Lentiviral Vectors

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According to the database "Gene therapy clinical trials worldwide" (http://www.wiley.co.uk/genetherapy/clinical/), out of 318 retroviral vector gene therapy clinical trials that have been completed, are ongoing or have been approved, only eleven trials involve the in vivo or ex vivo use of replication-incompetent lentiviral vectors. This number is expected to increase in the next years, because lentiviral vectors are on the rise from non-clinical safety assessment studies to clinical trials.

The development and use of lentiviral vectors for gene therapy has been started in 1996 when Naldini et al. published the first split genome vector system for human immunodeficiency virus (HIV) -1-derived lentiviral vectors (Naldini et al. 1996). Just like other retroviral vectors, lentiviral vectors stably integrate into the genome of transduced target cells. By integration of the vectors into the host cell genome, they allow persistence of the genetic modification in cells and long-term expression of the transferred gene. The commonly used γ-retroviral vectors, mostly derived from murine leukaemia virus (MLV) and its close relatives, depend on active cell division and breakdown of the cellular nuclear lamina for successful gene transfer. In contrast, lentiviral vectors also mediate gene transfer into non-proliferating cells. For *in vivo* gene therapy, this is a critical prerequisite for most tissues of interest, e.g. muscle, liver and brain, since these tissues usually consist of fully differentiated cells as well as non-replicating stem cells. In this regard, HIV-derived vectors have been shown to efficiently infect hepatic, glial, muscle, and retinal cells as well as human hematopoietic stem cells. Other lentivectors have been derived from HIV-2, SIVmac251, FIV, EIAV, CAEV and JDV (Delenda et al., 2004).

Although the precise mechanism whereby lentiviruses infect non-dividing cells is unknown, HIV-1-mediated gene transfer appears to be facilitated by the viral integrase, the matrix protein, and the accessory protein Vpr (Bukrinsky et al., 1993; Gallay et al., 1997; Heinzinger et al., 1994; von Schwedler U. et al., 1994). Vpr packaged into virus particles binds directly to the nuclear pore complex and affects the architecture and integrity of the nuclear membrane of infected cells (De Noronha et al., 2001). The precise role of the other elements remains to be elucidated, especially, since lentiviral vectors devoid of all accessory genes (including *vpr*) still infect non-dividing target cells (Zufferey et al., 1997). Yet, certain resting/quiescent cells in the G_0/G_{1a}-phase of the cell cycle, such as non-stimulated T-lymphocytes, monocytes, or non-stimulated HSC, are still refractory to gene transfer by HIV-1-derived lentiviral vectors and have to be stimulated to allow transduction.

The design and production of lentiviral vectors is similar to that of other retroviral vectors: The separation of critical components of the viral genome onto separate plasmids (split genome approach) allows the safe production of single-round infectious viral particles harbouring the respective retroviral/transfer vector RNA. For simple retroviruses, three components are needed for the generation of vector particles (schematically depicted in Fig. 1):

- *gag* and *pol* genes on the packaging construct, expressing structural and enzymatic components of the vectors
- an envelope (*env*) gene encoding a glycoprotein mediating cell-entry of the vector particles; it can be substituted by the glycoproteins of other viruses, e.g. that of the vesicular stomatitis virus (VSV-G). This substitution leads to pseudotyped vectors.
- a transfer vector: the only component encompassing the *cis*-acting sequences of the virus genome required for efficient synthesis and packaging of the vector RNA, its reverse

transcription and integration. This transfer vector includes the transgene to be transferred to and expressed in the target cells of interest.

Lentiviruses express additionally up to 4 accessory (*vif*, *vpr*, *vpx*, *vpu* or *nef*) and 2 regulatory (*tat* and *rev*) genes. As already pointed out, the accessory genes can be usually deleted from the vector system. However, either abundance of the regulatory proteins Tat and Rev in cells producing lentiviral vectors has to be confirmed by inclusion in the packaging construct or separate expression constructs in the vector system, or their function has to be substituted by other cis-acting elements in the transfer vector, e.g. heterologous promoters and constitutive transport elements (vector systems schematically depicted in Fig. 2).

Lentiviral vectors are usually produced via transient transfection of the vector components into packaging cells, since the generation of stable cell lines that produce vector particles has been hampered by toxicity of the packaging construct for the vector-producing cell. However, Ikeda et al. (2003) have described the generation of stable packing cells for lentiviral vectors, by using γ-retroviral vectors to express lentiviral *gag-pol* in these cells. Titers of up to 10^8 transducing particles per ml can be achieved with safe and advanced vector systems, which enable technically the treatment of patients in clinical trials.

One of the major problems of retroviral vectors turned out to be their oncogenic potential. The X-SCID trial published in 2000 (Cavazzana-Calvo et al., 2000), where γ-retroviral vectors were used, is generally regarded a success, because the conditions of most patients improved and patients benefitted from the therapy. However, the severe side effect was leukaemia induction in 4 of 15 patients. The integration of vectors into the genome of the host cell led to oncogenic cell transformation by activation of oncogene transcription near the integration site. The subsequent clonal selection of cells presumably suffering from additional genetic changes led to leukaemia-like phenotypes.

One focus has subsequently been the determination of the integration patterns of retroviral vectors. It turned out that retroviral integration is only semi-random. Most retroviruses and vectors derived thereof prefer transcriptionally active regions. Lentiviral vectors have a different integration pattern compared to γ-retroviruses: lentiviral vectors preferentially target transcription units, whereas γ-retroviruses target upstream regulatory genetic loci. Whether these differences make lentiviral vectors less likely to trigger oncogene expression than γ-retroviral vectors is under investigation.

A lot of progress has been made in the development of technical methods for the determination and improvement of vector safety. For example, LM-PCR and LAM-PCR are sensitive tools for investigating clonal dominance of certain integration sites or mapping of certain integration site populations, respectively. Furthermore, an *in vitro* immortalisation assay based on murine hematopoietic stem cells has been developed to evaluate and compare the risk of oncogene activation of different vectors. Thus, studies to evaluate the genotoxicity of vectors have become a substantial feature of gene therapy research.
The development of self-inactivating (SIN) vectors from lentiviral vectors further improves the safety. SIN vectors are modified in a way that the final integrated vector genome has deletions in both flanking LTRs which eliminate the viral promoter and enhancer elements from the integrated vector. The aforementioned *in vitro*-studies revealed that these SIN-vectors have a reduced genotoxic potential.

In November 2006, the first clinical trial utilizing lentiviral vectors has been published. It demonstrated successful gene delivery to patients´ T cells with good persistence *in vivo*. This

study used a conditionally replicating HIV-1 vector, which expresses antisense RNA against the HIV-1 envelope gene transcript. This vector is conditionally replicating since it is activated by accessory proteins that are provided only, if wild-type HIV-1 co-infects the cell. Immune functions improved in four of five patients with chronic HIV infection, who had failed to respond to at least two antiviral treatment approaches. No evidence for insertional mutagenesis was found after 21 to 36 months of observation (Levine et al., 2006).

Another target for lentiviral vectors, human globin gene therapy, provides a potential treatment for sickle cell disease and beta-thalassemia. A clinical trial of this treatment is currently under way in Paris using “lentiglobin” vectors (Bank, 2008). Current preclinical studies bearing further potential to be transformed into clinical trials using lentivectors address, for example, Wiskott-Aldrich-Syndrome, a rare X-linked recessive immune deficiency disease (Galy et al., 2008). HIV-1-derived vectors successfully transduce T lymphocytes and hematopoietic stem cells carrying the Wiskott-Aldrich-Syndrome protein and its endogenous promoter (Toscano et al., 2008).

Taken together, lentiviral vectors are regarded as a key element in advancing gene therapy due to their properties (Kohn, 2007).

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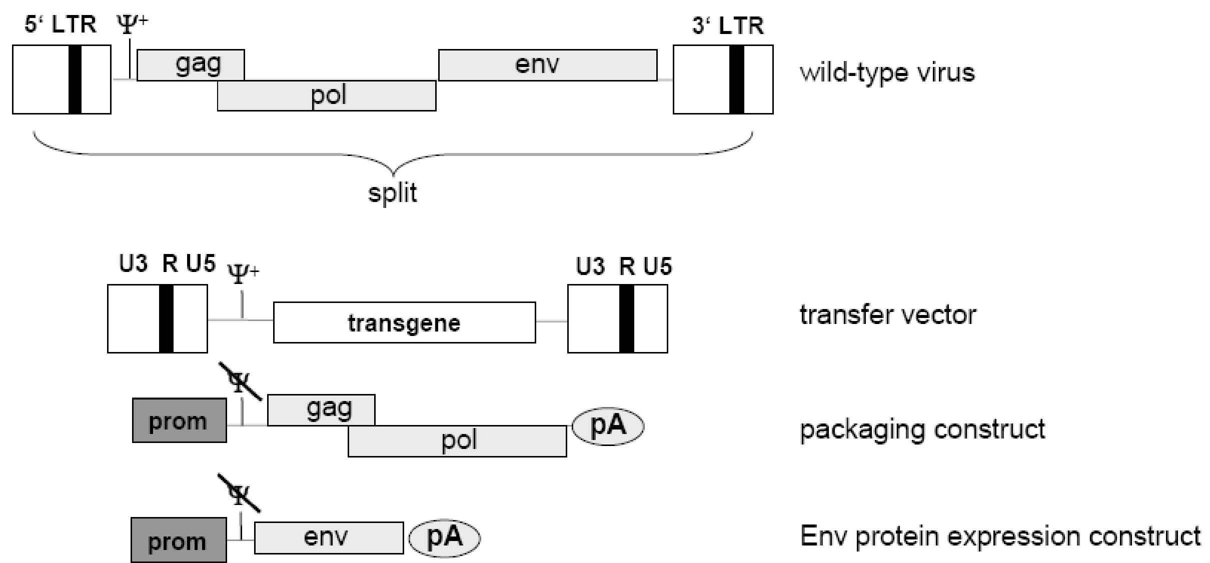
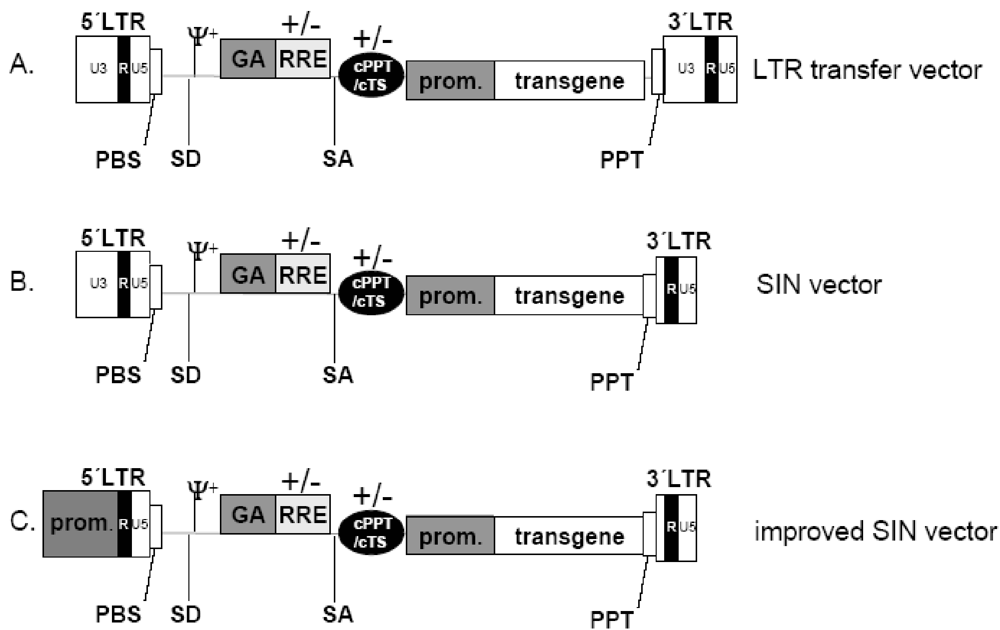


Figure 1: ***Design of Retroviral Vector Systems.*** Representative genomic structure of a replication-competent γ–retrovirus are shown including all viral genes and the flanking long terminal repeats (LTRs) with the 5´ LTR containing the functional viral promoter (U3). To construct a replication-incompetent vector system, the viral components are split into three separate plasmids comprising the packaging signal (Ψ)-negative packaging constructs for expression of viral capsid (Gag) and non-structural (Pol), as well as envelope (Env) proteins. The Ψ-positive transfer vector harbors the transgene and is marked by the presence of a packaging signal. To reduce sequence homologies, viral promoter and polyadenylation signals (pA) within the packaging constructs are replaced by heterologous elements displaying similar functions.

a) Lentiviral transfer vectors



b) Lentiviral packaging constructs:

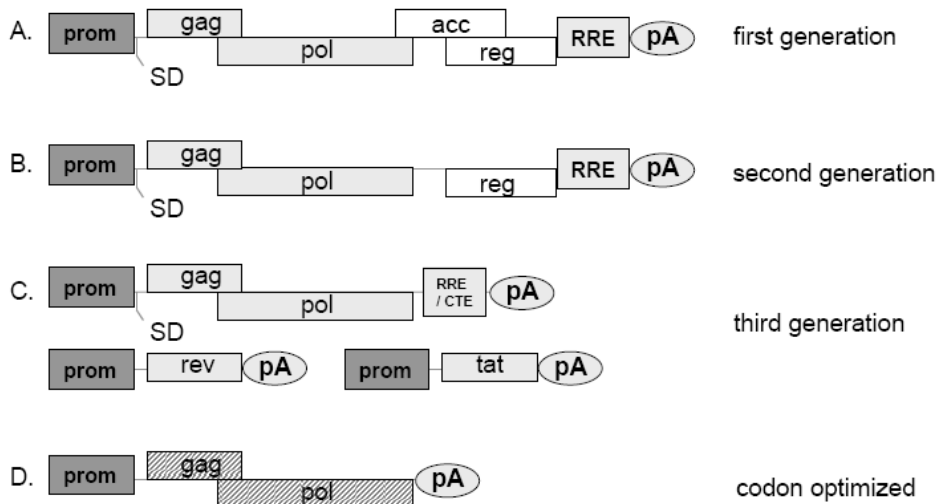


Figure 2. ***Design of Lentiviral Vector Systems*.** (a) The basic lentiviral (+ RRE and + cPPT) transfer vector (A) contains all sequences required for transfer and subsequent integration of the vector into the genome of the target cell. It is flanked by non-modified LTRs. (B) In contrast, a SIN-vector carries a deletion in the U3 region of the 3’ LTR. (C) Substitution of the 5’ U3 region with a constitutive heterologous promoter improves transcription of full-length viral RNA in virus producer cells. Upon infection and reverse transcription the deleted 3’ U3 region is copied to the 5’ end, rendering both LTRs transcriptionally inactive. PBS: primer binding site; ψ packaging signal; SD: splice donor; GA: gag coding region, RRE: Rev responsive element, cPPT/cTS: central polypurine tract/central termination sequence. SA: splice acceptor. (b) Schematic representation of typical first, second and third generation lentiviral packaging constructs. A strong constitutive promoter and polyadenylation signal (polyA) are used to replace the 5’ and 3’ LTR, respectively. The packaging signal ψ is deleted. In terms of development, the accessory genes (acc) and the regulatory genes (reg) are eliminated stepwise from the packaging construct. In third generation constructs the regulatory gene *rev* is expressed from a separate expression plasmid lacking homologous sequences. In case of using a RRE equivalent cytoplasmic transport element (CTE) Rev might be deleted. Similarly, Tat might be expressed from a separate plasmid or, in case a chimeric Tat-independent 3’ promoter is used, completely deleted.