



# CLINIGENE CURRENT GENE THERAPY WEEKLY

From May 24<sup>th</sup> to May 31<sup>st</sup> 2010

## Table of contents:

Inhibition of proliferation of rabbit lens epithelial cells by S-phase kinase-interacting protein 2 targeting small interfering RNA.....	3
Targeting HSV-1 virions for specific binding to epidermal growth factor receptor-vIII-bearing tumor cells.....	3
VEGF blockade decreases the tumor uptake of systemic oncolytic herpes virus but enhances therapeutic efficacy when given after virotherapy.....	4
Regeneration of pancreatic islets in vivo by ultrasound-targeted gene therapy.....	4
Ultrasound-assisted non-viral gene transfer to the salivary glands.....	5
Surface immobilization of hexa-histidine-tagged adeno-associated viral vectors for localized gene delivery.....	5
Progress and prospects: graft-versus-host disease.....	6
Development of the research on the application of chlorotoxin in imaging diagnostics and targeted therapies for tumors.....	6
PEG- and PDMAEG-graft-modified branched PEI as novel gene vector: synthesis, characterization and gene transfection.....	7
Multi- to Unilamellar Transitions in Catanionic Vesicles.....	7
Radius of gyration of plasmid DNA isoforms from static light scattering.....	8
Respiratory Syncytial Virus Engineered to Express CFTR Corrects the Bioelectric Phenotype of Human Cystic Fibrosis Airway Epithelium In Vitro.....	8
Switching of mesodermal and endodermal properties in hTERT-modified and expanded fetal human pancreatic progenitor cells.....	9
DNA as therapeutics; an update.....	9
Retinoic acid and dimethyl sulfoxide promote efficient delivery of transgenes to mouse skin by topically transdermal penetration.....	10
Titers of lentiviral vectors encoding shRNAs and miRNAs are reduced by different mechanisms that require distinct repair strategies.....	10
Intratumoral Neoadjuvant Immunotherapy Using IL-12 and Dendritic Cells Is an Effective Strategy to Control Recurrence of Murine Hepatocellular Carcinoma in Immunosuppressed Mice.....	11
Preclinical Studies for Gene Therapy of Duchenne Muscular Dystrophy.....	11
Rapid, Simple and Versatile Manufacturing of Recombinant Adeno-Associated Virus Vectors at Scale.....	12
AAV6 capsid tyrosine to phenylalanine mutations improve gene transfer to skeletal muscle.....	12
Kinetics and longevity of phiC31 integrase in mouse liver and cultured cells.....	13
Gene transfer of antisense b7.1 attenuates acute rejection against liver allografts in rats.....	13
The positive feedback system provides efficient and persistent transgene expression.....	14
Muscle-derived stem cells: isolation, characterization, differentiation, and application in cell and gene therapy.....	14
Toward delivery of multiple growth factors in tissue engineering.....	15

Gene therapy for adenosine deaminase deficiency.....	15
Gene therapy for primary immunodeficiencies.....	16
Bone marrow transplantation and alternatives for adenosine deaminase deficiency. ....	16
Triptolide T10 Enhances AAV-mediated Gene Transfer in Mice Striatum.....	16
Mesenchymal stem cells: a promising targeted-delivery vehicle in cancer gene therapy.....	17
Lentivirus-mediated gene transfer of interleukin 10 to the ovine and human cornea.....	17

**PMID:** 20508867 Mol Vis. 2010 May 25;16:907-15.

**Inhibition of proliferation of rabbit lens epithelial cells by S-phase kinase-interacting protein 2 targeting small interfering RNA.**

Su Y, Wang F, Yan Q, Teng Y, Cui H.

Department of Ophthalmology, First Clinic College of Harbin Medical University, Harbin, China.

**PURPOSE:** Improper proliferation of lens epithelial cells is causally related to posterior capsule opacification. In the present study, we investigated whether small interfering RNA (siRNA)-mediated gene silencing of S-phase kinase-interacting protein 2 (Skp2) can be employed to inhibit rabbit lens epithelial cell (rLEC) proliferation by increasing the p27(kip1) level. **METHODS:** A plasmid containing Skp2 siRNA was used to decrease the high constitutive level of Skp2 protein in rLECs, which can lead to consequent degradation of p27(kip1). Protein expression of Skp2 and p27(kip1) was detected by immunocytochemistry and western blot. Cell viability was measured using the tetrazolium reduction (3-(4,5-dimethylthiazolyl-2-)-2,5-diphenyltetrazoliumbromide [MTT]) assay. Cell proliferation was assayed by cell counts, immunocytochemistry, and western blot by using antibodies against proliferating cell nuclear antigen. **RESULTS:** Immunocytochemistry and western blot showed a decreased level of Skp2 and increased level of p27(kip1) in cells transfected with pSkp2 siRNA but not in vehicle transfection and uninfected cells. MTT assay showed that cell viability significantly declined in rLECs transfected with Skp2 siRNA. Skp2 siRNA transfected cells showed significantly less 59-bromodeoxyuridine- and proliferating cell nuclear antigen-positive staining compared with control cells. **CONCLUSIONS:** Skp2 siRNA inhibits cell proliferation and decreases cell viability of rLECs in vitro by suppression of p27(kip1) downregulation. Our findings suggest that siRNA-mediated gene silencing of Skp2 can be a novel gene therapy for posterior capsule opacification induced by LEC abnormal proliferation.

**PMID:** 20508670 Cancer Gene Ther. 2010 May 28. [Epub ahead of print]

**Targeting HSV-1 virions for specific binding to epidermal growth factor receptor-vIII-bearing tumor cells.**

Grandi P, Fernandez J, Szentirmai O, Carter R, Gianni D, Sena-Esteves M, Breakefield XO.

[1] Department of Neurology, Harvard Medical School, Boston, MA, USA [2] Department of Radiology, Harvard Medical School, Boston, MA, USA.

Oncolytic herpes simplex virus (HSV) vectors have been used in early phase human clinical trials as a therapy for recurrent malignant glioblastoma. This treatment proved safe but limited improvements in patient survival were observed. The potency of these vectors might be enhanced by targeting vector infectivity to tumor cells. Glioma tumors often express a mutant form (vIII) of the epidermal growth factor receptor (EGFR) resulting in the presence of a novel epitope on the cell surface. This epitope is specifically recognized by a single-chain antibody designated MR1-1. HSV-1 infection involves initial binding to heparan sulfate (HS) on the cell surface mediated primarily by the viral envelope, glycoprotein C (gC). Here we joined the MR1-1 single-chain antibody (scFv) to the gC sequence deleted for the HS-binding domain as a means of targeting viral attachment to EGFRvIII on glial tumor cells. Virions bearing MR1-1-modified gC had fivefold increased infectivity for EGFRvIII-bearing human glioma U87 cells compared to mutant receptor-deficient cells. Further, MR1-1/EGFRvIII-mediated infection was more efficient for EGFRvIII-positive cells than was wild-type virus for either positive or negative cells. Sustained infection of EGFRvIII+ glioma cells by MR1-1-modified gC-bearing oncolytic virus, as compared to wild-type gC oncolytic virus, was also shown in subcutaneous tumors in vivo using firefly luciferase as a reporter of infection. These data show that HSV tropism can be manipulated so that virions recognize a cell-specific binding site with increased infectivity for the target cell. The retargeting of HSV infection to tumor cells should enhance vector specificity, tumor cell killing and vector safety.

**PMID:** 20508601 Gene Ther. 2010 May 27. [Epub ahead of print]

**VEGF blockade decreases the tumor uptake of systemic oncolytic herpes virus but enhances therapeutic efficacy when given after virotherapy.**

Eshun FK, Currier MA, Gillespie RA, Fitzpatrick JL, Baird WH, Cripe TP.  
Division of Hematology/Oncology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA.

Effective therapies for metastatic sarcomas remain elusive. Oncolytic viruses have shown promise as anticancer agents, but their access to metastatic sites following systemic delivery is low. As systemic delivery of small-molecule chemotherapy is enhanced by previous treatment with antiangiogenic agents because of changes in intravascular-to-tumor interstitial pressure, we sought to determine whether antiangiogenic pretreatment increases the antitumor efficacy of systemic virotherapy by increasing virus uptake into tumor. Virus biodistribution and antitumor effects were monitored in tumor-bearing mice given antihuman vascular endothelial growth factor (VEGF) or antimouse VEGFR2 before or after an intravenous (i.v.) injection of virus. Without pretreatment, the average virus titers in the tumor samples amplified 1700-fold over 48 h but were undetectable in other organs. After antiangiogenic treatment, average virus titers in the tumor samples were unchanged or in some cases decreased up to 100-fold. Thus, antiangiogenic pretreatment failed to improve the tumor uptake of systemic oncolytic herpes simplex virus (oHSV), in contrast to previously reported enhanced uptake of small molecules. Superior tumor control because of the combined effects of virus and anti-VEGF was seen most dramatically when anti-VEGF was given after virus. Our data suggest that i.v. oHSV can treat distant sites of disease and can be enhanced by antiangiogenic therapy, but only when given in the proper sequence.

**PMID:** 20508600 Gene Ther. 2010 May 27. [Epub ahead of print]

**Regeneration of pancreatic islets in vivo by ultrasound-targeted gene therapy.**

Chen S, Shimoda M, Wang MY, Ding J, Noguchi H, Matsumoto S, Grayburn PA.  
Baylor Research Institute, Baylor University Medical Center, Dallas, TX, USA.

This study uses a novel approach to gene therapy in which plasmid DNA is targeted to the pancreas in vivo using ultrasound-targeted microbubble destruction (UTMD) to achieve islet regeneration. Intravenous microbubbles carrying plasmids are destroyed within the pancreatic microcirculation by ultrasound, achieving local gene expression that is further targeted to beta-cells by a modified rat insulin promoter (RIP3.1). A series of genes implicated in endocrine development were delivered to rats 2 days after streptozotocin-induced diabetes. The genes, PAX4, Nkx2.2, Nkx6.1, Ngn3 and Mafa, produced alpha-cell hyperplasia, but no significant improvement in beta-cell mass or blood glucose level 30 days after UTMD. In contrast, RIP3.1-NeuroD1 promoted islet regeneration from surviving beta-cells, with normalization of glucose, insulin and C-peptide levels at 30 days. In a longer-term experiment, four of six rats had a return of diabetes at 90 days, accompanied by beta-cell apoptosis on TUNEL staining. Pretreatment with the JNK inhibitor SP600125 successfully blocked beta-cell apoptosis and resulted in restoration of beta-cell mass and normalization of blood glucose level for up to 90 days. This technique allows in vivo islet regeneration, restoration of beta-cell mass and normalization of blood sugar, insulin and C-peptide in rats without viruses.

**PMID:  
20508599**

Gene Ther. 2010 May 27. [Epub ahead of print]

**Ultrasound-assisted non-viral gene transfer to the salivary glands.**

Passineau MJ, Zourelis L, Machen L, Edwards PC, Benza RL.

Division of Cardiovascular Medicine, Department of Medicine, Allegheny-Singer Research Institute, West-Penn Allegheny Health System, Pittsburgh, PA, USA.

We report a non-viral gene transfer method using ultrasound induced microbubble destruction to allow the uptake of plasmid gene transfer vectors to the cells of the mouse salivary gland. The Luciferase (Luc) reporter gene, driven by a cytomegalovirus (CMV) promoter, was delivered unilaterally to the submandibular salivary gland via retroductal cannulation and Luc expression was monitored with in vivo imaging. The CMV-Luc plasmid was delivered to the salivary gland in a carrier solution containing microbubbles composed of lipid-encased perfluoropropane gas, with two different concentrations of microbubbles used (100 and 15% volume/volume). An Adenoviral (Ad) vector using an identical CMV-Luc expression cassette was used as a positive control at two different dosages. Whereas ultrasound-assisted gene transfer (UAGT) with 100% microbubbles was weak and rapidly extinguished, UAGT with the 15% microbubble solution was robust and stable for 28 days. UAGT seems to be a practicable and promising method for non-viral gene delivery to the salivary glands.

**PMID:  
20508598**

Gene Ther. 2010 May 27. [Epub ahead of print]

**Surface immobilization of hexa-histidine-tagged adeno-associated viral vectors for localized gene delivery.**

Jang JH, Koerber JT, Gujraty K, Bethi SR, Kane RS, Schaffer DV.

[1] Department of Chemical Engineering, The Helen Wills Neuroscience Institute, University of California, Berkeley, CA, USA [2] Department of Chemical and Biomolecular Engineering, Yonsei University, Seoul, Korea.

Adeno-associated viral (AAV) vectors, which are undergoing broad exploration in clinical trials, have significant promise for therapeutic gene delivery because of their safety and delivery efficiency. Gene delivery technologies capable of mediating localized gene expression may further enhance the potential of AAV in a variety of therapeutic applications by reducing spread outside a target region, which may thereby reduce off-target side effects. We have genetically engineered an AAV variant capable of binding to surfaces with high affinity through a hexa-histidine metal-binding interaction. This immobilized AAV vector system mediates high-efficiency delivery to cells that contact the surface and thus may have promise for localized gene delivery, which may aid numerous applications of AAV delivery to gene therapy.

**PMID:**  
**20508597**

Gene Ther. 2010 May 27. [Epub ahead of print]

**Progress and prospects: graft-versus-host disease.**

Mastaglio S, Stanghellini MT, Bordignon C, Bondanza A, Ciceri F, Bonini C.

[1] Hematology and BMT Unit, Department of Oncology, San Raffaele Scientific Institute, Milano, Italy [2] Experimental Hematology Unit Cancer Immunotherapy and Gene Therapy Program, Division of Regenerative Medicine, Stem Cell and Gene Therapy, San Raffaele Scientific Institute, Milano, Italy [3] University Vita-Salute, Milano, Italy [4] MolMed SpA, Milano, Italy.

Graft-versus-host disease (GvHD) is one of the major complications of allogeneic hematopoietic stem cell transplantation, an otherwise highly effective therapeutic modality for patients affected by hematological diseases. The main inducers of GvHD are alloreactive donor T cells, which recognize host antigens presented by recipient cells. The critical role of lymphocytes in GvHD is well documented by the observation that T-cell depletion from the graft prevents GvHD. Unfortunately, the removal of donor lymphocytes from the graft increases the incidence of disease relapse and life-threatening infectious complications. Gene transfer technologies are promising tools to manipulate donor T-cell immunity to enforce graft-versus-tumor/graft-versus-infection while preventing or controlling GvHD. For this purpose, several cell and gene transfer approaches have been investigated at the preclinical level and implemented in clinical trials.

**PMID:**  
**20507737**

Chin J Cancer. 2010 Jun;29(6):626-30.

**Development of the research on the application of chlorotoxin in imaging diagnostics and targeted therapies for tumors.**

Wu XS, Jian XC, Yin B, He ZJ.

Department of Oral and Maxillofacial Surgery, Xiangya Hospital, Central South University, Changsha, Hunan 410008, P. R. China. jianxinchun@hotmail.com.

Precisely locating tumors always proves to be difficult. To find a molecule that can specifically bind to tumor cells is the key. Recently, chlorotoxin (CTX) has been proved to be able to bind to many kinds of tumor cells. The CTX receptor on the cell surface has been demonstrated to be matrix metalloproteinase-2 (MMP-2). Many researchers have combined CTX with other molecules, including 131I, Cy5.5, iron oxide nanoparticles coated by polyethylene glycol (NP-PEG), and so on, and thus synthesized various types of probes that can be detected by gamma-camera, single photon emission computed tomography (SPECT) or magnetic resonance imaging (MRI). With these methods, the binding degree of CTX could be assessed. These studies demonstrated that CTX has a highly specific binding ability, high stability, and security. CTX could also inhibit or kill the tumor cells. A nonviral nanovector has been developed for gene therapy. As a result, it gradually develops into a new method of diagnosis and targeted therapy of tumors. This article reviews the current progress on CTX including the origin, chemical construction, the mechanism of binding with tumor cells, and the application to tumor imaging diagnosis and therapy.

PMID:  
20507711

J Biomater Sci Polym Ed. 2010;21(8-9):1103-26.

**PEG- and PDMAEG-graft-modified branched PEI as novel gene vector: synthesis, characterization and gene transfection.**

Wen Y, Pan S, Luo X, Zhang W, Shen Y, Feng M.

School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510080, P. R. China.

The cytotoxicity of polyethylenimine (PEI) was a dominating obstacle to its application. Introduction of poly(ethylene glycol) (PEG) blocks to PEI is one of the strategies to alleviate the cytotoxicity of PEI. However, it is well known that the transfection efficiency of PEGylated PEI is decreased to some extent compared to the corresponding PEI. Thus, the aim of our study was to enhance the transfection efficiency of PEGylated PEI. A series of tri-block co-polymers, PEG-g-PEI-g-poly(dimethylaminoethyl L-glutamine) (PEG-g-PEI-g-PDMAEG), as novel vectors for gene therapy was synthesized and evaluated. PEG-g-PEI was first obtained by linking PEG and PEI using isophorone diisocyanate (IPDI) as coupling reagent. The anionic copolymerization of gamma-benzyl-L-glutamate N-carboxyanhydride (BLG-NCA) using PEG-g-PEI as a macro-initiator was carried out, followed by aminolysis with 2-dimethylaminoethylamine to obtain the target water-soluble tri-block co-polymer. The structures of the polymers were confirmed by FT-IR and <sup>1</sup>H-NMR. The influence of the molecular weight of PEI and the length of the PDMAEG chain on the physicochemical properties and transfection activity of polymer/DNA was evaluated. All PEI derivatives were revealed to compact plasmid DNA effectively to give polyplexes with suitable size (approx. 100 nm) and moderate zeta potentials (10-15 mV) at N/P ratios over 10. The PEG-g-PEI-g-PDMAEG tri-block co-polymers displayed particularly low cytotoxicity, even at high concentration, reflecting an improved safety profile compared to PEI 25k. Gene transfection efficiency of PEG-g-PEI-g-PDMAEG on HeLa in the presence and absence of serum was determined. Remarkably, the transfection activity of PEG-g-PEI (10k)-g-PDMAEG (PPP-4)/DNA polyplex formulations was nearly twofold higher than PEI 25k/DNA formulations in vitro, and the transfection efficiency was less affected by the presence of serum. These results indicated that the synthesized PEG-g-PEI-g-PDMAEG tri-block co-polymers are promising candidates as carriers for gene delivery.

PMID:  
20507136

J Phys Chem B. 2010 May 27. [Epub ahead of print]

**Multi- to Unilamellar Transitions in Catanionic Vesicles.**

Andreozzi P, Funari SS, La Mesa C, Mariani P, Ortore MG, Sinibaldi R, Spinozzi F.

Department of Chemistry, Universita La Sapienza, Rome, Italy, HASYLAB, Hamburg, Germany, SOFT-INFN, Universita La Sapienza, Rome, Italy, and Department of SAIFET & CNISM, Universita Politecnica delle Marche, Ancona, Italy.

Sodium dodecylsulfate (SDS) and cetyltrimethylammonium bromide (CTAB) dispersed in aqueous solution form catanionic vesicles. Depending on composition, such vesicles show different net charge, stability, and interaction capability, indicative of the strong impact that catanionic systems may have in gene therapy and drug delivery technologies. To reveal the interplay among composition, net charge, sensitivity to temperature changes, vesicle size, and inner structure, a series of experiments on catanionic vesicles prepared at different SDS/CTAB mole ratios was performed. Dynamic light scattering, small-angle X-ray scattering, and zeta-potential experiments allow one to characterize an unexpected critical phenomenon at the nanoscale level. On heating, vesicles increase in size, but at a critical temperature an abrupt vesicle size reduction has been observed, together with a transition from multi- to a unilamellar state. The critical temperature regularly depends on the SDS/CTAB mole ratio. The unilamellar state obtained upon heating is retained for weeks. These phenomena suggest a new way to produce stable unilamellar vesicles with tunable size and charge.

**PMID:** Biotechnol Bioeng. 2010 May 7. [Epub ahead of print]  
**20506212**

**Radius of gyration of plasmid DNA isoforms from static light scattering.**

Latulippe DR, Zydney AL.

Department of Chemical Engineering, The Pennsylvania State University, University Park, PA, 16802.

Despite the extensive interest in applications of plasmid DNA, there have been few direct measurements of the root mean square radius of gyration,  $R(G)$ , of different plasmid isoforms over a broad range of plasmid size. Static light scattering data were obtained using supercoiled, open-circular, and linear isoforms of 5.76, 9.80, and 16.8 kbp plasmids. The results from this study extend the range of  $R(G)$  values available in the literature to plasmid sizes typically used for gene therapy and DNA vaccines. The experimental data were compared with available theoretical expressions based on the worm-like chain model, with the best-fit value of the apparent persistence length for both the linear and open-circular isoforms being statistically identical at 46 nm. A new expression was developed for the radius of gyration of the supercoiled plasmid based on a model for linear DNA using an effective contour length that is equal to a fraction of the total contour length. These results should facilitate the development of micro/nano-fluidic devices for DNA manipulation and size-based separation processes for plasmid DNA purification.

**PMID:** J Virol. 2010 May 26. [Epub ahead of print]  
**20504917**

**Respiratory Syncytial Virus Engineered to Express CFTR Corrects the Bioelectric Phenotype of Human Cystic Fibrosis Airway Epithelium In Vitro.**

Kwilas AR, Yednak MA, Zhang L, Liesman R, Collins PL, Pickles RJ, Peebles ME.

Integrated Biomedical Science Graduate Program and Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH 43210; The Research Institute at Nationwide Children's Hospital, Columbus, OH 43205; CF/Pulmonary Research and Treatment Center, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599; and Laboratory of Infectious Diseases, NIAID, NIH, Bethesda, MD 20892.

Cystic fibrosis (CF) is the most common lethal recessive genetic disease in the Caucasian population. It is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene that is normally expressed in ciliated airway epithelial cells and the submucosal glands of the lung. Since the CFTR gene was first characterized in 1989, a major goal has been to develop an effective gene therapy for CF lung disease since this represents the potential for ameliorating morbidity and mortality. Respiratory syncytial virus (RSV) naturally infects the ciliated cells in the human airway epithelium. In addition, the immune response mounted against an RSV infection does not prevent subsequent infections, suggesting that an RSV-based vector might be effectively readministered. To test whether the large, 4.5 kb, CFTR gene could be expressed by a recombinant RSV and whether infectious virus could be used to deliver CFTR to ciliated airway epithelium derived from CF patients, we inserted the CFTR gene into four sites in a green fluorescent protein-expressing (rg)RSV genome to generate virus expressing four different levels of CFTR protein. Two of these four rgRSV-CFTR virus vectors were capable of expressing CFTR with little effect on viral replication. rgRSV-CFTR infection of primary human airway epithelial cultures derived from CF patients resulted in expression of CFTR protein that was properly localized at the luminal surface and corrected the chloride ion channel defect in these cells.

**PMID:** Stem Cell Res Ther. 2010 Mar 15;1(1):6.  
**20504287**

**Switching of mesodermal and endodermal properties in hTERT-modified and expanded fetal human pancreatic progenitor cells.**

Cheng K, Follenzi A, Surana M, Fleischer N, Gupta S.

Hepatology Division, Department of Medicine, Albert Einstein College of Medicine, Ullmann Bldg, Rm 625, 1300 Morris Park Avenue, Bronx, NY 10461, USA. kcheng@aecom.yu.edu.

**ABSTRACT: INTRODUCTION :** The ability to expand organ-specific stem/progenitor cells is critical for translational applications, although uncertainties often arise in identifying the lineage of expanded cells. Therefore, superior insights into lineage maintenance mechanisms will be helpful for cell/gene therapy. **METHODS :** We studied epithelial cells isolated from fetal human pancreas to assess their proliferation potential, changes in lineage markers during culture, and capacity for generating insulin-expressing beta cells. Cells were isolated by immunomagnetic sorting for epithelial cell adhesion molecule (EpCAM), and characterized for islet-associated transcription factors, hormones, and ductal markers. Further studies were performed after modification of cells with the catalytic subunit of human telomerase reverse transcriptase (hTERT). **RESULTS :** Fetal pancreatic progenitor cells efficiently formed primary cultures, although their replication capacity was limited. This was overcome by introduction and expression of hTERT with a retroviral vector, which greatly enhanced cellular replication in vitro. However, we found that during culture hTERT-modified pancreatic progenitor cells switched their phenotype with gain of additional mesodermal properties. This phenotypic switching was inhibited when a pancreas-duodenal homeobox (Pdx)-1 transgene was expressed in hTERT-modified cells with a lentiviral vector, along with inductive signaling through activin A and serum deprivation. This restored endocrine properties of hTERT-modified cells in vitro. Moreover, transplantation studies in immunodeficient mice verified the capacity of these cells for expressing insulin in vivo. **CONCLUSIONS :** Limited replication capacity of pancreatic endocrine progenitor cells was overcome by the hTERT mechanism, which should facilitate further studies of such cells, although mechanisms regulating switches between meso-endodermal fates of expanded cells will need to be controlled for developing specific applications. The availability of hTERT-expanded fetal pancreatic endocrine progenitor cells will be helpful for studying and recapitulating stage-specific beta lineage advancement in pluripotent stem cells.

**PMID:** Indian J Pharm Sci. 2009 Sep;71(5):488-98.  
**20502565**

**DNA as therapeutics; an update.**

Saraswat P, Soni RR, Bhandari A, Nagori BP.

Mahatma Gandhi Medical College and Hospital, RIICO Institutional Area, Sitapura, Jaipur-302 022, India.

Human gene therapy is the introduction of new genetic material into the cells of an individual with the intention of producing a therapeutic benefit for the patient. Deoxyribonucleic acid and ribonucleic acid are used in gene therapy. Over time and with proper oversight, human gene therapy might become an effective weapon in modern medicine's arsenal to help fight diseases such as cancer, acquired immunodeficiency syndrome, diabetes, high blood pressure, coronary heart disease, peripheral vascular disease, neurodegenerative diseases, cystic fibrosis, hemophilia and other genetic disorders. Gene therapy trials in humans are of two types, somatic and germ line gene therapy. There are many ethical, social, and commercial issues raised by the prospects of treating patients whose consent is impossible to obtain. This review summarizes deoxyribonucleic acid-based therapeutics and gene transfer technologies for the diseases that are known to be genetic in origin. Deoxyribonucleic acid-based therapeutics includes plasmids, oligonucleotides for antisense and antigene applications, deoxyribonucleic acid aptamers and deoxyribonucleic acidzymes. This review also includes current status of gene therapy and recent developments in gene therapy research.

**PMID:** Drug Deliv. 2010 May 26. [Epub ahead of print]  
**20500128**

**Retinoic acid and dimethyl sulfoxide promote efficient delivery of transgenes to mouse skin by topically transdermal penetration.**

Chen X, Zhang Y, Liu C, Zhang Y, Zhou X, Zhou T, Mao Y, Kan B, Wei YQ, Li J.  
State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, PR China.

Simple and efficient gene transfer to the skin would facilitate many local and systemic gene therapy applications. This study reports a novel approach that allows expression of plasmid DNA in epidermis and hair follicle cells with dimethyl sulfoxide (DMSO) after pre-treatment with depilation and retinoic acid (RA) for the purposes of gene therapy. This study investigated the transdermal efficacy of gene to mouse skin when utilizing DMSO after RA pre-treatment. Retinoic acid pre-treatment can increase the efficiency of transfection. This finding indicates that one can more effectively and much less expensively make use of genes therapy to treat diseases of the hair and skin.

**PMID:** RNA. 2010 May 24. [Epub ahead of print]  
**20498457**

**Titers of lentiviral vectors encoding shRNAs and miRNAs are reduced by different mechanisms that require distinct repair strategies.**

Liu YP, Vink MA, Westerink JT, Ramirez de Arellano E, Konstantinova P, Ter Brake O, Berkhout B.

Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands.

RNAi-based gene therapy is a powerful approach to treat viral infections because of its high efficiency and sequence specificity. The HIV-1-based lentiviral vector system is suitable for the delivery of RNAi inducers to HIV-1 susceptible cells due to its ability to transduce nondividing cells, including hematopoietic stem cells, and its ability for stable transgene delivery into the host cell genome. However, the presence of anti-HIV short hairpin RNA (shRNA) and microRNA (miRNA) cassettes can negatively affect the lentiviral vector titers. We show that shRNAs, which target the vector genomic RNA, strongly reduced lentiviral vector titers but inhibition of the RNAi pathway via saturation could rescue vector production. The presence of miRNAs in the vector RNA genome (sense orientation) results in a minor titer reduction due to Drosha processing. A major cause for titer reduction of miRNA vectors is due to incompatibility of the cytomegalovirus promoter with the lentiviral vector system. Replacement of this promoter with an inducible promoter resulted in an almost complete restoration of the vector titer. We also showed that antisense poly(A) signal sequences can have a dramatic effect on the vector titer. These results show that not all sequences are compatible with the lentiviral vector system and that care should be taken in the design of lentiviral vectors encoding RNAi inducers.

**PMID:**  
**20498356**

J Immunol. 2010 May 24. [Epub ahead of print]

**Intratumoral Neoadjuvant Immunotherapy Using IL-12 and Dendritic Cells Is an Effective Strategy to Control Recurrence of Murine Hepatocellular Carcinoma in Immunosuppressed Mice.**

Kayashima H, Toshima T, Okano S, Taketomi A, Harada N, Yamashita YI, Tomita Y, Shirabe K, Maehara Y.

Department of Surgery and Science.

Liver transplantation is accepted as an effective therapy for hepatocellular carcinoma (HCC). However, recurrence is one of the most fatal complications. The aim of this study is to evaluate the efficacy of intratumoral immunotherapy using IL-12 gene therapy and dendritic cell injection for the purpose of effective treatment for HCC under conditions of immunosuppression. We found that the combined immunotherapy significantly induced sustained and high amounts of intratumoral IL-12 and IFN-gamma proteins and that it induced high HCC-specific CTL activity under immunosuppression as compared with each monotherapy or control. The combined immunotherapy also exerted effective antitumor effects on the immunosuppressed host, resulting in significant suppression of growth of the s.c. established tumor and complete suppression of lung and liver metastasis, without rejection of a fully allogeneic skin graft. These antitumor effects were dependent on both T cells and NK cells. Noteworthy, the combined intratumoral immunotherapy and tumor resection (that is, neoadjuvant immunotherapy) resulted in achievement of tumor-free and long-term survival of the some immunosuppressed mice, even when the mice were challenged with i.v. injection of HCC at the time of tumor resection. In contrast, all of the mice treated with neoadjuvant immunotherapy using monotherapy or control therapy suffered from lung and liver metastasis. These results suggest that intratumoral neoadjuvant immunotherapy using IL-12 gene therapy and dendritic cell therapy is a potent effective strategy to control recurrence of HCC in patients after liver transplantation for HCC and may be applicable to general cancer treatment.

**PMID:**  
**20498332**

J Child Neurol. 2010 May 24. [Epub ahead of print]

**Preclinical Studies for Gene Therapy of Duchenne Muscular Dystrophy.**

Odom GL, Banks GB, Schultz BR, Gregorevic P, Chamberlain JS.

Department of Neurology, University of Washington School of Medicine, Seattle, Washington, USA.

The muscular dystrophies are a diverse group of genetic disorders without an effective treatment. Because they are caused by mutations in various genes, the most direct way to treat them involves correcting the underlying gene defect (ie, gene therapy). Such a gene therapy approach involves delivering a therapeutic gene cassette to essentially all the muscles of the body in a safe and efficacious manner. The authors describe gene delivery methods using vectors derived from adeno-associated virus that are showing great promise in preclinical studies for treatment of Duchenne muscular dystrophy. It is hoped that variations on these methods might be applicable for most, if not all, of the different types of muscular dystrophy.

**PMID:**  
**20497038**

Hum Gene Ther. 2010 May 24. [Epub ahead of print]

**Rapid, Simple and Versatile Manufacturing of Recombinant Adeno-Associated Virus Vectors at Scale.**

Lock M, Alvira M, Vandenberghe LH, Samanta A, Toelen J, Debyser Z, Wilson JM.

Penn, Gene Therapy Program, Wilson Lab, Philadelphia, Pennsylvania, United States, 19104; mlock@mail.med.upenn.edu.

Adeno-associated virus vector manufacturing at scale continues to hinder the application of AAV technology to gene therapy studies. While scalable systems based upon AAV-adenovirus, -herpesvirus and -baculovirus hybrids hold promise for clinical applications, they require time-consuming generation of reagents and are not highly suited to intermediate scale pre-clinical studies in large animals where several combinations of serotype and genome may need to be tested. Recently we observed that during production of many AAV serotypes, large amounts of vector are found in the culture supernatant, a relatively pure source of vector in comparison with cell-derived material. Here we describe a high yielding, recombinant AAV production process based upon PEI-mediated transfection of HEK 293 cells and iodixanol gradient centrifugation of concentrated culture supernatant. The entire process can be completed in one week and the steps involved are universal for a number of different AAV serotypes. Process conditions have been optimized such that final purified yields are routinely greater than  $1 \times 10^{14}$  genome copies per run, with capsid protein purity exceeding 90%. Initial experiments with vectors produced with the new process demonstrate equivalent or better transduction both in vitro and in vivo when compared to small scale, CsCl gradient-purified vectors. In addition, the iodixanol gradient purification process described effectively separates infectious particles from empty capsids, a desirable property for reducing toxicity and unwanted immune responses during pre-clinical studies.

**PMID:**  
**20497037**

Hum Gene Ther. 2010 May 24. [Epub ahead of print]

**AAV6 capsid tyrosine to phenylalanine mutations improve gene transfer to skeletal muscle.**

Qiao C, Zhang W, Yuan Z, Shin JH, Li J, Jayandharan GR, Zhong L, Srivastava A, Xiao X, Duan D.

University of North Carolina, Division of Molecular Pharmaceutics, 2072 Genetic Medicine Building, 120 Mason Farm Road, Chapel Hill, North Carolina, United States, 27599; cqiao@email.unc.edu.

Adeno-associated virus (AAV) vectors are the most efficient in vivo gene transfer tools for gene therapy applications. In recent years, efforts have been made in translating encouraging results from small animal models to human patients. However the large quantities of vector needed for clinical application remains a great challenge. Developing novel AAV vectors with enhanced infectivity may reduce high vector dose requirement for many applications such as gene therapy for muscular dystrophy. Selective mutation of AAV capsid surface-exposed tyrosine (Y) is a novel strategy to improve transduction efficiency. AAV6 has been considered as one of the most robust muscle gene delivery vehicles. Here, we hypothesize that AAV6 transduction efficiency can be further enhanced by mutating surface Y to F. We found that mutants AAV6-Y445F and AAV6-Y731F, especially the former, achieved more efficient gene transfer than the original AAV6 after intramuscular administration in mice. Expression of both firefly luciferase and alkaline phosphatase reporter genes increased up to 8-fold and DNA copy numbers in the muscle increased up to 6-fold. Our results suggest that tyrosine-mutant AAV6 vectors may represent powerful tools for testing muscle gene therapy in animal models and potentially in humans.

**PMID:** Hum Gene Ther. 2010 May 24. [Epub ahead of print]  
**20497035**

**Kinetics and longevity of phiC31 integrase in mouse liver and cultured cells.**

Chavez CL, Keravala A, Woodard LE, Hillman RT, Stowe TR, Chu JN, Calos MP.  
Stanford University School of Medicine, Genetics, 300 Pasteur Dr, Alway M318, Stanford, California, United States, 94305; clchavez@stanford.edu.

The phiC31 integrase system provides genomic integration of plasmid DNA that may be useful in gene therapy. For example, the phiC31 system has been used in combination with hydrodynamic injection to achieve long-term expression of factor IX in mouse liver. However, a concern is that prolonged expression of phiC31 integrase within cells could potentially stimulate chromosome rearrangements or an immune response. Western blot and immunofluorescence analyses were performed to investigate the duration of phiC31 integrase expression in mouse liver. Integrase was expressed within two to three hours after hydrodynamic injection of a plasmid expressing phiC31 integrase. Expression peaked between 8 and 16 hours and fell to background levels by 24-48 hours post-injection. Analysis of the amount of integrase plasmid DNA present in the liver over time suggested that the brief period of integrase expression could largely be accounted for by rapid loss of the bulk of the plasmid DNA, as well as silencing of plasmid expression. PCR analysis of integration indicated that phiC31 integrase carried out genomic integration of a co-delivered attB-containing plasmid by three hours after plasmid injection. Integrase was expressed for longer times and at higher levels in transfected cultured cells compared to liver. Inhibitor studies suggested that the enzyme had a short half-life and was degraded by the 26S proteasome. The short duration of integrase expression in liver and rapid integration reaction appear to be features favorable for use in gene therapy.

**PMID:** J Invest Surg. 2010 Apr;23(2):87-93.  
**20497010**

**Gene transfer of antisense b7.1 attenuates acute rejection against liver allografts in rats.**

Mi Y, Li R, Xu K, Jiang H, Sun X.  
Department of Hepatobiliary Surgery, Qilu Hospital of Shandong University, China.

**ABSTRACT** Objective: Blockade of CD80-CD28 costimulatory pathway induces unresponsiveness of T cells to alloantigens and protects allografts against immune rejection. The aim of this study was to investigate whether downregulating the expression of B7.1 (CD80) in the donor livers by antisense B7.1 gene transfer could attenuate the acute immune rejection against liver allografts in rats. Methods: The liver grafts from 60 Dark Agouti rats were intraportally perfused with antisense B7.1 expression vector, before they were transplanted into Lewis rats. Empty vector pcDNA3 served as control to be perfused into livers of another group of 60 Dark Agouti rats. The orthotopic liver transplantation was performed. The rats were randomly sacrificed at scheduled time points to collect liver allografts, or monitored to record the survival rate. The livers were histologically examined to calculate Banff rejection activity index, or subjected to Western blot analysis or immunohistochemistry for examining the expression of B7.1, or counting CD4+ and CD8+ cells. Results: Antisense gene transfer resulted in markedly downregulation of B7.1 in the donor livers, attenuated acute immune rejection against liver allografts, prolonged the survival time of rats, and decreased the number of infiltrating CD4+ and CD8+ cells in livers. Conclusions: Blocking expression of B7.1 in liver by antisense gene therapy may represent a potential strategy to attenuate acute rejection against liver allografts.

PMID:  
20496887

Mol Pharm. 2010 May 24. [Epub ahead of print]

**The positive feedback system provides efficient and persistent transgene expression.**

Ochiai H, Harashima H, Kamiya H.

The two-step transcriptional amplification (TSTA) system, using artificial transcription factors, effectively enhances transgene expression. In this study, a TSTA system-based positive feedback system was developed to achieve efficient and persistent transgene expression. A fusion protein of the sequence-specific DNA binding domain of yeast GAL4 and the transcriptional activation domain of herpes simplex virus VP16 (GAL4-VP16) was used as an "activator" to amplify the expression of the luciferase "reporter" gene. It was found that the introduction of five tandem copies of the GAL4 recognition sequence (G5) into both the upstream and downstream regions of the expression cassette synergistically enhanced the transgene expression. The upstream and downstream G5 sequences were introduced into the expression cassette of the activator itself, and into that of the reporter, to form the positive feedback loop that enabled continuous activator expression. This positive feedback system maintained the expression levels of the reporter for 4 days in HeLa cells and for a week in mouse liver, while those from the usual plasmids decreased by 30- and 50-fold, respectively. These results constitute the first evidence that the positive feedback system is a useful method for long-term transgene expression in cultured cells and in vivo. This system would be applicable to gene therapy, in vivo imaging, and biotechnology.

PMID:  
20495827

Cell Tissue Res. 2010 May 22. [Epub ahead of print]

**Muscle-derived stem cells: isolation, characterization, differentiation, and application in cell and gene therapy.**

Wu X, Wang S, Chen B, An X.

Shandong Medicinal Biotechnology Center, Shandong Academy of Medical Sciences, Jinan, Shandong Province, China, aoppolle@126.com.

Muscle tissue represents an abundant, accessible, and replenishable source of adult stem cells for cell-based tissue and genetic engineering. A population of cells isolated from muscle exhibits both multipotentiality and self-renewal capabilities. Satellite cells, referred to by many investigators as muscle stem cells, are myogenic precursors that are capable of regenerating muscle and that demonstrate self-renewal properties; however, they are considered to be committed to the myogenic lineage. Muscle-derived stem cells (MDSCs), which may represent a predecessor of the satellite cell, are considered to possess a higher regeneration capacity and to exhibit better cell survival and a broader range of multilineage capabilities. Remarkably, MDSCs are not only able to differentiate into mesodermal cell types including the myogenic, adipogenic, osteogenic, chondrogenic, endothelial, and hematopoietic lineages, but also possess the potential to break germ layer commitment and differentiate into ectodermal lineages including neuron-like cells under certain conditions. This article reviews the current preclinical studies and potential clinical applications of MDSC-mediated gene therapy and tissue-engineering and methods for MDSC isolation, differentiation, and molecular characterization.

**PMID:**  
**20493521**

Biomaterials. 2010 May 19. [Epub ahead of print]

**Toward delivery of multiple growth factors in tissue engineering.**

Chen FM, Zhang M, Wu ZF.

Department of Periodontology & Oral Medicine, School of Stomatology, Fourth Military Medical University, Xi'an 710032, Shaanxi, People's Republic of China.

Inspired by physiological events that accompany the "wound healing cascade", the concept of developing a tissue either in vitro or in vivo has led to the integration of a wide variety of growth factors (GFs) in tissue engineering strategies in an effort to mimic the natural microenvironments of tissue formation and repair. Localised delivery of exogenous GFs is believed to be therapeutically effective for replication of cellular components involved in tissue development and the healing process, thus making them important factors for tissue regeneration. However, any treatment aiming to mimic the critical aspects of the natural biological process should not be limited to the provision of a single GF, but rather should release multiple therapeutic agents at an optimised ratio, each at a physiological dose, in a specific spatiotemporal pattern. Despite several obstacles, delivery of more than one GF at rates mimicking an in vivo situation has promising potential for the clinical management of severely diseased tissues. This article summarises the concept of and early approaches toward the delivery of dual or multiple GFs, as well as current efforts to develop sophisticated delivery platforms for this ambitious purpose, with an emphasis on the application of biomaterials-based deployment technologies that allow for controlled spatial presentation and release kinetics of key biological cues. Additionally, the use of platelet-rich plasma or gene therapy is addressed as alternative, easy, cost-effective and controllable strategies for the release of high concentrations of multiple endogenous GFs, followed by an update of the current progress and future directions of research utilising release technologies in tissue engineering and regenerative medicine. Copyright © 2010 Elsevier Ltd. All rights reserved.

**PMID:**  
**20493400**

Immunol Allergy Clin North Am. 2010 May;30(2):249-60.

**Gene therapy for adenosine deaminase deficiency.**

Cappelli B, Aiuti A.

San Raffaele Telethon Institute for Gene Therapy, San Raffaele Scientific Institute, via Olgettina 58, Milan 20132, Italy.

In the last decade, gene therapy for adenosine deaminase deficiency has been developed as a successful alternative strategy to allogeneic bone marrow transplant and enzyme replacement therapy. Infusion of autologous hematopoietic stem cells, corrected ex vivo by retroviral vectors and combined to low-intensity conditioning regimen, has resulted in immunologic improvement, metabolic correction, and long-term clinical benefits. These findings have opened the way to applications of gene therapy in other primary immune deficiencies using novel vector technology.

**PMID:**  
**20493399**

Immunol Allergy Clin North Am. 2010 May;30(2):237-48.

**Gene therapy for primary immunodeficiencies.**

Fischer A, Hacein-Bey-Abina S, Cavazanna-Calvo M.

Developpement Normal et Pathologique du Systeme Immunitaire, INSERM U 768, Hopital Necker, 149 rue de sevres, Paris 75015, France. alain.fischer@inserm.fr

The concept of gene therapy emerged as a way of correcting monogenic inherited diseases by introducing a normal copy of the mutated gene into at least some of the patients' cells. Although this concept has turned out to be quite complicated to implement, it is in the field of primary immunodeficiencies (PIDs) that proof of feasibility has been undoubtedly achieved. There is now a strong rationale in support of gene therapy for at least some PIDs, as discussed in this article. Copyright (c) 2010 Elsevier Inc. All rights reserved.

**PMID:**  
**20493398**

Immunol Allergy Clin North Am. 2010 May;30(2):221-36.

**Bone marrow transplantation and alternatives for adenosine deaminase deficiency.**

Bobby Gaspar H.

Centre for Immunodeficiency, Molecular Immunology Unit, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK. h.gaspar@ich.ucl.ac.uk

Adenosine deaminase (ADA)-deficient severe combined immunodeficiency (SCID) comprises approximately 10% to 15% of all cases of SCID. The clinical effects of ADA deficiency are manifest most dramatically in the immune system, where it leads to severe lymphopenia. Although hematopoietic stem cell transplantation remains the mainstay of treatment for ADA-deficient SCID, 2 other treatment options are available, namely enzyme replacement therapy with PEG-ADA and autologous hematopoietic stem cell gene therapy. In this article the author reviews the available data on treatment by these different options, and offers an overview on when each of the different treatment options should be used.

**PMID:**  
**20493236**

Neurosci Lett. 2010 May 18. [Epub ahead of print]

**Triptolide T10 Enhances AAV-mediated Gene Transfer in Mice Striatum.**

Ren X, Zhang T, Hu J, Ding W, Wang X.

Department of Physiology and Key Laboratory of the Neurodegenerative Disorders of the Chinese Ministry of Education, Capital Medical University, Youanmen, Beijing 100069, P.R. China.

Adeno-associated virus (AAV) mediated gene transfer has been demonstrated to be an effective approach for treating Parkinson's disease (PD). Triptolide T10 is a monomeric compound isolated from tripterigium wilfordii Hook.f (Thunder God vine), a traditional Chinese herb for anti-inflammatory medications. In the present study, we co-administered T10 with recombinant AAV2 in SH-SY5Y human neuroblastoma cells and in the striatum of C57BL/6 mice, and then evaluated the AAV-mediated gene expression levels. The results have shown that T10 significantly augmented the expression of AAV-mediated gene in a dose-dependent fashion without detectable cytotoxicity. As growing evidence indicated that inflammation contributed to the progression of PD, and the anti-inflammatory effect of T10 was shown in our previous studies, our data of T10 to enhance AAV transduction suggest that T10 might be potentially used as a facilitating reagent for the AAV gene therapy applications in neurodegenerative diseases. Copyright © 2010. Published by Elsevier Ireland Ltd.

PMID:  
20493219

J Control Release. 2010 May 18. [Epub ahead of print]

**Mesenchymal stem cells: a promising targeted-delivery vehicle in cancer gene therapy.**

Hu YL, Fu YH, Tabata Y, Gao JQ.

Institute of Pharmaceutics, College of Pharmaceutical Sciences, Zhejiang University, P.R.China.

The targeting drug delivery systems (TDDS) have attracted extensive attention of researchers in recent years. More and more drug/gene targeted delivery carriers, such as liposome, magnetic nanoparticles, ligand-conjugated nanoparticles, microbubbles, etc., have been developed and under investigation for their application. However, the currently investigated drug/gene carriers have several disadvantages, which limit their future use in clinical practice. Therefore, design and development of novel drug/gene delivery vehicles has been a hot area of research. Recent studies have shown the ability of mesenchymal stem cells (MSCs) to migrate towards and engraft into the tumor sites, which make them a great hope for efficient targeted-delivery vehicles in cancer gene therapy. In this review article, we examine the promising of using mesenchymal stem cells as a targeted delivery vehicle for cancer gene therapy, and summarize various challenges and concerns regarding these therapies.

PMID:  
20491805

Clin Experiment Ophthalmol. 2010 Feb 28. [Epub ahead of print]

**Lentivirus-mediated gene transfer of interleukin 10 to the ovine and human cornea.**

Parker DG, Coster DJ, Brereton HM, Hart PH, Koldej R, Anson DS, Williams KA.

Department of Ophthalmology, Flinders University of South Australia, Adelaide, South Australia, Australia.

**Abstract Background:** Gene transfer to a donor cornea ex vivo can modulate corneal graft failure in experimental animal models. We compared a lentiviral vector (LV) carrying the transgene ovine interleukin 10 (IL10) with a comparable adenoviral vector (Ad) for its ability to transduce ovine and human corneas and to modulate ovine corneal allograft survival. **Methods:** The LV carrying the ovine IL10 gene was used to transduce ovine and human corneas in vitro. LV-mediated gene expression in corneal endothelium was assessed by real-time quantitative reverse-transcriptase polymerase chain reaction, at varying doses and duration of transduction. The effect of ex vivo transduction of the donor cornea with LV-SV40-IL10 was assessed following orthotopic corneal transplantation in outbred sheep. **Results:** Expression of IL10 mRNA in Ad-CMV-IL10-transduced ovine corneas was 10(3)-fold higher than in LV-SV40-IL10-transduced corneas ( $P < 0.0001$ ), and 10(7)-fold higher than in non-transduced controls. IL10 was secreted rapidly from Ad-CMV-IL10-transduced, organ-cultured corneas, peaking at 13-15 days. IL10 secreted from LV-SV40-IL10-transduced corneas increased 20-fold compared with controls, but had not reached a plateau at 15 days. Gene expression driven by LV-SV40-IL10 varied with vector dose and transduction time, but was less than with Ad-CMV-IL10 at both mRNA and protein levels. Gene expression driven by LV-SV40-IL10 was faster in the human cornea than the ovine cornea. Corneal allograft survival was prolonged by a median of 7 days in the LV-SV40-IL10-treated recipients, compared with the control group ( $P = 0.026$ ). **Conclusion:** Although lentiviral vectors show some promise for corneal gene therapy, they are less efficient than adenoviral vectors.