

Lentiviral vectors for immunization: an inflammatory field

Expert Rev. Vaccines 9(3), xxx-xxx (2010)

Mudita Pincha*,
Bala Sai Sundarasetty*
and Renata Stripecke†

†Author for correspondence
Lymphatic Cell Therapy
Laboratory, Department of
Hematology, Hemostaseology,
Oncology and Stem Cell
Transplantation, Hannover
Medical School, Carl-Neuberg-
Strasse 1, OE6860 – HBZ,
D-30625 Hannover, Germany
Tel.: +49 511 532 6999
Fax: +49 511 532 6975
stripecke.renata@
mh-hannover.de

*These authors contributed
equally to this manuscript.

Lentiviruses are retroviruses that are able to transduce both dividing and nondividing cells. Dendritic cells are key players in the innate and adaptive immune responses, and are natural targets for lentiviruses. Lentiviral vectors (LVs) have recently reached the clinical gene therapy arena, prompting their use as clinical vaccines. In recent years, LVs have emerged as a robust and practical experimental platform for gene delivery and rational genetic reprogramming of dendritic cells. Here, we present the *status quo* of the LV system for protective or therapeutic vaccine development. This vector system has been extensively evaluated for *ex vivo* and *in vivo* (immuno)gene delivery. Improvements of the LV design in order to further grant a higher biosafety profile for vaccine development are presented.

KEYWORDS: cancer • chronic infection • clinical development • dendritic cell • immunotherapy • lentiviral vector

Lentiviral vectors becoming mainstream

Retroviruses contain a diploid, positive-strand RNA genome. Their life cycle is characterized by their use of reverse transcriptase, converting the RNA genome to double-stranded DNA, which is then permanently integrated into the chromosomes of the host cell. Oncoretroviruses traditionally based on Moloney murine leukemia virus have been widely used as gene-transfer vectors, representing the vector system used in the majority of clinical gene therapy trials to date.

In contrast to oncoretroviruses, known for resulting in insertional mutagenesis, lentiviruses have so far not been reported to cause malignancies. HIV is the most well-characterized member of the lentiviridae family ('lenti' meaning 'slow'), which develops disease by profoundly affecting the function of the immune system. Entry of HIV-1 into cells is dependent on the presence of CD4 plus a chemokine coreceptor (notoriously CCR5 or CXCR4), restricting the infection to dendritic cells (DCs), T cells and macrophages. In addition to the *gag*, *pol* and *env* structural proteins expressed by oncoretroviruses, lentiviruses contain additional regulatory (*tat* and *rev*) and pathogenicity-enhancing 'accessory' genes (*vif*, *vpr*, *vpu* and *nef*).

Lentiviruses can infect nonproliferating cells due to the lentiviral pre-integration complex, which allows recognition by the cell nuclear

import machinery. Pioneer work describing the capability of engineered replication-deficient lentiviral vectors (LVs) in permanently infecting nondividing cells, such as macrophages and neurons, dates from over a decade ago [1]. Since then, the development of a wide variety of LVs has boomed, including simian immunodeficiency virus (SIV), feline immunodeficiency virus and equine infectious anemia virus. HIV-based lentiviruses comprise the fastest progressing LV platform and have already progressed to gene therapy clinical trials.

The most currently employed HIV-derived lentiviral packaging system used for research and for clinical development has been named 'third-generation self-inactivating LV'. The features attributed to this designation are: first, *Tat* and the four accessory genes of HIV were deleted from the viral packaging system, consisting of four plasmids used to transfect the 293T packaging cell line (FIGURE 1) [2]; second, inclusion of a 400-nucleotide deletion in the 3' long terminal repeat (LTR), which is copied to the 5' LTR upon reverse transcription, thereby abolishing the 5' LTR promoter activity and reducing the risk of vector mobilization with the wild-type virus [3]. Since the original lentivirus envelope protein (glycoprotein [gp]120) restricts the host range, is unstable and makes production of LVs more complex, vectors are usually pseudotyped

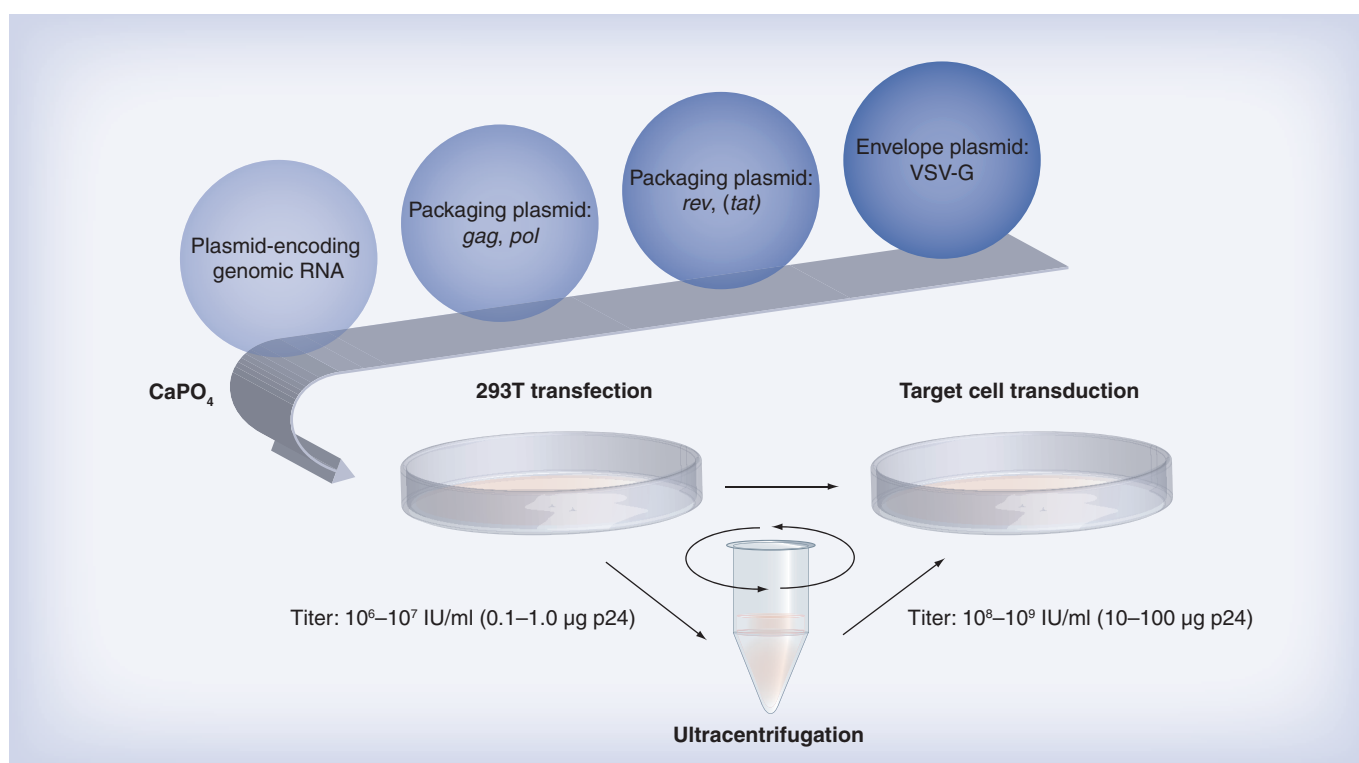


Figure 1. Laboratory production of commonly employed third-generation lentiviral vectors.
VSV-G: Vesicular stomatitis virus G.

(i.e., coated with a heterologous envelope protein) with vesicular stomatitis virus glycoprotein (VSV-G). VSV-G is a highly stable protein that is reported to bind to cell surface phospholipids rather than a specific cellular protein receptor, thereby achieving a wide host range.

The production protocols of LVs can vary widely in terms of the plasmids used for expression of the lentiviral genomic RNA and viral structural proteins, the transfection methods (using calcium phosphate or liposomes), the cell culture system (adherent or in suspension) and the concentration methods (ultracentrifugation or chromatography). Nevertheless, the 'life cycle' of LVs is quite uniform: it starts from a DNA plasmid containing the lentiviral backbone (and within a promoter and gene of interest) (FIGURE 2). The plasmid is transfected into packaging cells, which in turn is transcribed into a genomic lentiviral RNA, which is encapsidated in the virus particle (the other structural proteins are provided by three other plasmids used in the transfection). The virus containing the viral genomic RNA infects the target cell, and the genomic RNA becomes reverse-transcribed and integrates in the genome. In this process, the upstream 5' LTR loses its transcriptional function and therefore the gene of interest is transcribed solely through an internal (mostly from other viruses or cell-specific) promoter.

The use of lentiviral vectors for basic and applied research has increased considerably since the year 2000, as a result of their proven efficiency and commercial availability of the packaging vectors or ready-to-use virus. Along with their intensified usage, concerns about the biological risk of these novel vectors in generation

of replication-competent lentivirus became an issue in several institutions. Thus, in 2006, the Office of Biotechnology Activities at the US NIH published the guideline 'Biosafety Considerations for Research with Lentiviral Vectors' [101]. According to these guidelines, biosafety level 2 (BL2) containment is generally considered appropriate in research laboratories employing 'advanced' LVs (such as the third-generation pseudotyped self-inactivating LV described above). In addition, the presence of oncogenes in the transfer vector or production of large quantities of concentrated LVs should be ranked at higher biosafety levels such as 'enhanced' BL2 or BL3.

The use of LVs for vaccination purposes is so far an experimental, yet relatively expanding field, progressively finding a direction towards clinical implementation. The pioneer publications describing the feasibility of LVs for gene delivery into DCs for vaccination were comprehensively reviewed by Dullaers and Thielemans in 2006 [4]. Methodological aspects of LV production and DC transduction at the laboratory scale were described previously by Stripecke in 2009 [5]. This current review is focused on the *status quo* of LVs for immunotherapy and vaccine development, particularly at the preclinical stage.

Ex vivo transduction of DCs & their precursors with LVs **Efficiency & optimization of ex vivo gene delivery**

Viral gene transfer into *ex vivo* differentiated DCs was initially reported for adenoviral vectors (AdV), which are also capable of transducing nonreplicating cells such as DCs [6]. A drawback for efficient adenoviral transduction is the need for high multiplicity of infection ([MOI] = 100-1000), which can yield cytopathic and

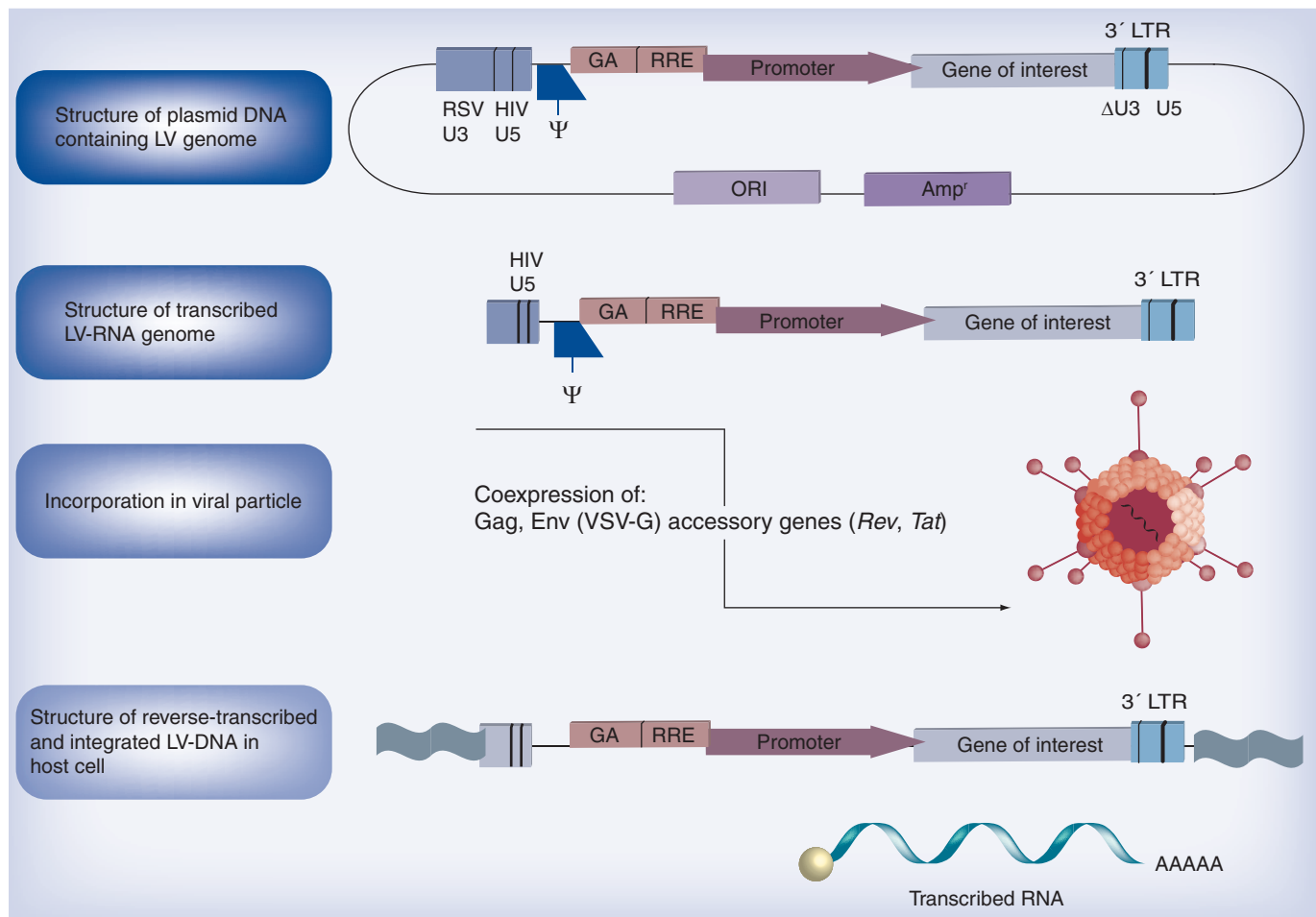


Figure 2. Lentiviral vector life cycle.

GA: Truncated GAG; LTR: Long terminal repeat; LV: Lentiviral vector; ORI: Origin of replication; RRE: Rev responsive element; RSV: Rous sarcoma virus; VSV-G: Vesicular stomatitis virus G.

cytotoxic effects to DCs, along with off-target immune responses (i.e., against adenoviral proteins). Thus, the commonly used AdV are themselves highly immunogenic in humans, which may hamper immune responses to weaker 'self' tumor antigens [7]. By contrast, lentiviral vectors offer persistent, nontoxic and nonimmunogenic gene delivery into DCs. The effectiveness of different viral vectors (AdV, LV, mouse mammary leukemia virus and equine anemia virus) on DC phenotype and function was characterized by Tan *et al.* [8]. AdV and LV provided the highest transduction and expression of a reporter gene (green fluorescent protein [GFP]).

Work by several groups has demonstrated that the transduction of human or mouse DCs or their precursors with LVs expressing markers such as GFP or truncated nerve growth factor receptor (tNGFR) consistently resulted in high gene delivery (10 to >90% of transduced cells expressing marker gene) (TABLE 1) [9–13]. Although viral titer determination and transduction methods varied among the groups, at a MOI of 10, more than 50% of the DC population became stably transduced. Transgene expression was persistent for several weeks [12] and did not alter the DC viability [11], immunophenotype [10], the ability to differentiate into mature DCs [10] or the capability of stimulating autologous T-cell responses [9,11].

DCs transduced with LVs for cancer immunotherapy

Data accumulated from the past few years have demonstrated that LV-transduced DCs for expression of tumor-associated antigens (TAAs) effectively activate cytotoxic T cells (CTLs), and in some instances, Th1 responses were also documented (TABLE 2). Melanoma-associated antigens, which are well characterized in terms of effectiveness and safety profile, are highly represented in most of the lentiviral gene transfer studies to date, such as tyrosinase-related protein 2 (TRP-2) [14–16], MAGE-3 [11,17], melanoma antigen recognized by T cells (MART-1) [16,18] and tyrosinase [19]. DCs transduced with LV and expressing those antigens effectively induce T-cell melanoma-specific responses. Lentiviral dose used in DC transductions for effective T-cell activation was in the MOI range of 5–100, thus considerably lower than adenoviral vectors. Nevertheless, under these transduction conditions, protection against tumor challenge in tumor models was in general 100% and, in some cases, therapeutic efficacy against pre-implanted tumors was also observed [14,20].

Therefore, development of lentivirally transduced DCs for melanoma immunotherapy has in recent years been highly pursued in different laboratories worldwide and, despite bio-safety issues described in more detail later, is proving to be a

Table 1. Overview of lentiviral dose and transduction efficiency of dendritic cells.

Gene	Cell target	Lentiviral vector dose	Transduction efficiency (%)	Ref.
<i>GFP</i>	Human DCs	MOI of 5	20–50	[9]
<i>tNGFR</i>	Human DCs	MOI of 15	80	[17]
<i>GFP</i>	Human DCs	MOI of 30–100	100	[19]
<i>GFP</i> and <i>tNGFR</i>	Human and mouse DCs	MOI of 130	81	[11]
<i>GFP</i>	Human monocytes	5 µg p24 equivalent/ml	55–83	[12]
<i>GFP</i>	Human DCs	1 × 10 ⁵ to 2 × 10 ⁸ IU/ml	30–60	[37]
<i>GFP</i>	Mouse BM cells	5 µg p24 equivalent/ml	>90	[34]
<i>GFP</i>	Human PBMC	MOI of 5–50	10–27	[13]

BM: Bone marrow; DC: Dendritic cell; GFP: Green fluorescent protein; MOI: Multiplicity of infection; PBMC: Peripheral blood mononuclear cell.

reliable system (TABLE 2). In this context, Dullaers *et al.* have compared a fairly established method of mRNA electroporation, which theoretically bypasses the safety issues related with viral gene delivery, with LV transduction [11]. This study showed that lentivirus-transduced DCs were superior in both obtaining high levels of transgene expression (fourfold higher) and capacity of the transduced cells at stimulating T-cell responses such as secretion of the Th1 cytokine IL-12 (tenfold higher). *In vivo*, LV-transduced DCs induced a higher CTL response in comparison with mRNA transfection.

Preclinical evaluation of LV-transduced DC vaccines against other tumor types, such as hepatoma (employing antigens such as Sca-2, GP38 and RABP1), have been successfully described [20]. Mossoba *et al.* have explored the use of DCs transduced for expressing the antigen erbB2tr, a HER-2/neu homolog, for immunotherapy of breast and prostate cancer [21]. In this study, even low doses of DC (2 × 10³ cells) demonstrated a considerable degree of anti-tumor protection, whereas higher doses (2 × 10⁵ cells) provided complete long-term protection with a prime–boost strategy.

DCs transduced with LVs for immunization against infectious diseases

In recent years, several groups have explored lentivirally transduced DCs for prophylactic or therapeutic vaccination against chronic or incurable infectious diseases such as HIV [9], influenza (flu) [22], lymphocytic choriomeningitis virus (LCMV) [23,24], SIV [25] and hepatitis C virus (TABLE 3) [26]. For these studies, the range of LV dose used for transduction varied between an MOI of 1 and an MOI of 50. Although effective CTL responses have been observed for all these model systems, data regarding protection against the actual pathogen challenge remains scarce, reflecting the fact that surrogate mouse models are mostly used, and pointing to the need for other types of animal models (humanized mice, or lower or higher primates) that enable challenge with the cognate viral pathogen.

Expression of immunomodulatory factors to enhance DCs for immunization

In addition to the conventional DC transduction for gene delivery of antigenic proteins into DCs by LVs, some reports have demonstrated delivery of immunomodulatory genes enhancing DC differentiation or maturation. A one-hit lentiviral transduction approach for codelivery of the factors required for DC differentiation plus tumor antigen was developed for human and mouse systems [12,16]. Coexpression of GM-CSF and IL-4 in monocytes and mouse bone marrow cells was sufficient to induce their self-differentiation into self-maintaining and long-lived (>3 weeks) DCs [12,16]. Lentivirus-induced DCs displayed efficient antigen-specific, MHC class I-restricted stimulation of CD8⁺ T cells, and resulted

in 100% therapeutic efficacy in a melanoma mouse model [16].

Lentiviral expression of CD40L in *ex vivo*-grown DCs resulted in potent DC maturation phenotype and function [10], whereas expression of gp34/OX40L promoted increased allogeneic CD4⁺ T-cell responses (TABLE 4) [27].

Chemokines and cytokines that enhance T-cell function/survival (such as IL-15 and IL-7) and that potentiate DC maturation (such as IFN-α and IFN-γ) are some of the immunomodulatory molecules warranted for future rational programming of DCs.

In vivo direct administration of LVs

Biodistribution of LV injected into mice via different routes

Lentiviral vectors have recently emerged as powerful tools for efficient and long-term gene delivery *in vivo*, either through local or systemic administration. This is largely due to the fact that even quiescent cells (which comprise the majority of the cells in the tissues of the adult body) are susceptible to LV infection and permanent integration. Thus, direct administration of LVs driving expression of marking genes from different types of promoters has been repeatedly documented (TABLE 5). Biodistribution studies of LVs administered intravenously into immunodeficient mice have shown that VSV-G-pseudotyped LV resulted in remarkable and persistent *GFP* gene transfer into the liver and spleen [28–30] and also in some cases the bone marrow [30]. An important achievement for the effective *in vivo* gene delivery was the inclusion of the central polypurine–polypurimidine tract sequence (cPPT–CTS), which significantly increased virus infectivity. The use of cPPT–CTS-containing vectors has consistently demonstrated that injections of 10⁸–10⁹ infective particles in the tail vein produce high levels of cells transduced in the spleen (12%) and liver (8%) [29]. For the liver, hepatocytes and Kupffer cells were the most frequently transduced cell types, whereas for the spleen, DCs and B cells were frequently transduced [29]. *GFP* expression could be observed for several weeks in the organs of immunodeficient mice with no signs of toxicity.

Subsequent studies have explored the use of immune-competent animals for analyses of the LV biodistribution, albeit with the obvious caveat that the most often used marking gene, *GFP*, is very immunogenic in mice, and thus GFP⁺ cells are efficiently immune rejected [31]. Despite this limitation, intravenous LV administration regularly yielded between 0.3 and 13% of GFP-expressing antigen-presenting cells (APCs) of the spleen (defined by coexpression of CD11c and/or MHCII) within the first week (TABLE 5) [32–35]. Notably, studies employing firefly luciferase as gene reporter and noninvasive optical imaging analyses demonstrated persistency of expression for several months [34].

A remarkable observation was that LVs pseudotyped with VSV-G demonstrated a natural tropism to infect MHCII⁺ cells, although this viral envelope is of broad tropism [34]. Since the tropism is not absolute, targeting the transgene expression specifically to DCs was further explored by the use of DC-specific promoters, such as MHCII (HLA-DR) [34] or Dectin-2 [35]. Kimura *et al.* explored LV containing the MHCII promoter and followed the biodistribution *in vivo* using both GFP (for flow cytometry) and luciferase (for optical imaging analyses) [34]. The study compared the MHCII promoter with the cytomegalovirus (CMV) potent ubiquitous promoter. The CMV promoter led to short-term expression of luciferase in the MHCII⁺ spleen cells of immunocompetent mice (1 month) in comparison with the MHCII promoter (>3 months). Notably, when the melanoma antigen TRP2 was expressed downstream of the CMV promoter, CTL and antimelanoma protective responses were evident and potent. Unexpectedly, the vector expressing TRP2 downstream of the MHCII promoter failed to immunize mice [34].

Intriguingly, systemic delivery of LVs was capable of persistent gene expression for long periods (>2 months) in APCs *in vivo*, despite the short lifespan of DCs (1–2 weeks). Thus, the cell turnover allowing long-term expression of the LV transgene in DCs was investigated by Arce *et al.* [36]. Upon systemic LV delivery, an increase in marking gene was observed in myeloid DC (mDC) and plasmacytoid DC (pDC) populations after 30 days in the spleen. Bromodeoxyuridine incorporation assays demonstrated that splenic mDC and pDC precursors with proliferation capabilities provided the long-term antigen expression in LV-immunized mice.

Although systemic LV immunization could lead to more potent vaccination effects, this approach is less practical than the clinical biosafety perspective due to the lack of clinical track records

Table 2. Lentiviral vector-mediated gene delivery of tumor-associated antigens to dendritic cells and immune effects.

Tumor/antigen	Cell target	Lentiviral vector dose or concentration	Effectors	Protection/therapy	Ref.
Melanoma/MAGE-3 (li80)	Human and mouse DCs	MOI of 130	CTL	NA	[11]
Melanoma/human tyrosinase	Human DCs	MOI of 30–100	CTL	NA	[19]
Melanoma/MAGE-3 (li-MAGE-3)	Human DCs	MOI of 15	CTL	NA	[17]
Melanoma/MART-1	Human DCs	MOI of 30	CTL	NA	[18]
Melanoma/Mouse TRP-2	Mouse DCs	MOI of 10	NA	Therapeutic effect against B16 melanoma (57%)	[14]
Melanoma/Mouse TRP-2	Mouse DCs	MOI of 10	T-cell proliferation	Protection against B16 melanoma (100%)	[15]
Melanoma/mouse TRP-2, human MART-1	Mouse BM cells	5 µg p24 equivalent/ml	CTL	Protection and therapeutic effect against B16 melanoma (100%)	[34]
Hepatoma/Sca2, GP38 and RABPI	Mouse DCs	MOI of 20–40	CTL	Therapeutic effect against 1MEA7R hepatoma cells (prolonged survival)	[20]
Prostrate/kinase-deficient form of mouse erbB2	Mouse DCs	5 × 10 ⁶ to 3.6 × 10 ⁸ IU	Humoral, CTL	Protection against RM-1-erbB2 cell line (100%)	[21]

CTL: Cytotoxic T lymphocyte; BM: Bone marrow; DC: Dendritic cell; MOI: Multiplicity of infection; NA: Not available.

using integrating viruses as vaccines. Therefore, some groups have also explored subcutaneous LV administration, resulting in the observation that approximately 1% of the CD11c⁺ cells homing in the adjacent lymph nodes were transduced [35,37,38].

Proof-of-concept studies regarding the immunostimulatory activity of LVs expressing the ovalbumin model antigen

During the initial period of testing administration of LVs as vaccines, several groups have employed ovalbumin (OVA) as a model antigen (TABLE 6). From the perspective of setting up basic immunology studies, there are several practical reasons for using OVA: first, a MHC class I-binding immunodominant epitope within OVA capable of eliciting HLA-A2.1 restricted CTLs has been largely used as a read-out of antigen-specific responses in HLA-A2.1 transgenic mice [39]; second, the functional ability of the CTL response can be assessed by rejection of syngeneic tumor cells

Table 3. Lentiviral vector-mediated gene delivery of viral antigens to dendritic cells and immune effects.

Antigen	Cell target	Lentiviral vector dose or concentration	Effectors	Protection/therapy	Ref.
Envelope depleted HIV	Human DCs	MOI of 5–50	CTL	NA	[9]
Influenza matrix protein-derived peptide (fused with CD8 α leader sequence)	Human DCs	MOI of 2–20	CTL	NA	[22]
LCMV gp (33–41)	Mouse DCs	MOI of 1–10	CTL	NA	[23]
Fusion protein: ubiquitin + GFP + LCMV epitope	Mouse DCs	MOI of 5	CTL	Protection against peripheral LCMV infection (100%)	[24]
SIV Gag	Human and simian DCs	2 \times 10 ⁶ normalized RT units per 1 \times 10 ⁵ DCs	CTL	NA	[48]
HCV structural and nonstructural clusters	Human DCs and monocytes	5 μ g p24 equivalent/ml	CTL	NA	[26]

CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; GFP: Green fluorescent protein; gp: Glycoprotein; HCV: Hepatitis C virus; LCMV: Lymphocytic choriomeningitis virus; MOI: Multiplicity of infection; NA: Not available; RT: Reverse transcriptase; SIV: Simian immunodeficiency virus;

expressing OVA (such as EL-4 cells grown in C57BL/6 mice) [40]; third, transgenic mice expressing T-cell receptors reactive against MHC class I and class II OVA epitopes (OT-I and OT-II) are conveniently available. With these tools, several parameters of immunization, such as vaccine strength and requirement of CD8⁺ and CD4⁺ T-cell responses, can be readily compared among different laboratories. In a study by Dullaers *et al.*, OVA was fused with a domain of the invariant chain in order to target OVA towards the MHC class II presentation pathway [41]. The approach of using DCs transduced *ex vivo* with LVs was compared side-by-side

Table 4. Lentiviral vector-mediated gene delivery of immunomodulatory factors to dendritic cells and immune effects.

Gene	Cell target	LV dose	Effectors	Protection/therapy	Ref.
<i>GM-CSF, IL-4, FLT3L</i>	Human monocytes	5–10 μ g p24 equivalent/ml	CTL	NA	[12]
<i>GM-CSF, IL-4</i>	Mouse BM cells	5–10 μ g p24 equivalent/ml	CTL	Protection and therapeutic effect against B16 melanoma (100%)	[16]
<i>CD40L</i>	Human DCs	MOI of 50	CTL	NA	[10]
<i>gp34/OX40L</i>	Human DCs	MOI of 10–50	Helper T cells	NA	[27]

BM: Bone marrow; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; LV: Lentiviral vector; MOI: Multiplicity of infection; NA: Not available.

with direct subcutaneous LV administration *in vivo*. The latter approach proved to be superior both in terms of eliciting a stronger CTL response for a longer period of time (30 days) and in inducing memory CTL responses. Both *ex vivo* and *in vivo* approaches required CD4⁺ T-cell help. In a therapeutic setting, direct administration of LVs was more effective than LV-transduced DCs at prolonging survival of the mice. Similarly, Rowe *et al.* have studied the importance of CD4⁺ T-cell responses in eliciting an effective immune response by using different fusion forms of OVA capable of targeting the MHC class II presentation pathway [33]. These studies confirmed that a domain of the invariant chain fused to OVA provided the highest CD4⁺ T-cell response and resulted in a higher effector T-cell response. In an elegant study, cutaneous delivery of LVs expressing OVA showed that skin DCs comprising of Langerhans cells and dermal DCs of CD8^{-low}Dec2⁺ phenotype were the predominant *in vivo*-transduced APCs, which could migrate to the lymph nodes for induction of potent and durable CTL responses [42].

Ovalbumin is nevertheless a xen-antigen that stimulates very potent immune responses in immunocompetent mice, unlike weaker (self) tumor antigens. Therefore, notwithstanding the validity of these enthusiastic proof-of-concept studies targeting the class II antigen presentation, their relevance in preclinical anti-tumor or antiviral immunizations remains to be confirmed.

LV vaccines for cancer immunotherapy

Owing to the vast availability of wellcharacterized melanoma-associated antigens, preclinical models testing LV vaccines against melanoma are vastly overrepresented. *In vivo* tumor studies using the B16 cell line for implanting local or metastatic melanoma into immunocompetent C57BL/6 mice models is a reproducible preclinical animal model explored by various laboratories. Chapatte *et al.* have compared peptide vaccination versus LV vaccination for the generation of anti-MART-1 responses, in which they concluded that LV vaccination was far more efficient in generating a CTL response and also a robust memory response, leading to long-term protection [43]. Intravenous or subcutaneous LV administration has consistently resulted in potent CTL responses specific against several antigens such as MART-1 [43,44], the testis antigen NY-ESO-1 [18,32] and TRP-2

[34,37]. Immunization of mice with LV expressing TRP-2, an anti-apoptotic self-antigen expressed in different tumors, resulted in protection and therapeutic efficacy against melanoma, glioma and breast cancer development (TABLE 7) [34,37]. Recently, in a study by Liu *et al.*, lentiviral expression of a mutated form of TRP-1 elicited a CTL response against wild-type TRP-1, resulting in effective protective and therapeutic antimelanoma immunity [45].

LV vaccines against infectious diseases: HIV

It is noticeable that several efforts for the use of LV vaccines against infectious diseases are converging towards treatment or protection against HIV (TABLE 8). The explanation from the biosafety perspective is logical: if an individual already has HIV or is highly exposed to HIV, an HIV-derived vector (vaccine) is regarded as an 'attenuated' nonreplicating virus. Therefore, not surprisingly, the first clinical trial performed with LV worldwide was applied to HIV gene therapy, exploring antisense technology to knock out HIV in CD4⁺ T cells [46]. A recent interesting review covering LVs as therapeutics against HIV was recently published by Lemiale and Korokhov [47]. The expanding interaction between the academic and private sectors is prompting the development of LV vaccines against HIV as one of the fronts for large-scale clinical trials. Concurrently, preclinical data led to optimism: several routes of LV administration have been explored in mice, leading to consistent and persistent anti-HIV/SIV immune responses [48,49].

Other recombinant viruses explored clinically in comparison

Adenovirus

Accumulated published or communicated preclinical and clinical experiences have reported expression of tumor antigens into DCs mediated by adenoviral vectors for cancer immunotherapy. Adenoviral modification of DC can safely induce T-cell responses, but few clinical responses have been achieved so far [50].

In the infectious diseases field, several vaccination trials using recombinant viral vaccines have been initiated for protection against HIV. One large trial called STEP explored three adenoviral vectors that individually expressed gag, pol or

nef protein [51,52]. The trial was suspended before completion when an interim analysis indicated that vaccination increased the risk of infection in subjects with prior immunity against Ad5 vector. The reason for the increased susceptibility still remains unknown and no apparent benefits in terms of preventing and reducing HIV infection were observed. Unfortunately, the STEP

Table 5. Administration of lentiviral vectors *in vivo* and gene delivery efficacy.

Gene/promoter	LV dose	Injection route and mouse strain	Tissues/cells transduced, persistency and percentage	Ref.
<i>GFP/CMV</i>	1 × 10 ⁹ IU	Portal vein Nude	Liver, mostly non-hepatocytes and Kupffer cells (day 21: 26%), Hepatocytes (day 21: 5.3%)	[28]
<i>GFP/CMV</i>	1 × 10 ⁹ IU	iv. SCID, BALB/c	SCID: Liver hepatocytes and nonparenchymal cells (day 7: 8% ± 6%); Spleen 20%: mostly DCs, MHCII ⁺ /CD11c ⁺ (day 7: 20%) BALB/c: Spleen CD11c ⁺ (day 4: 12%)	[29]
<i>GFP/CMV</i>	0.7–1 × 10 ⁹ IU (7–10 µg p24 equivalents)	iv. SCID, C57BL/6	SCID: Spleen (day 7: 30%) C57BL/6: Spleen (day 7: 0.3%)	[30]
<i>GFP/SFFV</i>	5 × 10 ⁷ IU	iv. C57BL/6HLA-A2 transgenic	Spleen: CD11c ⁺ (day 9: 0.3–0.4%)	[32]
<i>GFP/Ubq C</i>	1 × 10 ⁷ IU	sc. C57BL/6	Lymph node: CD11c ⁺ (day 3: <1%)	[37]
<i>GFP/SFFV</i>	1–3 × 10 ⁸ IU	iv. C57BL/6	Spleen: mDCs 1.2%, pDCs 1.3%, B cells 4.1% (day 5)	[33]
<i>GFP/CMV, MHCII</i>	1 × 10 ⁸ IU (2 µg p24 equivalents)	iv. C57BL/6	Spleen: CMV: MHCII ⁺ (day 7: 0.8%) MHCII: MHCII ⁺ (day 7: 0.4%)	[34]
<i>LUC/CMV, MHCII</i>	1 × 10 ⁸ IU (2 µg p24 equivalents)	iv. C57BL/6	Spleen, liver: up to 3 months	
<i>GFP/Dectin-2, SFFV</i>	3 × 10 ⁸ IU	sc., iv. C57BL/6	sc.: Lymph node: SFFV: CD11c ⁺ (1%) Dectin-2: CD11c ⁺ (0.27%) (day 5) iv.: Spleen: SFFV: CD11c ⁺ (13.3%) Dectin-2: CD11c ⁺ cells (4.7%) (day 10)	[35]
<i>GFP/Ubq C</i>	5 × 10 ⁷ TU	sc. C57BL/6	Lymph node: CD11c ⁺ cells (day 3: 1.9%)	[38]
<i>GFP/Ubq C</i>	1 × 10 ⁸ IU	sc., iv. C57BL/6	sc.: Lymph nodes: MHCII ^{hi} CD11c ^{hi} (day 5: 2.3%) iv.: Spleen: MHCII ^{hi} CD11c ^{hi} (4%), CD4 ⁺ (3.5%), CD8 ⁺ (1.8%) (day 30)	[36]

CMV: Cytomegalovirus; DC: Dendritic cell; GFP: Green fluorescent protein; iv.: Intravenous; LV: Lentiviral vector; MOI: Multiplicity of infection; NA: Not available; sc.: Subcutaneous; SCID: Severe combined immunodeficient; SFFV: Spleen focus-forming virus.

Table 6. *In vivo* immunization with lentiviral vectors expressing ovalbumin as a model antigen.

Antigen (promoter)	LV dose	Injection route and mouse strain	Effector cells	Protective and therapeutic effects	Ref.
OVA fused with invariant chain (CMV)	1 × 10 ⁷ 293T TU	sc. footpad C57BL/6 OT-II transgenic	CTL Memory CTL	Therapeutic immunity p < 0.001	[41]
OVA-cyt/li/Tfr: cytoplasmic or fused to invariant chain or transferring protein respectively (SFFV)	1 × 10 ⁷ IU	iv. C57BL/6 OT-II transgenic	CTL (OVA-li > OVA, OVA-Tfr, OVAcyt) CD4 ⁺ T-cell response	Protective immunity (OVA-li p = 0.00 > OVA p = 0.0023)	[33]
OVA (CMV)	1 × 10 ⁶ TU	sc. footpad C57BL/6 OT-I transgenic	CTL	NA	[42]
OVA I vs NI vector (Ubq C)	900 ng p24	sc. footpad C57BL/6 OT-I and OT-II transgenic	CTL (I > NI) antigen-specific serum Ig	Protective immunity	[71]
OVA-li/MKK6/v-FLIP I vs NI vector (SFFV)	150 ng of RT	sc. footpad C57BL/6 OT-I transgenic	CTL (I > NI)	Therapeutic immunity	[70]
OVA-li fused to invariant chain (SFFV)	1 × 10 ⁸ IU	iv. or sc. C57BL/6 OT-I transgenic	CTL	NA	[36]

CMV: Cytomegalovirus; CTL: Cytotoxic T lymphocyte; I: Intergrating; iv.: Intravenous; NA: Not available; NI: Nonintegrating; OT: Ovalbumin T-cell receptor; OVA: Ovalbumin; LV: Lentiviral vector; RT: Reverse transcriptase; sc.: Subcutaneous; SFFV: Spleen focus-forming virus; TU: Transduction units; Ubq C: Ubiquitin C; v-FLIP: Viral FADD-like IL-1 β -converting enzyme inhibitory protein.

vaccination study was a setback and led to severe criticism of current approaches in the design of effective vaccines against HIV [53,54].

Poxvirus

Poxviruses were the first recombinant viral vectors used for vaccination by Edward Jenner as early as the 1790s (reviewed by Pastoret [55]) and remain one of the hallmark success stories of immunology for the eradication of smallpox disease. Since then, they have been extensively characterized and used in several clinical trials.

Poxviruses have also been used for generating an immune response in a cancer immunotherapy setting. Pre-existing immunity against poxviruses in humans is an issue that needs to be addressed to improve the performance of these vectors in the clinic [56,57]. In order to overcome this setback, avipoxviruses, which are known for their replication incompetence in humans, are being considered. They have been used for encoding several types of cancer antigens, such as carcinoembryonic antigen (CEA) and mucin (MUC)-1 [58–60] (which are overexpressed

in colorectal and gastric carcinomas). Vaccinia and Fowlpox viruses expressing CEA and MUC-1 in a prime–boost vaccination study showed antigen-specific T-cell responses (five out of eight patients) [59]. Poxviral delivery of CEA, MUC-1 and TRICOM (a set of costimulatory molecules: B7.1, ICAM-1 and lymphocyte function-associated antigen-3) led to enhanced levels of antigen-specific IFN- γ secretion [60].

Recombinant Vaccinia virus expressing prostate-specific antigen (PSA) showed stabilized levels of PSA in 42% of the patients studied [61]. Prime vaccination with Vaccinia virus expressing PSA and B7.1 followed by boost vaccination with Fowlpox virus expressing PSA has shown elevated levels of PSA-specific T cells [62].

A melanoma immunotherapy study using Vaccinia and Fowlpox virus expressing tyrosinase demonstrated that 13% of patients achieved T-cell response to individual tyrosinase peptides, whereas 25% showed T-cell responses to full-length tyrosinase protein. Nevertheless, the vaccine failed to produce any long-term clinical benefit [63].

Overall, these studies demonstrate that although poxviruses are considered to be relatively safe for clinical use, pre-existing viral immunity and possible deleterious effects of poxviruses on DCs, such as inhibition of migration and interference with the chemokine receptor switch [64], could

present a hindrance to developing vaccines.

Since the majority of the human population is not infected by HIV, it is anticipated that pre-existing immunity against HIV-derived vectors would not act as a limiting factor on vaccine development. In addition, as several groups have demonstrated, DCs transduced with LV vectors did not display abnormal migration to lymphatic organs. Thus, these considerations may be of relevance for LV as a potential superior platform for novel vaccines.

Expert commentary

Considering the risk-versus-benefit ratio, one could speculate that DCs transduced with LV *ex vivo* are likely to reach the clinical stage of development sooner than direct LV administration, since there is better control for the APCs and not other cells to become genetically modified. Unlike hematopoietic stem cells presenting with extensive self-renewal capacity, monocytes and DCs are differentiated cells, and therefore their risk of developing malignancies is low. Of note, gene therapy with LV-modified hematopoietic stem cells has already had some prime-time in the clinic in a

Phase I trial for adrenoleukodystrophy [65], with no biosafety concerns so far. Thus, the development of clinical LV-modified DC vaccines will soon follow.

Unlike the *ex vivo* approach, the implications of human *in vivo* vaccination with LVs are not known, as these vectors have not yet been sufficiently explored in clinical trials. Concurrent novel developments in LV design are ongoing in order to address a critical milestone for the clinical translation of LV vaccines: safety. Some of the recent technological innovations that will impact the LV vaccine field are described in the following sections.

Development of integration-deficient LV

A high priority for recombinant vectors in the vaccination field is to exclude insertional mutagenesis in order to minimize malignancies, an issue previously observed in *ex vivo* gene therapy clinical trials using retroviral vectors [66]. A straightforward approach to avoid genotoxicity is to minimize integration of genetic vectors into the genome. Mutations that disable the integrase protein in the lentivirus particle are able to prevent integration of the provirus reverse-transcribed DNA [67] (for an excellent review on this topic, see [68]). Nonintegrated lentiviral DNA can support transcription, but the episomal viral DNA eventually gets lost during cell replication, leading to continuous transgene expression decline in dividing cells. By contrast, for growth-arrested cells, unintegrated HIV-1 vectors can produce steady high-level transgene expression [67]. Thus, this system substantially reduces the risk of insertional mutagenesis and would be highly relevant for therapeutic applications of genetically modified DCs, which are terminally differentiated, nondividing cells. Recent studies have shown that integration-deficient (ID)-LV can also mediate stable transduction and sustained transgene expression *in vivo* in rodent ocular and brain tissues [69]. Two recent reports have used ID-LV as a vaccine, with OVA as the model antigen. Karwacz *et al.* have reported that both LV and ID-LV were capable of transgene expression for up to 30 days and were also capable of generating a strong CTL response [70]. Higher doses of ID-LV were used in comparison to the LV counterparts (250 ng RT ID-LV, versus 10 ng RT ILV, thus almost 25-fold higher). LVs generated a partial therapeutic response, whereas only ID-LV were capable of prolonging survival. This limitation could be overcome by the use of DC activators like mitogen-activated protein kinase kinase 6/ viral FADD-like IL-1 β -converting enzyme inhibitory protein. A humoral response in the context of a hepatitis

Table 7. Administration of lentiviral vectors *in vivo* for immunization against melanoma.

Antigen (promoter)	LV dose	Injection route and mouse strain	Effector cells	Protective and therapeutic effects	Ref.
Human HLA-Cw3, MART-1 (CMV)	2.5 × 10 ⁷ EFU	sc. C57BL/6 B6D2F1 (for Cw3)HLA-A2 transgenic (for MART-1)	Cw3: CTL Melan A: CTL	NA	[44]
NY ESO 1 (CMV) Vaccinia virus boost	5 × 10 ⁵ , 5 × 10 ⁶ , 5 × 10 ⁷ IU	iv. C57BL/6 HLA-A2 transgenic	CTL with boost (all doses) Without boost: no response	NA	[32]
TRP-2 hsp70 (Ubq c)	1.6 × 10 ⁷ PFU	sc. C57BL/6, rat Her-2/neu transgenic BALB/c-neu transgenic	CTL	Therapeutic immunity: prolonged survival (B16, GL-26, BALB/c neu transgenic)	[37]
TRP-2 (CMV vs MHCII)	1 × 10 ⁸ IU (2 μg p24 equivalent)	iv. C57BL/6	CMV: CTL MHCII: None	Protection: CMV: 35% MHCII: None	[34]
MART-1 (CMV)	4 × 10 ⁶ EFU	sc. C57BL/6 HLA-A2 transgenic	CTL, memory CTL	NA	[43]
NY ESO 1 (Dectin-2) Vaccinia virus boost	1 × 10 ⁶ , 1 × 10 ⁷ , 1 × 10 ⁸ IU	iv. C57BL/6	CTL, epitope-specific CD4 ⁺ T cell	NA	[35]
wtTRP1 and mutated TRP-1 (CMV)	2.5 × 10 ⁷ TU	sc. C57BL/6	CTL (μTRP1 > wtTRP1)	Protective and therapeutic immunity (p < 0.01)	[45]

CMV: Cytomegalovirus; CTL: Cytotoxic T lymphocyte; EFU: Expression-forming unit; iv.: Intravenous; LV: Lentiviral vector; MART: Melanoma antigen recognized by T cells; NA: Not available; PFU: Plaque-forming unit; sc.: Subcutaneous; TRP: Tyrosinase-related protein; TU: Transduction unit; Ubq C: Ubiquitin C.

B virus viral antigen was also reported. Hu *et al.* have demonstrated the use of ID-LVs in generating antigen-specific CTL response and protective immunity [71].

Restricting LV entry by modified envelopes

Despite the natural tropism of VSV-G-pseudotyped LV to infect proliferating DC precursors *in vivo* resulting in a potent and persistent immunization [34,36], attempts to engineer the VSV-G glycoprotein in order to target the virus to a specific receptor on the cell surface have not so far been successful. In a recent report, Yang and collaborators took advantage of the natural tropism of Sindbis virus towards DCs [38]. They inserted a modification in the envelope that abrogated binding to heparin sulphate, restricting binding to DC-specific intercellular adhesion molecule-3-grabbing non-integrin (SIGN). *In vivo* testing of LV pseudotyped with the mutated Sindbis envelope showed more specific gene delivery to the

Table 8. Administration of lentiviral vectors *in vivo* for immunization against viral pathogens.

Antigen (promoter)	LV dose	Injection route and mouse strain	Immune response	Ref.
HIV env (CMV)	1 × 10 ⁷ RT	iv. BALB/c	CTL, humoral IgG	[48]
SIV gag (CMV)	1 × 10 ⁷ RT	im. BALB/c	CTL, humoral IgG	[48]
HIV polyepitopes: HLA-A2 restricted (13); HLA-A7 restricted (12) (CMV)	HLA-A2: 1 × 10 ⁷ IU HLA-A7: 2 × 10 ⁷ IU	ip. C57BL/6 Transgenic HLA-A2 HLA-B7	CTL	[49]
Hepatitis B virus surface antigen Integrating vs nonintegrating (SFFV)	75 ng of RT	im. BALB/c	CTL (I > NI) IgG response	[70]

CMV: Cytomegalovirus virus; CTL: Cytotoxic T lymphocyte; EFU: Expression-forming unit; env: Envelope protein; I: Integrating; im.: Intramuscular; ip.: Intraperitoneal; iv.: Intravenous; LV: Lentiviral vector; NI: Nonintegrating; PFU: Plaque-forming units; RT: Reverse transcriptase; SFFV: Spleen focus-forming virus; SIV: Simian immunodeficiency virus; TU: Transduction unit.

DC-SIGN⁺ CD11c⁺ DC population using GFP as a reporter both *in vitro* and *in vivo* (3.2% of total CD11c⁺ with modified envelope to 1.9% with VSV-G). These engineered viruses were also capable of producing an effective CTL response (using OVA as a model antigen) and resulted in protective and therapeutic responses.

Inclusion of a suicide gene in the 'gene cargo'

'Suicide genes' are genes that lead to the expression of catalytic proteins capable of converting a specific prodrug into a toxic product capable of inducing cell death. The herpes simplex virus thymidine kinase (HSV-TK) enzyme has high activity to catalyze the phosphorylation of the prodrug ganciclovir (GCV) into GCV triphosphate. GCV triphosphate is a nucleotide analog, which is incorporated in the genome during DNA synthesis, causing DNA chain termination and apoptosis. A clinical example of HSV-TK gene transfer to schedule the elimination of therapeutic cells is the retroviral genetic modification of T cells used in donor lymphocyte infusion to control graft-versus-host disease in allograft T-cell transplants. In addition, the intercellular transfer of GCV metabolites, from HSV-TK⁺ cells through gap junction channels to HSV-TK⁻ neighboring cells, results in the 'bystander effect'. HSV-TK-negative cells can therefore be efficiently killed by proximity. Clinical applications of this 'bystander effect' include the use of oncolytic virus-expressing TK for elimination of glioma [72] or prostate cancer [73].

Expression of multicistrons for coordinated expression of genes for DC differentiation/maturation/antigen presentation & forced self-elimination

Rational programming of DCs *in vivo* will require antigen presentation in fully differentiated and mature DCs (to avoid tolerogenic effects), along with the possibility of forced scheduled elimination

of DCs after immunization. Development of tri- and tetra-cistronic LV designs is possible if the open reading frames are interspaced by '2A' elements [PINCHA ET AL., MANUSCRIPT IN PREPARATION]. 2A elements from different viruses corresponding to 18 amino acid peptide sequences are encoded between different open reading frames, which serve as cotranslational cleavage sites between two protein products. Viruses use these 2A peptides (consensus motif 2A, Asp-Val/Ile-Glu-X-Asn-Pro-Gly; 2B, Pro) to mediate multicistronic expression and they can be 'adapted' to genetic engineering approaches [74].

Five-year view: steps for the clinical development of LV vaccines

During the next 5 years, the deeper understanding of the risk factors predisposing lentivirally modified DCs or LV vaccines to unwanted side effects will become an important topic. The main concern with the use of HIV-derived lentiviral vector system

clinically is the occurrence of replication-competent lentivirus in treated patients, which could potentially cause AIDS. As discussed previously, a Phase I gene therapy clinical trial with HIV-derived lentiviral vectors in HIV patients has been reported [46] with no side effects so far. Therefore, this and other pioneering Phase I gene therapy clinical trials with LV will be pivotal to establish the confidence that most currently available generations of lentiviral vectors are safe and do not (re)generate HIV.

The second concern is the genotoxicity of LV, a topic currently considered as highly relevant. Analyses of LV integration sites in *ex vivo*-transduced human macrophages [75] and CD4⁺ T cells [76] have recently demonstrated that lentivirus tends to integrate within active genes but, unlike retroviruses, LVs have not demonstrated a particular 'preference' of integration upstream proto-oncogenes. Genotoxicity of LV-modified DCs or LV vaccines administered directly into mice has not been reported so far, but cannot be excluded. Therefore, the development of ID-LV and inclusion of suicide genes in the transfer vector offer two independent direct safeguards, as discussed above.

Another possible threat is that long-term immune stimulation would lead to chronic inflammation. Although the mainstream immunologist would argue that this is a positive effect for immunization, in recent years it has become a paradigm that chronic infections ultimately cause an exhaustion of the immune system, a scenario enabling escape of mutant forms of the pathogens to thrive. Thus, some parameters still warrant better elucidation in mouse models, such as the long-term effects of LV antigen delivery in memory responses, host immune competence, autoimmunity and therapeutic efficacy.

Finally, affordable and pharmacologically tested good manufacturing practice grade LVs for large-scale vaccination in the next 5 years' trials is still an intangible wish. Although some companies

are starting to produce LV for experimental gene therapy trials, the costs of production are still too high for prophylactic vaccination trials. Therapeutic trials against advanced cancer or lethal infections will be first in line. Ultimately, following the example of AdV used for Phase III prophylactic vaccination against HIV, close cooperation between the public and private sectors will be required for the development of LV in large batches. Once this hallmark in translational biomedicine is achieved, there will be reason to be optimistic that Phase I trials with LV vaccines will not be too delayed.

Financial & competing interests disclosure

This work was supported by the Deutsche Forschungsgemeinschaft, Germany (DFG: Rebirth Excellence Cluster EXC 62/1 and SFB 738), Deutsche Jose Carreras Stiftung and Deutsche Krebshilfe (to Renata Stripecke). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Effective gene delivery into dendritic cells is achieved with lentiviral vectors *in vitro* and *in vivo*.
- Prophylactic and therapeutic effects are obtained with dendritic cells/lentiviral vectors (LVs) or direct LV administration in mouse tumor models.
- LVs have recently been used in gene therapy clinical trials.
- Risk factors such as the occurrence of replication-deficient LV or genotoxicity in transduced dendritic cells are unlikely but cannot be excluded.
- New vector modalities for vaccination will focus on integration-deficient LV carrying an additional suicide gene. Vector targeting through engineered envelopes or cell-specific promoters are also strategies to avoid off-target gene delivery.
- Production methods for the large-scale production of LV will have to be developed for vaccination trials.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

- Naldini L, Blomer U, Gallay P *et al*. *In vivo* gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* 272(5259), 263–267 (1996).
- Dull T, Zufferey R, Kelly M *et al*. A third-generation lentivirus vector with a conditional packaging system. *J. Virol.* 72(11), 8463–8471 (1998).
- Zufferey R, Dull T, Mandel RJ *et al*. Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery. *J. Virol.* 72(12), 9873–9880 (1998).
- Dullaers M, Thielemans K. From pathogen to medicine: HIV-1-derived lentiviral vectors as vehicles for dendritic cell based cancer immunotherapy. *J. Gene Med.* 8(1), 3–17 (2006).
- Stripecke R. Lentiviral vector-mediated genetic programming of mouse and human dendritic cells. *Methods Mol. Biol.* 506, 139–158 (2009).
- Diao J, Smythe JA, Smyth C, Rowe PB, Alexander IE. Human PBMC-derived dendritic cells transduced with an adenovirus vector induce cytotoxic T-lymphocyte responses against a vector-encoded antigen *in vitro*. *Gene Ther.* 6(5), 845–853 (1999).
- Molnar-Kimber KL, Sterman DH, Chang M *et al*. Impact of preexisting and induced humoral and cellular immune responses in an adenovirus-based gene therapy Phase I clinical trial for localized mesothelioma. *Hum. Gene Ther.* 9(14), 2121–2133 (1998).
- Tan PH, Beutelspacher SC, Xue SA *et al*. Modulation of human dendritic-cell function following transduction with viral vectors: implications for gene therapy. *Blood* 105(10), 3824–3832 (2005).
- Gruber A, Kan-Mitchell J, Kuhlen KL, Mukai T, Wong-Staal F. Dendritic cells transduced by multiply deleted HIV-1 vectors exhibit normal phenotypes and functions and elicit an HIV-specific cytotoxic T-lymphocyte response *in vitro*. *Blood* 96(4), 1327–1333 (2000).
- Koya RC, Kasahara N, Favaro PM *et al*. Potent maturation of monocyte-derived dendritic cells after CD40L lentiviral gene delivery. *J. Immunother.* 26(5), 451–460 (2003).
- Dullaers M, Breckpot K, van Meirvenne S *et al*. Side-by-side comparison of lentivirally transduced and mRNA-electroporated dendritic cells: implications for cancer immunotherapy protocols. *Mol. Ther.* 10(4), 768–779 (2004).
- Koya RC, Weber JS, Kasahara N *et al*. Making dendritic cells from the inside out: lentiviral vector-mediated gene delivery of granulocyte-macrophage colony-stimulating factor and interleukin 4 into CD14⁺ monocytes generates dendritic cells *in vitro*. *Hum. Gene Ther.* 15(8), 733–748 (2004).
- Masurier C, Boutin S, Veron P, Bernard J, Danos O, Davoust J. Enhanced lentiviral transduction of monocyte-derived dendritic cells in the presence of conditioned medium from dying monocytes. *Hum. Gene Ther.* 18(2), 161–170 (2007).
- Metharom P, Ellem KA, Schmidt C, Wei MQ. Lentiviral vector-mediated tyrosinase-related protein 2 gene transfer to dendritic cells for the therapy of melanoma. *Hum. Gene Ther.* 12(18), 2203–2213 (2001).
- Metharom P, Ellem KA, Wei MQ. Gene transfer to dendritic cells induced a protective immunity against melanoma. *Cell. Mol. Immunol.* 2(4), 281–288 (2005).
- Koya RC, Kimura T, Ribas A *et al*. Lentiviral vector-mediated autonomous differentiation of mouse bone marrow cells into immunologically potent dendritic cell vaccines. *Mol. Ther.* 15(5), 971–980 (2007).
- Breckpot K, Heirman C, De Greef C, van der Bruggen P, Thielemans K. Identification of new antigenic peptide presented by HLA-Cw7 and encoded by several MAGE genes using dendritic cells transduced with lentiviruses. *J. Immunol.* 172(4), 2232–2237 (2004).

- 18 Lopes L, Fletcher K, Ikeda Y, Collins M. Lentiviral vector expression of tumour antigens in dendritic cells as an immunotherapeutic strategy. *Cancer Immunol. Immunother.* 55(8), 1011–1016 (2006).
- 19 Lizee G, Gonzales MI, Topalian SL. Lentivirus vector-mediated expression of tumor-associated epitopes by human antigen presenting cells. *Hum. Gene Ther.* 15(4), 393–404 (2004).
- 20 Wang B, He J, Liu C, Chang LJ. An effective cancer vaccine modality: lentiviral modification of dendritic cells expressing multiple cancer-specific antigens. *Vaccine* 24(17), 3477–3489 (2006).
- 21 Mossoba ME, Walia JS, Rasaiah VI *et al.* Tumor protection following vaccination with low doses of lentivirally transduced DCs expressing the self-antigen erbB2. *Mol. Ther.* 16(3), 607–617 (2008).
- 22 Dyall J, Latouche JB, Schnell S, Sadelain M. Lentivirus-transduced human monocyte-derived dendritic cells efficiently stimulate antigen-specific cytotoxic T lymphocytes. *Blood* 97(1), 114–121 (2001).
- 23 Zarei S, Leuba F, Arrighi JF, Hauser C, Piguet V. Transduction of dendritic cells by antigen-encoding lentiviral vectors permits antigen processing and MHC class I-dependent presentation. *J. Allergy Clin. Immunol.* 109(6), 988–994 (2002).
- 24 Zarei S, Abraham S, Arrighi JF *et al.* Lentiviral transduction of dendritic cells confers protective antiviral immunity *in vivo*. *J. Virol.* 78(14), 7843–7845 (2004).
- 25 Buffa V, Negri DR, Leone P *et al.* Evaluation of a self-inactivating lentiviral vector expressing simian immunodeficiency virus gag for induction of specific immune responses *in vitro* and *in vivo*. *Viral Immunol.* 19(4), 690–701 (2006).
- 26 Jirmo AC, Koya RC, Sundarasetty BS *et al.* Monocytes transduced with lentiviral vectors expressing hepatitis C virus non-structural proteins and differentiated into dendritic cells stimulate multi-antigenic CD8⁺ T cell responses. *Vaccine* 28(4), 922–933 (2010).
- 27 Kobayashi M, Takaori-Kondo A, Fukunaga K, Miyoshi H, Uchiyama T. Lentiviral gp34/OX40L gene transfer into dendritic cells facilitates alloreactive CD4⁺ T-cell response *in vitro*. *Int. J. Hematol.* 79(4), 377–383 (2004).
- 28 Pfeifer A, Kessler T, Yang M *et al.* Transduction of liver cells by lentiviral vectors: analysis in living animals by fluorescence imaging. *Mol. Ther.* 3(3), 319–322 (2001).
- 29 Vanden Driessche T, Thorrez L, Naldini L *et al.* Lentiviral vectors containing the human immunodeficiency virus type-1 central polypurine tract can efficiently transduce nondividing hepatocytes and antigen-presenting cells *in vivo*. *Blood* 100(3), 813–822 (2002).
- 30 Follenzi A, Battaglia M, Lombardo A, Annoni A, Roncarolo MG, Naldini L. Targeting lentiviral vector expression to hepatocytes limits transgene-specific immune response and establishes long-term expression of human antihemophilic factor IX in mice. *Blood* 103(10), 3700–3709 (2004).
- 31 Stripecke R, Carmen Villacres M, Skelton D, Satake N, Halene S, Kohn D. Immune response to green fluorescent protein: implications for gene therapy. *Gene Ther.* 6(7), 1305–1312 (1999).
- 32 Palmowski MJ, Lopes L, Ikeda Y, Salio M, Cerundolo V, Collins MK. Intravenous injection of a lentiviral vector encoding NY-ESO-1 induces an effective CTL response. *J. Immunol.* 172(3), 1582–1587 (2004).
- 33 Rowe HM, Lopes L, Ikeda Y *et al.* Immunization with a lentiviral vector stimulates both CD4 and CD8 T cell responses to an ovalbumin transgene. *Mol. Ther.* 13(2), 310–319 (2006).
- 34 Kimura T, Koya RC, Anselmi L *et al.* Lentiviral vectors with CMV or MHCII promoters administered *in vivo*: immune reactivity versus persistence of expression. *Mol. Ther.* 15(7), 1390–1399 (2007).
- 35 Lopes L, Dewannieux M, Gileadi U *et al.* Immunization with a lentivector that targets tumor antigen expression to dendritic cells induces potent CD8⁺ and CD4⁺ T-cell responses. *J. Virol.* 82(1), 86–95 (2008).
- 36 Arce F, Rowe HM, Chain B, Lopes L, Collins MK. Lentiviral vectors transduce proliferating dendritic cell precursors leading to persistent antigen presentation and immunization. *Mol. Ther.* 17(9), 1643–1650 (2009).
- 37 Kim JH, Majumder N, Lin H, Watkins S, Falo LD Jr, You Z. Induction of therapeutic antitumor immunity by *in vivo* administration of a lentiviral vaccine. *Hum. Gene Ther.* 16(11), 1255–1266 (2005).
- 38 Yang L, Yang H, Rideout K *et al.* Engineered lentivector targeting of dendritic cells for *in vivo* immunization. *Nat. Biotechnol.* 26(3), 326–334 (2008).
- 39 Rotzschke O, Falk K, Stevanovic S, Jung G, Walden P, Rammensee HG. Exact prediction of a natural T cell epitope. *Eur. J. Immunol.* 21(11), 2891–2894 (1991).
- 40 Moore MW, Carbone FR, Bevan MJ. Introduction of soluble protein into the class I pathway of antigen processing and presentation. *Cell* 54(6), 777–785 (1988).
- 41 Dullaers M, van Meirvenne S, Heirman C *et al.* Induction of effective therapeutic antitumor immunity by direct *in vivo* administration of lentiviral vectors. *Gene Ther.* 13(7), 630–640 (2006).
- 42 He Y, Zhang J, Donahue C, Falo LD Jr. Skin-derived dendritic cells induce potent CD8⁺ T cell immunity in recombinant lentivector-mediated genetic immunization. *Immunity* 24(5), 643–656 (2006).
- 43 Chapatte L, Colombetti S, Cerottini JC, Levy F. Efficient induction of tumor antigen-specific CD8⁺ memory T cells by recombinant lentivectors. *Cancer Res.* 66(2), 1155–1160 (2006).
- 44 Esslinger C, Chapatte L, Finke D *et al.* *In vivo* administration of a lentiviral vaccine targets DCs and induces efficient CD8⁺ T cell responses. *J. Clin. Invest.* 111(11), 1673–1681 (2003).
- 45 Liu Y, Peng Y, Mi M *et al.* Lentivector immunization stimulates potent CD8 T cell responses against melanoma self-antigen tyrosinase-related protein 1 and generates antitumor immunity in mice. *J. Immunol.* 182(10), 5960–5969 (2009).
- 46 Levine BL, Humeau LM, Boyer J *et al.* Gene transfer in humans using a conditionally replicating lentiviral vector. *Proc. Natl Acad. Sci. USA* 103(46), 17372–17377 (2006).
- **First report on clinical use of LV.**
- 47 Lemiale F, Korokhov N. Lentiviral vectors for HIV disease prevention and treatment. *Vaccine* 27(25–26), 3443–3449 (2009).
- **Interesting review and discussion regarding use of LV for HIV therapy and prevention.**
- 48 Buffa V, Negri DR, Leone P *et al.* A single administration of lentiviral vectors expressing either full-length human immunodeficiency virus 1 (HIV-1)(HXB2) Rev/Env or codon-optimized HIV-1(JR-FL) gp120 generates durable immune responses in mice. *J. Gen. Virol.* 87(Pt 6), 1625–1634 (2006).
- 49 Iglesias MC, Mollier K, Beignon AS *et al.* Lentiviral vectors encoding HIV-1 polyepitopes induce broad CTL responses *in vivo*. *Mol. Ther.* 15(6), 1203–1210 (2007).
- 50 Ribas A. Genetically modified dendritic cells for cancer immunotherapy. *Curr. Gene Ther.* 5(6), 619–628 (2005).

- 51 Buchbinder SP, Mehrotra DV, Duerr A *et al.* Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* 372(9653), 1881–1893 (2008).
- 52 Mulligan MJ. Advances in human clinical trials of vaccines to prevent HIV/AIDS and other HIV prevention interventions. *Curr. Infect. Dis. Rep.* 11(5), 399–406 (2009).
- 53 Steinbrook R. One step forward, two steps back – will there ever be an AIDS vaccine? *N. Engl. J. Med.* 357(26), 2653–2655 (2007).
- 54 Sekaly RP. The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? *J. Exp. Med.* 205(1), 7–12 (2008).
- 55 Pastoret PP, Vanderplasschen A. Poxviruses as vaccine vectors. *Comp. Immunol. Microbiol. Infect. Dis.* 26(5–6), 343–355 (2003).
- 56 Essajee S, Kaufman HL. Poxvirus vaccines for cancer and HIV therapy. *Expert Opin. Biol. Ther.* 4(4), 575–588 (2004).
- 57 Jacobs BL, Langland JO, Kibler KV *et al.* Vaccinia virus vaccines: past, present and future. *Antiviral Res.* 84(1), 1–13 (2009).
- 58 McAneny D, Ryan CA, Beazley RM, Kaufman HL. Results of a Phase I trial of a recombinant vaccinia virus that expresses carcinoembryonic antigen in patients with advanced colorectal cancer. *Ann. Surg. Oncol.* 3(5), 495–500 (1996).
- 59 Kaufman HL, Kim-Schulze S, Manson K *et al.* Poxvirus-based vaccine therapy for patients with advanced pancreatic cancer. *J. Transl. Med.* 5, 60 (2007).
- 60 Gulley JL, Arlen PM, Tsang KY *et al.* Pilot study of vaccination with recombinant CEA-MUC-1-TRICOM poxviral-based vaccines in patients with metastatic carcinoma. *Clin. Cancer Res.* 14(10), 3060–3069 (2008).
- 61 Eder JP, Kantoff PW, Roper K *et al.* A Phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. *Clin. Cancer Res.* 6(5), 1632–1638 (2000).
- 62 Arlen PM, Gulley JL, Todd N *et al.* Antiandrogen, vaccine and combination therapy in patients with nonmetastatic hormone refractory prostate cancer. *J. Urol.* 174(2), 539–546 (2005).
- 63 Lindsey KR, Gritz L, Sherry R *et al.* Evaluation of prime/boost regimens using recombinant poxvirus/tyrosinase vaccines for the treatment of patients with metastatic melanoma. *Clin. Cancer Res.* 12(8), 2526–2537 (2006).
- 64 Humrich JY, Thumann P, Greiner S *et al.* Vaccinia virus impairs directional migration and chemokine receptor switch of human dendritic cells. *Eur. J. Immunol.* 37(4), 954–965 (2007).
- 65 Cartier N, Hacein-Bey-Abina S, Bartholomae CC *et al.* Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. *Science* 326(5954), 818–823 (2009).
- 66 Hacein-Bey-Abina S, Garrigue A, Wang GP *et al.* Insertional oncogenesis in four patients after retrovirus-mediated gene therapy of SCID-X1. *J. Clin. Invest.* 118(9), 3132–3142 (2008).
- 67 Saenz DT, Loewen N, Peretz M *et al.* Unintegrated lentivirus DNA persistence and accessibility to expression in nondividing cells: analysis with class I integrase mutants. *J. Virol.* 78(6), 2906–2920 (2004).
- 68 Wanisch K, Yanez-Munoz RJ. Integration-deficient lentiviral vectors: a slow coming of age. *Mol. Ther.* 17(8), 1316–1332 (2009).
- **Recent review covering basic science and applications of integration-deficient LV.**
- 69 Yanez-Munoz RJ, Balaggan KS, MacNeil A *et al.* Effective gene therapy with nonintegrating lentiviral vectors. *Nat. Med.* 12(3), 348–353 (2006).
- 70 Karwacz K, Mukherjee S, Apolonia L *et al.* Nonintegrating lentivector vaccines stimulate prolonged T-cell and antibody responses and are effective in tumor therapy. *J. Virol.* 83(7), 3094–3103 (2009).
- 71 Hu B, Yang H, Dai B, Tai A, Wang P. Nonintegrating lentiviral vectors can effectively deliver ovalbumin antigen for induction of antitumor immunity. *Hum. Gene Ther.* 20(12), 1652–1664 (2009).
- 72 Pulkkanen KJ, Yla-Herttuala S. Gene therapy for malignant glioma: current clinical status. *Mol. Ther.* 12(4), 585–598 (2005).
- 73 Nasu Y, Saika T, Ebara S *et al.* Suicide gene therapy with adenoviral delivery of *HSV-tK* gene for patients with local recurrence of prostate cancer after hormonal therapy. *Mol. Ther.* 15(4), 834–840 (2007).
- 74 de Felipe P. Skipping the co-expression problem: the new 2A “CHYSEL” technology. *Genet. Vaccines Ther.* 2(1), 13 (2004).
- **Informative review about multicistronic RNA containing interspacing 2A elements.**
- 75 Barr SD, Ciuffi A, Leipzig J, Shinn P, Ecker JR, Bushman FD. HIV integration site selection: targeting in macrophages and the effects of different routes of viral entry. *Mol. Ther.* 14(2), 218–225 (2006).
- 76 Wang GP, Levine BL, Binder GK *et al.* Analysis of lentiviral vector integration in HIV+ study subjects receiving autologous infusions of gene modified CD4+ T cells. *Mol. Ther.* 17(5), 844–850 (2009).

Website

- 101 Biosafety Considerations for Research with Lentiviral Vectors
http://oba.od.nih.gov/oba/rac/Guidance/LentiVirus_Containment/pdf/Lenti_Containment_Guidance.pdf

Affiliations

- Mudita Pincha, MS
 Department of Hematology, Hemostaseology, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany
 Tel.: +49 511 532 6999
 Fax: +49 511 532 6975
pincha.mudita@mh-hannover.de
- Bala Sai Sundarasetty, MS, MTech
 Department of Hematology, Hemostaseology, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany
 Tel.: +49 511 532 6999
 Fax: +49 511 532 6975
balasai.sundarasetty@mh-hannover.de
- Renata Stripecke, Prof., PhD
 Lymphatic Cell Therapy Laboratory, Department of Hematology, Hemostaseology, Oncology and Stem Cell Transplantation, Hannover Medical School, Carl-Neuberg-Strasse 1, OE6860 – HBZ, D-30625 Hannover, Germany
 Tel.: +49 511 532 6999
 Fax: +49 511 532 6975
stripecke.renata@mh-hannover.de