



Introduction for “Safety Considerations for Retroviral Vectors: A Short Review”

Donald E. Mosier

The Scripps Research Institute, La Jolla, California

Author’s Note

This mini-review was initially written in 2002 as an institutional training document and was subsequently distributed to interested individuals on the Biosafety Discussion List (BIOSAFTY@mitvma.mit.edu). Two events prompted its updating and publication for a wider audience. First, there were two cases of leukemia associated with retroviral-mediated gene therapy in children with severe combined immune deficiency, which have resulted in a cessation of gene therapy trials in the United States and Europe. Secondly, we have continued to receive requests for this review, and it has resurfaced from secondary sources, so we were pleased to respond to Karen Byers’ request to submit the review to *Applied Biosafety*. Finally, we continue to encounter researchers who assume that retrovirus vectors must be safe because they are just little pieces of nucleic acid that you can buy from commercial vendors.

Abstract

Retroviral vectors are becoming standard tools in cell biology as well as potential therapeutic agents for human disease. Many investigators have come to believe that retroviral vectors are safe, but both current biosafety guidelines and distributors of vectors recommend using the vectors under Biosafety level-2 (BSL-2) containment, with certain experiments requiring BSL-3 practices. This short review considers the dangers posed by the large variety of retroviral vectors and gives the rationale for safety evaluation.

I. Introduction

The safety of retrovirus vectors for use in human clinical trials has been an issue since the promise of “gene therapy” was first recognized. Howard Temin, the co-discoverer of reverse transcriptase and a Nobel laureate, wrote about the safety of retrovirus vectors in 1990 (Temin, 1990). He said, “Although [gene therapy] involves recombinant DNA technologies and modified retroviruses, proper design of the vectors and delivery systems removes most potential foreseen risks. Furthermore, even in the very remote possibility that there is a non-therapeutic biological effect of the treatment, it is unlikely to be a harmful one. Thus, safety considerations should not hold up further human trials of retrovirus vectors.”

However, a preclinical trial of retrovirus vectors in bone marrow transplantation of primates performed shortly after this optimistic statement had a sobering outcome (Donahue et al., 1992; Vanin et al., 1994). Three of the 10 monkeys developed fatal lymphomas following transplantation with retrovirus-transduced, autologous bone marrow progenitor (CD34⁺) cells. The explanation for the death of these animals was that replication competent retroviruses (RCR) had arisen by two distinct recombination events during vector production. These viruses infected monkey T lymphocytes and induced tumors by insertional mutagenesis (Vanin et al., 1994). One event involved recombination between vector coding sequences and the helper packaging sequences, resulting in RCR formation. The second RCR was generated by a second recombination event involv-

ing the first RCR and endogenous murine retroviral sequences in the packaging cell line (Vanin et al., 1994). Despite many improvements in the design of retroviral vectors and packaging cell lines, generation of RCR still occurs (Chong, Starkey, & Vile, 1998; Otto et al., 1994). The dual aims of producing very high titers of infectious retrovirus vectors for efficient transduction of target cells and preventing rare recombination events are inherently at odds. Recombination between two RNA vectors appears to occur when the two different viral RNAs are packaged into the same virus particle, and occurs at a frequency of about 10^4 per virus replication cycle (Stuhlmann & Berg, 1992). Reducing regions of homology between packaging and vector sequences can reduce but not eliminate the risk of recombination (Sheridan et al., 2000). Other safety modifications, such as deletions in parts of the viral genome (the long terminal repeat or LTR) that reduce the probability of replication of an RCR in the producer cell line, may not have the same effect when that virus infects human cells (Reuss et al., 2001a; Reuss et al., 2001b). A candidate HIV vaccine vector with deletions in the LTR as well as two other genes recovered virulence after 2 months in culture. The cause was the duplication of the LTR sequence binding the Sp1 transcription factor, allowing more efficient replication of the “crippled” virus (Berkhout et al., 1999). The goal of engineering safe retroviral vectors, which seemed so close in 1990, has proven to be remarkably difficult.

Despite these safety concerns, there has been no evidence of the generation of RCR in patients who have participated in clinical trials of gene therapy involving retrovirus-based vectors (Wilson, Ng, & Miller, 1997). Although this has been taken to indicate that current vectors are safe, this is not necessarily the case. The low number of virally transduced cells found in these patients, as well as the silencing of transcription from integrated vector proviruses, has limited both the clinical benefit and the risk of vector rescue by recombination (Kohn, 2001). The safety of retroviral vectors for both introduction into humans and for use in basic research continues to be an important issue. A recent report (Li et al., 2002) documents that mouse stem cells marked by a clinically used mouse retrovirus vector caused leukemia in recipient animals because of two rare events:

insertional mutagenesis and cooperation between the activated host gene and the introduced transgene. Unfortunately, a similar event in human gene therapy trials has brought clinical use of retroviral vectors to a halt. Infants with Severe Combined Immunodeficiency (SCID) because of mutations in the IL-2 receptor common gamma chain (IL2RG) were successfully treated by transduction of autologous stem cells using retrovirus vectors encoding normal IL2RG. Two of these infants developed leukemias caused by insertion of the retrovirus sequence adjacent to the LMO2 oncogene (Check, 2003; Hacein-Bey-Abina et al., 2003a; Hacein-Bey-Abina et al., 2003b). A recent review summarizes the postulated mechanism for leukemia induction in these patients (McCormack & Rabbitts, 2004). In retrospect, the warnings from preclinical studies should have been heeded.

Acceptance of some low level risk may be justified when attempting to treat a life threatening disease. For example, we know that radiation or chemotherapy for cancer increases the risk of secondary cancers many years later. This is viewed as an acceptable risk/benefit ratio given the dire consequences of not treating the primary cancer. However, use of retrovirus vectors in a research setting has a less tangible immediate benefit, and it is necessary to consider the low level risks more seriously.

II. Generation of Retrovirus Vectors

A recent review has summarized production of the many variants of retrovirus vectors (Hu & Pathak, 2000). Production of retroviral vectors has a common strategy, although details may vary. Retroviruses package RNA molecules into virus particles. Normally, the double stranded RNA retrovirus genome is packaged into virions, but retrovirus packaging cell lines (also known as helper cells) are constructed in order to package other RNA molecules (Figure 1). These RNA molecules have limited retroviral sequences and commonly express a messenger RNA of interest (the “vector sequence”) as well as a selectable marker such as a drug-resistance gene. Figure 1 illustrates a typical scenario in which a packaging cell line is stably transfected with two partial (split) retroviral genomes. One construct contains

the gag/pol region that encodes proteins required for virus particle assembly and reverse transcription (copying the double-stranded RNA insert into DNA), and the second construct contains the env gene that encodes the proteins needed for virus binding to, and entry into, target cells. The viral RNA encoding these functions is not packaged into virus particles because the RNA sequences needed for binding to gag proteins (the packaging signal, or Ψ) have been deleted. The vector sequence containing the packaging signal is transfected into the packaging cells, and inclusion of the packaging signal in the construct insures that the vector sequence is packaged into virus particles.

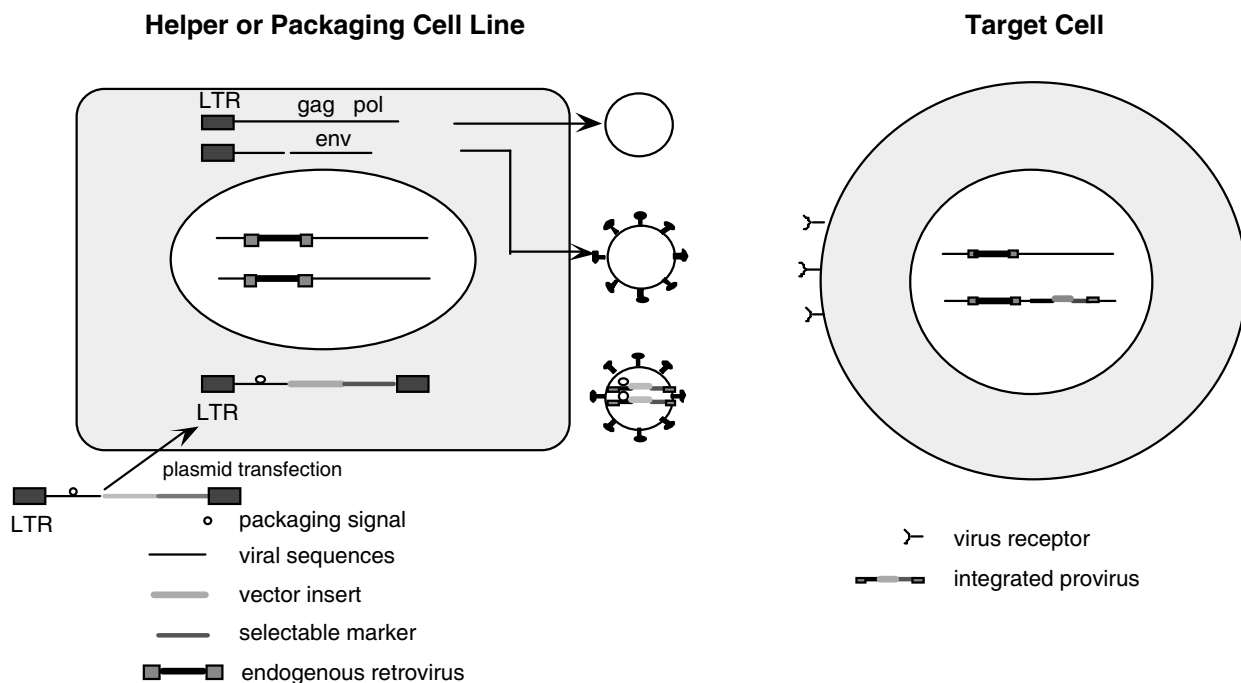
Virus particles are harvested from packaging cell lines transfected with a vector sequence, and these particles are used to "transduce" the vector sequence (as well as the retrovirus RNA) into target cells bearing the appropriate receptors for the retroviral or other viral envelope expressed on the virus particles.

"Transduction" is in essence a one-time infection since the viral particles are infectious, but their genetic information is insufficient to generate new infectious virus *unless* some rare rescue event takes place.

Because none of the retrovirus genomes expressed in packaging cell lines is intact, no replication competent viruses are produced unless a rare and specific recombination event generates an intact retroviral genome. The virus particles are "infectious" for only one replication cycle. They can bind and enter target cells expressing appropriate receptors, although very low levels of virus entry may occur in the absence of specific receptor binding (Pizzato et al., 2001). The vector sequence is reverse transcribed into DNA, and the two retroviral LTR and the viral integrase mediate integration of the vector sequence into the target cell DNA. The integrated vector DNA becomes a permanent part of the target cell genome, and it is thus possible that rescue

Figure 1

Typical construction of packaging (helper) cell lines and retroviral vectors. Dangers associated with retrovirus vectors involve regeneration of replication competent retrovirus by recombination with vector or endogenous retrovirus sequences in either the packaging cell line or the target cell. Vector sequences integrate into target cell DNA and may rarely generate mutations or alter host gene expression in ways that predispose to cancer or other disorders.



of RCR by recombination with endogenous retroviral elements or exogenous retroviral infection (e.g., HIV-1) can occur many years after the initial transduction of target cells.

Generation of replication competent retroviruses in target cells or tissues is the primary risk associated with the use of retroviral vectors. Assessment of this risk is the first task in determining the safety of retroviral vectors. The target cell range of the vector is also a safety issue. Incorporation of a virus envelope that can infect cells from multiple species increases the risk of both RCR generation and the potential danger of any resulting virus, which could spread from one species to another. The species' tropism of various retroviral envelopes and their cellular receptors are listed in Table 1 (Overbaugh, Miller, & Eiden, 2001). Most of these cellular receptors for retroviruses are widely distributed in mammalian species, including humans. In addition to

retrovirus envelopes, the packaging of RNA in particles with the envelope protein of vesicular stomatitis virus (VSV-G protein; VSV-G pseudotyping) provides a broad target cell range because most cell types express the phospholipids to which VSV-G protein binds (von Laer et al., 2000).

III. Safety Assessment

A number of issues should be reviewed when assessing the safety of retrovirus vectors. These are summarized in Table 2.

Recombination between vector and packaging sequences, between vector and endogenous retroviruses, or between vector and exogenous retroviruses may generate new replication competent retroviruses (RCR). Homology between vectors and other retroviral sequences increases the risk of RCR. Therefore, the sequence of the packaging compo-

Table 1

Receptors and Species Tropism for Retroviral Vector Envelopes (Overbaugh, Miller, & Eiden, 2001)

Retrovirus	Genus	Receptor	Type	Function	Tropism
MoMLV	Gammaretrovirus	CAT-1	TM14	amino acid transport	ecotropic. mouse
X-MLV	Gammaretrovirus	XPR1	TM8	unknown	xenotropic. human, others
P-MLV	Gammaretrovirus	XPR1	TM8	unknown	polytropic, mouse & human
A-MLV	Gammaretrovirus	Pit-2	TM10-13	phosphate transport	amphotropic, mouse & human
GALV	Gammaretrovirus	Pit-1	TM10-13	phosphate transport	primate & human
HERV-W	Gammaretrovirus	RDR	TM9-10	amino acid transport	human
SRV-1-5	Gammaretrovirus	RDR	TM9-10	amino acid transport	primate
HIV-1, HIV-2	Lentivirus	CD4, CCR5/ CXCR4	TM1, TM7	MHCII binding, chemokine receptor	human
SIV-1	Lentivirus	CD4, CCR5, others	TM1, TM7	MHCII binding, chemokine receptor	primate. human
FIV-1	Lentivirus	CXCR4, HS	TM7	chemokine receptor	feline, human

Abbreviations: MoMLV, Moloney Murine Leukemia Virus; X-MLV, xenotropic MLV; P-MLV, polytropic MLV; A-MLV, amphotropic MLV; GALV, gibbon ape Leukemia Virus; HERV-W, human endogenous retrovirus group W; SRV 1-5, simian retroviruses type 1-5; HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; FIV, feline immunodeficiency virus; CAT-1, cationic amino acid transporter 1; XPR-1, xenotropic, polytropic receptor 1; Pit-1/2, phosphate transporter 1 or 2; RDR, RD-114 and D-type retrovirus receptor; CCR5, C-C chemokine receptor 5; CXCR4, CXC chemokine receptor 4; HS, heparan sulfate; TM, transmembrane

Table 2

Safety Assessment of Retrovirus Vectors

A. Test for replication competent retroviruses (RCR) [see Table 3]

1. Test virus vectors for growth on cell lines appropriate for envelope of vector.
2. Assess virus sequences for homology. Greater homology increases risk of RCR.

B. Characteristics of packaging cell line

1. Origin of species
2. Endogenous retrovirus sequences and expression
3. Vector sequences and expression
4. Cell tropism of envelope expressed in vector virions

C. Characteristics of target cells

1. Origin of species
2. Endogenous retrovirus sequences and expression
3. Susceptibility to exogenous retroviruses

D. Infectious titer of vector

1. Very high titered vector may exceed safety testing limits for RCR.
2. More virus replication cycles increase risk of recombination.

E. Fate of retroviral vector transduced cells

1. Introduction into animals or humans?
2. Mixing with cells or tissues from different species?
3. Possible interaction with exogenous retroviruses; e.g., HIV-1, X-MLV?

F. Genes expressed in vector

1. Does the vector encode oncogenes, or genes that might alter growth regulation or impact immunity?
2. Does the vector encode genes from infectious organisms that might be pathogenic if expressed (e.g., HIV-1 nef), or might recombine with exogenous infectious agents?

nents and the vector should be examined. Many packaging cell lines have been constructed, but the twin goals of safety and high output of infectious virus often conflict. The ability of the vector to infect target cells is determined by the type of virus envelope expressed. Vectors that infect humans should not be generated unless it is essential to target human cells. Even retroviral vectors expressing the mouse ecotropic envelope protein can become potentially infectious for human cells by recombination events with mouse endogenous retrovirus genes encoding amphotropic, polytropic, or xenotropic envelopes (Table 1).

Introduction of vector-infected cells into animals or humans increases the risk of recombination events because subsequent virus infections or activation of endogenous retroviruses can rescue RNA transcribed from integrated proviral vector sequences. In fact, rescue of lentivirus vectors by ex-

ogenous HIV-1 infection has been demonstrated in tissue culture (Evans & Garcia, 2000) and may limit the choice of lentivirus vectors for clinical use. Experiments in which cells from two species are intermixed (xenotransplantation) pose special risks for interactions between endogenous viruses and retroviral vectors (van der Laan et al., 2000). Porcine tissue harbors pig endogenous retroviruses (PERV) that are replication competent and have been shown to be infectious for human cells (van der Laan et al., 2000). Rescue of vector sequences in human cells could thus be mediated not only by human retroviruses but also by PERV in a xenotransplantation setting.

The infectious titer of the retroviral vector and the duration of the planned experiments are an issue, since recombination events are rare and their probability increases with both time and the number of virus replication cycles. Safety measures to prevent

generation of RCR may not always work. For example, self-inactivating retrovirus (SIN) vectors involve a deletion in the 3' long terminal repeat (LTR) that should block reverse transcription (Zufferey et al., 1998). However, examples of such HIV-1 constructs have reverted to replication competence by deletion of additional LTR sequences (Vicenzi et al., 1994). Endogenous human retrovirus sequences produce active reverse transcriptase (Berkhout, Jebbink, & Zsiros, 1999) and envelope in human cells (An, Xie, & Chen, 2001). Expression of these proteins can lead to copying of vector sequences and pseudotype formation (packaging of vector sequences in particles with human endogenous retrovirus (HERV-W) envelope and spread vector or infectious virus sequences to new target cells (An, Xie, & Chen, 2001).

A third consideration in risk assessment is the nature of the vector-coding sequence. Marker genes such as green fluorescent protein (GFP) pose no special risk (unless one is concerned with turning the proverbial green thumb into a literal green thumb). However, vectors that include genes involved in oncogenesis, growth regulation, innate or adaptive immunity, or infectious diseases obviously carry a greater risk. A strong oncogene (e.g., *ras*) in a vector that is later rescued into an RCR by recombination events would recreate a human version of mouse leukemia viruses.

The devastating HIV-1 pandemic resulted from the cross-species transmission of a primate retrovirus (Hahn et al., 2000). Continued vigilance when using retroviral vectors is essential to prevent generation of RCR with potential pathogenic potential. The widespread availability of retrovirus vectors for laboratory

use and the generation of "safer" vectors appears to have resulted in a sense of false security, particularly among first-time users of vectors. Biosafety level 2 containment and careful monitoring of long-term or animal experiments for the emergence of vector-derived RCR are necessary to ensure safety (Table 3). In some cases, the vector construct and/or the nature of the experiment may dictate BSL-3 practices used in conjunction with BSL-2 containment. The experiments described by van der Laan et al. (2000), in which human and porcine cells were transplanted to immunodeficient mice, provided the opportunity for intermixing of retroviruses from three species. These experiments were conducted using BSL-3 practices and containment. Understanding the factors associated with the risks of RCR generation listed in Table 2 will aid in the design of safer experiments and vectors, and should promote better monitoring of experiments with higher risk.

Acknowledgements

The author wishes to thank Richard Gulizia and Carolyn Keierleber for their thoughtful review of this manuscript. The author is chairperson of the Institutional Biosafety Committee at Scripps; RG is Director of BSL-3 laboratories; and CK is Director of Environmental & Health Services and Biosafety Officer.

References

An, D. S., Xie, Y., & Chen, I. S. (2001). Envelope gene of the human endogenous retrovirus HERV-W

Table 3
Methods for Testing for Replication Competent Retroviruses

Vector Envelope	Cell Line	Positive Result
A-MLV, P-MLV, X-MLV	Feline PG-4(Bassin et al., 1982) 14 day M. dunni cell expansion + PG-4 (Li, Blair, & Thorner, 1999)	Infectious foci Infectious foci
A-MLV, P-MLV, X-MLV	M. dunni LacZ reporter line (Forestell et al., 1996)	Blue foci (with substrate)
Lentivirus with VSV-G	Permissive human or other species cell line	Capsid p24 (or p27) antigen accumulation by ELISA

- encodes a functional retrovirus envelope. *Journal of Virology*, 75, 3488-3489.
- Bassin, R. H., Ruscetti, S., Ali, I., Haapala, D. K., & Rein, A. (1982). Normal DBA/2 mouse cells synthesize a glycoprotein which interferes with MCF virus infection. *Virology*, 123, 139-151.
- Berkhout, B., Jebbink, M., & Zsiros, J. (1999). Identification of an active reverse transcriptase enzyme encoded by a human endogenous HERV-K retrovirus. *Journal of Virology*, 73, 2365-2375.
- Berkhout, B., Verhoef, K., van Wamel, J. L., & Back, N. K. (1999). Genetic instability of live, attenuated human immunodeficiency virus type 1 vaccine strains. *Journal of Virology*, 73, 1138-1145.
- Check, E. (2003). Harmful potential of viral vectors fuels doubts over gene therapy. *Nature*, 423, 573-574.
- Chong, H., Starkey, W., & Vile, R. G. (1998). A replication-competent retrovirus arising from a split-function packaging cell line was generated by recombination events between the vector, one of the packaging constructs, and endogenous retroviral sequences. *Journal of Virology*, 72, 2663-2670.
- Donahue, R. E., Kessler, S. W., Bodine, D., McDonagh, K., Dunbar, C., Goodman, S., Agricola, B., Byrne, E., Raffeld, M., Moen, R., et al. (1992). Helper virus induced T cell lymphoma in nonhuman primates after retroviral mediated gene transfer. *Journal of Experimental Medicine*, 176, 1125-1135.
- Evans, J. T., & Garcia, J. V. (2000). Lentivirus vector mobilization and spread by human immunodeficiency virus. *Human Gene Therapy*, 11, 2331-2339.
- Forestell, S. P., Dando, J. S., Bohnlein, E. & Rigg, R. J. (1996). Improved detection of replication-competent retrovirus. *Journal of Virology Methods*, 60, 171-178.
- Hacein-Bey-Abina, S., von Kalle, C., Schmidt, M., Le Deist, F., Wulffraat, N., McIntyre, E., Radford, I., Villeval, J. L., Fraser, C. C., Cavazzana-Calvo, M., & Fischer, A. (2003a). A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *New England Journal of Medicine*, 348, 255-256.
- Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M. P., Wulffraat, N., Leboulch, P., Lim, A., Osborne, C. S., Pawliuk, R., Morillon, E., Sorensen, R., Forster, A., Fraser, P., Cohen, J. I., de Saint Basile, G., Alexander, I., Wintergerst, U., Frebourg, T., Aurias, A., Stoppa-Lyonnet, D., Romana, S., Radford-Weiss, I., Gross, F., Valensi, F., Delabesse, E., Macintyre, E., Sigaux, F., Soulier, J., Leiva, L. E., Wissler, M., Prinz, C., Rabbitts, T. H., Le Deist, F., Fischer, A., & Cavazzana-Calvo, M. (2003b). LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science*, 302, 415-419.
- Hahn, B. H., Shaw, G. M., De Cock, K. M., & Sharp, P. M. (2000). AIDS as a zoonosis: Scientific and public health implications. *Science*, 287, 607-614.
- Hu, W. S., & Pathak, V. K. (2000). Design of retroviral vectors and helper cells for gene therapy. *Pharmacology Review*, 52, 493-511.
- Kohn, D. B. (2001). Gene therapy for genetic hematological disorders and immunodeficiencies. *Journal of Internal Medicine*, 249, 379-390.
- Li, Z., Blair, M., & Thorner, L. (1999). PG-4 cell plaque assay for xenotropic murine leukemia virus. *Journal of Virology Methods* 81, 47-53.
- Li, Z., Dullmann, J., Schiedlmeier, B., Schmidt, M., von Kalle, C., Meyer, J., Forster, M., Stocking, C., Wahlers, A., Frank, O., Ostertag, W., Kuhlcke, K., Eckert, H. G., Fehse, B., & Baum, C. (2002). Murine leukemia induced by retroviral gene marking. *Science*, 296, 497.
- McCormack, M., & Rabbitts, T. (2004) Activation of the T-cell oncogene LMO2 after gene therapy for x-linked severe combined immunodeficiency. *New England Journal of Medicine*, 350, 913-922.

- Otto, E., Jones-Trower, A., Vanin, E. F., Stambaugh, K., Mueller, S. N., Anderson, W. F., & McGarrity, G. J. (1994). Characterization of a replication-competent retrovirus resulting from recombination of packaging and vector sequences. *Human Gene Therapy*, 5, 567-575.
- Overbaugh, J., Miller, A. D., & Eiden, M. V. (2001). Receptors and entry cofactors for retroviruses include single and multiple transmembrane-spanning proteins as well as newly described glycoposphatidylinositol-anchored and secreted proteins. *Microbiology & Molecular Biology Reviews*, 65, 371-389.
- Pizzato, M., Blair, E., Fling, M., Kopf, J., Tomassetti, A., Weiss, R., & Takeuchi, Y. (2001). Evidence for nonspecific adsorption of targeted retrovirus vector particles to cells. *Gene Therapy*, 8, 1088-1096.
- Reuss, F. U., Berdel, B., Ploss, M. & Heber, R. (2001a). Replication of enhancer-deficient amphotropic murine leukemia virus in human cells. *Proceedings of the National Academy of Science USA*, 4, 4.
- Reuss, F. U., Heber, R., Ploss, A., & Berdel, B. (2001b). Amphotropic murine leukemia virus replication in human mammary epithelial cells and the formation of cytomegalovirus-promoter recombinants. *Virology*, 291, 91-100.
- Sheridan, P. L., Bodner, M., Lynn, A., Phuong, T. K., DePolo, N. J., de la Vega, D. J., Jr., O'Dea, J., Nguyen, K., McCormack, J. E., Driver, D. A., Townsend, K., Ibanez, C. E., Sajjadi, N. C., Greengard, J. S., Moore, M. D., Respass, J., Chang, S. M., Dubensky, T. W., Jr., Jolly, D. J., & Sauter, S. L. (2000). Generation of retroviral packaging and producer cell lines for large-scale vector production and clinical application: Improved safety and high titer. *Molecular Therapy*, 2, 262-275.
- Stuhlmann, H., & Berg, P. (1992). Homologous recombination of copackaged retrovirus RNAs during reverse transcription. *Journal of Virology*, 66, 2378-2388.
- Temin, H. M. (1990). Safety considerations in somatic gene therapy of human disease with retrovirus vectors. *Human Gene Therapy*, 1, 111-123.
- van der Laan, L. J., Lockey, C., Griffeth, B. C., Frasier, F. S., Wilson, C. A., Onions, D. E., Hering, B. J., Long, Z., Otto, E., Torbett, B. E., & Salomon, D. R. (2000). Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. *Nature*, 407, 90-94.
- Vanin, E. F., Kaloss, M., Broscius, C., & Nienhuis, A. W. (1994). Characterization of replication-competent retroviruses from nonhuman primates with virus-induced T-cell lymphomas and observations regarding the mechanism of oncogenesis. *Journal of Virology*, 68, 4241-4250.
- Vicenzi, E., Dimitrov, D. S., Engelman, A., Migone, T. S., Purcell, D. F., Leonard, J., Englund, G., & Martin, M. A. (1994). An integration-defective U5 deletion mutant of human immunodeficiency virus type 1 reverts by eliminating additional long terminal repeat sequences. *Journal of Virology*, 68, 7879-7890.
- von Laer, D., Corovic, A., Vogt, B., Herwig, U., Roscher, S., Fehse, B., & Baum, C. (2000). Amphotropic and VSV-G-pseudotyped retroviral vectors transduce human hematopoietic progenitor cells with similar efficiency. *Bone Marrow Transplant*, 25(suppl 2), S75-79.
- Wilson, C. A., Ng, T. H., & Miller, A. E. (1997). Evaluation of recommendations for replication-competent retrovirus testing associated with use of retroviral vectors. *Human Gene Therapy*, 8, 869-874.
- Zufferey, R., Dull, T., Mandel, R. J., Bukovsky, A., Quiroz, D., Naldini, L., & Trono, D. (1998). Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *Journal of Virology*, 72, 9873-9880.