



# CLINIGENE CURRENT GENE THERAPY WEEKLY

From April 19<sup>th</sup> to April 26<sup>th</sup> 2010

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**PMID:**  
**20414973**

J Cell Mol Med. 2010 Jan;14(1-2):290-302.

**Genetic transfer of fusion proteins effectively inhibits VCAM-1-mediated cell adhesion and transmigration via inhibition of cytoskeletal anchorage.**

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The adhesion of leukocytes to endothelium plays a central role in the development of atherosclerosis and thus represents an attractive therapeutic target for anti-atherosclerotic therapies. Vascular cell adhesion molecule-1 (VCAM-1) mediates both the initial tethering and the firm adhesion of leukocytes to endothelial cells. Our work evaluates the feasibility of using the cytoskeletal anchorage of VCAM-1 as a target for gene therapy. As a proof of concept, integrin  $\alpha$ 5 $\beta$ 3-mediated cell adhesion with clearly defined cytoskeletal anchorage was tested. We constructed fusion proteins containing the intracellular domain of  $\beta$ 3 placed at various distances to the cell membrane. Using cell adhesion assays and immunofluorescence, we established fusion constructs with competitive and dominant negative inhibition of cell adhesion. With the goal being the transfer of the dominant negative mechanism towards VCAM-1 inhibition, we constructed a fusion molecule containing the cytoplasmic domain of VCAM-1. Indeed, VCAM-1 mediated leukocyte adhesion can be inhibited via transfection of DNA encoding the designed VCAM-1 fusion protein. This is demonstrated in adhesion assays under static and flow conditions using CHO cells expressing recombinant VCAM-1 as well as activated endothelial cells. Thus, we are able to describe a novel approach for dominant negative inhibition of leukocyte adhesion to endothelial cells. This approach warrants further development as a novel gene therapeutic strategy that aims for a locally restricted effect at atherosclerotic areas of the vasculature.

PMID:  
20414916

ChemMedChem. 2010 Apr 22. [Epub ahead of print]

**Gene Therapy in HIV-Infected Cells to Decrease Viral Impact by Using an Alternative Delivery Method.**

Gonzalo T, Clemente MI, Chonco L, Weber ND, Díaz L, Serramía MJ, Gras R, Ortega P, de la Mata FJ, Gómez R, Lopez-Fernández LA, Muñoz-Fernández MA, Jiménez JL.

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The ability of dendrimer 2G-[Si{O(CH<sub>2</sub>)(<sub>2</sub>)N(Me)(<sub>2</sub>) (+)(CH<sub>2</sub>)(<sub>2</sub>)NMe(<sub>3</sub>) (+)(I(-))(<sub>2</sub>)}](<sub>8</sub>) (NN16) to transfect a wide range of cell types, as well as the possible biomedical application in direct or indirect inhibition of HIV replication, was investigated. Cells implicated in HIV infection such as primary peripheral blood mononuclear cells (PBMC) and immortalized suspension cells (lymphocytes), primary macrophages and dendritic cells, and immortalized adherent cells (astrocytes and trophoblasts) were analyzed. Dendrimer toxicity was evaluated by mitochondrial activity, cell membrane rupture, release of lactate dehydrogenase, erythrocyte hemolysis, and the effect on global gene expression profiles using whole-genome human microarrays. Cellular uptake of genetic material was determined using flow cytometry and confocal microscopy. Transfection efficiency and gene knockdown was investigated using dendrimer-delivered antisense oligonucleotides and small interfering RNA (siRNA). Very little cytotoxicity was detected in a variety of cells relevant to HIV infection and erythrocytes after NN16 dendrimer treatment. Imaging of cellular uptake showed high transfection efficiency of genetic material in all cells tested. Interestingly, NN16 further enhanced the reduction of HIV protein 24 antigen release by antisense oligonucleotides due to improved transfection efficiency. Finally, the dendrimer complexed with siRNA exhibited therapeutic potential by specifically inhibiting cyclooxygenase-2 gene expression in HIV-infected nervous system cells. NN16 dendrimers demonstrated the ability to transfect genetic material into a vast array of cells relevant to HIV pathology, combining high efficacy with low toxicity. These results suggest that NN16 dendrimers have the potential to be used as a versatile non-viral vector for gene therapy against HIV infection.

**PMID:**  
**20414325**

Cancer Gene Ther. 2010 Apr 23. [Epub ahead of print]

**Bleomycin/interleukin-12 electrochemogenetherapy for treating naturally occurring spontaneous neoplasms in dogs.**

Reed SD, Fulmer A, Buckholz J, Zhang B, Cutrera J, Shiomitsu K, Li S.  
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On the basis of superior outcomes from electrochemogenetherapy (ECGT) compared with electrochemotherapy in mice, we determined the efficacy of ECGT applied to spontaneous canine neoplasms. Intralesional bleomycin (BLM) and feline interleukin-12 DNA injection combined with translesional electroporation resulted in complete cure of two recurrent World Health Organization stage T(2b)N(0)M(0) oral squamous cell carcinomas (SCCs) and one T(2)N(0)M(0) acanthomatous ameloblastoma. Three remaining dogs, which had no other treatment options, had partial responses to ECGT; one had mandibular T(3b)N(2b)M(1) melanoma with pulmonary and lymph node metastases; one had cubital T(3)N(0)M(1) histiocytic sarcoma with spleen metastases; and one had soft palate T(3)N(0)M(0) fibrosarcoma. The melanoma dog had decrease in the size of the primary tumor before recrudescence and euthanasia. The histiocytic sarcoma dog had resolution of the primary tumor, but was euthanized because of metastases 4 months after the only treatment. The dog with T(3)N(0)M(0) fibrosarcoma had tumor regression with recrudescence. Treatment was associated with minimal side effects and was easy to perform, was associated with repair of bone lysis in cured dogs, improved quality of life for dogs with partial responses and extended overall survival time. ECGT seems to be a safe and resulted in complete responses in SCC and acanthomatous ameloblastoma.

**PMID:**  
**20414324**

Cancer Gene Ther. 2010 Apr 23. [Epub ahead of print]

**Enhanced growth inhibition of metastatic lung tumors by intravenous injection of ATRA-cationic liposome/IL-12 pDNA complexes in mice.**

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Interleukin 12 (IL-12) is a proinflammatory cytokine with antitumor activity. All-trans-retinoic acid (ATRA) exerts antitumor effects by regulating a variety of gene expressions, including tumor necrosis factor receptor 1 (TNFR1), increases the number of TNFR1 and potentiates TNF-alpha-induced apoptosis in cancer cells. In this study, ATRA-incorporated cationic liposome (ATRA-cationic liposome)/IL-12 plasmid DNA (pDNA) complexes were prepared to improve therapeutic efficacy of cationic liposome/IL-12 pDNA complexes in a mouse model of metastatic lung tumor after intravenous injection. IL-12 production in lungs by ATRA-cationic liposome/IL-12 pDNA complexes was comparable with that by cationic liposome/IL-12 pDNA complexes. The number of metastatic tumor cells (colon26/Luc) was quantitatively evaluated by measuring luciferase activity. ATRA-cationic liposome/IL-12 pDNA complexes reduced the number of colon26/Luc cells and tumor nodules in lungs. ATRA-cationic liposome/IL-12 pDNA complexes significantly prolonged the survival time of mice, whereas cationic liposome/IL-12 pDNA only slightly prolonged it. ATRA-cationic liposome/IL-12 pDNA complexes increased the TNFR1 mRNA upregulation and the number of apoptotic cells in the lung. Moreover, reduced serum alanine transaminase (ALT) and aspartate transaminase (AST) activities were observed in mice treated with ATRA-cationic liposome/IL-12 pDNA complexes. These results suggest that intravenous injection of ATRA-cationic liposome/IL-12 pDNA complexes is an effective method for the treatment of lung metastasis in mice.

**PMID:**  
**20412688**

Comp Med. 2010;60(2):130-5.

**Guinea pig adenovirus infection does not inhibit cochlear transfection with human adenoviral vectors in a model of hearing loss.**

Hankenson FC, Wathen AB, Eaton KA, Miyazawa T, Swiderski DL, Raphael Y.  
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Routine surveillance of guinea pigs maintained within a barrier facility detected guinea pig adenovirus (GPAdV) in sentinel animals. These guinea pigs served as models of induced hearing loss followed by regeneration of cochlear sensory (hair) cells through transdifferentiation of nonsensory cells by using human adenoviral (hAV) gene therapy. To determine whether natural GPAdV infection affected the ability of hAV vectors to transfect inner ear cells, adult male pigmented guinea pigs (n = 7) were enrolled in this study because of their prolonged exposure to GPAdV-seropositive conspecifics. Animals were deafened chemically (n = 2), received an hAV vector carrying the gene for green fluorescent protein (hAV-GFP) surgically without prior deafening (n = 2), or were deafened chemically with subsequent surgical inoculation of hAV-GFP (n = 3). Cochleae were evaluated by using fluorescence microscopy, and GFP expression in supporting cells indicated that the hAV-GFP vector was able to transfect inner ears in GPAdV-seropositive guinea pigs that had been chemically deafened. Animals had histologic evidence of interstitial pneumonia, attributable to prior infection with GPAdV. These findings confirmed that the described guinea pigs were less robust animal models with diminished utility for the overall studies. Serology tests confirmed that 5 of 7 animals (71%) were positive for antibodies against GPAdV at necropsy, approximately 7 mo after initial detection of sentinel infection. Control animals (n = 5) were confirmed to be seronegative for GPAdV with clinically normal pulmonary tissue. This study is the first to demonstrate that natural GPAdV infection does not negatively affect transfection with hAV vectors into guinea pig inner ear cells, despite the presence of other health complications attributed to the viral infection.

PMID:  
20412454

Oral Dis. 2010 Apr 19. [Epub ahead of print]

**Synthetic radiation-inducible promoters mediated HSV-TK/GCV gene therapy in the treatment of oral squamous cell carcinoma.**

Yu DS, Zhao W, Huang HZ, Hu XW, Liu XQ, Tang HK.

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Oral Diseases (2010) doi: 10.1111/j.1601-0825.2010.01655.x Objective: To investigate the therapeutic effect of herpes simplex virus thymidine kinase (HSV-TK) gene mediated by synthetic radiation-inducible promoters in the treatment of oral squamous cell carcinoma (OSCC) in vitro and in vivo. Methods: The plasmids pcDNA3.1(+)-E6-HSV-TK were constructed, in which the HSV-TK genes were mediated by synthetic radiation-inducible promoters. The recombinant plasmids were transfected into the Tca8113 cells and golden hamster buccal carcinoma, respectively. Low-dose radiotherapy was used to upregulate the HSV-TK genes expression. HSV-TK mRNA was assayed by RT-PCR. Apoptosis and proliferating cell nuclear antigen were detected respectively by in situ end-labeling and immunohistochemical method. Results: Compared with control group, the comparative survival rate of Tca8113 cells in HSV-TK/GCV/IR group was markedly decreased and the golden hamster buccal carcinoma in HSV-TK/GCV/IR group was obviously suppressed. Up-regulation of HSV-TK gene expression was found in the Tca8113 cells and in the golden hamster buccal carcinoma resulting from exposure to low-dose irradiation. The apoptosis indexes in Tca8113 cells or golden hamster buccal carcinoma with irradiation were markedly higher than those without irradiation. At the same time, the proliferation indexes in Tca8113 cells or golden hamster buccal carcinoma with irradiation were markedly lower than those without irradiation. Conclusion: The results indicate that the synthetic radiation-inducible promoters can serve as a molecular switch to adjust the expression of HSV-TK gene in the treatment of OSCC, and low-dose induction radiation can significantly improve therapeutic efficiency.

**PMID:**  
**20412417**

J Periodontal Res. 2010 Apr 19. [Epub ahead of print]

**Effects of adenoviral-mediated coexpression of bone morphogenetic protein-7 and insulin-like growth factor-1 on human periodontal ligament cells.**

Yang L, Zhang Y, Dong R, Peng L, Liu X, Wang Y, Cheng X.

Key Laboratory for Oral Biomedical Engineering of the Ministry of Education, School & Hospital of Stomatology, Wuhan University, Wuhan, PR China.

Yang L, Zhang Y, Dong R, Peng L, Liu X, Wang Y, Cheng X. Effects of adenoviral-mediated coexpression of bone morphogenetic protein-7 and insulin-like growth factor-1 on human periodontal ligament cells. J Periodont Res 2010; doi: 10.1111/j.1600-0765.2009.01268.x. (c) 2010 John Wiley & Sons A/S Background and Objective: Bone morphogenetic protein-7 (BMP-7) and insulin-like growth factor-1 (IGF-1) are important in periodontal reconstruction. However, their synergistic effect in periodontal regeneration by gene delivery has not been reported. In this study, gene delivery of these two growth factors to human periodontal ligament cells (hPDLCs) was examined for its effects on cell proliferation and differentiation. Material and Methods: Recombinant adenoviruses containing both human BMP-7 and IGF-1 cDNA created by introducing the internal ribosome entry site (IRES) sequence were used to transfer the genes into hPDLCs. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and cell cycle analysis were used to observe their effects on cell proliferation, while alkaline phosphatase activity measurement, RT-PCR and in vivo tests were conducted to investigate their effects on cell differentiation. Results: The proliferation of hPDLCs transduced by adenoviruses coexpressing BMP-7 and IGF-1 was suppressed while their differentiation ability was enhanced. There was a synergism of BMP-7 and IGF-1 in up-regulating alkaline phosphatase activity and mRNA levels of collagen type I and Runx2. Implantation in vivo with scaffolds illustrated that the transduced cells exhibited osteogenic differentiation and formed bone-like structures. Conclusion: The combined delivery of BMP-7 and IGF-1 genes using an IRES-based strategy synergistically enhanced differentiation of hPDLCs. It is suggested that this could be a new potential method in gene therapy for periodontal reconstruction.

**PMID:**  
**20410932**

Gene Ther. 2010 Apr 22. [Epub ahead of print]

**Therapeutic levels of erythropoietin (EPO) achieved after gene electrotransfer to skin in mice.**

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Gene electrotransfer refers to gene transfection by electroporation and is an effective non-viral method for delivering naked DNA into cells and tissues. This study presents data from gene electrotransfer with erythropoietin (EPO) to mouse skin. Nine-week-old female NMRI mice received one, two or three intradermal injections of 50 mug EPO plasmid and were subsequently electroporated. With plate electrodes and 100 mug of EPO, a significant increase in hemoglobin ( $P < 0.01$ ) was observed compared with controls. The level of hemoglobin peaked after 5 weeks but stayed significantly elevated for more than 3 months. Serum EPO was significantly increased ( $P < 0.001$ ) 24 h after the transfection and remained significantly different compared with controls until the maximum level of serum EPO was reached after 2 weeks. Eight weeks after the transfection serum EPO returned to baseline. In this study, we have established that gene electrotransfer to skin of even small amounts of DNA can lead to systemically therapeutic levels of protein. This means that in addition to DNA vaccinations, there is a potential utility for electroporation in alleviating systemic diseases such as cancer and protein deficiency disorders.

PMID:  
20410931

Gene Ther. 2010 Apr 22. [Epub ahead of print]

**Phosphorothioate cap analogs increase stability and translational efficiency of RNA vaccines in immature dendritic cells and induce superior immune responses in vivo.**

Kuhn AN, Diken M, Kreiter S, Selmi A, Kowalska J, Jemielity J, Darzynkiewicz E, Huber C, Türeci O, Sahin U.

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Vaccination with in vitro transcribed RNA coding for tumor antigens is considered a promising approach for cancer immunotherapy and has already entered human clinical testing. One of the basic objectives for development of RNA as a drug is the optimization of immunobioavailability of the encoded antigen in vivo. By analyzing the effect of different synthetic 5' mRNA cap analogs on the kinetics of the encoded protein, we found that m(2)(7,2'-O)Gpp(S)pG (beta-S-ARCA) phosphorothioate caps, in particular the D1 diastereoisomer, profoundly enhance RNA stability and translational efficiency in immature but not mature dendritic cells. Moreover, in vivo delivery of the antigen as beta-S-ARCA(D1)-capped RNA species is superior for protein expression and for efficient priming and expansion of naive antigen-specific T cells in mice. Our findings establish 5' mRNA cap analogs as yet another module for tuning immunopharmacological properties of recombinant antigen-encoding RNA for vaccination purposes.

PMID:  
20410930

Gene Ther. 2010 Apr 22. [Epub ahead of print]

**Development of a nonintegrating Rev-dependent lentiviral vector carrying diphtheria toxin A chain and human TRAF6 to target HIV reservoirs.**

Wang Z, Tang Z, Zheng Y, Yu D, Spear M, Iyer SR, Bishop B, Wu Y.

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Persistence of human immunodeficiency virus (HIV) despite highly active antiretroviral therapy (HAART) is a lasting challenge to virus eradication. To develop a strategy complementary to HAART, we constructed a series of Rev-dependent lentiviral vectors carrying diphtheria toxin A chain (DT-A) and its attenuated mutants, as well as human tumor necrosis factor receptor-associated factor 6 (TRAF6). Expression of these suicide genes following delivery through viral particles is dependent on Rev, which exists only in infected cells. Among these toxins, DT-A has been known to trigger cell death with as little as a single molecule, whereas two of the attenuated mutants in this study, DT-A(176) and DT-A(DeltaN), were well tolerated by cells at low levels. TRAF6 induced apoptosis only with persistent overexpression. Thus, these suicide genes, which induce cell death at different expression levels, offer a balance between efficacy and safety. To minimize possible mutagenesis introduced by retroviral integration in nontarget cells, we further developed a nonintegrating Rev-dependent (NIRD) lentiviral vector to deliver these genes. In addition, we constructed a DT-A-resistant human cell line by introducing a human elongation factor 2 mutant into HEK293T cells. This allowed us to manufacture the first high-titer NIRD lentiviral particles carrying DT-A to target HIV-positive cells. Gene Therapy advance online publication, 22 April 2010; doi:10.1038/gt.2010.53.

PMID:  
20410929

Gene Ther. 2010 Apr 22. [Epub ahead of print]

**Early exposure of high-dose interleukin-4 to tumor stroma reverses myeloid cell-mediated T-cell suppression.**

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Myeloid-derived suppressor cells (MDSCs) inhibit T-cell activity and promote tumor growth in tumor-bearing hosts. We sought to determine how to prevent the generation of these cells and modulate anti-tumor immunity at different times during tumor growth. Interleukin-4 (IL-4), a cytokine closely associated with the differentiation of myeloid cells, was expressed locally at the tumor site with its dose and expression time tightly regulated by a tet-off system. Early exposure of high-dose IL-4 to the tumor stromal cells effectively prevented the generation of myeloid suppressor cells and led to a T-cell-mediated tumor rejection. However, IL-4 had no effect a few days after tumor growth, when myeloid suppressor cells had been generated and T cells were tolerized. Importantly, coinoculation of IL-4 receptor (IL-4R)-deficient tumor cells with IL-4R competent, but not IL-4R-deficient myeloid cells led to IL-4-mediated tumor regression in IL-4R-deficient mice, indicating that IL-4 acts directly on myeloid cells. These results show a novel way to prevent T cells from MDSC-induced suppression, with important indications for cancer therapy.

PMID:  
20410928

Gene Ther. 2010 Apr 22. [Epub ahead of print]

**Efficient gene transfer using the human JC virus-like particle that inhibits human colon adenocarcinoma growth in a nude mouse model.**

Chen LS, Wang M, Ou WC, Fung CY, Chen PL, Chang CF, Huang WS, Wang JY, Lin PY, Chang D.

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The JC virus (JCV) may infect human oligodendrocytes and consequently cause progressive multifocal leukoencephalopathy (PML) in patients with immune deficiency. In addition, the virus has also been detected in other human tissues, including kidney, B lymphocytes, and gastrointestinal tissue. The recombinant major structural protein, VP1, of JCV is able to self-assemble to form a virus-like particle (VLP). It has been shown that the VLP is capable of packaging and delivering exogenous DNA into human cells for gene expression. However, gene transfer is not efficient when using in vitro DNA packaging methods with VLPs. In this study, a novel in vivo DNA packaging method using the JCV VLP was used to obtain high efficiency gene transfer. A reporter gene, the green fluorescence protein, and a suicide gene, the herpes simplex virus thymidine kinase (tk), were encapsidated into VLPs in *Escherichia coli*. The VLP was used to specifically target human colon carcinoma (COLO-320 HSR) cells in a nude mouse model. Intraperitoneal administration of ganciclovir in the tk-VLP-treated mice greatly reduced tumor volume. These findings suggest that it will be possible to develop the JCV VLP as a gene delivery vector for human colon cancer therapy in the future.

PMID:  
20410927

Gene Ther. 2010 Apr 22. [Epub ahead of print]

**Improvement of lentiviral vector-mediated gene transduction by genetic engineering of the structural protein Pr55(Gag).**

Aoki T, Shimizu S, Urano E, Futahashi Y, Hamatake M, Tamamura H, Terashima K, Murakami T, Yamamoto N, Komano J.

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The lentiviral vector is a promising tool for human gene therapy because of its ability to transduce genes into many cell types. However, one of the technical problems associated with the lentiviral vector is that lentiviral titers in current production systems are relatively low compared with the other viral vectors. In this study, we provide genetic evidence that the attachment of heterologous myristoylation (myr) signals on the amino-terminus of human immunodeficiency virus type 1 Pr55(Gag) (Gag) can increase the viral yield up to 10-fold, leading to the enhancement of gene transduction in many cell lines. The myr signal Gag constructs behaved similarly to the wild-type Gag in targeting to detergent-resistant membrane compartments, Vps4-dependence for viral budding, and virion morphology. However, the myr signal Gag constructs showed improved oligomerization efficiency as measured by bioluminescence resonance energy transfer in living cells, contributing to increased viral production and efficient activation of the viral protease responsible for virion maturation. The genetically modified Gag represents the next generation lentiviral vector, and should contribute to the success of many lentiviral vector applications.

PMID:  
20410926 [

Gene Ther. 2010 Apr 22. [Epub ahead of print]

**Retargeted adenoviral cancer gene therapy for tumour cells overexpressing epidermal growth factor receptor or urokinase-type plasminogen activator receptor.**

Harvey TJ, Burdon D, Steele L, Ingram N, Hall GD, Selby PJ, Vile RG, Cooper PA, Shnyder SD, Chester JD.

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We have assessed the ability of bispecific fusion proteins to improve adenovirus-mediated transfer of therapeutic and marker transgenes. We constructed an expression vector that can be easily modified to synthesize a variety of fusion proteins for retargeting adenoviral gene therapy vectors to cell surface markers, which are differentially expressed between normal and cancer cells. Adenoviral transduction can be improved in a number of tumour cell lines which overexpress EGFR (epidermal growth factor receptor) or uPAR (urokinase-type plasminogen activator receptor), but which have only low levels of endogenous hCAR (human coxsackie B and adenovirus receptor) expression. Up to 40-fold improvement in beta-galactosidase transgene expression was seen using an EGFR retargeting protein, and up to 16-fold using a second fusion protein targeting uPAR. In vitro, our uPAR retargeting fusion protein improved the sensitivity to adenoviral herpes simplex virus thymidine kinase/ganciclovir by an order of magnitude, whereas in vivo, our EGFR retargeting protein is able to significantly delay tumour growth in rodent animal models in a dose-dependent manner. The 'cassette' design of our fusion protein constructs offers a flexible method for the straightforward synthesis of multiple adenoviral retargeting proteins, directed against a variety of tumour-associated antigens, for use in clinical trials.

**PMID:**  
**20410313**

J Gen Virol. 2010 Apr 21. [Epub ahead of print]

**High-titer retroviral vector system for efficient gene delivery into human and mouse cells of hematopoietic and lymphocytic lineages.**

Wu C, Lu Y.

University of Hawaii.

Genetically modified cells of hematopoietic and lymphocytic lineages would provide potentially curative treatments for a wide range of inherited and acquired diseases. However, this application is limited in mouse models by low efficiency of lentiviral vectors. To facilitate the rapid production of high-titer helper-free retroviral vectors for enhanced gene delivery, multiple modifications to a proto-type moloney murine leukemia virus (MoMLV)-derived vector system were made including adaptation of the vector system to SV40 ori/Ag T-mediated episomal replication in packaging cells, replacement of the MoMLV 5' U3 promoter with a series of stronger composite promoters, and addition of an extra polyadenylation signal downstream of the 3' LTR. These modifications enhanced vector production by 2-3 logs. High-titer vector stocks were tested for their ability to infect a variety of cells derived from humans and mice, including primary monocyte-derived-macrophage cultures. While lentiviral vector was significantly restricted at integration level, MoMLV based vector showed effective gene transduction of mouse cells. This high-titer retroviral-vector system represents a useful tool for efficient gene delivery into human and mouse hematopoietic and lymphocytic cells, with particular application in using mice as a small animal model for novel gene therapy tests.

**PMID:**  
**20410274**

J Virol. 2010 Apr 21. [Epub ahead of print]

**Self-Inactivating Alpharetroviral Vectors with a Split-Packaging Design.**

Suerth JD, Maetzig T, Galla M, Baum C, Schambach A.

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Accidental insertional activation of proto-oncogenes, and potential vector mobilization pose serious challenges for human gene therapy using retroviral vectors. Comparative analyses of integration sites of different retroviral vectors have elucidated distinct target site preferences, highlighting vectors based on alpharetroviral Rous Sarcoma Virus (RSV) as the ones with the most neutral integration spectrum. Alpharetroviral vector systems so far are mainly based on single constructs containing viral coding sequences and intact long terminal repeats (LTR). Even though they are considered to be replication-incompetent in mammalian cells, the transfer of intact viral genomes is unacceptable for clinical applications, due to the risk of vector mobilization and the potentially immunogenic expression of viral proteins, which we minimized by setting up a split-packaging system expressing the necessary viral proteins in trans. Moreover, intact LTRs containing transcriptional elements are capable of activating cellular genes. By removing most of these transcriptional elements we were able to generate a self-inactivating alpharetroviral vector, whose LTR-transcriptional activity is strongly reduced, and whose transgene expression can be driven by an internal promoter of choice. Codon-optimization of the alpharetroviral gag/pol expression construct and further optimization steps allowed the production of high-titer self-inactivating vector particles in human cells. We demonstrate proof-of-principle for the versatility of alpharetroviral SIN vectors for genetic modification of murine and human hematopoietic cells at a low multiplicity of infection.

**PMID:**  
**20410262**

J Virol. 2010 Apr 21. [Epub ahead of print]

**Inhibition of In Vivo HIV Infection In Humanized Mice By Gene Therapy of Human Hematopoietic Stem Cells with a Lentiviral Vector Encoding a Broadly Neutralizing Anti-HIV Antibody.**

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Due to the inherent immune evasion properties of the HIV envelope, broadly neutralizing HIV-specific antibodies capable of suppressing HIV infection are rarely produced by infected individuals. We examined the feasibility of utilizing genetic engineering to circumvent the restricted capacity of individuals to endogenously produce broadly neutralizing HIV-specific antibodies. We constructed a single lentiviral vector that encoded the heavy and light chains of 2G12, a broadly neutralizing anti-HIV human antibody, and that efficiently transduced and directed primary human B cells to secrete 2G12. To evaluate the capacity of this approach to provide protection from in vivo HIV infection, we used the humanized NOD/SCID/gammac(null) mouse model which becomes populated with human B cells, T cells and macrophages after transplantation with human hematopoietic stem cells (hu-HSC) and develops in vivo infection after inoculation with HIV. The plasma of the irradiated NOD/SCID/gammac(null) mice transplanted with hu-HSC transduced with the 2G12-encoding lentivector contained 2G12 antibody, likely secreted by progeny human lymphoid and/or myeloid cells. After intraperitoneal inoculation with high-titer HIV-1JR-CSF, mice engrafted with 2G12-transduced hu-HSC displayed marked inhibition of in vivo HIV infection as manifested by a profound 70-fold reduction in plasma HIV RNA levels and almost 200-fold reduction in HIV-infected human cell numbers in mouse spleens, as compared to control hu-HSC-transplanted NOD/SCID/gammac(null) mice inoculated with equivalent high-titer HIV-1JR-CSF. These results support the potential efficacy of this new gene therapy approach of using lentiviral vectors encoding a mixture of broadly neutralizing HIV antibodies for the treatment of HIV infection, particularly infection with multiple drug-resistant isolates.

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**Polycationic nanoparticles as nonviral vectors employed for gene therapy in vivo.**

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Currently, polycationic nanoparticles (polycation) as nonviral vectors are promising to overcome the defects of traditional viral vectors. This review focuses on the recent progress and challenges to improve the transfection efficacy and circulation time of polyplexes (complexes between polycation and pDNA or oligonucleotides such as siRNA) in vivo.

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### **Toward Brain Tumor Gene Therapy Using Multipotent Mesenchymal Stromal Cell Vectors.**

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Gene therapy of solid cancers has been severely restricted by the limited distribution of vectors within tumors. However, cellular vectors have emerged as an effective migratory system for gene delivery to invasive cancers. Implanted and injected multipotent mesenchymal stromal cells (MSCs) have shown tropism for several types of primary tumors and metastases. This capacity of MSCs forms the basis for their use as a gene vector system in neoplasms. Here, we review the tumor-directed migratory potential of MSCs, mechanisms of the migration, and the choice of therapeutic transgenes, with a focus on malignant gliomas as a model system for invasive and highly vascularized tumors. We examine recent findings demonstrating that MSCs share many characteristics with pericytes and that implanted MSCs localize primarily to perivascular niches within tumors, which might have therapeutic implications. The use of MSC vectors in cancer gene therapy raises concerns, however, including a possible MSC contribution to tumor stroma and vasculature, MSC-mediated antitumor immune suppression, and the potential malignant transformation of cultured MSCs. Nonetheless, we highlight the novel prospects of MSC-based tumor therapy, which appears to be a promising approach.

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### **Hybrid Lentiviral Vectors.**

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Lentiviral vectors have remarkable cell entry and gene delivery properties that make them highly attractive for gene therapy. However, all integration-competent gene delivery systems have come under scrutiny for possible adverse insertional events. Circumventing the risk of insertional mutagenesis, integration-deficient human immunodeficiency virus (HIV)-1-derived vectors have been shown to support durable transcription of transgenes in certain nonmitotic cell lineages. In mitotic cell populations, such nonintegrated viral forms are lost during cell division and so have time-limited effects. Hybrid lentiviral vectors that harness the cell entry properties of HIV to facilitate carriage of alternative DNA modification systems into cells may allow durable genetic modification with more favorable integration profiles. Thus, systems, which have previously been plasmid-based such as those based on nuclease-enhanced homologous recombination (HR) and artificial transposons, have been incorporated into the viral genome to allow them to "hitch-hike" into cells that are difficult to transfect. Here, we review recent progress in the development of such hybrid lentiviral systems and consider potential applications of such vectors.

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**Biomaterial-based technologies for brain anticancer therapeutics and imaging.**

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Treating malignant brain tumors represents one of the most formidable challenges in oncology. Contemporary treatment of brain tumors has been hampered by limited drug delivery across the blood-brain barrier (BBB) to the tumor bed. Biomaterials are playing an increasingly important role in developing more effective brain tumor treatments. In particular, polymer (nano)particles can provide prolonged drug delivery directly to the tumor following direct intracerebral injection, by making them physiochemically able to cross the BBB to the tumor, or by functionalizing the material surface with peptides and ligands allowing the drug-loaded material to be systemically administered but still specifically target the tumor endothelium or tumor cells themselves. Biomaterials can also serve as targeted delivery devices for novel therapies including gene therapy, photodynamic therapy, anti-angiogenic and thermotherapy. Nanoparticles also have the potential to play key roles in the diagnosis and imaging of brain tumors by revolutionizing both preoperative and intraoperative brain tumor detection, allowing early detection of pre-cancerous cells, and providing real-time, longitudinal, non-invasive monitoring/imaging of the effects of treatment. Additional efforts are focused on developing biomaterial systems that are uniquely capable of delivering tumor associated antigens, immunotherapeutic agents or programming immune cells in situ to identify and facilitate immune-mediated tumor-cell killing. The continued translation of current research into clinical practice will rely on solving challenges relating to the pharmacology of nanoparticles but it is envisioned that novel biomaterials will ultimately allow clinicians to target tumors and introduce multiple, pharmaceutically relevant entities for simultaneous targeting, imaging, and therapy in a unique and unprecedented manner.

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**Helper-independent piggyBac plasmids for gene delivery approaches: Strategies for avoiding potential genotoxic effects.**

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Efficient integration of functional genes is an essential prerequisite for successful gene delivery such as cell transfection, animal transgenesis, and gene therapy. Gene delivery strategies based on viral vectors are currently the most efficient. However, limited cargo capacity, host immune response, and the risk of insertional mutagenesis are limiting factors and of concern. Recently, several groups have used transposon-based approaches to deliver genes to a variety of cells. The piggyBac (pB) transposase in particular has been shown to be well suited for cell transfection and gene therapy approaches because of its flexibility for molecular modification, large cargo capacity, and high transposition activity. However, safety considerations regarding transposase gene insertions into host genomes have rarely been addressed. Here we report our results on engineering helper-independent pB plasmids. The single-plasmid gene delivery system carries both the piggyBac transposase (pBt) expression cassette as well as the transposon cargo flanked by terminal repeat element sequences. Improvements to the helper-independent structure were achieved by developing new plasmids in which the pBt gene is rendered inactive after excision of the transposon from the plasmid. As a consequence, potentially negative effects that may develop by the persistence of an active pBt gene posttransposition are eliminated. The results presented herein demonstrate that our helper-independent plasmids represent an important step in the development of safe and efficient gene delivery methods that should prove valuable in gene therapy and transgenic approaches.

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**Potential Cancer Gene Therapy by Baculoviral Transduction.**

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Many different types of therapeutic genes, ranging from suicide genes, tumor suppressor genes, to genes encoding tumor-specific antigens, have been successfully delivered by insect baculoviral vectors to treat tumours in animal models. These encouraging results observed to date underscore the potential for using the non-human baculovirus to combat human cancer. The present review outlines the advances in this area and highlights the challenges behind translating the findings from research with baculoviral vectors into clinical practice.