiviral effect ($p < 0.07$) on HIV rebound in these well-controlled patients (Deeks *et al.*, 2002). These trials, together with another nongene transfer HIV cellular therapy trial not discussed here (Levine *et al.*, 2002), helped to establish the safety of multiple infusions of activated T cells in HIV patients.

More recently, Morgan and colleagues reported long-term engraftment with T cells engineered to express an antisense TAR element or Rev M10 (Morgan *et al.*, 2005). Robust antiviral effects were documented, particularly in patients with high viral loads. Macpherson *et al.* have reported persistent engraftment of T cells for more than 4 years after treatment of syngeneic CD4⁺ T cells with an MLV vector expressing an *anti-tat* ribozyme (Macpherson *et al.*, 2005). This study was similar to the Rev M10 study in that cells were transduced either with an empty vector or a vector expressing an anti-HIV payload. In contrast to Ranga *et al.* (1998) and Morgan *et al.* (2005), however, Macpherson *et al.* reported no selective advantage of the HIV-resistant cells when compared with control cells.

The most recent HIV gene therapy trial evaluated a retroviral vector (M87o) that encodes the membrane-anchored antiviral peptide C46. C46 comprises 46 amino acids and is derived from the second heptad repeat of the HIV-1 envelope glycoprotein gp41 and inhibits fusion of the viral and cellular membranes during virus entry (Egelhofer *et al.*, 2004). A pilot clinical trial was carried out by von Laer, van Lunzen, and colleagues in 10 patients with late-stage HIV/AIDS and HAART failure, who were given an infusion of CD4⁺ T cells transduced with the retroviral vector. Initial results from the study indicate that the approach is safe, and that although a significant rise in CD4⁺ T cell counts was seen, viral loads were not affected. Gene marking could be detected throughout the 1-year follow-up.

Stem cells

Human hematopoietic progenitor cells (HPCs) represent an important cellular target for HIV/AIDS gene therapy. In contrast to T cells, the genetic modification of stem cells offers the potential to interfere with HIV replication in the multiple cell types that are the targets of HIV infection such as monocytes, macrophages, dendritic cells, and microglial cells, all of which are derived from HPCs. Furthermore, HPC therapy has the potential to improve the T cell receptor repertoire after thymopoiesis and the production of genetically resistant cells, a particular advantage for patients with severe T cell depletion. However, at present use of HPCs as a cellular vehicles for HIV gene therapy is limited by several factors including low gene transfer efficiency of MLV vectors, silencing of anti-HIV sequences, and impaired engraftment of primitive multipotential

cells after transplantation (Case *et al.*, 1999; Ellis, 2005). Some of these limitations can be addressed by improved vector technology and encouraging results have been reported, as described below.

An early study of MLV-transduced CD34⁺ marrow cells expressing an RRE decoy resulted in the marking of 0.001–0.003% of peripheral blood cells, which dropped even lower after 6 months (Kohn *et al.*, 1999). This study indeed helped to establish the tolerability of this approach, but the long-term persistence of the cells was disappointing. A separate study with MLV vectors in the setting of bone marrow transplantation for hematologic malignancies in patients with HIV reported persistence to more than 2 years, perhaps because of the improved engraftment due to the myeloablative conditioning (Kang *et al.*, 2002).

Later studies have shown more promise, even in the absence of conditioning before HPC transplantation. CD34⁺ cells isolated from mobilized peripheral blood and transduced with an MLV vector expressing a ribozyme against *tat* demonstrated that HIV patients are capable of significant levels of thymic maturation, supporting the important hypothesis that the CD4⁺ T cell compartment can be protected through HPC modification (Amado *et al.*, 2004). A second study performed in two children used the dominant negative Rev M10 gene or a control MLV vector for modification of autologous bone marrow CD34⁺ cells. Vector-modified cells were rarely detected after about 3 months, but intriguing results showed the reemergence of vector-modified cells in peripheral blood mononuclear cells (PBMCs) after an increase in HIV replication (Podsakoff *et al.*, 2005). Only cells modified with the anti-HIV gene were detected, which provides *in vivo* evidence for HIV-induced selection of gene-modified cells.

CHALLENGES AND FUTURE DIRECTIONS

Many approaches have been developed for inhibition of HIV in tissue culture; however, few of these have been translated for clinical evaluation. After more than a decade of investigation into these trials, a successful treatment for HIV gene therapy remains on the horizon. Contrary to what some may consider slow progress in HIV gene therapy, the field has advanced in a meaningful way through the observations made in past trials regarding cellular persistence as it relates to the cellular vehicle, the dose and antiviral payload, and the safety of cellular therapy with retroviral and lentiviral vectors.

Although there have been some encouraging results, the major difficulty remains in understanding the mechanism that might lead to success in the clinic. Without understanding such a mechanism, it remains difficult to optimize with intention the antiviral payload, vector, or cellular vehicle and/or dose to improve the chances of success. In light of this, investigators must continue to test novel approaches to explore the potential mechanisms. Possible *in vivo* mechanisms of action (MOAs) that may lead to clinical success include (1) selective outgrowth of HIV-resistant cells to such a point that overall HIV replication is thwarted (Lund *et al.*, 1997; von Laer *et al.*, 2006), (2) generation of an expanding HIV-resistant T cell population through spread of conditionally replicating HIV vectors (Weinberger *et al.*, 2003), or (3) protection and/or boosting of critical HIV-spe-

cific immunity by HIV-resistant helper cells. A combination of these approaches may be required for success. Considering the MOA provides insight into why careful thought should go into the design of the vector, or the site of action of the payload, depending on the hypothesized MOA of the therapy.

To explore the first MOA, one could accelerate the outgrowth of HIV-resistant cells *in vivo*. This can be done in several ways; the immediately obvious approach is to increase engraftment of the cellular dose either via repeat doses (T cells) or by optimizing conditioning before stem cell engraftment. Another way to test this MOA when using T cells as the vehicle would be to create an environment for homeostatic expansion of modified cells by “creating room” in the body by pretreatment conditioning (using antibodies or chemotherapy), or through the strategic timing of leukapheresis and infusion. Alternatively, *in vivo* selection could be performed for transduced cells expressing the anti-HIV sequences. Methylguanine methyltransferase (MGMT) has been used for selection of transduced HPCs when used in combination with bischloroethyl nitrosourea (BCNU) and benzylguanine (BG) (Davis *et al.*, 1997). Whereas BCNU has shown toxicity, the more recently available temozolimide, an oral agent that has been used clinically for prolonged periods, has shown no serious adverse events. Therefore, *in vivo* selection could be a viable approach for improving the percentage of HIV-resistant cells in the body (Neff *et al.*, 2003; Davis *et al.*, 2004).

With the development of HIV-based lentiviral vectors (Naldini *et al.*, 1996), it may be advantageous for these vectors to mobilize and spread their anti-HIV sequences throughout the T cell population in the body (Dropulic *et al.*, 1996; Bukovsky *et al.*, 1999). The now completed VIRxSYS (Gaithersburg, MD)–University of Pennsylvania (Philadelphia, PA) clinical trial that used a conditionally replicating HIV-1-derived lentiviral vector expressing long antisense against the HIV envelope provides some intriguing data relating to this MOA. Interestingly, mobilization of the lentiviral vector via packaging by the endogenous HIV was detected in the serum of these patients shortly after dosing, in the pattern of a short burst without subsequent detection of mobilized vector at later time points. A model describing the potential for conditionally replicating anti-HIV vectors to overcome wild-type infection *in vivo* has been described (Weinberger *et al.*, 2003). Further investigations in two follow-on trials are ongoing in an effort to evaluate whether the mobilization is related to an antiviral effect, and whether it occurs in lymphoid tissues such as the gut, which is a primary site of HIV production (Veazey *et al.*, 1998).

The safety of retroviral and lentiviral vectors for HIV/AIDS has been satisfactory to date and establishment of a credible safety database will be necessary for incorporation of these vectors into the practice of medicine. Although insertional oncogenesis was observed in one trial using retroviral vectors, this event is thought to have required the colluding circumstances of a signaling payload and the cellular target for the leukemia to develop. On the basis of the adverse results with CD34⁺ marrow cells in severe combined immunodeficiency (SCID), it is likely that therapy involving HPCs may be more susceptible to the risks of insertional mutagenesis than therapy involving mature T cells. The extensive safety record of gene therapy with T cells, along with the demonstration of long-lived “central memory” and “stem cell” T cells, has increased the rationale

for T cell-based approaches (Fearon *et al.*, 2001; Luckey *et al.*, 2006). Thus, research will likely switch to lentiviral vectors in the future as the supply of clinical-grade vectors improves. Establishment of the safety of lentiviral vectors is in its infancy, but preclinical data demonstrating the lack of enhancer activity and an altered insertional profile suggest that these vectors are likely to be safer than retroviral vectors.

Gene therapy for HIV continues to march forward with promise, and is sparking the evaluation of novel payloads and dosing approaches that can be applied in various areas of translational medicine in gene therapy. Progress for the development of a gene therapy for HIV/AIDS has been slow, but it is presently the only approach that promises a lasting cure for the disease.

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Address reprint requests to:
 Dr. Boro Dropulic
 Lentigen
 1450 South Rolling Road
 Baltimore, MD 21227

E-mail: boro.dropulic@Lentigen.com

or

Dr. Carl June
 Abramson Family Cancer Research Institute
 University of Pennsylvania
 421 Curie Boulevard
 Philadelphia, PA 19104

E-mail: cjune@mail.med.upenn.edu

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