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# Surface Modification of Retroviral Vectors for Gene Therapy

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## 1. Introduction

If biomedicine is considered to be the use of biological or botanical agents in medicine then it is certainly the oldest kind of therapy. Since drugs, however, it is no longer the most prolifically used, at least in the developed world. Will this change, and if so will it be in the near or more distant future? It has been discussed amongst experts in the industry for some time that, despite ever-increasing funding and new high-tech methods of discovery and screening, amounts of new drugs and drug leads are declining. In part, this has opened the way for biologics. The frontrunner is of course antibody technologies, mainly monoclonals, the use of which has exploded over the last 5 years to the point where most of the major pharma players are involved or are getting involved in what has now become a multi-billion dollar industry. Antibodies have reached the point of being a well trusted and accepted form of medical product across the industry and communities from bench to bedside.

Many believe the next similar success story will be for cell therapy, probably some differentiated form of stem cells. Although it has been happening for 20 years, it is only in the past 5 years that the amount of patients being treated with cells, as a therapy, has increased into the thousands per year. The vast majority of these are autologous cell treatments, undertaken by hospitals and private clinics on a patient to patient basis using the patients' own cells to treat a wide variety of diseases and conditions. These are not officially approved medical products. However, there are several non-pharmaceutical giant companies now in clinical trials, notable amongst these are Geron in the USA, Mesoblast in Australia and ReNeuron in the UK, although there are some interesting endeavors in Southeast Asia too, e.g. Medipost in Korea. All of these are undergoing various stages and sizes of clinical trials for diverse indications, some of which are showing already very encouraging results. It is predicted that within the next few years one or more of these potential products will reach the market as an approved medical product. Having said this, other than showing ongoing safety and efficacy in the trials, some hurdles do remain in the industry as whole, such as up-scaling for mass production of the cells which is considered necessary for generating an off-the-shelf product.

If this trend continues, it is only reasonable to assume that gene therapy will follow, most probably some years after cell therapy has been more widely accepted, but possibly in parallel to some extent, as there are also a number of human gene therapy trials already in progress. So, how significant will retroviral (RV) and lentiviral (LV) vectors be in this future gene therapy industry? One approach to answer this is to look at how popular they have

been up to now, both in terms of previous and ongoing trials as well as the interest that the research community has in them compared to other forms of gene therapy tools.

In order to assess this one must firstly consider all forms of gene delivery tools, as this is a pre-requisite for gene therapy. In general, gene delivery can be achieved either by viral or non-viral means. Mostly, when non-viral gene delivery is discussed, it involves the use of plasmid DNA, although there is much interest in other forms of nucleic acids such as bacterial artificial chromosomes (BACs) (for recent review on BACs see Tunster et al. 2011). Nucleic acids can be transduced into the patients' cells by physical or chemical mechanisms. Physical methods include obvious ways like direct injection, or aerosolisation/inhalation but also more ingenious techniques like the gene gun or using sound waves, such as ultrasound (Passineau et al. 2011) or by electroshock, which can intriguingly even work *in vivo* (De Vry et al. 2010; Kaneda 2010). Such non-viral means have classically been considered to be safer because viral vectors may cause insertional mutagenesis, revert to wild-type via recombinations during preparation or *in vivo* with other viruses (infections or endogenous viruses). But, at the same time, non-viral means are also considered to be far less effective, as once the nucleic acid is inside the cell it is only by chance that it will enter the nucleus and be expressed. Both of these views are changing somewhat though. From a safety perspective, as techniques for producing and detecting virus vectors are advancing and people's understandings of viral vector safety is improving, and, from an efficiency perspective, as chemical and nanomaterial technologies improve.

It boils down to a risk/benefit calculation and viral based methods are still generally considered to be far more efficient tools, and as such, outweighing the risks. Mammalian cells could be transformed with virus-based methods as early as the 1960's, yet virus related vectorology, with respect to gene delivery, only started to strongly emerge in the early 1980's (Figure 1), showing that this is somewhat a matter of definition. In any case, adenoviruses (AdV) have always been and still are the most popularly researched and used vectors. One would expect that over the years, the number of different types of viruses and new viruses being researched as vectors would increase, and this is indeed the case (Fig. 1, see "others" from 1 in 1985 to over 400 in 2010). However, one trend that was not as expected is that the amount of studies on herpes simplex (HSV) as a vector has not increased. In other words, in 1980 the first talk of using viruses in connection with gene delivery was concerning HSV (Anderson et al. 1980), by 1985 a few publications related to RV, but the most were still HSV related, and surprisingly this number has almost not changed up to 2010 where still only approximately 20 publications related to using HSV as a vector for gene therapy or delivery. Is it fair to conclude from this that they may simply be unsuitable for the job? Further investigation would be required to make such a claim but it may be reasonable to suggest that if more successful steps had been made, then increasing popularity would be inevitable. Most surprising, however, is that over the last 10 years, publications of research and use of viruses as vectors seems to have reached a plateau, and in the last four years, have begun to decrease (see Figure 1, All). This may be a sign that that some technologies are being absorbed by industrial processes, e.g. patent before publication, trade secrets. Such translational activities, although more practical, are less academically novel and may reduce the number of research papers.

Most relevant perhaps for this chapter on surface modifications of enveloped viruses and their application, is the changing relationship of interest between RV and LV vectors in the research community over the last ten years (Figure 1, compare RV and LV).

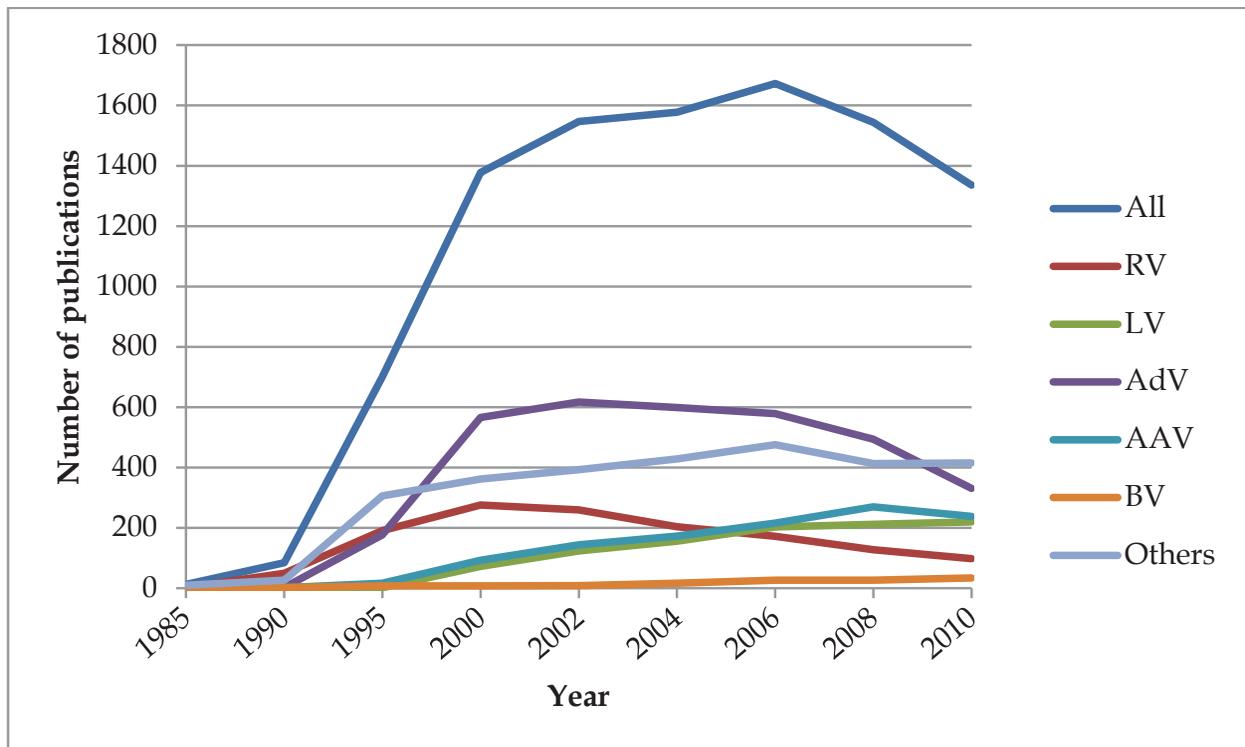


Fig. 1. The trend of scientific publications in the area of viral vectors for gene delivery over the last 25 years. A study was undertaken using the scientific reference collating programme, Endnote. The method used was to search and cross-reference various terms. For example, both “retrovirus” and “retroviral” were searched together with either “gene delivery” or “gene therapy” and “vector” and each were cross-referenced with each other, which allowed not only to be sure that the topic in question was relating to gene delivery/therapy with viral vectors, and that nothing was missed, but it also gave an idea of publications which were using both types of system, i.e. that these were not counted twice. The same was done for lentivirus, adenovirus and adeno-associated virus vectors.

Key: All = total number of all viral vectors for gene delivery and includes the following: RV = retroviral vectors; LV = lentiviral vectors; AdV = adenovirus vectors; AAV = adeno-associated virus vectors; BV = baculoviral vectors; Others = vectors based on the following viruses: alphaviruses (e.g. Sindbis, Semliki Forest, Ross River, Venezuelan equine encephalitis), vaccinia viruses, poxviruses, infectious bursal disease, herpes simplex, Sendai/hemagglutinating virus of Japan, measles, transmissible gastroenteritis virus, human and avian influenza, oncolytic vesicular stomatitis, papilloma viruses, lymphocytic choriomeningitis, rabies, hepatitis B/C, combinations of viruses (e.g. RV-LV, AdV-RV) as well as replicons, amplicons, retroelements and virally related transposons.

Although the actual numbers are not huge, there is clearly a significant shift in the focus of interest from RV vectors to LV vectors. When RV vectors are discussed, this means mainly the two forms of murine leukemia virus (MLV), the Friend and Moloney strains, although, studies also include vector work with mouse mammary tumour virus (MMTV) and Rous sarcoma virus (RSV) (Gunzburg 2003). When LV vectors are discussed, this means mainly human immunodeficiency virus type-1 (HIV-1), although again, studies also include vector work with simian and feline immunodeficiency viruses (SIV and FIV). There may be many reasons for this shift in interest in the scientific community. One is certainly that LV vectors,

in contrast to RV vectors, offer the ability to transduce non-dividing cells (Sakoda et al. 2007). This is generally seen as an advantage, however, it may not be desirable in all gene delivery cases, as it may be useful to target only dividing cells (e.g. in cancer therapy). Another reason is likely to be the sheer amount of interest in HIV as its affliction on humans continues to globally expand, i.e. relatively large amounts of finance available and research undertaken to understand the virus for human medicine not only leads to drug targets but also to ingenious ways of using the virus as a vector for gene delivery or therapy, e.g. self-inactivating vectors to increase safety (Liehl et al. 2007).

Other than RV, LV, AdV and AAV, there are many types of viruses being developed as gene delivery vectors, all with limited impact, but it seems in more recent years that the numbers of these studies are no longer increasing (see Figure 1, Others). One explanation may be that this correlates with the amount of new viruses being discovered. Either man has discovered most viral entities already or a future technological breakthrough will allow discovery of viral or viral related entities of a new dimension. It may also be surprising to some to see how many “nasty” viruses are being used to make vectors. For example, HSV BACs were developed around 2000 mainly for studying viral genetics, but more recently they are being explored for gene therapy (Warden et al. 2011). Other ongoing examples include measles, influenza and papilloma viruses, the latter of which cause cancer. When considering this, however, one just has to remember what has happened with HIV over the last 15 years. First suggestions of using HIV vectors for human medicine were met with serious concern in the general media, and even scientists were skeptical, but now they are well accepted and even approved for use in human clinical trials (Sheridan 2011, also see below).

Some publications relate to expression of viral genes within other more common types of vector system, e.g. the thymidine kinase gene of the HSV (HSV-TK) expressed using a RV or AdV vector, so it is somewhat a matter of definition as to whether they are included as virus based gene delivery systems. For this study, such cases were not included. In some cases there were also hits for viral components such as the envelope proteins of vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV) and amphotropic MLV (see also Table 2), but this is relating to pseudotyping, and, although there are a few genuine cases of scientists developing true gene delivery vectors from these viruses, the pseudotyping cases were not counted in this study. Pseudotyping as a method for viral surface modification is discussed later in this chapter.

Finally, how has all the viral vector research culminated into gene therapy trials in the clinic? The majority of trials that have been undertaken and are currently ongoing (around 14 human trials) are using AdV and AAV (for a recent review see Sheridan 2011). More relating to the topic of this chapter, however, are past and ongoing successes for enveloped viral vector gene therapies, i.e. RV and LV vectors. Most worthy of mention are the studies, human trials and treatments made by Alain Fischer, Marina Cavazzana-Calvo and Salima Hacein-Bey-Abina. Children who were suffering from severe combined immunodeficiency (SCID-X1) had their T-cells treated *ex vivo* with a RV vector (Moloney MLV based) to replace a defective part of the gene for the interleukin-2 receptor (Cavazzana-Calvo et al. 2000; Hacein-Bey-Abina et al. 2008). Although four of the twenty patients treated developed leukemia (caused by insertional mutagenesis and activation of a proto-oncogene), they were able to be treated for this secondary issue, and seventeen from twenty have had their immunodeficiency corrected over the follow-up period of almost 10 years now (Cavazzana-Calvo et al. 2010). This is a great success in which the benefit clearly outweighed the risk, as without alternative treatment (the only one being a bone marrow transplant with only 25%

chance of success), the outcome would have been fatal within a few years at best. Other ongoing studies in the area of primary immunodeficiency are being undertaken by San Raffaele in Italy, also using a Moloney MLV vector (Aiuti et al. 2002), although it is unclear if more human trials are planned at present. Two USA companies have ongoing RV vector treatments in trials, namely Neurologix for Parkinson's disease (Kaplitt et al. 2007; Lewitt et al. 2011) and Tocagen for a form of brain cancer, glioblastoma multiforme. The Tocagen trial is of particular interest as it is the first study in humans using a replication competent RV. All RV and LV vectors previously used in trials (even the vast majority used in research) are non-replicating or self-inactivating, which means after one round of infection/gene delivery that the virus is "dead" and cannot replicate further. It has been classically developed this way for safety reasons, unfortunately, as it turns out, the *in vivo* infection efficiency of such vectors may never be good enough for many applications. The MLV based replication competent vector developed by Tocagen and its research associates (Anliker et al. 2010) has the therapeutic gene, cytosine deaminase (CD), inserted into the viral genome in a stable position where the virus cannot easily reject it, even after multiple rounds of infection (Logg et al. 2001; Paar et al. 2007). As the RV vector is limited to replicating in dividing cells, and only very few cells in the brain are dividing at a speed comparable to the tumour, it is the ideal setting. Also, the prodrug used for the treatment, 5-FU, can only pass the blood-brain barrier in its non-activated form, i.e. once activated to 5-FU by the CD gene product at the cancer location, it cannot pass back into the rest of the body, where there are faster dividing cells which could be affected. Once the infected tumour is destroyed, so in turn are the replicating viruses in this so called suicide-gene therapy system. This trial is being followed by many and with great interest.

Concerning clinical trials with LV, three companies are worth mentioning. Bluebird Bio (formerly Genetix Pharmaceuticals) in the USA, recently completed 2 trials with three patients in total who underwent *ex vivo* treatment of their own hematopoietic stem cells with a LV vector delivering corrective genes for either cerebral adrenoleukodystrophy (Cartier et al. 2009) or beta-thalassaemia (Cavazzana-Calvo et al. 2010). Although both showed positive results, no public announcement on further trials has been announced as yet. Lentigen, also in USA, are currently undertaking a pilot study through the University of Pennsylvania for several kinds of leukemia and lymphoma, whereby, once again, the patients T-cells will be modified *ex vivo*, but in this case the modification should turn-on the T-cells enabling them to attack and trigger the destruction of the cancerous B cells once re-implanted (ClinicalTrials.gov Identifier No. NCT00891215). Oxford BioMedica are currently undertaking Phase I/II trial for Parkinson's disease by injecting their LV vector technology via stereotactic injection directly into the striatum of patients' brains. Three genes which are necessary for dopamine production should be delivered/expressed and the results are predicted to be published soon (EudraCT No. 2007-001109).

## 2. Methods to modify surfaces of RV/LV vectors

### 2.1 Introduction - proteins of retroviral surfaces

When retroviral or lentiviral (RV/LV) particles exit infected cells, they are surrounded by a lipid membrane, termed the envelope, derived from the infected cells. The envelope contains both virus-derived and cellular proteins (VP and CP, respectively), which may perform distinct functions for the virus. Since proteins displayed on the envelope are the first to make contact with neighboring molecules, they are often involved in virus-cell and

virus-medium interactions. In order to complete their life cycle, viruses are depending on this communication with their surroundings, specifically the soluble factors (i.e. antibodies or proteins of the complement system) contained in the liquid environment and elements displayed on the surface of potential host cells (i.e. the receptors found on the cell surface required for binding and entry of the virus).

This is maybe best demonstrated by the VPs found in the lipid membrane of the RV/LV particle, termed envelope or Env proteins. The function of these proteins is to initiate cell entry (for more details see chapter 3.5.). The RV/LV envelope proteins may be replaced by surface glycoproteins of different virus species in a process termed pseudotyping (Bischof and Cornetta 2010; Cronin et al. 2005). The resulting viral pseudotypes can be of interest for gene therapy applications (see section 2.2. for details). Apart from the virus encoded Env proteins, CP are also incorporated into the viral envelopes. Theoretically, this may happen as a consequence of three processes: (i) interaction of host proteins with viral proteins (type 1 incorporation), (ii) incorporation due to directed colocalisation (type 2 incorporation) and (iii) random incorporation (type 3 incorporation). The incorporation of the cytoplasmic protein cyclophilin A to HIV-1 particles is an example of a type 1 incorporation. The protein associates with the viral Gag proteins and is subsequently incorporated with a similar efficiency (Hammarstedt and Garoff 2004). A similar situation was shown for the tumor susceptibility gene 101 (Tsg-101) product, which presumably has a role in RV/LV particle release (Garrus et al. 2001; Pornillos et al. 2003). Additionally, in 1995 the incorporation of complement regulatory factors such as CD55 (decay accelerating factor) and CD59 (protectin) into viral envelopes was described (Breun et al. 1999; Saifuddin et al. 1994; Saifuddin et al. 1995; Saifuddin et al. 1997). The levels of these proteins are high enough to ensure protection from the human complement system, a part of the innate immune system (Breun et al. 1999; Saifuddin et al. 1997). Both the glycosylphosphatidylinositol-anchored CD55 and CD59 molecules are enriched in membrane microdomains, often termed lipid rafts. Interestingly, it has been demonstrated that a range of viral species including retroviruses and lentiviruses may use lipid rafts as sites of viral assembly (Metzner et al. 2008a). Thus co-localisation of these molecules at the site of viral budding would lead to their incorporation into viral envelopes. Thus, this may constitute a type 2 incorporation. The co-localisation of molecules at membrane microdomains may also form the framework for pseudotyping events (Briggs et al. 2003; Metzner et al. 2008a; Pickl et al. 2001) (see section 2.2.). Discriminating between type 2 and type 3 incorporation events may be difficult, since directed co-localisation is not easy to demonstrate and the concept of membrane microdomains is controversial (Shaw 2006). However, there is reason to believe that most incorporation events happen passively (Hammarstedt and Garoff 2004), i.e. concentration of proteins is not increased compared to normal membrane composition. To maintain a proteome not altogether dissimilar from the cellular membrane may generally be beneficial for the virus, since this would contribute to immune-camouflage, i.e. "hiding" the virus from the host's immune system.

A special case is the exclusion of host proteins from the viral particles. Several cases have been described including proteins such as CD45 (Esser et al. 2001), CD4 and the HIV co-receptors CXCR4 and CCR5 (Lallos et al. 1999). Excluding receptors and co-receptors from budding viral particles can be beneficial for the virus, since premature receptor engagement and induction of fusion can be avoided. The mechanisms responsible for exclusion of proteins from viral envelopes may be that the formation of a network of viral Gag proteins inhibits access of proteins with large cytoplasmic domains (such as CD45) or multiple

transmembrane passes (such as the HIV co-receptors). Alternatively, involvement of excluded proteins in larger complexes may prevent them from being incorporated (such as CD4-p56lck). Recently, proteomics approaches have been used to identify host proteins found in viral envelopes to get an overview of incorporation (see Table 1) and to date, a long list of proteins have been identified in viral particle envelopes (Chertova et al. 2006; Segura et al. 2008b) including a range of molecules involved in cellular adhesion. Viruses may profit from these molecules, as they can provide additional or indeed, initial anchoring before specific interactions between Env and the cognate cell-membrane viral receptors.

Protein	Virus	Function	Comment	Reference
Tsg-101	HIV-1	MVB sorting; HIV budding	Interaction with Gag	Hammarstedt and Garoff, 2004; Pornillos et al.; 2002
CD55	HIV-1, MLV	Complement regulation	GPI-anchored	Saifuddin et al., 1995; Plewa et al., 2005
CD59	HIV-1, MLV	Complement regulation	GPI-anchored	Saifuddin et al., 1995; Plewa et al., 2005
CD14	HIV-1	Lipopolysaccharide receptor	GPI-anchored	Chertova et al., 2006
CD29 (Integrin $\beta$ 1)	HIV-1, MLV	Adhesion	Random?	Chertova et al., 2006; Segura et al., 2008
CD44	HIV-1	Adhesion	Random?	Chertova et al., 2006
CD54 (ICAM 1)	HIV-1	Adhesion	Random?	Chertova et al., 2006
CD48	HIV-1	Signaling	Random?	Chertova et al., 2006
CD45	HIV-1	Signaling	Excluded	Esser et al., 2001
CD4	HIV-1	HIV receptor	Excluded	Lallos et al., 1999
CXCR4	HIV-1	HIV co-receptor	Excluded	Lallos et al., 1999
CCR5	HIV-1	HIV co-receptor	Excluded	Lallos et al., 1999

Table 1. Examples for cellular proteins incorporated to RV/LV envelopes. Tsg tumor suppressor gene 101; MVB multivesicular bodies; ICAM intercellular adhesion molecule.

Surface modification of RV/LV particles can be broadly separated into five categories (see Figure 2): (i) pseudotyping, (ii) generation of fusion proteins, (iii) post translational modification of proteins with lipophilic residues, most notably glycosylphosphatidylinositol (GPI) anchors, (iv) utilization of adaptor molecules, or (v) direct chemical modifications. These types of modification will be discussed in sections 2.2. to 2.6., while the purpose of such modifications and possible applications will be discussed in section 3. Special attention will be given to the “non-classical” methods for modification of viral surfaces discussed in section 2.4. to 2.6.

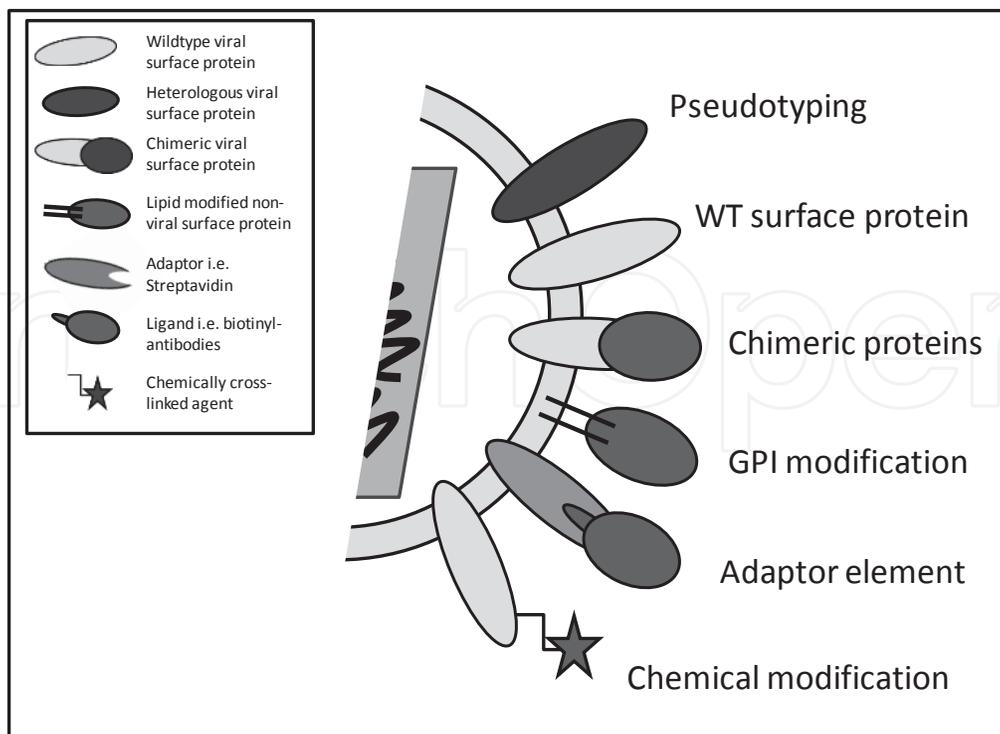


Fig. 2. Overview of strategies for modification of RV/LV vector surfaces.

## 2.2 Pseudotyping

The phenomenon that surface (glyco-)proteins of one virus species can be displayed on the surface of another retrovirus, or indeed other viral species has been termed “pseudotyping”. It was first described more than 55 years ago, after the observation that cells infected with two different viruses, can give rise to phenotypically mixed particles (Granoff and Hirst 1954; Zavada 1982). Interestingly, surface glycoproteins from different families of viruses can be exchanged or mixed in such a manner (see Table 2), indicating some degree of similarity between these molecules or the mechanisms by which they are incorporated into the virions. Because of this compatibility, a limited set of modifications can be introduced to retroviral particles by displaying surface molecules of other viral species. Since the primary function of viral surface glycoproteins is to mediate binding and entry to host cells, the replacement of retroviral Env molecules in most cases changes the infectious range (the tropism) of the vector, because different receptors are engaged. This phenomenon can be exploited for gene therapy approaches, as it allows for example, the broadening or redirection of the virus’ host range (see Table 2). For example, HIV-1 based LV vectors, naturally having a very limited, highly specific tropism, infecting only CD4-expressing cells, can be redirected from these cells by replacing the Env proteins of HIV-1 with those of the vesicular stomatitis virus (VSV), thus generating a vector with a more diverse tropism (Bischof and Cornetta 2010). VSV-G, the surface glycoprotein of VSV, is probably the most often used molecule for pseudotyping applications. In addition to changing tropism, VSV-G also enhances virion stability (Burns et al. 1993) but is toxic to cells if expressed constitutively making it challenging to generate stable packaging cell lines. To overcome this, tetracycline-inducible promoters are frequently used to generate stable virus-producing cell lines (Ory et al. 1996). Additionally VSV-G pseudotypes are highly susceptible to inactivation by human serum complement (DePolo et al. 2000) and require additional

treatment e.g. by polyethylene glycol (PEG) modification or PEGylation (Croyle et al. 2004). Other examples for pseudotyping are described in table 2. It is not in all cases that pseudotyping is used to transfer new cell binding properties to the RV/LV vector. Recent approaches employ pseudotyping to transfer fusion properties of surface glycoproteins from Sindbis (Morizono et al. 2009a; H. Yang et al. 2008a), Influenza (Lin et al. 2001; H. Yang et al. 2008a) or measles virus (Anliker et al. 2010; Frecha et al. 2011). In these molecules, fusion activity is not strictly dependent on receptor binding. In such cases the original binding activity may be abrogated and replaced with a binding function of choice. This constitutes a “mixed” modification, since both, pseudotyping and generation of chimeric proteins are employed to generate vector particles (see also sections 2.3 and 3.5).

Viral Surface Protein	Virus Family	Vector Background	Targeting Preference	References
MLV amphotropic	Retroviridae	HIV-1, SIV	broad, fibroblasts	Strang et al., 2004
GALV	Retroviridae	HIV-1	hematopoietic, cancer	Diaz et al., 2000; Sandrin et al., 2002
RD114	Retroviridae	HIV-1, SIV	hematopoietic	Sandrin et al., 2002; Zhang et al., 2004
LCMV	Arenaviridae	HIV-1, EIAV, SIV	CNS, glioma, pancreas	Miletic et al., 2004; Kobinger et al., 2004
Sindbis	Togaviridae	HIV-1	broad	Morizono et al. 2009
Ross River	Togaviridae	HIV-1, FIV	Liver (non-hepatocyte)	Kang et al. 2002
VSG-G	Rhabdoviridae	HIV-1, HIV-2, SIV, FIV, EIAV	CNS, Liver, retina	Watson et al. 2002; Park et al. 2003; Auricchio et al. 2001
Ebola	Filoviridae	HIV-1, FIV	Lung, muscle	Kobinger et al. 2004; MacKenzie et al. 2002
Rabies	Rhabdoviridae	HIV-1, EIAV	CNS	Stein et al. 2005
Influenza A	Orthomyxoviridae	HIV-1, EIAV, SIV	Lung	McKay et al. 2006
Measles	Paramyxoviridae	HIV-1	broad (Edmonston strain)	Anliker et al. 2010
Sendai	Paramyxoviridae	HIV-1, SIV	Liver, Lung	Kowolik et al. 2002; Sinn et al. 2005
Hepatitis C	Flaviviridae	HIV-1	Liver	Hsu et al. 2003

Table 2. Examples for the use of pseudotyping events for gene therapy approaches. MLV murine leukemia virus; SIV simian immunodeficiency virus; GALV gibbon ape leukemia virus; LCMV lymphocytic choriomeningitis virus; EIAV equine infectious anemia virus; FIV feline immunodeficiency virus; VSV-G vesicular stomatitis virus glycoprotein.

The mechanisms behind pseudotyping are believed to be related to the presence of membrane subdomains in cellular membranes which function as sites for viral assembly and/or budding of a range of different viral species (Briggs et al. 2003; Metzner et al. 2008a; Pickl et al. 2001). Since viruses from quite different taxonomical origins are assembled at such sites, incorporation of proteins from different virus backgrounds can occur. Consequently, pseudotyping can be viewed as a form of type 2 incorporation. Another prerequisite for pseudotyping, is that wild-type RV/LV Env proteins are not required for functional assembly of virions, inferring that the absence of the native glycoproteins does not interfere with particle production. Alternatively, direct or indirect (via a cellular intermediate) protein interaction may be responsible for pseudotyping. Recently, an indirect interaction model has been proposed using a scanning electron microscopy approach (Jorgenson et al. 2009) thus supporting involvement of type 1 incorporation events. To date, a large number of combinations have been tried and have been assessed in terms of their targeting potential in gene therapy approaches (see Table 2). Recent reviews provide a more extensive overview of pseudotyping of RV/LV vectors (Bischof and Cornetta 2010; Cronin et al. 2005).

### 2.3 Fusion proteins

Hybrid proteins consisting of amino acid sequence elements derived from more than one original polypeptide are termed fusion or chimeric proteins. To avoid confusion with proteins such as viral surface glycoproteins exhibiting membrane fusion activity, the latter term will be used. The technique of fusing protein parts by manipulation on the DNA level has been widely used to study the spatial distribution or kinetics of expression by utilizing fluorescent marker proteins such as green fluorescent protein (GFP). Fusing retroviral envelope proteins with molecules of interest allows for a more widespread modification of viral surfaces, since it is not limited by the availability of naturally occurring viral glycoproteins. In the case of chimeric proteins, the residual RV/LV Env molecule part is used as a sorting signal, directing the chimeric molecule to sites of budding. The advantage of this method is that theoretically no limit is placed on the type of amino acid sequence introduced and that incorporation to the viral particle is in most cases efficient (Ryu, 2008). The fused parts may be complete ligands (Kasahara et al. 1994), peptides (Gollan and Green 2002; Morizono et al. 2009a) or single-chain antibodies (Anliker et al. 2010; Somia et al. 1995). However, limits have been shown to apply to this technique, as structural or functional elements are typically disturbed by their introduction, leading to loss of infectivity, since cellular uptake is inhibited at the level of envelope-cell membrane fusion (Galanis et al. 2001; Ryu et al. 2008; Zhao et al. 1999). When a chimeric Env molecule, containing a CD33 specific single-chain antibody, was generated to target CD33 positive cells, the collected data indicated, that the chimeric protein could not initiate fusion of the virus and cell membranes during infection (Zhao et al. 1999). The most widely accepted explanation for this is that the chimeric proteins are unable to undergo a mandatory conformational change which activates the fusion activity (see also section 3.5.). Thus, the use of such modifications for targeting applications, i.e. the induction of cellular uptake upon engagement of the chimeric protein with a cognate receptor, is limited. Inclusion of wild-type RV Env proteins to targeted vectors can help to increase infectivity (Tai et al. 2003). This, however, may come at a cost for specificity of targeting. Studies conducted on chimeric Moloney murine leukemia virus (MoMLV) Env proteins suggest, that addition of as little as two point mutations might rescue targeted infection (Zavorotinskaya and Albritton 2001). More recent data suggests

that such mutations may need to be determined depending on the characteristics of the insert as well as the insertion site (Ryu, 2008).

However, for purposes other than targeting, such as labeling of viral particles or tagging for enrichment purposes, this technique still holds appeal. A GFP-Env chimeric protein, containing the sequence of enhanced GFP fused to the N-terminus of the amphotropic 4070A Env of MLV has been generated and in this case viral particles retained their natural infection range (Spitzer et al. 2003). The construct was used to stain viral receptor-carrying cells and may be used for monitoring the dynamics of virus-cell interactions (Spitzer et al., 2003). An interesting aspect is, that the “virion-targeting functions” of chimeric proteins may not necessarily have to be provided by RV/LV Env proteins, but could also be provided by the use of cellular proteins present or enriched in viral membranes such as Tsg-101 (see Table 2). Alternatively, virion-targeting function may be provided by GPI-anchoring (see section 2.4.) or indeed, by making use of mixed modifications using fusion proteins of non-RV/LV glycoproteins, such as Sindbis virus (Morizono et al. 2009b; Pariente et al. 2007) influenza (Lin et al. 2001; L. Yang et al. 2006b) or measles virus (Anliker et al. 2010; Frecha et al. 2008) with engineered novel binding properties. In these cases the wild-type binding specificity was destroyed and replaced with molecules conferring specific targeting to molecules such as integrin (Morizono et al. 2009a) or the B lymphocyte marker CD20 (Anliker et al. 2010). In these cases, membrane fusion activity is inhibited to a lesser degree by the change in binding properties. For Sindbis and influenza, virus fusion is triggered by a decrease in pH. First *in vivo* experiments indicated that such vectors can be used for targeting applications (Anliker et al. 2010; L. Yang et al. 2006b) (see also section 3.5.)

## 2.4 Glycosylphosphatidylinositol (GPI) modification

GPI anchoring is a type of post-translational modification occurring in eukaryotic cells and probably constitutes the most complex and metabolically challenging way of attaching proteins to lipid membranes. Proteins targeted for GPI anchoring contain a GPI signaling sequence (GSS) at the C-terminal end. The GSS is recognized in the endoplasmatic reticulum by the transamidase enzyme complex where it is cleaved at the omega site, the point at which the preformed GPI anchor is attached. The biochemical pathway for synthesis of the GPI anchors is complex and chemical structures of GPI anchors vary to a great degree (Ikezawa 2002) however a common backbone structure is observed: Linkage of the GPI anchor to the C-terminal end of the protein is achieved by an amide bond to phosphoethanolamine. The following central three mannose residues are linked via a non-acetylated glucosamine to the phosphoinositol part, which in turn is associated to the lipid residues, usually acyl or aryl fatty acid chains or sphingolipids e.g. ceramide (Ikezawa 2002). GPI anchored proteins have a variety of different functions. In addition to the mentioned complement regulatory activity of CD55 and CD59, GPI-linked proteins serve as hydrolytic enzymes like acetylcholinesterase and placental alkaline phosphatase (Ikezawa, 2002) or are involved in signal transduction like Thy1 (Haeryfar and Hoskin 2004). They share several unique properties: GPI-linked (or “glypiated”) proteins are targeted to the outer surface of the cell membrane (Ferguson 1999; Nosjean et al. 1997) and are frequently associated with dynamic membrane microdomains also known as lipid rafts (Legler et al. 2005). As mentioned previously, they have been suggested as the site of viral assembly for certain enveloped viruses e.g. HIV-1 (Briggs et al. 2003; D. H. Nguyen and Hildreth 2000). GPI proteins can also be found in serum and other body fluids both with intact or absent GPI anchors (Landi et al. 2003). Processes that release GPI-linked proteins into the medium with

intact GPI anchors are reversible and it has been shown in a variety of *in vitro* and *in vivo* systems that GPI-linked proteins can be re-inserted into cell membranes (Dunn et al. 1996; Kooyman et al. 1995; Rifkin and Landsberger 1990; Rooney et al. 1993; Rooney et al. 1996; Vakeva et al. 1994). Transfer has been demonstrated for CD59 from erythrocytes to endothelial cells (Kooyman et al. 1995) as well as for trypanosomal variant surface glycoprotein (VSG) to erythrocytes of infected patients (Rifkin and Landsberger 1990). Therefore GPI-anchored proteins can be considered to be “hypermobile”. This hypermobility allows for the re-integration of purified GPI-anchored proteins to lipid membranes of cells (Legler et al. 2005; Medof et al. 1996) and viruses (Metzner et al. 2008b). This process has been termed cellular or viral painting, respectively.

Technically, introducing a GPI anchor to a protein is achieved in the same way fusion proteins are made: Following genetic engineering, the recombinant protein is translated and the amino acid sequence describing the GSS is included to the nascent polypeptide, thus artificially GPI-anchored proteins are produced. A range of recombinant GPI-anchored proteins have been produced including glypiated GFP and CD4 (for a review see Metzner et al. 2008a). GPI-anchored proteins can be employed for the modification of RV/LV vectors by two distinct pathways: (i) after transfection of virus producing cells, facilitated by the co-localisation of glypiated proteins at the site of viral budding and (ii) by viral painting, re-introducing purified GPI-anchored proteins to mature viral particles as a result of the GPI-anchored protein hypermobility.

Co-transfection of plasmid vectors carrying genes for the production of retroviral vectors with constructs expressing the GPI-anchored proteins or super-transfection of pre-existing virus producing cell lines, leads to the formation of viral particles displaying GPI-anchored molecules on their envelopes. These particles acquire novel properties as a consequence of the incorporation of the GPI-anchored protein e.g. super-transfection of the murine retroviral producer cell line PALSG/S with the human GPI-anchored protein CD59, yields viral particles that are resistant to the activity of complement in human serum (Breun et al. 1999). These results suggested for the first time that incorporation of recombinantly expressed GPI-anchored proteins into the envelopes of viral vectors is possible and that these modifications can be useful for gene therapy approaches. In two more recent studies, co-transfection approaches successfully produced virus-like particles (VLPs) containing glypiated proteins from mammalian (Kueng et al. 2007) or insect cells (Skountzou et al. 2007). In both cases, recombinant GPI-anchored different cytokine species were generated i.e. interleukin-2 (IL-2) and granulocyte-macrophage colony stimulating factor (GM-CSF). In the first study, it was demonstrated that the GPI-anchored cytokines are functional and elicit cellular responses such as differentiation and proliferation with similar efficiency as their soluble counterparts when co-cultured with the appropriate target cells (Kueng et al. 2007); and in the second it was described that GPI-anchored cytokines engineered onto VLPs based on simian immunodeficiency virus (SIV) can enhance immunogenicity of the VLPs. In both cases a modulation of the immune responses was achieved by displaying GPI-anchored cytokine (Kueng et al. 2007; Skountzou et al. 2007). The major advantage of this approach is that stable transfection of RV/LV producer cell lines co- or super-transfected with GPI-anchored proteins can provide a reproducible long-term source of modified viral particles. In addition, no post-exit steps that may reduce infectivity of the vectors are required.

The second method to modify RV/LV vectors is by using GPI-anchored proteins that have been extracted and purified from cells and can be re-inserted after incubation with enveloped viruses. This was first described for the GPI-linked model protein CD59his which

associates to viral vectors based on MLV and HIV-1 (Metzner et al. 2008b). The association is specific and painted virus particles remain infectious after insertion of the GPI-linked protein, albeit at reduced efficiencies caused by the duration of the painting process, rather than the actual introduction of GPI-anchored molecules into the viral outer shell (Metzner et al. 2008b). Estimates of the number of GPI-anchored proteins painted onto retroviral particles were in the range of the numbers observed for Env molecules per virion and are thus similar to that achieved after incorporation of hybrid proteins produced in co-transfection experiments (Skountzou et al. 2007). The main advantage of this approach is flexibility. Different GPI-anchored proteins can be attached to a range of enveloped viral particles without repeated genetic manipulations of the virus-producing cells. This also means a considerable gain of time, compared to transfection-based methods. Additionally, the amounts of protein deposited at the viral surface are controllable and only a limited amount of information about the genetic requirements of the virus is necessary for modification. Viral painting may be the method of choice for modification of enveloped viral particles in all situations where a degree of flexibility is favourable, e.g. in response to genetic heterogeneity in gene therapy approaches or in response to high antigen variability for vaccination. Also when genetic modification of virus producing cell lines is difficult, e.g. when applying toxic proteins or when genetically or biochemically poorly defined virus species are the targets of modification. Viral painting is an example of post-exit surface modification, since fully formed viral particles are the target for modification. While increasing the flexibility of such approaches, the time invested in modification after exit from cells most likely will result in loss of titer (Metzner et al. 2008b) depending on the duration of the modification steps. Thus, keeping post-exit incubation times as short as possible is vital.

## 2.5 Using adaptors structures

Another strategy to modify viral particles is to introduce adaptor molecule onto the particles which in turn can mediate association of other molecules. These adaptors can either be soluble, non-covalently attached molecules or membrane bound factors. Soluble adaptors have been used to enable targeting strategies in gene therapy. In these cases bispecific molecules or assemblies were used, contacting specifically a molecule present on the virus and another on the cells about to become infected. These bispecific adaptors or bridge complexes can take different forms. For example, two different antibodies, modified with biotin, can be linked via avidin or streptavidin (Roux et al. 1989) thus providing specificity for viral surface proteins and the target molecule on the cell. Such a system has been proposed as early as 1989, showing directed infection of MHC class I and II expressing cells with murine retroviruses (Roux et al. 1989). This system is highly flexible and versatile, since a wide range of antibodies which can be biotinylated are available. Pre-treatment of the viral vector with the anti-viral antibody would effectively neutralize the viral particle, thus increasing the safety of the application. Alternatively, a receptor/ligand chimeric protein, in which the binding partner for the viral attachment protein is coupled to a ligand, binding to the target molecule on the cell surface may be used. Vectors pseudotyped with Avian sarcoma and leukosis virus (ASLV) have been used for implementing such strategies. The chimeric bridge protein consisted of the extracellular domains of the cellular receptor for ASLV, fused to ligands such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) or heregulin, thus targeting cells expressing the respective receptors (Snitkovsky et al. 2000; Snitkovsky et al. 2001; Snitkovsky and Young 2002). Since these

receptors are commonly overexpressed on tumor cells, the approach is already of some medical relevance. Instead of the ligands, also single-chain antibodies may be used. An approach which has been used for targeting cells expressing a tumor-specific form of the EGF receptor (Snitkovsky et al. 2000).

When using membrane associated adaptors, in most cases, avidin or streptavidin engineered to contain a trans-membrane domain are utilised, due to their extraordinarily strong affinity to biotin and the comparative ease with which biotin can be attached to a wide range of compounds from DNA to antibodies. Avidin and streptavidin molecules are available in a wide range of modifications, tailor-made for different applications (Laitinen et al. 2007). Again, mixed modifications may be used, by generating fusion proteins of avidin/streptavidin with viral surface proteins (M.U. Kaikkonen et al. 2008; M. U. Kaikkonen et al. 2009) or even GPI-anchors (Pinaud et al. 2009). The main advantage of this system is its flexibility, since factors attached to avidin or streptavidin can be exchanged. However, similar to viral painting, post-exit modification steps may be necessary, which could potentially reduce infectivity of RV/LV vectors. Such a system has been implemented by fusing avidin and streptavidin with the transmembrane domain of VSV-G (M. U. Kaikkonen et al. 2009). The binding of biotin to such vectors was demonstrated and they could be used for dual imaging and for targeting application (see sections 3.2. and 3.5.). Other approaches lead to the biotinylation of the lentiviral vector. This can be achieved by direct chemical modification (G. Yang et al. 2006a) (see also section 2.6.) or after addition of a biotin-adaptor peptide (BAP), a site for specific enzymatic biotin ligation (G. D. Chen et al. 2010a; Nesbeth et al. 2006) (see also section 2.6.). The bacterial enzyme, biotin ligase, has to be provided as a form of metabolic engineering to allow the modification of the BAP-containing protein. Both a cellular protein, low-affinity nerve growth factor (Nesbeth et al. 2006) and a viral protein, Sindbis virus glycoprotein (Morizono et al. 2009b), have been modified in such a way to generate novel LV vectors. The latter in fact constitutes pseudotyping of an LV vector with a chimeric envelope molecule, containing an adaptor element, added by enzyme-mediated covalent chemical modification, thus mixing four different strategies to modify viral RV/LV vectors. Alternatively, membrane proteins binding antibodies may be used to modify viral surfaces. Insertion of immunoglobulin G-binding domains (the ZZ domain of staphylococcal protein A) into the Env protein of MLV vectors allowed for the binding of specific antibodies directed against the EGF receptor HER2. However, infectivity was significantly reduced, as can be expected (Tai et al., 2003). A similar approach utilizes a fusion of the same antibody binding domain with Sindbis envelope glycoproteins (L. Yang et al. 2006b). The main disadvantage of adaptor systems is that an additional, separate element is necessary for the system to work, thus introducing a new level of complexity. Additionally, adaptors may dissociate from one or the other binding partner, especially if antibody binding domains are used. Competition from serum antibodies *in vivo* may significantly enhance dissociation (Morizono et al. 2009b). Pre-treatment of viral vectors with the adaptor at least allows administering the virus/adaptor complex as a single entity (Boerger et al. 1999). However, adaptor association in most cases requires post-exit procedures, which can again contribute to loss of infectivity.

## 2.6 Direct chemical modification

Another option would be the direct chemical linkage of substrates to viral surfaces. While successful modification of polymers and polypeptides to adenoviruses and adeno-associated viruses has been achieved (Croyle et al. 2000; Croyle et al. 2002) it has been difficult to carry

out such modifications on RV/LV particles and attempts have been rare. A successful example is the attachment of monomethoxy-poly(ethylene)glycol (PEG) to VSV-G pseudotyped LV vectors (Croyle et al. 2004). In this case an activated form of PEG is covalently attached to lysine residues on proteins displayed on the virus. PEGylation reduces the susceptibility of these vectors for the human and murine complement system, while maintaining transduction efficiencies (Croyle et al. 2004), thus manipulating the host immune system (see also section 3.4.). In another, early attempt, MoMLV was modified by chemical addition of carbohydrate (galactose) moieties in order to change viral tropism (Neda et al. 1991). Introduction of these residues was supposed to specifically infect hepatocytes expressing asialoglycoprotein receptors recognizing the carbohydrate moieties on the viral vectors. However, the modification resulted in severely reduced infectivity of RV/LV particles. Direct chemical biotinylation of retroviral vectors has also been demonstrated, using sulfo-N-hydroxysuccinimide-biotin MoMLV derived vectors (G. Yang et al. 2006). For this approach neutravidin was covalently linked to poly-lysine. The resulting compound was then associated to the biotinylated vector. The aim of the study was to allow transduction of human cells with ecotropic MLV vectors, which normally cannot infect human cells. In this case, progeny of modified viruses would lack the modification, hence infection of neighboring cells, even if replication competent vectors were generated, would not be possible. This could contribute to safety of gene therapy approaches. Another strategy to biotinylate viral surfaces includes the potential for chemical display of biotin using a metabolic engineering approach as was described in section 2.5, i.e. that the introduction of biotin-adaptor peptides to viral surface glycoproteins allows for the specific biotinylation of these proteins by a secreted biotin ligase, conferring the possibility for avidin, streptavidin or neutravidin linkage. These adaptors can in turn be used for attachment of secondary biotinylated compounds (Morizono et al. 2009b). Alternatively, desthiobiotin can be metabolically introduced to RV/LV vectors (R. Chen et al. 2010b). In this case, the binding to avidin and its derivatives will be easier to reverse, owing to lower affinity. More recently, developments in bioorthogonal chemistry could bring new impetus to the field. Bioorthogonal chemistry describes the possibility to allow controlled, specific chemical reactions amidst the background of a biological system i.e. in cell culture. Specifically, cell surfaces can be modified by oxidation of sialic acids present on glycosylated surface proteins by periodate, generating reactive aldehyde groups, which in turn can be modified by conjugation of aminoxy-functionalised compounds (Zeng et al. 2009). When this technique was applied to cells producing VSV-G pseudotyped MoMLV, resulting viral particles carried the modification (S. Wong and Kwon 2011). They used this to introduce aminoxy-biotin and could subsequently associate magnetic particles to the virus, facilitating purification and concentration of virus preparations (Wong and Kwon 2011; see also section 3.3.). This approach may also be applied to viral particles post-exit. Direct chemical modification of herpesvirus particles with radioactive labels has also been demonstrated and was used for biodistribution studies (Schellingerhout and Bogdanov 2002). Biological chemistry, by developing bioorthogonal methods, appears to have great potential for novel types of modification.

### 3. Applications for surface modification of RV

#### 3.1 General aspects

The purpose of modifying the surface of RV/LV vectors is to facilitate more efficient delivery of the gene of interest to desired sites of expression (see Figure 3). Changes to the viral surface

can facilitate this in a number of ways, such as helping to produce more efficient vector stocks by enabling purification and concentration of viral vector stocks (thereby helping to increase transduction efficiencies) (see section 3.3.), by ensuring optimal interactions with the host organism, especially the host's immune system (see 3.4.), or by limiting viral entry to a subset of host cells (see 3.5.). In its simplest form, physical vector surface modification leads to a more efficient way of detecting the viral particles, due to the association of labeling molecules, thus allowing for a controlled administration and delivery regime (see 3.2.). All of these aspects will also help to increase safety of gene therapy approaches, by eliminating potentially toxic or immunogenic contaminants and reducing adverse and off-target effects. Suitability of modification types for each application will be discussed in the following sections. Another important aspect of course is that multifunctional modifications can – and should – be applied to allow implementation of different applications such as easy detection by labeling and purification/concentration in one modification step.

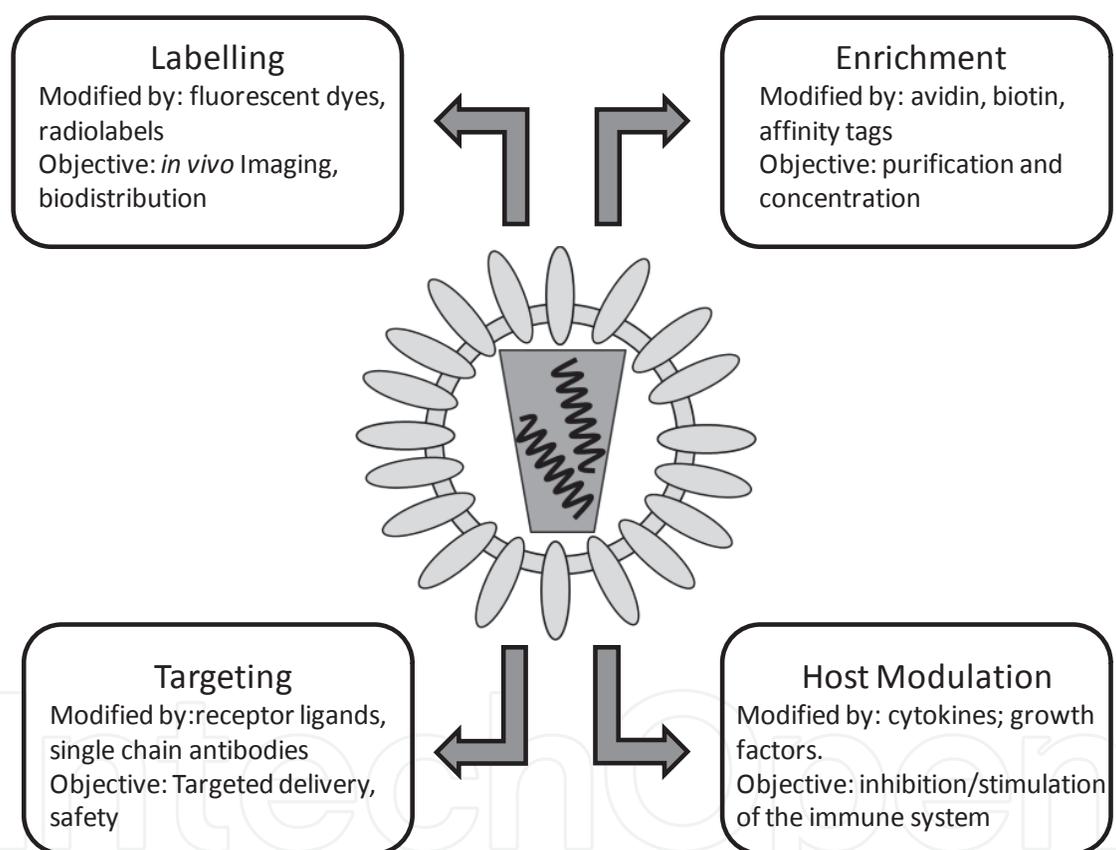


Fig. 3. Overview of applications for surface modifications of RV/LV vectors in gene therapy.

### 3.2 Imaging/biodistribution

Efficiency of gene therapy will be depending on administration regime and the subsequent distribution of vectors in the patient. Therefore, these events need to be controlled, already at the pre-clinical stages to assess vector performance i.e. in animal experiments. *In vivo* transgene expression analysis, as was used for the detection of specific targeting of LV vectors (Pariente et al. 2007) is not ideal, since the information yielded by measurement of transduction efficiency or, more generally, transgene expression is different from data describing physical particle distribution. Vector particles may be trapped or degraded long

before transduction, subsequently not contributing to transgene expression. Thus detection of the physical vector particles is required (in addition to and in parallel to transgene expression analysis) and may need to be facilitated by labeling of the RV/LV vector particle. Generally, labeling approaches should have only minimal to no influence on the performance of the RV/LV vector and on the host or target organism (low toxicity and low immunogenicity). Another requirement is the stability of label attachment. Loss of label from viral particles would not only lead to decrease of signal, but also to an increase of background noise. Finally, detection of the label should be easy and easily achievable *in vivo*. Small, chemically inert substances, giving a strong and localized signal, enabling non-invasive detection methods will be the ideal tags. For practical reasons, a “one size fits all” approach to labeling may be preferred. Flexible and versatile procedures which can be applied to a broad range of RV/LV vectors are called for. All mentioned aspects will obviously influence choice of technique for labeling and subsequently, location and type of label.

RV/LV particles can be labeled at the level of the capsid, i.e. using chimeric proteins of capsid proteins and fluorescent proteins (Lampe et al. 2007; Lehmann et al. 2005). While loss of label will be less of a concern in such circumstances, changing the geometry of the capsid might very well have an influence on capsid and/or viral assembly. Modification can also be carried out at the level of viral surfaces, where they are much more susceptible to dissociation or degradation. So far viral particles are mainly labeled by incorporation of chimeric proteins of viral structural proteins and (fluorescent) marker proteins (Lampe et al. 2007; Lehmann et al. 2005), owing to a high degree of stability of association caused by the covalent association. However, fluorescent proteins are comparatively big, increasing the chance for steric hindrance and thus influence on vector performance. Detection of fluorescence signal may allow for a limited depth of penetration only. Standard immunohistochemistry methods use non-protein fluorophore-labelled secondary antibody, and while acceptable for *in vitro* purposes, it cannot provide the stability of a covalent bond and therefore may not be suitable for *in vivo* applications. For the same reason, most adaptor-based systems will also not be suitable for labeling, with the possible exception of systems using membrane-bound avidin/biotin (M. U. Kaikkonen et al. 2009), due to the extraordinarily strong association, which is comparable to covalent interactions. The use of GPI-anchored marker proteins provides an alternative, as the modification pathway does not interfere with viral structural components, i.e. the envelope surface protein required for target cell binding and infection. Infection efficiency is not disturbed by painting (Metzner et al. 2008b). The use of viral painting technology can provide the additional advantage of flexibility. Incorporation of label is mostly independent of viral producer cell lines and can be performed relatively quickly (Metzner et al. 2008b; Metzner et al. 2008a). However, still comparatively large polypeptide molecules are attached to the viruses and the degree of stability of interaction still needs to be determined. Direct chemical attachment seems to have good prospects in this area, since small molecules can be attached to virus particles covalently, in a relatively small amount of time (R. Chen et al. 2010b; S. Wong and Kwon 2011). However, such methods still need to be assessed in terms of their *in vivo* applications. So far fluorescent molecules were considered as primary detection labels. While fluorescence detection, together with luminescence has been used for pre clinical *in vivo* detection approaches, it will not be suitable for application in human patients due to low penetration depth (Shah et al. 2004). Alternatively, detection based on magnetic resonance imaging (MRI) and two radiological methods, positron emission tomography (PET) and single photon emission computer tomography (SPECT), may be used (for a review on

molecular imaging techniques in gene therapy see Shah et al. 2004; Raty et al. 2007). While MRI has been recently utilized for *in vivo* transgene expression, using ferritin as the reporter gene (Hasegawa et al. 2010; M. U. Kaikkonen et al. 2009), SPECT shows promising qualities, combining high sensitivity with comparatively low cost equipment and reagents, in analyzing physical particle distribution. Such an approach has been utilized to study biodistribution of herpes- (Schellingerhout and Bogdanov 2002) and baculovirus (Raty et al. 2007) *in vivo*. In these cases the radiolabels were attached to the virus either by direct chemical modification (Schellingerhout and Bogdanov 2007) or by adding a biotinylated radiolabel to streptavidin modified viral particles (Raty et al. 2007). Dual *in vivo* imaging of rats was demonstrated for LVvectors coding for transferring and displaying streptavidin adaptors (M. U. Kaikkonen et al. 2009). In this case, enrichment of iron in cells upon transferrin expression was used to enable MRI, while radiolabels were attached to the displayed streptavidin molecules to allow for SPECT detection. For this study, deposition of adaptor molecules on viral particles was additionally used for targeting applications, demonstrating the versatile nature of adaptor systems. However, also approaches using fluorescent labeling molecules may additionally have a function in enrichment or concentration prior to transduction i.e. viral particles modified with proteins containing 6xhis-tagged proteins can be enriched by using established immobilized metal ion affinity chromatography (IMAC) (Gaberc-Porekar and Menart 2001; Magnusdottir et al. 2009) or magnetic purification techniques (Franzreb et al. 2006) (see also section 3.3.).

### 3.3 Purification/concentration

Another important issue in gene therapy is generation of suitable vector preparations in terms of viral titer, purity, speed and costs. Cheap methods which allow for quick concentration and purification of RV/LV vectors after harvesting from producing cell lines are of great importance. Contaminants from producing cell cultures may inhibit transduction of target cells (Rodrigues et al. 2007). Additionally, with clinical use of vectors as a mid- to long-term aim, vector preparations need to be compliant with regulators' standards. In nearly all cases, purification and concentration of viruses starts with a micro-filtration step, using 0.45  $\mu\text{m}$  filters to remove cells and cellular debris from culture supernatants. When large amounts of dead cells are present in the supernatant, centrifugation may be preferred for removal of micro-level contaminants. Subsequently, RV/LV particles are still concentrated and purified in most cases by ultra-centrifugation, often utilizing sucrose gradients to prevent mechanical damage to viruses. These methods are time-consuming and require the use of expensive equipment, i.e. high velocity centrifuges. Additionally, not all RV/LV vectors tolerate ultra-centrifugation well. Sucrose may need to be removed from preparations afterwards, adding another preparatory step. Additionally, high sucrose content can harm virus by change in osmotic pressure. Taken together these aspects lead to often quite significant reductions of infectivity (Rodrigues et al. 2007). Using VSV-G pseudotypes as well as ultra-centrifugation-resistant virus strains can improve yield after ultra-centrifugation (Burns et al. 1993). While acceptable for laboratory scale preparation, ultra-centrifugation is unsatisfactory for larger scale preparations. Alternatively viruses may be concentrated and purified by ultra-filtration or dialysis protocols, separating virus and contaminants according to size using semi-permeable membranes. Conventional column based methods lead to problems regarding large-scale preparations, but use of tangential flow devices can circumvent this aspect (Geraerts et al. 2005; Kuiper et al. 2002).

What these methods have in common is that they do not rely on any modification of the virus. This is partially also true for purification strategies involving chromatography applications e.g. size exclusion or ion exchange approaches, both of which have been used for purification and concentration purposes (Rodrigues et al. 2007). Affinity chromatography is a very powerful tool for removal of contaminants. Using naturally occurring affinity tags for purification and concentration such as heparin can work well (Segura et al. 2008a; Segura et al. 2010). In such an application the tag should interfere minimally with the virus activity, be easily accessible for the purification matrix and, in most cases, a transient interaction would be preferred, allowing for removal of virus under mild conditions. An additional advantage would be, if the same strategy would be applicable for a broad spectrum of vectors. After all, the key property of a purification label would be its affinity and specificity for the purification matrices used. Approaches utilising either biotin-streptavidin interactions or IMAC have been reported (Williams et al. 2005a; Williams et al. 2005b; Ye et al. 2004). In the latter strategy, the affinity of complexed nickel or cobalt ions to stretches of histidine aminoacids is exploited. Viruses may be modified for application of the streptavidin/biotin system in numerous ways, as described in sections 2.5 and 2.6. Direct chemical modification was chosen to biotinylate retroviral vectors prior to purification using a streptavidin coated stationary phase (Chan et al. 2005; Williams et al. 2005a; Williams et al. 2005b). As a result of the strong interaction of biotin and avidin/streptavidin, removal of viral particles from the purification matrix can require harsh conditions, which will reduce yield and infectivity of viral preparations. While IMAC was used successfully on viral vectors containing histidine tags in the Env protein (Ye et al., 2004), immediate dialysis of resulting samples is necessary to remove the chemicals necessary for desorption (imidazole, EDTA). These compounds would also need to be removed prior to any clinical application, as well as metal ions potentially leaking from the purification matrix, since both may lead to adverse side effects in patients (Rodrigues et al. 2007). In addition to chromatography based methods, attachment of magnetic particles to the viral vectors may enable purification and concentration. Recently, attempts have been made to attach micro- or nanoparticles with magnetic properties to modified viral surfaces (R. Chen et al. 2010b; M. U. Kaikkonen et al. 2009; Nesbeth et al. 2006; S. Wong and Kwon 2011). Again, avidin-biotin interactions were exploited to attach the magnetic particles. Such approaches are interesting due to their potential for up-scaling. Ideally, magnetic nanoparticles could be designed in such a way, that they may not have to be removed from viruses, but may serve additional function as contrast agents in detection via MRI. Generally, adaptor approaches can be considered most useful for labeling purposes, if only due to their versatility. Indeed, when an adaptor is present on a viral vector, it may as well be used for purification purposes in addition to the primary aim of modification, i.e. transduction targeting. Alternatively, quick post-exit approaches, such as viral painting with GPI-anchored proteins may be used, giving a great degree of flexibility, as they can be applied to a wide range of viral preparations, and again more than one objective may be achieved by a single modification i.e. by the use of a histidine-tagged, GPI-anchored immunomodulatory protein.

### 3.4 Modulation of host functions

Depending on the administration protocol planned, RV/LV vectors used in gene therapy may have to find their way to the target cells. During this journey, they will share contacts with both soluble and cell bound components of the host. Elements performing functions in the

host's immune system will be of special interest, since in most patients, gene therapy vectors, including RV/LV vectors, will encounter an intact immune system. Navigating the immune system will be vital to any successful gene therapy approach. Interaction of virus particles with host molecules including immunological reactions are often mediated by molecules located in the envelope, thus modifying the envelope with immunologically competent molecules, e.g. cytokines or growth factors, allows for the manipulation of surrounding immune responses. This specifically includes protection of viral vectors from unwanted immune reactions such as complement activity. Immunoprotection can help to ensure efficient delivery to target cells by eliminating premature inactivation of vectors in gene therapy approaches. One example is the pegylation of VSV-G pseudotyped vectors (Croyle et al. 2004), as discussed in section 2.6. Other approaches to protect from complement activity include the introduction of complement regulatory factors such as CD55 or CD59 (Breun et al. 1999), if they are not already part of the envelope protein contents. In this case the presence of naturally occurring GPI-anchored proteins was exploited.

Protection from the complement or neutralizing antibodies is only one aspect of immunomodulation. Stimulation of immune responses may be a desired effect to augment therapeutic effect i.e. in cancer gene therapy or vaccine strategies. For example, presentation of antigen on the surface of virus-like particles (VLPs) is possible via the use of GPI-anchored molecules. GPI-anchored cytokines engineered onto simian immunodeficiency virus (SIV) can enhance immunogenicity of the VLPs (Skountzou et al. 2007). VLPs can be used to modulate the immune system in several ways (Kueng et al. 2011). These aspects may be of more importance in the development of vaccines or specific adjuvants enhancing vaccine efficacy, but variations may also prove useful for gene therapy approaches. For example, early acting cytokines such as interleukin 6, stem cell factor or thrombopoietin, have been shown to enhance gene transfer using RV/LV vectors to haematopoietic stem cells (Santoni de Sio et al. 2006; Zielske and Gerson 2003). Attachment of such factors to viral surfaces as discussed above can help to achieve stronger local effects. This may be considered also in the context of "targeting by activation", whereby efficient transduction is dependent on activation of the target cell by the ligand/receptor interaction (Verhoyen and Cosset 2004; see also section 3.5.).

A further aspect of modulation of host cell function is inducing differentiation e.g. for tissue engineering purposes. Proof of principle was shown through differentiation of monocytes to dendritic cells (Kueng et al. 2007). Again, the GPI-anchored cytokines used were functional and elicited cellular responses such as differentiation and proliferation with similar efficiency as their soluble counterparts when co-cultured with the appropriate target cells. The major advantage of this approach is that stable transfection of RV/LV producer cell lines co- or super-transfected with GPI-anchored proteins can provide a long-term source of modified viral particles reproducibly. In addition, no post-exit steps that may reduce infectivity of viral vectors are required. Alternatively, viral painting may be used in cases where flexibility is required. Immunomodulation appears to be an area of great possibilities, allowing potentially for the "fine tuning" of gene therapy approaches, by enhancing distribution or transgene expression and providing additional beneficial side effects such as increased tumor cell killing.

### 3.5 Infection targeting

A key element of successful and efficient gene therapy is the ability to target only a certain subset of cells for treatment after systemic administration. This constitutes both, a measure

to ensure safety, since (ideally) no non-target cells should become genetically modified, and enhance efficacy, since (again, ideally) all vector particles present should recognize and infect target cells. Targeted infection is an especially important feature when using replication-competent vectors. Such vectors can replicate in infected cells and produce progeny virus which in turn can infect new target cells. Whilst highly efficient, i.e. in the case of tumor gene therapy, safety of course is an important issue, as a form of viremia is part of the delivery strategy, which needs to be tightly controlled. Additionally, infection targeting is equally important for all *in vivo* gene therapy approaches (as opposed to *ex vivo* approaches, where infection can be limited by other means), especially upon systemic administration. Here, viral vectors are introduced to the patient and non-specific infection is a definitive risk, especially considering integration of viral DNA into the host genome and the associated potential for insertional mutagenesis.

In the case of RV/LV vectors, the viral glycoproteins located in the envelope function as recognition and entry devices to allow access to the target cells. The so called Env proteins consist of two subunits, the surface (SU) and transmembrane (TM), both with distinct functions. The Env protein complex is a hexamer consisting of 3 copies of each of the TM and SU subunits. SU mediates the first contact to the host cells by engaging the viral receptor and, eventually co-receptors. The binding specificity of the SU subunit therefore determines the host cell range of the virus. Upon this first contact, TM activates fusogenic properties, which allow viral and cellular membranes to fuse, resulting in viral entry. The interaction of SU and TM is highly sensitive to changes in SU and already small changes can disturb the activation of the TM activity (Zhao et al. 1999). Subsequently, modifications introduced to the Env proteins are tolerated badly, quite often leading to severe reduction in infectivity. Nevertheless, modification of these properties is crucial to achieve infection or transduction targeting – one of the most important goals of viral gene therapy. The choice of method being used for targeting applications may also be influenced by the specific target molecule, cell or tissue and the target's distribution in the organism, since access to the targets will add additional obstacles to delivery/targeting, for example regarding the stability of the interaction between viral vector, targeting molecule and target. In targeting approaches, specificity is the most important parameter.

A range of different strategies have been tested to change the infection tropism including the use of glycoproteins from heterologous viral species (pseudotyping) or chimeric envelope glycoproteins (Env fusion proteins) as well as bridging molecules (adaptors) (Waehler et al. 2007). The application of pseudotyping for transduction targeting (Croyle et al. 2004; Engelstadter et al. 2001; Miller et al. 1991) is limited by the range of available glycoproteins with useful infection tropisms. A more versatile strategy is the use of chimeric envelope proteins in which parts of the protein responsible for receptor binding are replaced with peptides (Gollan and Green 2002), ligands (Cosset et al. 1995; Kasahara et al. 1994) or single-chain antibodies (Anliker et al. 2010; Somia et al. 1995) conferring binding to the desired receptors for viral entry. Additionally, adaptors, which are capable of associating with both the viral glycoprotein and the cellular receptor, can be used to mediate between virus and target cell (Boerger et al. 1999; Snitkovsky et al. 2001).

Another concept is targeting by activation. Retroviral vectors displaying the amphotropic MLV Env containing the original receptor binding domain and elements coding for IL2 (Maurice et al. 1999) or hepatocyte growth factor (HGF) (T. H. Nguyen et al. 1998) were generated. These vectors would allow binding of and entry to a broad range of cells. However, only upon cell activation due to binding of IL2 or HGF to their cognate receptor would lead to

significant transduction, as introducing proliferation enabled the progress of MLV provirus to the nucleus and subsequent integration and transgene expression (Maurice et al. 1999). Although lentiviral vector can infect non-dividing cells, blocks to transduction occur, for example in monocytes (Kootstra et al. 2000; Neil et al. 2001) and resting T cells (Dardalhon et al. 2001). Activation targeting can help to overcome such blocks. Problems encountered in such approaches include differentiation of stimulated cells or background infection in rapidly dividing cells (Verhoeven and Cosset 2004). What these approaches have in common is that they often lead to significantly reduced infection rates (Galanis et al. 2001).

Progress in transduction targeting has been made by separating binding and fusion properties (Lin et al. 2001; L. Yang et al. 2006b). This is possible as in several viral species, binding and fusion properties are independent features and fusion activity is triggered by different stimuli, i.e. low pH after endocytosis (L. Yang et al. 2006b). Most promising candidates for the use as heterologous fusogenic proteins in RV are genetically engineered variants of the Sindbis virus (SIN) glycoprotein (Morizono et al. 2005; L. Yang et al. 2006b; L. Yang et al. 2008b; H. Yang et al. 2008a; Ziegler et al. 2008), the influenza virus hemagglutinin (Lin et al. 2001; L. Yang et al. 2006b) and measles virus surface glycoproteins (Anliker et al. 2010). In such a case, the use of adaptor systems may help to construct flexible targeting systems, as well as deposition of specific binding factors by using GPI-anchored proteins, especially in cases where binding and entry of viral particles are mediated by independent proteins, i.e. *in trans* (Lin et al. 2001; L. Yang et al. 2006b). The same basic viral particle can be modified with a range of binding properties to suit the specific needs of the applications. Antibody molecules, for example, in the form of single-chain antibody molecules, engineered to contain a GPI anchor, can provide a vast range of binding specificities. Summing up, the use of non RV/LV viral surface glycoproteins capable of inducing virus/cell fusion independent of specific binding taken together with a flexible such as an adaptor system or viral painting seems currently the most promising candidates for targeting applications.

#### 4. Outlook and discussion

Due to their average size of 100 nm, RV and LV particles can be considered as bionanotechnological devices. Modification thus becomes the – in the field of nanotechnology – more commonly used term “functionalisation”. Multiple modifications could lead to using viral vectors as multifunctional platforms for biomedicine combined with other nanotechnological elements. For example, magnetic micro- and nanoparticles are already commercially available to allow the purification of proteins containing tags such as the histidine tag or Flag tag. GPI-anchored proteins used for painting of enveloped viral vectors have also been engineered to contain histidine tags, allowing the particles to be coated with GPI-anchored protein and attached to the viral vectors by means of the GPI-anchor. Or, alternatively, biolinker proteins such as streptavidin can potentially be modified at the genetic level to contain a GPI signal sequences, and as such, after production in a suitable expression system, can be purified as reactive reagents to link up with biotinylated nanoparticles. In both cases the recombinant GPI-anchored protein acts as a linker or interface between the organic viral particles and inorganic nanomaterials, either magnetic or fluorescent capabilities (or both). With such a system in place to functionalise the surface of viral vectors without affecting the viral infectivity pathway, one can imagine many biomedical applications such as targeting of gene therapy vectors *in vivo* to tumours using magnetic force and tracking via bioimaging techniques based on magnetic field, i.e. MRI or

high-sensitivity camera *in vivo* fluorescence imaging. However, with all of this in mind one must not forget the safety issues as the effects of such materials cannot always be predicted in biological systems, an issue that has already emerged for carbon nanotubes which would not have been expected to be as toxic as has proven to be the case in many instances (Patlolla et al. 2010; van der Zande et al. 2011). Sometimes the speed of development can lead to rashness when translating into the clinic, a fate that already befell gene therapy in some regards (Sheridan 2011), so the same mistakes should be avoided when mixing together such new technologies with clinically used viral vectors (Subbiah et al. 2010).

## 5. Conclusions / summary

RV/LV vectors are already proving to be useful delivery vehicles for gene therapy applications but in order to establish more efficient vectors for *in vivo* delivery, the viral surface can potentially be modified to provide better means of preparation, purification, concentration, detection, tracking, imaging, and targeting in order to not only successfully manufacture the product but also to navigate interactions within the patient and infect pre-defined target cells selectively. To achieve this aim several techniques may be employed for surface engineering or RV/LV vectors. Table 3 provides a concise overview of these techniques and the applications for which they have been used and may conceivably be used in the future. Pseudotyping has been used for targeting applications, however, can only make use of a limited amount of targeting options. Chimeric proteins have been used for labeling and targeting applications, and can be useful for immunomodulation approaches. Enrichment may be achieved as a secondary objective. Modifications employing GPI-anchored proteins may prove to be versatile and efficient; however, further research will be necessary. A similar assessment can be made for the use of membrane-bound adaptor systems and direct chemical modifications. Finally, combining different strategies will allow broadening possibilities for the surface modification of viral vectors considerably.

Modification	Comment	Labelling	Enrichment	Modulation	Targeting
Pseudotyping	limited possibilities				X
Chimeric Protein	versatile, decreased infectivity	X	(x)	(x)	X
GPI Modifications	via budding versatile, time-consuming		(x)	X	(x)
	via painting flexible, quick, post-exit additional	(x)	(x)	(x)	(x)
Adaptors	soluble component, post-exit	(x)	(x)		X
	membraneous flexible, versatile	X	X	(x)	X
Chemical Modification	flexible, quick, maybe post-exit	X	X	X	X

Table 3. Methods for engineering RV/LV vector surfaces and potential applications. X applications mentioned in the text; (x) potential for future applications.

## 6. References

- Aiuti, A., et al. (2002), 'Immune reconstitution in ADA-SCID after PBL gene therapy and discontinuation of enzyme replacement', *Nat Med*, 8 (5), 423-5.
- Anderson, W. F., et al. (1980), 'Replication and expression of thymidine kinase and human globin genes microinjected into mouse fibroblasts', *Proc Natl Acad Sci U S A*, 77 (9), 5399-403.
- Anliker, B., et al. (2010), 'Specific gene transfer to neurons, endothelial cells and hematopoietic progenitors with lentiviral vectors', *Nat Methods*, 7 (11), 929-35.
- Auricchio, A., et al. (2001), 'Exchange of surface proteins impacts on viral vector cellular specificity and transduction characteristics: the retina as a model', *Hum Mol Genet*, 10 (26), 3075-81.
- Azzouz, M., et al. (2004), 'Lentivector-mediated SMN replacement in a mouse model of spinal muscular atrophy', *J Clin Invest*, 114 (12), 1726-31.
- Bischof, D. and Cornetta, K. (2010), 'Flexibility in cell targeting by pseudotyping lentiviral vectors', *Methods Mol Biol*, 614, 53-68.
- Boerger, A. L., Snitkovsky, S., and Young, J. A. (1999), 'Retroviral vectors preloaded with a viral receptor-ligand bridge protein are targeted to specific cell types', *Proc Natl Acad Sci U S A*, 96 (17), 9867-72.
- Breun, S., et al. (1999), 'Protection of MLV vector particles from human complement', *Biochem Biophys Res Commun*, 264 (1), 1-5.
- Briggs, J. A., Wilk, T., and Fuller, S. D. (2003), 'Do lipid rafts mediate virus assembly and pseudotyping?', *J Gen Virol*, 84 (Pt 4), 757-68.
- Burns, J. C., et al. (1993), 'Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells', *Proc Natl Acad Sci U S A*, 90 (17), 8033-7.
- Cartier, N., et al. (2009), 'Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy', *Science*, 326 (5954), 818-23.
- Cavazzana-Calvo, M., et al. (2000), 'Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease', *Science*, 288 (5466), 669-72.
- (2010), 'Transfusion independence and HMG A2 activation after gene therapy of human beta-thalassaemia', *Nature*, 467 (7313), 318-22.
- Chan, L., et al. (2005), 'Conjugation of lentivirus to paramagnetic particles via nonviral proteins allows efficient concentration and infection of primary acute myeloid leukemia cells', *J Virol*, 79 (20), 13190-4.
- Chen, G. D., et al. (2010a), 'Concentration and purification of human immunodeficiency virus type 1 virions by microfluidic separation of superparamagnetic nanoparticles', *Anal Chem*, 82 (2), 723-8.
- Chen, R., et al. (2010b), 'Affinity recovery of lentivirus by diaminopelargonic acid mediated desthiobiotin labelling', *J Chromatogr B Analyt Technol Biomed Life Sci*, 878 (22), 1939-45.
- Chertova, E., et al. (2006), 'Proteomic and biochemical analysis of purified human immunodeficiency virus type 1 produced from infected monocyte-derived macrophages', *J Virol*, 80 (18), 9039-52.
- Cosset, F. L., et al. (1995), 'Retroviral retargeting by envelopes expressing an N-terminal binding domain', *J Virol*, 69 (10), 6314-22.

- Cronin, J., Zhang, X. Y., and Reiser, J. (2005), 'Altering the tropism of lentiviral vectors through pseudotyping', *Curr Gene Ther*, 5 (4), 387-98.
- Croyle, M. A., Yu, Q. C., and Wilson, J. M. (2000), 'Development of a rapid method for the PEGylation of adenoviruses with enhanced transduction and improved stability under harsh storage conditions', *Hum Gene Ther*, 11 (12), 1713-22.
- Croyle, M. A., et al. (2002), 'PEGylation of E1-deleted adenovirus vectors allows significant gene expression on readministration to liver', *Hum Gene Ther*, 13 (15), 1887-900.
- (2004), 'PEGylation of a vesicular stomatitis virus G pseudotyped lentivirus vector prevents inactivation in serum', *J Virol*, 78 (2), 912-21.
- Dardalhon, V., et al. (2001), 'Lentivirus-mediated gene transfer in primary T cells is enhanced by a central DNA flap', *Gene Ther*, 8 (3), 190-8.
- De Vry, J., et al. (2010), 'In vivo electroporation of the central nervous system: a non-viral approach for targeted gene delivery', *Prog Neurobiol*, 92 (3), 227-44.
- DePolo, N. J., et al. (2000), 'VSV-G pseudotyped lentiviral vector particles produced in human cells are inactivated by human serum', *Mol Ther*, 2 (3), 218-22.
- Diaz, R. M., et al. (2000), 'A lentiviral vector expressing a fusogenic glycoprotein for cancer gene therapy', *Gene Ther*, 7 (19), 1656-63.
- Dunn, D. E., et al. (1996), 'A knock-out model of paroxysmal nocturnal hemoglobinuria: Pig-a(-) hematopoiesis is reconstituted following intercellular transfer of GPI-anchored proteins', *Proc Natl Acad Sci U S A*, 93 (15), 7938-43.
- Engelstadter, M., et al. (2001), 'Targeted gene transfer to lymphocytes using murine leukaemia virus vectors pseudotyped with spleen necrosis virus envelope proteins', *Gene Ther*, 8 (15), 1202-6.
- Esser, M. T., et al. (2001), 'Differential incorporation of CD45, CD80 (B7-1), CD86 (B7-2), and major histocompatibility complex class I and II molecules into human immunodeficiency virus type 1 virions and microvesicles: implications for viral pathogenesis and immune regulation', *J Virol*, 75 (13), 6173-82.
- Ferguson, M. A. (1999), 'The structure, biosynthesis and functions of glycosylphosphatidylinositol anchors, and the contributions of trypanosome research', *J Cell Sci*, 112 ( Pt 17), 2799-809.
- Franzreb, M., et al. (2006), 'Protein purification using magnetic adsorbent particles', *Appl Microbiol Biotechnol*, 70 (5), 505-16.
- Frecha, C., et al. (2008), 'Stable transduction of quiescent T cells without induction of cycle progression by a novel lentiviral vector pseudotyped with measles virus glycoproteins', *Blood*, 112 (13), 4843-52.
- (2011), 'Measles virus glycoprotein-pseudotyped lentiviral vector-mediated gene transfer into quiescent lymphocytes requires binding to both SLAM and CD46 entry receptors', *J Virol*.
- Gaberc-Porekar, V. and Menart, V. (2001), 'Perspectives of immobilized-metal affinity chromatography', *J Biochem Biophys Methods*, 49 (1-3), 335-60.
- Galanis, E., Vile, R., and Russell, S. J. (2001), 'Delivery systems intended for in vivo gene therapy of cancer: targeting and replication competent viral vectors', *Crit Rev Oncol Hematol*, 38 (3), 177-92.
- Garrus, J. E., et al. (2001), 'Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding', *Cell*, 107 (1), 55-65.

- Geraerts, M., et al. (2005), 'Upscaling of lentiviral vector production by tangential flow filtration', *J Gene Med*, 7 (10), 1299-310.
- Gollan, T. J. and Green, M. R. (2002), 'Redirecting retroviral tropism by insertion of short, nondisruptive peptide ligands into envelope', *J Virol*, 76 (7), 3558-63.
- Granoff, A. and Hirst, G. K. (1954), 'Experimental production of combination forms of virus. IV. Mixed influenza A-Newcastle disease virus infections', *Proc Soc Exp Biol Med*, 86 (1), 84-8.
- Gunzburg, W. H. (2003), 'Retroviral gene therapy--where now?', *Trends Mol Med*, 9 (7), 277-8.
- Hacein-Bey-Abina, S., et al. (2008), 'Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1', *J Clin Invest*, 118 (9), 3132-42.
- Haeryfar, S. M. and Hoskin, D. W. (2004), 'Thy-1: more than a mouse pan-T cell marker', *J Immunol*, 173 (6), 3581-8.
- Hammarstedt, M. and Garoff, H. (2004), 'Passive and active inclusion of host proteins in human immunodeficiency virus type 1 gag particles during budding at the plasma membrane', *J Virol*, 78 (11), 5686-97.
- Hasegawa, S., Furukawa, T., and Saga, T. (2010), 'Molecular MR imaging of cancer gene therapy: ferritin transgene reporter takes the stage', *Magn Reson Med Sci*, 9 (2), 37-47.
- Hsu, M., et al. (2003), 'Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles', *Proc Natl Acad Sci U S A*, 100 (12), 7271-6.
- Ikezawa, H. (2002), 'Glycosylphosphatidylinositol (GPI)-anchored proteins', *Biol Pharm Bull*, 25 (4), 409-17.
- Jorgenson, R. L., Vogt, V. M., and Johnson, M. C. (2009), 'Foreign glycoproteins can be actively recruited to virus assembly sites during pseudotyping', *J Virol*, 83 (9), 4060-7.
- Kaikkonen, M. U., et al. (2009), '(Strept)avidin-displaying lentiviruses as versatile tools for targeting and dual imaging of gene delivery', *Gene Ther*, 16 (7), 894-904.
- Kaikkonen, M.U., et al. (2008), 'Avidin-displaying lentiviruses as versatile tools for targeting and dual-imaging of gene delivery', : *Consert Lab Course Lentiviral Vectors* (Evry, France).
- Kaneda, Y. (2010), 'Update on non-viral delivery methods for cancer therapy: possibilities of a drug delivery system with anticancer activities beyond delivery as a new therapeutic tool', *Expert Opin Drug Deliv*, 7 (9), 1079-93.
- Kang, Y., et al. (2002), 'In vivo gene transfer using a nonprimate lentiviral vector pseudotyped with Ross River Virus glycoproteins', *J Virol*, 76 (18), 9378-88.
- Kaplitt, M. G., et al. (2007), 'Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial', *Lancet*, 369 (9579), 2097-105.
- Kasahara, N., Dozy, A. M., and Kan, Y. W. (1994), 'Tissue-specific targeting of retroviral vectors through ligand-receptor interactions', *Science*, 266 (5189), 1373-6.
- Kobinger, G. P., et al. (2004), 'Transduction of human islets with pseudotyped lentiviral vectors', *Hum Gene Ther*, 15 (2), 211-9.
- Kootstra, N. A., Zwart, B. M., and Schuitemaker, H. (2000), 'Diminished human immunodeficiency virus type 1 reverse transcription and nuclear transport in primary macrophages arrested in early G(1) phase of the cell cycle', *J Virol*, 74 (4), 1712-7.

- Kooyman, D. L., et al. (1995), 'In vivo transfer of GPI-linked complement restriction factors from erythrocytes to the endothelium', *Science*, 269 (5220), 89-92.
- Kowolik, C. M. and Yee, J. K. (2002), 'Preferential transduction of human hepatocytes with lentiviral vectors pseudotyped by Sendai virus F protein', *Mol Ther*, 5 (6), 762-9.
- Kueng, H. J., Schmetterer, K. G., and Pickl, W. F. (2011), 'Lipid rafts, pseudotyping, and virus-like particles: relevance of a novel, configurable, and modular antigen-presenting platform', *Int Arch Allergy Immunol*, 154 (2), 89-110.
- Kueng, H. J., et al. (2007), 'General strategy for decoration of enveloped viruses with functionally active lipid-modified cytokines', *J Virol*, 81 (16), 8666-76.
- Kuiper, M., et al. (2002), 'Purification of a functional gene therapy vector derived from Moloney murine leukaemia virus using membrane filtration and ceramic hydroxyapatite chromatography', *Biotechnol Bioeng*, 80 (4), 445-53.
- Laitinen, O. H., et al. (2007), 'Brave new (strept)avidins in biotechnology', *Trends Biotechnol*, 25 (6), 269-77.
- Lallos, L. B., et al. (1999), 'Exclusion of HIV coreceptors CXCR4, CCR5, and CCR3 from the HIV envelope', *AIDS Res Hum Retroviruses*, 15 (10), 895-7.
- Lampe, M., et al. (2007), 'Double-labelled HIV-1 particles for study of virus-cell interaction', *Virology*, 360 (1), 92-104.
- Landi, A. P., et al. (2003), 'Determination of CD59 protein in normal human serum by enzyme immunoassay, using octyl-glucoside detergent to release glycosyl-phosphatidylinositol-CD59 from lipid complex', *Immunol Lett*, 90 (2-3), 209-13.
- Legler, D. F., et al. (2005), 'Differential insertion of GPI-anchored GFPs into lipid rafts of live cells', *Faseb J*, 19 (1), 73-5.
- Lehmann, M. J., et al. (2005), 'Actin- and myosin-driven movement of viruses along filopodia precedes their entry into cells', *J Cell Biol*, 170 (2), 317-25.
- Lewitt, P. A., et al. (2011), 'AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial', *Lancet Neurol*, 10 (4), 309-19.
- Liehl, B., et al. (2007), 'Simian immunodeficiency virus vector pseudotypes differ in transduction efficiency and target cell specificity in brain', *Gene Ther*, 14 (18), 1330-43.
- Lin, A. H., et al. (2001), 'Receptor-specific targeting mediated by the coexpression of a targeted murine leukemia virus envelope protein and a binding-defective influenza hemagglutinin protein', *Hum Gene Ther*, 12 (4), 323-32.
- Logg, C. R., et al. (2001), 'A uniquely stable replication-competent retrovirus vector achieves efficient gene delivery in vitro and in solid tumors', *Hum Gene Ther*, 12 (8), 921-32.
- Magnusdottir, A., et al. (2009), 'Enabling IMAC purification of low abundance recombinant proteins from E. coli lysates', *Nat Methods*, 6 (7), 477-8.
- Maurice, M., et al. (1999), 'Efficient gene delivery to quiescent interleukin-2 (IL-2)-dependent cells by murine leukemia virus-derived vectors harboring IL-2 chimeric envelope glycoproteins', *Blood*, 94 (2), 401-10.
- McKay, T., et al. (2006), 'Influenza M2 envelope protein augments avian influenza hemagglutinin pseudotyping of lentiviral vectors', *Gene Ther*, 13 (8), 715-24.
- Medof, M. E., Nagarajan, S., and Tykocinski, M. L. (1996), 'Cell-surface engineering with GPI-anchored proteins', *Faseb J*, 10 (5), 574-86.

- Metzner, C., et al. (2008a), 'Rafts, anchors and viruses - A role for glycosylphosphatidylinositol anchored proteins in the modification of enveloped viruses and viral vectors', *Virology*.
- (2008b), 'Association of glycosylphosphatidylinositol-anchored protein with retroviral particles', *Faseb J*, 22 (8), 2734-9.
- Miletic, H., et al. (2004), 'Selective transduction of malignant glioma by lentiviral vectors pseudotyped with lymphocytic choriomeningitis virus glycoproteins', *Hum Gene Ther*, 15 (11), 1091-100.
- Miller, A. D., et al. (1991), 'Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus', *J Virol*, 65 (5), 2220-4.
- Morizono, K., et al. (2009a), 'Redirecting lentiviral vectors by insertion of integrin-targeting peptides into envelope proteins', *J Gene Med*, 11 (7), 549-58.
- (2009b), 'A versatile targeting system with lentiviral vectors bearing the biotin-adaptor peptide', *J Gene Med*, 11 (8), 655-63.
- (2005), 'Lentiviral vector retargeting to P-glycoprotein on metastatic melanoma through intravenous injection', *Nat Med*, 11 (3), 346-52.
- Neda, H., Wu, C. H., and Wu, G. Y. (1991), 'Chemical modification of an ecotropic murine leukemia virus results in redirection of its target cell specificity', *J Biol Chem*, 266 (22), 14143-6.
- Neil, S., et al. (2001), 'Postentry restriction to human immunodeficiency virus-based vector transduction in human monocytes', *J Virol*, 75 (12), 5448-56.
- Nesbeth, D., et al. (2006), 'Metabolic biotinylation of lentiviral pseudotypes for scalable paramagnetic microparticle-dependent manipulation', *Mol Ther*, 13 (4), 814-22.
- Nguyen, D. H. and Hildreth, J. E. (2000), 'Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts', *J Virol*, 74 (7), 3264-72.
- Nguyen, T. H., et al. (1998), 'Amphotropic retroviral vectors displaying hepatocyte growth factor-envelope fusion proteins improve transduction efficiency of primary hepatocytes', *Hum Gene Ther*, 9 (17), 2469-79.
- Nosjean, O., Briolay, A., and Roux, B. (1997), 'Mammalian GPI proteins: sorting, membrane residence and functions', *Biochim Biophys Acta*, 1331 (2), 153-86.
- Ory, D. S., Neugeboren, B. A., and Mulligan, R. C. (1996), 'A stable human-derived packaging cell line for production of high titer retrovirus/vesicular stomatitis virus G pseudotypes', *Proc Natl Acad Sci U S A*, 93 (21), 11400-6.
- Paar, M., et al. (2007), 'Effects of viral strain, transgene position, and target cell type on replication kinetics, genomic stability, and transgene expression of replication-competent murine leukemia virus-based vectors', *J Virol*, 81 (13), 6973-83.
- Pariente, N., et al. (2007), 'A novel dual-targeted lentiviral vector leads to specific transduction of prostate cancer bone metastases in vivo after systemic administration', *Mol Ther*, 15 (11), 1973-81.
- Park, F. (2003), 'Correction of bleeding diathesis without liver toxicity using arenaviral-pseudotyped HIV-1-based vectors in hemophilia A mice', *Hum Gene Ther*, 14 (15), 1489-94.
- Passineau, M. J., et al. (2011), 'Ultrasound-assisted non-viral gene transfer to the salivary glands', *Gene Ther*, 18 (4), 424.

- Patlolla, A., Patlolla, B., and Tchounwou, P. (2010), 'Evaluation of cell viability, DNA damage, and cell death in normal human dermal fibroblast cells induced by functionalized multiwalled carbon nanotube', *Mol Cell Biochem*, 338 (1-2), 225-32.
- Pickl, W. F., Pimentel-Muinos, F. X., and Seed, B. (2001), 'Lipid rafts and pseudotyping', *J Virol*, 75 (15), 7175-83.
- Pinaud, F., et al. (2009), 'Dynamic partitioning of a glycosyl-phosphatidylinositol-anchored protein in glycosphingolipid-rich microdomains imaged by single-quantum dot tracking', *Traffic*, 10 (6), 691-712.
- Pornillos, O., et al. (2003), 'HIV Gag mimics the Tsg101-recruiting activity of the human Hrs protein', *J Cell Biol*, 162 (3), 425-34.
- Raty, J. K., et al. (2007), 'Non-invasive Imaging in Gene Therapy', *Mol Ther*, 15 (9), 1579-86.
- Rifkin, M. R. and Landsberger, F. R. (1990), 'Trypanosome variant surface glycoprotein transfer to target membranes: a model for the pathogenesis of trypanosomiasis', *Proc Natl Acad Sci U S A*, 87 (2), 801-5.
- Rodrigues, T., et al. (2007), 'Purification of retroviral vectors for clinical application: biological implications and technological challenges', *J Biotechnol*, 127 (3), 520-41.
- Rooney, I. A., Heuser, J. E., and Atkinson, J. P. (1996), 'GPI-anchored complement regulatory proteins in seminal plasma. An analysis of their physical condition and the mechanisms of their binding to exogenous cells', *J Clin Invest*, 97 (7), 1675-86.
- Rooney, I. A., et al. (1993), 'Physiologic relevance of the membrane attack complex inhibitory protein CD59 in human seminal plasma: CD59 is present on extracellular organelles (prostasomes), binds cell membranes, and inhibits complement-mediated lysis', *J Exp Med*, 177 (5), 1409-20.
- Roux, P., Jeanteur, P., and Piechaczyk, M. (1989), 'A versatile and potentially general approach to the targeting of specific cell types by retroviruses: application to the infection of human cells by means of major histocompatibility complex class I and class II antigens by mouse ecotropic murine leukemia virus-derived viruses', *Proc Natl Acad Sci U S A*, 86 (23), 9079-83.
- Ryu, B. Y., et al. (2008), 'The block to membrane fusion differs with the site of ligand insertion in modified retroviral envelope proteins', *J Gen Virol*, 89 (Pt 4), 1049-58.
- Saifuddin, M., et al. (1994), 'Host cell components affect the sensitivity of HIV type 1 to complement-mediated virolysis', *AIDS Res Hum Retroviruses*, 10 (7), 829-37.
- (1997), 'Human immunodeficiency virus type 1 incorporates both glycosyl phosphatidylinositol-anchored CD55 and CD59 and integral membrane CD46 at levels that protect from complement-mediated destruction', *J Gen Virol*, 78 ( Pt 8), 1907-11.
- (1995), 'Role of virion-associated glycosylphosphatidylinositol-linked proteins CD55 and CD59 in complement resistance of cell line-derived and primary isolates of HIV-1', *J Exp Med*, 182 (2), 501-9.
- Sakoda, T., et al. (2007), 'Lentiviral vector-mediated gene transfer to endothelial cells compared with adenoviral and retroviral vectors', *Prep Biochem Biotechnol*, 37 (1), 1-11.
- Sandrin, V., et al. (2002), 'Lentiviral vectors pseudotyped with a modified RD114 envelope glycoprotein show increased stability in sera and augmented transduction of primary lymphocytes and CD34+ cells derived from human and nonhuman primates', *Blood*, 100 (3), 823-32.

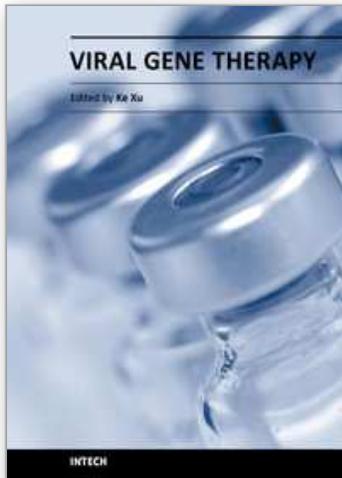
- Santoni de Sio, F. R., et al. (2006), 'Proteasome activity restricts lentiviral gene transfer into hematopoietic stem cells and is down-regulated by cytokines that enhance transduction', *Blood*, 107 (11), 4257-65.
- Schellingerhout, D. and Bogdanov, A. A., Jr. (2002), 'Viral imaging in gene therapy noninvasive demonstration of gene delivery and expression', *Neuroimaging Clin N Am*, 12 (4), 571-81, vi-vii.
- Segura, M. M., Kamen, A., and Garnier, A. (2008a), 'Purification of retrovirus particles using heparin affinity chromatography', *Methods Mol Biol*, 434, 1-11.
- Segura, M. M., et al. (2010), 'New protocol for lentiviral vector mass production', *Methods Mol Biol*, 614, 39-52.
- (2008b), 'Identification of host proteins associated with retroviral vector particles by proteomic analysis of highly purified vector preparations', *J Virol*, 82 (3), 1107-17.
- Shah, K., et al. (2004), 'Molecular imaging of gene therapy for cancer', *Gene Ther*, 11 (15), 1175-87.
- Shaw, A. S. (2006), 'Lipid rafts: now you see them, now you don't', *Nat Immunol*, 7 (11), 1139-42.
- Sheridan, C. (2011), 'Gene therapy finds its niche', *Nat Biotechnol*, 29 (2), 121-8.
- Skountzou, I., et al. (2007), 'Incorporation of glycosylphosphatidylinositol-anchored granulocyte- macrophage colony-stimulating factor or CD40 ligand enhances immunogenicity of chimeric simian immunodeficiency virus-like particles', *J Virol*, 81 (3), 1083-94.
- Snitkovsky, S. and Young, J. A. (2002), 'Targeting retroviral vector infection to cells that express heregulin receptors using a TVA-heregulin bridge protein', *Virology*, 292 (1), 150-5.
- Snitkovsky, S., et al. (2001), 'Targeting avian leukosis virus subgroup A vectors by using a TVA-VEGF bridge protein', *J Virol*, 75 (3), 1571-5.
- (2000), 'A TVA-single-chain antibody fusion protein mediates specific targeting of a subgroup A avian leukosis virus vector to cells expressing a tumor-specific form of epidermal growth factor receptor', *J Virol*, 74 (20), 9540-5.
- Somia, N. V., Zoppe, M., and Verma, I. M. (1995), 'Generation of targeted retroviral vectors by using single-chain variable fragment: an approach to in vivo gene delivery', *Proc Natl Acad Sci U S A*, 92 (16), 7570-4.
- Spitzer, D., et al. (2003), 'Green fluorescent protein-tagged retroviral envelope protein for analysis of virus-cell interactions', *J Virol*, 77 (10), 6070-5.
- Strang, B. L., et al. (2004), 'Characterization of HIV-1 vectors with gammaretrovirus envelope glycoproteins produced from stable packaging cells', *Gene Ther*, 11 (7), 591-8.
- Subbiah, R., Veerapandian, M., and Yun, K. S. (2010), 'Nanoparticles: functionalization and multifunctional applications in biomedical sciences', *Curr Med Chem*, 17 (36), 4559-77.
- Tai, C. K., et al. (2003), 'Antibody-mediated targeting of replication-competent retroviral vectors', *Hum Gene Ther*, 14 (8), 789-802.
- Tunster, S. J., Van De Pette, M., and John, R. M. (2011), 'BACs as tools for the study of genomic imprinting', *J Biomed Biotechnol*, 283013.

- Vakeva, A., et al. (1994), 'High-density lipoproteins can act as carriers of glycoposphoinositol lipid-anchored CD59 in human plasma', *Immunology*, 82 (1), 28-33.
- van der Zande, M., et al. (2011), 'Carbon nanotubes in animal models: a systematic review on toxic potential', *Tissue Eng Part B Rev*, 17 (1), 57-69.
- Verhoeven, E. and Cosset, F. L. (2004), 'Surface-engineering of lentiviral vectors', *J Gene Med*, 6 Suppl 1, S83-94.
- Waehler, R., Russell, S. J., and Curiel, D. T. (2007), 'Engineering targeted viral vectors for gene therapy', *Nat Rev Genet*, 8 (8), 573-87.
- Warden, C., Tang, Q., and Zhu, H. (2011), 'Herpesvirus BACs: past, present, and future', *J Biomed Biotechnol*, 2011, 124595.
- Watson, D. J., et al. (2002), 'Targeted transduction patterns in the mouse brain by lentivirus vectors pseudotyped with VSV, Ebola, Mokola, LCMV, or MuLV envelope proteins', *Mol Ther*, 5 (5 Pt 1), 528-37.
- Williams, S. L., Eccleston, M. E., and Slater, N. K. (2005a), 'Affinity capture of a biotinylated retrovirus on macroporous monolithic adsorbents: towards a rapid single-step purification process', *Biotechnol Bioeng*, 89 (7), 783-7.
- Williams, S. L., et al. (2005b), 'Affinity recovery of Moloney Murine Leukaemia Virus', *J Chromatogr B Analyt Technol Biomed Life Sci*, 820 (1), 111-9.
- Wong, L. F., et al. (2004), 'Transduction patterns of pseudotyped lentiviral vectors in the nervous system', *Mol Ther*, 9 (1), 101-11.
- Wong, S. and Kwon, Y. J. (2011), 'Synthetically functionalized retroviruses produced from the bioorthogonally engineered cell surface', *Bioconjug Chem*, 22 (2), 151-5.
- Yang, G., et al. (2006a), 'Retrovirus molecular conjugates: a versatile and efficient gene transfer vector system for primitive human hematopoietic progenitor cells', *Cancer Gene Ther*, 13 (5), 460-8.
- Yang, H., et al. (2008a), 'Gamma-retroviral vectors enveloped with an antibody and an engineered fusogenic protein achieved antigen-specific targeting', *Biotechnol Bioeng*, 101 (2), 357-68.
- Yang, L., et al. (2006b), 'Targeting lentiviral vectors to specific cell types in vivo', *Proc Natl Acad Sci U S A*, 103 (31), 11479-84.
- (2008b), 'Engineered lentivector targeting of dendritic cells for in vivo immunization', *Nat Biotechnol*, 26 (3), 326-34.
- Ye, K., et al. (2004), 'Tagging retrovirus vectors with a metal binding peptide and one-step purification by immobilized metal affinity chromatography', *J Virol*, 78 (18), 9820-7.
- Zavada, J. (1982), 'The pseudotypic paradox', *J Gen Virol*, 63 (Pt 1), 15-24.
- Zavorotinskaya, T. and Albritton, L. M. (2001), 'Two point mutations increase targeted transduction and stabilize vector association of a modified retroviral envelope protein', *Mol Ther*, 3 (3), 323-8.
- Zeng, Y., et al. (2009), 'High-efficiency labeling of sialylated glycoproteins on living cells', *Nat Methods*, 6 (3), 207-9.
- Zhang, X. Y., La Russa, V. F., and Reiser, J. (2004), 'Transduction of bone-marrow-derived mesenchymal stem cells by using lentivirus vectors pseudotyped with modified RD114 envelope glycoproteins', *J Virol*, 78 (3), 1219-29.
- Zhao, Y., et al. (1999), 'Identification of the block in targeted retroviral-mediated gene transfer', *Proc Natl Acad Sci U S A*, 96 (7), 4005-10.

- Ziegler, L., et al. (2008), 'Targeting lentiviral vectors to antigen-specific immunoglobulins', *Hum Gene Ther*, 19 (9), 861-72.
- Zielske, S. P. and Gerson, S. L. (2003), 'Cytokines, including stem cell factor alone, enhance lentiviral transduction in nondividing human LTCIC and NOD/SCID repopulating cells', *Mol Ther*, 7 (3), 325-33.

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The development of technologies that allow targeting of specific cells has progressed substantially in recent years for several types of vectors, particularly viral vectors, which have been used in 70% of gene therapy clinical trials. Particular viruses have been selected as gene delivery vehicles because of their capacities to carry foreign genes and their ability to efficiently deliver these genes associated with efficient gene expression. This book is designed to present the most recent advances in viral gene therapy

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