Review Article

HIV-Derived Lentiviral Vectors: Current Progress toward Gene Therapy and DNA Vaccination

M. Reza Sadaie[*1] and Suresh K. Arya[2]

Department of Microbiology and Immunology, Georgetown University Medical Center, 3900 Reservoir Road, Washington DC, 20007, USA, [2]Basic Research Laboratory, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892. USA

ABSTRACT

Lentiviral vectors are promising gene delivery tools capable of transducing a variety of dividing and non-dividing cells, including pluripotent stem cells which are refractory for transduction by murine retroviruses. Although there is a growing debate on the safety of lentiviral vectors for gene transfer, in particular for those derived from human immunodeficiency viruses, type one (HIV-1) and type two (HIV-2), these vectors are envisioned to possess several advantages. Importantly, they can be utilized not only for transducing specific target cells or for in *vivo* gene therapy of HIV infection and acquired immunodeficiency syndrome (AIDS), but also in a pseudotype recombinant form can be used for different target cells including neurological and cancer cells. For HIV-2, the most compelling advantages are: (i) its reduced ability to recombine with resident HIV-1 genome; (ii) its ability to induce in recipients antibodies which can be distinguished from host immune response to HIV-1; (iii) HIV-2 is apparently less pathogenic; and (iv) may downregulate HIV-1 expression. This review will summarize new developments on HIV-1 vectors, while focusing on alternate strategies toward developing HIV-2-based vectors. *Iran. Biomed. J. 2: 95-103, 1998*

INTRODUCTION

Human immunodeficiency viruses, HIV-1 and HIV-2, are among the most studied of the lentivirus group, and a great deal is known about their gene structure and function. They are thus ideal candidates for vector development [1, 2]. Recent reports of the successful use of HIV-1 derived transfer vectors to transduce non-dividing neuronal cells *in vivo* [3-5] and CD34^{+} hematopoietic cells *in vitro* [6] have heightened the interest in lentivirus vectors. These studies generally used pseudovirions encasing HIV-1 vector into heterologous vesicular stomatitis virus (VSV) envelop glycoprotein G. The non-primate feline immunodeficiency virus (FIV) lentiviral vector, also pseudotyped with VSV-G, offers a promising new way of delivering genes to different human cell types [7]. However, a transduced FIV vector if used in primates may raise its own safety concern in that such vector may

enable wild type FIV infecting cats in the same environment to break species barrier and thus infect primates, resulting possibly in a novel disease. Moreover, the loss of specificity of targeting CD4 receptor and possible toxicity of VSV-G protein and its possible immune rejection *in vivo* are drawbacks for both VSV-HIV and VSV-FIV approaches. Efforts to create homologous packaging cell lines [8, 9] must thus continue to be pursued.

Our effort is directed towards developing HIV-2-derived retroviral vectors [1, 2, 10]. These vectors are likely to possess a number of advantages, particularly for gene therapy of HIV infection and acquired immunodeficiency syndrome (AIDS). These include: (i) *tropism* for human CD4^{+} cells, targets of HIV infection *in vivo;* (ii) *regulated expression* modulated by the regulatory gene-response element loops; (iii) vector *DNA integration* for likely persistent expression; (iv) the

---

*Corresponding Author;

ability to induce in recipients *distinct antibodies* which can readily be distinguished from the natural immune response to resident or infecting (pandemic) HIV-1; and finally (v) the ability to affect expression in *both dividing and non-dividing* cells. These are some of the distinct advantages over the murine retroviruses which require target cell mitosis, infect indiscriminately many different cell types, and are unregulated and less potent in expressing the desired gene.

It is tempting to note that HIV vector combined with packaging systems could be used for *ex vivo* gene delivery or for direct administration *in vivo.* Furthermore, such vector itself could be used for DNA vaccination as well as non-replicative genome-free vector virions as a vaccine for immunotherapy. Moreover, among the human lentiviruses, HIV-2 appears to be better suited for gene transfer than HIV-1 because: (i) it is less pathogenic and thus safer during design and production; (ii) in contrast to HIV-1, the desirable nuclear import function is segregated from the undesirable cell cycle arrest function in HIV-2 [11-13]; and (iii) because of the limited relatedness between HIV-1 and HIV-2 at the genetic level [14], HIV-2 vector is less likely to recombine with the resident HIV-1 genome in HIV-1 infection. This will minimize the risk of any enhanced pathogenicity of the recombinant in the individual and of transmission to the bystanders; (iv) HIV-2 itself may downregulate HIV-1 expression [15-17]. These intriguing properties of HIV-2 vectors, relative to the known features of HIV-1 as well as those of murine retroviral vectors, are summarized in Table 1.

We previously reported the parameters of optimal design and genetic configuration of multigenic lentiviral vectors [1]. We addressed such issues as the effect on expression of the (i) order of the placement of two or more genes in a transcriptional unit (positional effects), (ii) transcription of one or more genes as part of the same or different transcriptional unit (unit effect), (iii) transcription from the same or opposite DNA strand (polarity effects), (iv) independent internal initiation of translation in a polycistronic message (cistron effects), and (v) the choice of the promoter, primary or internal (promoter effect). Similar studies have been also reported to murine retroviral vectors.

These studies and others indicated that the strength of a lentiviral promoter depended not only on its structure but also on the position of the inserted genes and target cell in an unpredictable manner [1, 18, 19]. We also found that the LTR-driven gene expression in the presence of Tat was much stronger than expression driven by the CMV promoter [1]. Given that the transcriptional and translational apparatuses engage a myriad of cellular factors, which may be more limiting in one cell-type than the other, the cell-type dependent differential expression is perhaps not surprising in retrospect. However, these studies were needed to establish guidelines for future development of effective gene therapy vectors specifically derived from the lentiviruses.

Strategies for developing HIV-2 packaging cells. In addition to the design of a transfer vector that will shuttle transgene with the potential for regulation and for high titer encapsidation, a successful vector system requires the creation of a packaging cell line that will encapsidate vector RNA but not the viral RNA encoding the packaging components and thus be helper virus-free. Since packaging is governed by specific sequence elements, the opposing requirements-inclusion in the transfer vectors and exclusion from the packaging vectors necessitates a detailed knowledge of the packaging signal. Our initial studies of the leader sequence of HIV-2 showed that this sequence contains a packaging signal downstream of the splice donor site, but that it was not the sole determinant of RNA encapsidation [9]. Our more recent studies, this time with a different HIV-2 genotype (namely, ROD) than the previously studied ST genotype, confirmed the contributory role of downstream sequences for encapsidation, and further revealed that: (a) deletion of both the upstream and downstream elements was needed for curtailing helper virus production, (b) the magnitude of the effect of the specific sequence elements depended on the cell type, and (c) the replacement of the 3'-LTR with a cassette containing heterologous transcriptional terminator and drug selection marker gene further ensured helper virus-free phenotype without additional loss of expression of the packaging components [2]. A recent report has suggested that the upstream sequence element is the major determinant of packaging under certain conditions [20].

To obtain a helper virus-free packaging cell line, it is thought advisable to split the viral genome into two or more parts in a way that will minimize the synthesis of full length virus genome and the components of the packaging machinery will be encoded by different

Table 1. Salient features of lentiviral vectors compared to 'retroviral' vectors.

Type	Source	Advantages	Disadvantages
MLV	Murine	- Safe - Simple genetic structure - Broad cell type tropism - Less potent promoter	- Requires target cell mitosis (refractory to non-dividing cells) - Unregulated gene expression
FIV	Feline	- Serologically distinguishable from HIV-1 and HIV-2	- Safety? - Breaking species barrier?
FIV/VSV	Pseudotype (feline/human)		- Loss of specific tropism to $CD4^+$ cells - Toxicity of VSV-G protein - Possible immune rejection
HIV-1 and HIV-2	Human	- Stable transduction - Regulated gene expression - Tropism for human cells - Target cell specificity - Transduction of dividing and non-dividing cells	- Safety? - DNA integration
HIV-1	Human		-Genetic recombination with infecting HIV-1 -Nuclear import/cell cycle arrest functions encoded in one gene (*vpr*)
HIV-2	Human	- Downregulates HIV-1 - Less pathogenic than HIV-1 - Less likely to recombine with HIV-1 - Safer for production - Serologically distinguishable from HIV-1 - Nuclear import/cell cycle arrest functions are distinct; encoded by two separate genes (*vpx & vpr*) - Replicates better in non-human primate animal models	
HIV-1/-2 & VSV	Pseudotype (human)	- Broad cell tropism	- Loss of specific tropism

templates. To further promote helper virus-free phenotype, the split genomes are modified to delete the packaging signal, thus disenabling them for encapsidation. To minimize possible toxic effects of over-expression of packaging components, a desirable vector system will contain inducible elements attached to the promoter such as a heterologous tetracycline-inducible operator and response elements (Fig. 1). This will allow a controlled expression of the packaging components which may be needed for a large scale production of recombinant virions of recombinant vectors which may be needed for a large scale production of recombinant virions at a plant scale level.

Based on accumulated knowledge on HIV lentiviral replication and its control, a three component HIV-2 vector system should allow the production of 'vector virus'. The general approach has been to split and modify HIV-2 genome to 2 or 3 non-overlapping subclones and test their ability to package the 'transfer vector' designated X and Y genes in Figure 2. The new generation transfer vector is likely to contain a modified leader sequence region to increase its stability and expression. One way to obtain a lentiviral vector with a stable RNA processing and gene expression capability is to insert the cis-acting regulatory sequence, namely RRE or rev-responsive element (Fig. 2), thus to increase its ability to express and encapsidate vector RNA.

In general, a homologous lentiviral packaging system typically consists of packaging cells transfected with an HIV-1 or HIV-2 transfer vector. Cells are then tested for efficiency of the packaging. We believe that the lymphocytic cells such as CEMss, because of their increased susceptibility to HIV infection, should serve ideal reservoir to express all viral structural proteins which are then needed to wrap around a desired transfer vector after a direct DNA-mediated transfection of the transfer vector. On the other hand, because of their higher transfection efficiency, epithelioid 293 cells may serve as suitable cells as 'pre-packaging' cells. Of course, these cells can be modified to express the viral CD4 receptor which will then allow a multiple replication cycle of the transfer vector, thus resulting in amplification of 'vector virus' (Fig. 3).

Other strategies for vector design and production. The concern over the presence of HIV regulatory and accessory genes, sometimes called virulence genes, in HIV-based vectors for gene therapy has been debated for several years. Most of these concerns hover around the possible toxic side effects by these genes; they can provide hot spots for recombination, facilitate production of infectious virus, and/or increase pathogenicity. In an attempt to overcome this potential concern, packaging vectors with deletions of the viral regulatory and accessory genes *(tat, vif, vpr, vpu, and nef)* have been designed [21]. The design is based on the premise that the accessory genes are redundant for functions needed for transduction of non-dividing cells. Although the vectors packaged with such minimal vectors were capable of transducing at least non-dividing cells, their general applicability remains uncertain. Thus, inclusion of the regulatory gene loop may be an important parameter in the design of a regulatable vector. This may then allow an effective expansion or `mobilization' of a therapeutic lentiviral vector *in vivo*.

To improve the titer of HIV-1-based vector, protocols have been devised to concentrate the particles without damage. One such protocol to achieve about two orders of magnitude enrichment involved a combination of sulfonated cellulose column and a subsequent centrifugation of a mixture of the vector preparation and the target cells at transduction [22]. Moreover, CD4-enriched PBMCs were stably transduced without any

apparent detection of replication competent wild type HIV [22].

The development of HIV-1 packaging cell line, which can synthesize virions, after stable transfection with an HIV-1 or HIV-2 transfer vector, was reported [8]. The vector produced by this cell line was capable of transducing CD4$^+$ T cells and macrophages [9]. However, because of phenotypic instability, long term utility of the cell line is uncertain. This study also noted that HIV vectors *per se* inhibited HIV infection of transduced cells [23], and further substantiated our previously reported observation [15-17]. It is noteworthy that our strategy employs clones derived from a noncytopathic variant of HIV-2 isolate, which will be a very important criterion in establishing an effective, as well as a safe, lentiviral vector system. As previously noted, use of HIV-2, as compared with HIV-1, minimizes the risk of recombination with the resident HIV-1 genomes in HIV-1 infection [1, 2, 15, 16].

In the usual design, the expression of packaging components is RRE-dependent and is trans-regulated by Rev. To make packaging independent of RRE/Rev, RRE has been substituted with a cis-acting constitutive transport element from a simian retrovirus [9]. Although the supernatant of such packaging cells stably transfected with this new vector was capable of transducing CD4$^+$ cells and primary macrophages [9], the vector titer was low. Thus, further modifications are needed to effectively render this packaging line as a high efficiency vector producer. It is possible that the modification of RRE in the latter study contributed to a reduced gene expression from the packaging vector. However, a recent study has reported that the replication of clinical isolates of HIV-1 from HIV-1-positive mothers is significantly inhibited in infants cord blood-derived CD34$^+$ stem cells, at tissue culture level, transduced with a recombinant retroviral vector expressing a ribosome against the U5 region of HIV-1 [24]. These studies further lend the credence to the feasibility of using a gene-based therapy for preventing the spread of HIV.

A variety of model genes has been tested as a proof-of-principle of gene modification. For example, a minigene consisting of a fusion of a ribosome, spliceosomal U 1 small nuclear RNA (snRNA) and the 5' splice site of the Rev pre-mRNA has resulted in co-localization of anti-HIV ribosome to the nucleus in human T cell clones and were shown to be very efficient in inhibiting viral
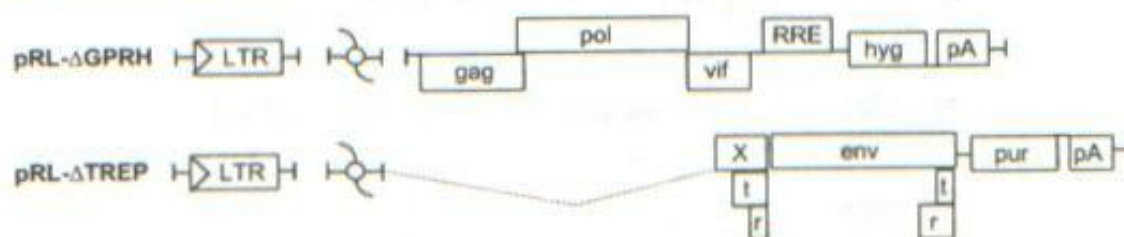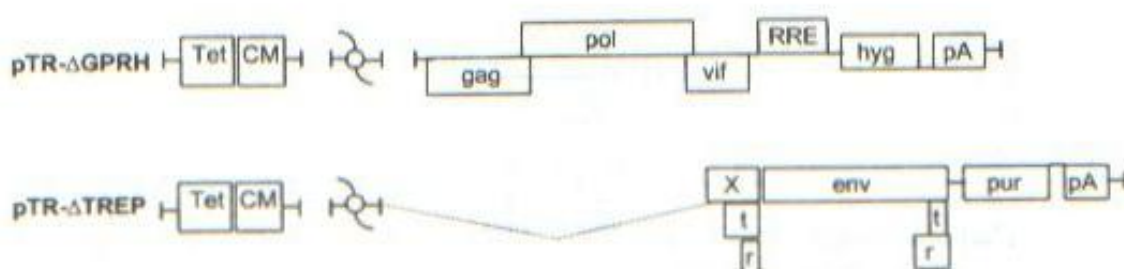
Fig. 1. HIV-2 packaging vectors. This figure shows two sets of HIV-2 'split-genome' packaging vectors. (a) Tat-inducible vectors: pRL- GPRH, a proviral deletion mutant clone with intact 5'LTR, main splice donor site, structural *gag-pol* genes and accessory *vpr* gene and rev-responsive element (RRE), but it lacks the packaging sequences, and the *env* gene and 3' LTR which are replaced by the antibiotic selection gene, hygromycin (hyg), and a heterologous polyadenylation signal (pA). The clone pRL-TREP, also with a deletion of the packaging sequence, has a large deletion of the structural genes, contains intact envelop (env), regulatory (tat and rev), and antibiotic selection, puromycin (pur), genes, and a heterologous polyadenylation site. (b) Tetracycline-inducible vectors: pTR-GPRH and pTR-TREP, are same vectors as in (a) above, except that the 5'LTR gene is replaced by a tetracycline inducible gene fused to a heterologous human cytomegalovirus (CM) promoter. The boxes represent approximations of the size and position of each gene, thus are not drawn to scale.

replication [25]. An HIV-1-based vector containing an HIV-1 *gag* dominant negative mutant and a human ribonuclease, eosinophilderived neurotoxin (EDN) was reported to successfully inhibit HIV-1 replication in cell lines [26]. However, it remains to be seen whether a protective gene such as EDN, when it encounters infectious virions, will function safely or will potentially exert harmful effects on normal bystander cells.

Tissue Culture transduction and functional studies. When murine retroviral-based vectors expressing the antisense RNAs of HIV-1 *pol, vif,* and *env* genes and the 3' long terminal repeat (LTR) were used to transduce a CD4$^+$ T-cell line, the most efficient inhibition of HIV-1 replication was observed with the *env* antisense RNA, followed by the *pol* complementary sequence, with a 2- to 3-logl0 reductions in p24 antigen production and a considerable reduction of HIV-1 steady-state RNA levels [27]. However, this vector system inhibited HIV-1 to much lesser degree in primary CD4$^+$ lymphocytes, presumably due to the inability of murine retroviral vectors to effectively transduce human primary cells. Furthermore, a comparative analysis indicated that expressed antisense sequences have more pronounced antiviral efficacy than the transdominant Rev-M10 protein, perhaps making these antisense RNAs promising protective genes against HIV-1.

HIV-2 can downregulate different HIV-1 in T cells, including human primary peripheral blood mononuclear cells (PBMCs) [15-17]. Arya and co-workers have proposed that the observed inhibition may be mediated through competition between HIV-1 and HIV-2 for cellular factors, possibly involving the long terminal repeats (LTRs) [15-17]. As a prelude to clinical trials, animal model studies demonstrating the inhibitory effectiveness of HIV-2 over HIV-1 *in vivo* need to be undertaken.

Preclinical and clinical studies. Non-human primates serve as animal models for HIV disease to
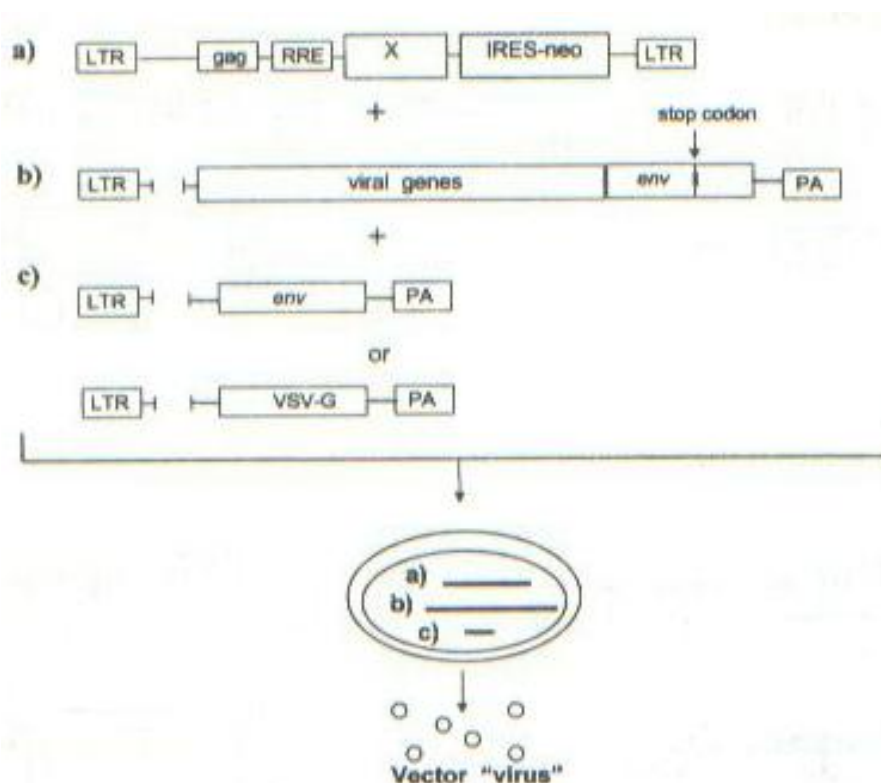
Fig. 2. Schematic presentation of a 'three-component' lentiviral vector. The three-component clones (a-c) illustrate overall structure and design of second generation HIV-2 based lentiviral vectors. (a) The 'transfer vector' containing a multigene which consists of a gene truncated gag leader sequence (gag), cis-acting rev-response element (RRE), therapeutic gene (X), internal ribosomal entry site (IRES) and selectable marker neomycin (neo); (b) The 'packaging vector' expressing HIV-2 structural proteins (viral genes) including intact gag (capsid and core proteins), but envelop which contains an stop codon, and (c) The 'auxiliary packaging vector' supplying viral envelop protein.

study the *in vivo* safety and efficacy of gene therapy vectors and/or DNA vaccination. The use of a vector in a suitable non-human primate model will shorten the time needed to obtain a preclinical proof-of-concept for clinical trials. For example, a recent study used a combination of anti-sense *tat* and *rev* genes [28]. In this study, CD4-enriched lymphocytes from rhesus macaques were transduced with retrovirally mediated gene transfer with a vector expressing an antisense *tat/rev* gene. Animals infused with lymphocytes *ex vivo* transduced with the antisense vector demonstrated a significant reduction in viral load in both peripheral blood and lymph nodes, and they showed a normal range of CD4$^+$ cell level and normal lymph node architecture [28].

Convenient animal models for HIV-2 infection exist, which can be used for preclinical evaluation of safety and effectiveness of HIV-2 vectors. This may not be the case for HIV-1 vectors. Similarly designed SIV vectors or SIV-HIV pseudotype vectors (SHIV) in simian model may offer alternate opportunities to generate important preclinical data using non-human primates. Some strains of SIV have been shown to cause disease in limited number of days; for example, SIV/PBj isolate causing an acutely

lethal syndrome in both pig-tailed and cynomolgus macaques in 6-10 days after intravenous inoculation [29, 30].

An important consideration should be given to create gene therapy vectors from appropriate recombinant viruses, such as SIV/HIV chimera (SHIV), to use in animal models. A variety of chimeric SHIVs containing core antigen of SIV, and *env, tat, rev, vpu* and *nef* of HIV-1 were reported recently [e.g., ref. 31, 32, 33]. SHIV in which RT gene of SIV was replaced by RT gene of HIV-1 (RT-SHIV) replicated similarly to SIV [34]. SHIV and it's variants reportedly induced AIDS-like disease in macaques [34, 35]. Moreover, some variants, such as SHIV-4 adapted to replicate in macaques and rhesus monkeys, induced fatal pathogenesis in rhesus within 6 months [33, 36]. In an attempt to increase their virulence and pathogenicity in monkeys, other chimeric SHIVs were constructed that used *env* of clinical HIV-1 isolates [37, 38]. Such studies were carried out with an intention of adapting SHIV to grow in a more relevant animal model, such as baboon. SHIV variants, with SIV core and HIV-1 envelope, reportedly required monkey cells expressing human CD4 expression, but not human coreceptor (CCR-5 or CXCR-4) which
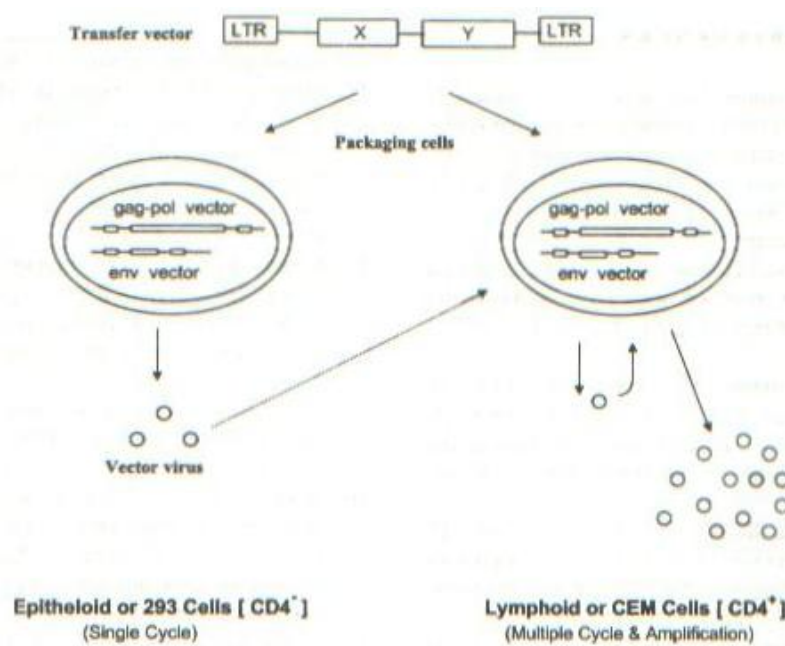
Fig. 3. Vector production by transfection of stable packaging cell lines. This figure shows a two-pronged approach for producing a typical dual gene transfer vector, i.e., X and Y genes flanked by viral LTR. The left column represents epithelioid 293 cells with an anticipated low production level of 'virus vector' because these cells are CD4-negative [CD41. Shown on the right column are CEM cells which will release a high level of 'virus vector' because these cells are CD4-positive [CD4$^+$] and will allow multiple cycles of replication of virus, resulting in amplification of the 'transfer vector' and subsequent release of 'vector virus'.

are required for HIV-1 [39]. This study indicated that SHIV is relatively a convenient model for preclinical studies; an advantage that will minimize further engineering of monkey cells to express human coreceptors. However, different strains of HIV-2 were shown to successfully infect baboon and induce AIDS-like symptoms [40, 41, 42]. Furthermore, in a fashion similar to the non-pathogenic life HIV-2 virus as a potential vaccine [43], HIV-2 gene therapy vectors could be used as either non-replicative or minimally replicative virions as models for vaccine. Thus, it would be important to consider HIV-2, alone or in the context of SHIV, in devising the future generation HIV-derived gene therapy vectors needed for studies in non-human primates.

Limited clinical trials with HIV-1 infected individuals using viral proteins as transdominant effectors have been undertaken. In one such trial, a mutant inhibitory form of an essential HIV-1 protein, Rev-M10, was administered to some AIDS patients by an *ex vivo* non-viral means. The gene-modified cells could not be traced in the patients beyond 8 weeks [44]. When the same gene was transduced using a murine retroviral vector, the

transduced cells, in 3 patients tested, survived and the recombinant protein were expressed for up to 6 months, suggesting that retroviral delivery of an antiviral gene may potentially prolong survival of CD4$^+$ cells [45]. However, a murine retroviral vector fails to transduce non-dividing cells which account for a significant reservoir of HIV-1 target cells. On the other hand, HIV-1-based vectors have also been shown *in vitro* to transduce cell cycle-arrested cells, blocked at the G2 or Gl/S phases, with a transduction efficiency comparable to that in proliferating cells [46].

Conclusion. A lentiviral vector system capable of transducing a desired therapeutic gene has been developed, which successfully transduces both dividing and non-dividing human target cells in culture. Future studies are likely to focus on customized transfer vectors containing desired genes, while focusing on optimizing the vector design and production procedures to generate a large amount of recombinant virions which are stable and safe for human use, and yet effective in terms of preventing and curing a disease.

REFERENCES

1. Sadaie, M.R., Zamani, M., Whang, S., Sistron, N. and Arya, S.K. (1998) Towards developing HIV-2 lentivirus-based retroviral vectors for gene therapy: Dual gene expression in the context of HIV-2 LTR and Tat. *J. Med. Virol. 54: 118-128.*

2. Arya, S.K., Zamani, M. and Kundra, P. (1998) Human immunodeficiency virus type 2 lentivirus vectors for gene transfer: Expression and potential for helper virus-free packaging. *Hum. Gen. Ther. 9: 1371-1380.*

3. Naldini, L., Blomer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F.H., Verma, I.M. and Trono, D. (1996) *In vivo* gene delivery and stable transduction of non-dividing cells by a lentiviral vector. *Science 272: 263-267.*

4. Blomer, U., Naldini, L., Kafari, T., Trono, D., Verma, I.M. and Gage, F.H. (1997) Highly efficient and sustained gene transfer in adult neurons with a lentivirus vector. *J. Virol. 71: 6641-6649.*

5. Zuffery, R., Nagy, D., Mandel, R.J., Naldini, L. and Trono, D. (1997) Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo. Nature Biotechnology. 15: 871-875.*

6. Akkina, R.K., Walton, R.M., Chen, M.L., Li, Q.X., Planelles, V., and Chen, I.S.Y. (1996) High-efficiency gene transfer into CD34$^+$ cells with a human immunodeficiency virus type 1-based retroviral vector pseudotyped with vesicular stomatitis virus envelop glycoprotein. *J. Gen. Viral. 70: 2581-2585.*

7. Poeschla, E.M., Wong-Staal, F. and Looney, D.J. (1998) Efficient transduction of nondividing human cells by feline immunodeficiency virus lentiviral vectors. *Nature Medicine 4: 354-357.*

8. Corbeau, P., Kraus, G. and Wong-Staal, F. (1996) Efficient gene transfer by a human immunodeficiency virus type I (HIV-I)-derived vector utilizing a stable HIV packaging cell line. *Proc. Natl. Acad. Sci. USA 93: 14070-14075.*

9. Corbeau, P., Kraus, G. and Wong-Staal, F. (1998) Transduction of human macrophages using a stable HIV-1/HIV-2-derived gene delivery system. *Gene Ther. 5:99-104.*

10. Garzino-Demo, A., Gallo, R.C. and Arya, S.K. (1995) Human immunodeficiency virus type 2 (HIV-2): Packaging signal and associated negative regulatory element. *Hum. Gen. Ther. 6: 177-184.*

11. Di Marzio, P., Choe, S., Ebright, M., Knoblauch. R. and Landau, N.R. (1995) Mutational analysis of cell cycle arrest, nuclear localization and virion packaging of human immunodeficiency virus type 1Vpr. *J. Viral. 69: 7909-7916.*

12. Mahalingam, S., Ayyavoo, V., Patel, M., KieberEmmons, T. and Weiner, D.B. (1997) Nuclear import, virion incorporation, and cell cycle arrest/differentiation are mediated by distinct functional domains of human immunodeficiency virus type 1 Vpr. J. *Virol. 71: 6339-6347.*

13. Fletcher, T.M., Brichacek, B., Sharova, N., Newman, M.A., Stivahtis, G., Sharp, P.M., Emerman, M., Hahn. B.H. and Stevenson, M. (1996) Nuclear import and cell cycle arrest functions of the HIV-1 Vpr protein are encoded by two separate genes in HIV-2/SIVsm. *EMBO J. 15: 6155-6165.*

14. Kumar, P., Hui, H.X., Kappes, J.C., Haggarty, B.S., Hoxie, J.A., Arya, S.K., Shaw, G.M. and Hahn, B.H. (1990) Molecular characterization of an attenuated human immunodeficiency virus type 2 isolate. *J. Virol. 64: 890-901.*

15. Arya, S. K., Zamani, M. and Gallo, R. C. (1994) HIV-2 inhibits HIV-1. *AIDS Res. Hum. Retrovir. 10: 5158.*

16. Arya, S.K. and Gallo, R.C. (1996) Human immunodeficiency virus (HIV) type-2 mediated inhibition of HIV type 1: A new approach to gene therapy of HIV infection. *Proc. Natl. Acad. Sci. USA 93: 4486-4491.*

17. Al-Harthi, L., Owais, M. and Arya, S.K. (1998) Molecular inhibition of HIV type 1 by HIV type 2: Effectiveness in peripheral blood mononuclear cells. *AIDS Res. Hum. Retrovir. 14: 59-64.*

18. Li, M., Hantzopoulos, P.A., Banerjee, D., Zhao, S.C., Schweitzer, B.I., Gilboa, E. and Bertino, J.R. (1992) Comparison of the expression of a mutant dihydrofolate reductase under control of different internal promoters in retroviral vectors. *Hum. Gen. Ther. 3: 381-390.*

19. Correll, P.H., Colilla, S. and Karlsson, S. (1994) Retroviral vector design for long-term expression in murine hematopoietic cells *in vivo. Blood 84: 1812-1822.*

20. McCann, E.M. and Lever, A.M. (1997) Location of cis-acting signal important for RNA encapsidation in the leader sequence of human immunodeficiency virus type 2. *J. Virol. 71: 4133-4137.*

21. Kim, V.N., Mitrophanous, K., Kingsman, S.M. and Kingsman, A.J. (1998) Minimal requirement for a lentivirus vector based on human immunodeficiency virus type 1. *J. Virol. 72: 811-816.*

22. Matsuoka, H., Miyake, K. and Shimada, T. (1998) Improved methods of HIV vector mediated gene transfer. *Int. J. Hematol. 67: 267-273.*

23. Corbeau, P. and Wong-Staal, F. (1998) Anti-HIV effects of HIV vectors. *Virology 243: 268-274.*

24. Li, X., Gervaix, A., Kang, D., Law, P., Spector, S.A., Ho, A.D. and Wong-Staal, F. (1998). Gene therapy targeting cord blood-derived CD34$^+$ cells from HIV-exposed infants: preclinical studies. *Gene Ther. 5: 233-239.*

25. Michienzi, A., Conti, L., Varano, B., Prislei, S., Gessani, S. and Bozzoni I. (1998) Inhibition of human immunodeficiency virus type 1 replication by nuclear chimeric anti-HIV ribozymes in a human T lymphoblastoid cell line. *Hum. Gene Ther. 9: 621-628.*

26. Cara, A., Rybak, S.M., Newton, D.L., Crowley, R., Rottschafer, S.E., Reitz, M.S. Jr; and Gusella, G.L. (1998) Inhibition of HIV-1 replication by combined expression of gag dominant negative mutant and a human ribonuclease in a tightly controlled HIV-1 inducible vector. *Gene Ther.* 5: 65-75.

27. Veres, G., Junker, U., Baker, J., Barske, C., Kalfoglou, C., Ilves, H., Escaich, S., Kaneshima, H. and Bohnlein, E. (1998) Comparative analyses of intracellularly expressed antisense RNAs as inhibitors of human immunodeficiency virus type 1 replication. *J. Virol. 72: 1894-901.*

28. Donahue, R.E., Bunnell, B.A., Zink, M.C., Metzger, M.E., Westro, R.P., Kirby, M.R., Unangst T., Clements, J.E. and Morgan, R.A. (1998) Reduction in SIV replication in rhesus macaques infused with autologous lymphocytes engineered with antiviral genes. *Nat. Med. 4: 181-186.*

29. Tao, B. and Fultz, P. (1995) Molecular and biological analyses of quasispecies during evolution of a virulent simian immunodeficiency virus, SIVsmmPBj14. *J Virol. 69: 2031-2037.*

30. Lewis, M.G., Zack, P.M., Elkins, W.R. and Jahrling, P.B. (1992) Infection of rhesus and cynomolgus macaques with a rapidly fatal SIV (SIVSMM/PBj) isolate from sooty mangabeys. *AIDS Res. Hum. Retrovir. 8:1631-1639.*

31. Karlsson, G.B., Halloran, M., Li, J., Park, I.W., Gomila, R., Reimann, K.A., Axthelm, M.K., Iliff, S.A., Letvin, N.L. and Sodroski, J. (1997) Characterization of molecularly cloned simian-human immunodeficiency viruses causing rapid CD4+ lymphocyte

depletion in rhesus monkeys. *J Virol. 71: 4218-4225*.

32. Shibata, R., Maldarelli, F., Siemon, C., Matano, T., Parta, M., Miller, G., Fredrickson, T. and Martin, M.A. (1997) Infection and pathogenicity of chimeric simian-human immunodeficiency viruses in macaques: determinants of high virus loads and CD4 cell killing. *J. Infect. Dis. 176: 362-373*.

33. McCormick-Davis, C., Zhao, L.J., Mukherjee, S., Leung, K., Sheffer, D., Joag, S.V., Narayan, 0. And Stephens EB. (1998) Chronology of genetic changes in the vpu, env, and Nef genes of chimeric simian-human immunodeficiency virus (strain HXB2) during acquisition of virulence for pig-tailed macaques. *Virology 248: 275-283*.

34. Balzarini, J., De Clercq, E. and Uberla, K. (1997) SIV/HIV-1 hybrid virus expressing the reverse transcriptase gene of HIV- 1 remains sensitive to HIV-1-specific reverse transcriptase inhibitors after passage in rhesus macaques. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 15: 1-4*.

35. Harouse, J.M., Tan, R.C., Genie, A., Dailey, P., Marx, P.A., Luciw, P.A. and Cheng-Mayer, C. (1998) In vitro infection of primate PBMC with simian/human immunodeficiency virus, SHIV(SF33A): correlation to in vivo outcome. *J. Med. Primatol. 27 : 81-6*.

36. Joag, S.V., Li, Z., Foresman, L., Pinson, D.M., Raghavan, R., Zhuge, W., Adany I., Wang, C., Jia, F., Sheffer, D., Ranchalis, J., Watson, A. and Narayan, O. (1997) Characterization of the pathogenic KU-SHIV model of acquired immunodeficiency syndrome in macaques. *AIDS Res. Hum. Retrovir. 13: 635-645*.

37. Ranjbar, S., Jones, S., Stott, E.J. and Almond, N. (1997) The construction and evaluation of SIV/HIV chimeras that express the envelope of European HIV type 1 isolates. *AIDS Res. Hum. Retrovir. 13: 797-800*.

38. Klinger, J.M., Himathongkham, S., Legg, H., Luciw, P.A. and Barnett, S.W. (1998) Infection of baboons with a simian immunodeficiency virus/HIV-1 chimeric virus constructed with an HIV-1 Thai subtype E envelope. *AIDS 12: 849-857*.

39. Chackerian, B., Long, E.M. and Luciw PA. (1997) Human immunodeficiency virus type 1 coreceptors participate in postentry stages in the virus replication cycle and function in simian immunodeficiency virus infection. *J. Virol. 71: 3932-3939*.

40. Castro, B.A., Nepomuceno, M., Lerche, N.W., Eichberg, J.W. and Levy, J.A. (1991) Persistent infection of baboons and rhesus monkeys with different strains of HIV-2. *Virology 184: 219-226*.

41. Barnett, S.W., Murthy, K.K., Herndier, B.G. and Levy, J.A. (1994) An AIDS-like condition induced in baboons by HIV-2. *Science 266: 642-646*.

42. Barnett, S.W., Legg, H.S., Sun, Y., Klinger, J., Blackbourn, D.J., Locher, C.P., Levy, J.A. (1996) Molecular cloning of the human immunodeficiency virus subtype 2 strain HIV-2UC2. *Virology 222: 257-261*.

43. Looney, D.J., McClure, J., Kent, S.J., Radaelli, A., Kraus, G., Schmidt, A., Steffy, K., Greenberg, P., Hu, S.L., Morton, W.R. and Wong-Staal, F. (1998) A minimally replicative HIV-2 live-virus vaccine protects M. nemestrina from disease after HIV-2(287) challenge. *Virology 242: 150-160*.

44. Woffendin, C., Ranga, U., Yang, Z., Xu, L. and Nabel, G.J. (1996) Expression of a protective gene prolongs survival of T-cells in human immunodeficiency virus-infected patients. *Proc. Natl. Acad. Sci. USA 93: 2889-2894*.

45. Ranga, U., Woffendin, C., Verma, S., Xu, L., June, C.H., Bishop, D.K., and Nabel, G.J. (1998) Enhanced T cell engraftment after retroviral delivery of an antiviral gene in HIV-infected individuals. *Proc. Natl. Acad. Sci. USA 95: 1201-206*.

46. Miyake, K., Suzuki, N., Matsuoka, H., Tohyama, T. and Shimada, T. (1998) Stable integration of human immunodeficiency virus-based retroviral vectors into the chromosomes of non-dividing cells. *Hum. Gene Ther. 9: 467-475*.