



# CLINIGENE CURRENT GENE THERAPY WEEKLY

From March 15<sup>th</sup> to March 22<sup>nd</sup> 2010

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**20168352**

Cancer Gene Ther. 2010 Feb 19. [Epub ahead of print]

**Gene-modified tumor vaccine secreting a designer cytokine Hyper-Interleukin-6 is an effective therapy in mice bearing orthotopic renal cell cancer.**

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Although Renal Cell Cancer (RCC) is known to be immunogenic, clinical efficacy of various immunotherapeutic approaches remains unsatisfactory. Novel targeted therapies showing cytostatic rather than cytotoxic activity are unable to cure RCC patients. In our studies, we evaluated the therapeutic efficacy of whole-cell vaccine based on irradiated murine RENCA cells genetically modified to secrete designer cytokine-Hyper-IL6 (H6)-comprising IL-6 and soluble IL-6 receptor. An orthotopic RCC model based on a subcapsular implantation of RENCA cells into kidneys of Balb/C mice was employed. The efficacy of RENCA-H6 vaccine was compared with control vaccine (RENCA-wt) in relation to naive (non-immunized) animals. Three sets of vaccination experiments were carried out in a (i) protective, (ii) palliative and (iii) adjuvant (following nephrectomy) setting. The influence of vaccination on survival of RCC-bearing animals was analyzed. Specificity of vaccine-induced immune response was studied using model antigen-GFP. RCC-bearing animals immunized with RENCA-H6 vaccine showed prolonged survival compared with other groups. In palliative and adjuvant settings the survival RENCA-H6-immunized animals exceeded 75%. Administration of RENCA-H6 inhibited formation and recruitment of Treg cells (CD4+CD25+Foxp3+) and increased maturation of DCs. RENCA tumors in RENCA-H6- vaccinated animals contained large populations of NK cells and activated CD4+, CD8+ T cells. In addition, in mice vaccinated with RENCA-H6 cells large population of CD4+ and CD8+ memory cells (CD62Llow) were detected. In the orthotopic RCC model, RENCA-H6 vaccine showed high therapeutic potential, which resulted from modulation of numerous immunological mechanisms.

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Cancer Gene Ther. 2010 Feb 19. [Epub ahead of print]

**Potent antitumor effects of combined therapy with a telomerase-specific, replication-competent adenovirus (OBP-301) and IL-2 in a mouse model of renal cell carcinoma.**

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OBP-301 (a telomerase-specific, replication-competent adenovirus with hTERT promoter) was constructed in a previous study and it showed a strong anticancer effect by inducing cell lysis in human lung and prostate cancer cells. This study investigated the effectiveness of a combination therapy of OBP-301 and interleukin-2 (IL-2) in a mouse model of renal cell carcinoma (RCC). The cell-killing effect of OBP-301 was confirmed in vitro in the RENCA cancer cells. In in vivo experiment, luciferase-expressing RENCA cells were implanted in the left kidney and lung of BALB/c mice to prepare the RCC metastatic model. The animals were randomly divided into four treatment groups: PBS, IL-2 alone, OBP-301 alone and the combination. The analyses of orthotopic tumor weight, lung metastasis and luciferin-stained tumor images 14 days after each treatment showed significant tumor growth inhibition in the combination group in comparison with that in the OBP-301- or IL-2-treated groups. In addition, the percentage of regulatory T-cells (Tregs) in the combination group was significantly suppressed in comparison with that in the PBS and single-agent treatment groups. The outcomes of this study suggest that tumor-specific oncolytic immunovirotherapy may become an attractive strategy for the treatment of human RCC.

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Cancer Gene Ther. 2010 Feb 19. [Epub ahead of print]

**Treatment of metastatic neuroblastoma with systemic oncolytic virotherapy delivered by autologous mesenchymal stem cells: an exploratory study.**

García-Castro J, Alemany R, Cascalló M, Martínez-Quintanilla J, Del Mar Arriero M, Lassaletta A, Madero L, Ramírez M.

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Treatment of metastatic tumors with engineered adenoviruses that replicate selectively in tumor cells is a new therapeutic approach in cancer. Systemic administration of these oncolytic adenoviruses lack metastatic targeting ability. The tumor stroma engrafting property of intravenously injected mesenchymal stem cells (MSCs) may allow the use of MSCs as cellular vehicles for targeted delivery. In this work, we study the safety and the efficacy of infusing autologous MSCs infected with ICOVIR-5, a new oncolytic adenovirus, for treating metastatic neuroblastoma. Four children with metastatic neuroblastoma refractory to front-line therapies received several doses of autologous MSCs carrying ICOVIR-5, under an approved preliminary study. The tolerance to the treatment was excellent. A complete clinical response was documented in one case, and the child is in complete remission 3 years after this therapy. We postulate that MSCs can deliver oncolytic adenoviruses to metastatic tumors with very low systemic toxicity and with beneficial antitumor effects.

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20167102

BMC Biotechnol. 2010 Feb 18;10(1):16. [Epub ahead of print]

**Immunohistochemical detection of transgene expression in the brain using small epitope tags.**

Lobbestael E, Reumers V, Ibrahim A, Paesen K, Thiry I, Gijssbers R, Van den Haute C, Debysers Z, Baekelandt V, Taymans JM.

**ABSTRACT:** **BACKGROUND:** In vivo overexpression of proteins is a powerful approach to study their biological function, generate disease models or evaluate gene therapy approaches. In order to investigate an exogenously expressed protein, specific and sensitive detection is essential. Unfortunately, antibodies that allow histological detection of the protein of interest are not always readily available. The use of an epitope tag fused to the protein can circumvent this problem as well as provide the possibility to discriminate endogenous from overexpressed proteins. In order to minimize impact on the bioactivity and biodistribution of the overexpressed protein, preference is given to small tags. **RESULTS:** In the present study, we evaluated several small epitope tags together with corresponding anti-tag antibodies for the detection of overexpressed proteins in rat brain, using eGFP as a reference. We generated several lentiviral vectors encoding eGFP with different N-terminally fused small epitope tags (AU1, flag, 3flag, HA, myc and V5). After confirmation of their functionality in cell culture, we injected these lentiviral vectors stereotactically into the striatum of rats and prepared paraformaldehyde fixed floating sections for immunohistochemical analysis. Using multiple antibodies and antibody dilutions per epitope tag, we extensively assessed the efficiency of several anti-tag antibodies for chromogenic immunohistochemical detection of the epitope tagged eGFPs by determining the proportion of immunoreactivity detected by anti-tag antibodies compared to anti-GFP antibody. Using fluorescence immunohistochemistry and confocal microscopy, we also quantified the proportion of eGFP-positive cells detected by anti-tag antibodies. Our results show that all the examined small epitope tags could be detected by anti-tag antibodies both in cell extracts as well as in vivo, although to varying degrees depending on the tag and antibody used. Using the presented protocol, V5/anti-V5 and HA/HA11 tag/antibody combinations provided the most sensitive detection in brain tissue. We confirmed the applicability of these optimized in vivo tag detection conditions for a difficult to detect protein, firefly luciferase (fLuc), using lentiviral vector constructs expressing V5 tagged and 3flag tagged fLuc protein. **CONCLUSIONS:** We show here that several small epitope tags are useful for immunohistochemical detection of exogenous proteins in vivo. Our study also provides a generic methodology which is broadly applicable for the detection of overexpressed transgenes in mammalian brain tissue.

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20167077

BMC Biotechnol. 2010 Feb 18;10(1):15. [Epub ahead of print]

**Characterization of a molecular switch system that regulates gene expression in mammalian cells through a small molecule.**

Taylor JL, Rohatgi P, Spencer HT, Doyle DF, Azizi B.

**ABSTRACT:** **BACKGROUND:** Molecular switch systems that activate gene expression by a small molecule are effective technologies that are widely used in applied biological research. Nuclear receptors are valuable candidates for these regulation systems due to their functional role as ligand activated transcription factors. Previously, our group engineered a variant of the retinoid X receptor to be responsive to the synthetic compound, LG335, but not responsive to its natural ligand, 9-cis-retinoic acid. **RESULTS:** This work focuses on characterizing a molecular switch system that quantitatively controls transgene expression. This system is composed of an orthogonal ligand/nuclear receptor pair, LG335 and GRQCIMFI, along with an artificial promoter controlling expression of a target transgene. GRQCIMFI is composed of the fusion of the DNA binding domain of the yeast transcription factor, Gal4, and a retinoid X receptor variant. The variant consists of the following mutations: Q275C, I310M, and F313I in the ligand binding domain. When introduced into mammalian cell culture, the switch shows luciferase activity at concentrations as low as 100 nM of LG335 with a 6.3 +/- 1.7-fold induction ratio. The developed one-component system activates transgene expression when introduced transiently or virally. **CONCLUSIONS:** We have successfully shown that this system can induce tightly controlled transgene expression and can be used for transient transfections or retroviral transductions in mammalian cell culture. Further characterization is needed for gene therapy applications.

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Int J Colorectal Dis. 2010 Feb 18. [Epub ahead of print]

**Adenovirus-mediated stem cell leukemia gene transfer induces rescue of interstitial cells of Cajal in ICC-loss mice.**

Li F, Zhang L, Li C, Ni B, Wu Y, Huang Y, Zhang G, Wang L, Zhang A, He Y, Fu T, Tong W, Liu B.

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**OBJECTIVE:** Interaction of c-Kit and its ligand stem cell factor (SCF) is necessary for appropriate development and survival of interstitial cells of Cajal (ICC) in the intestine. Blockade of c-Kit will cause ICC loss in vivo. Stem cell leukemia (SCL) gene acts as a positive regulator of upstream transcription of c-Kit expression. This study aimed to explore whether the restoration of c-Kit expression promoted by SCL gene transfer could rescue ICC in vivo. **MATERIALS AND METHODS:** A modified ICC-loss mouse model was created by continual administration of anti-c-Kit antibody (ACK2) to obtain a steady status of ICC loss, and a recombinant adenovirus vector containing SCL gene (Ad-SCL) was designed to rescue ICC in these mice. Western blot analysis and immunofluorescence labeling assays were performed to analyze the SCL and c-Kit expression in vitro and in vivo. The distribution and configuration of ICC were observed with immunohistochemistry and electromicroscope. **RESULTS:** Western blot analysis and immunofluorescence labeling assays showed that SCL gene was successfully delivered to cultured HeLa and ICC cells in vitro. Moreover, significantly increased c-Kit expression could be detected in the colon of Ad-SCL-infected ICC-loss mice. Furthermore, rescue of the ICC network and ICC with typical ultrastructural features could be detected in Ad-SCL-infected ICC-loss mice at day 37. **CONCLUSIONS:** Ad-SCL was able to enhance c-Kit expression, reactivate the c-Kit/SCF pathway, and rescue ICC in ICC-loss mice. Since loss and defects of ICC are associated with many human gut motility disorders, Ad-SCL may be of potential use in gene therapy of these patients.

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**20164859**

**Lack of humoral immune response to the tetracycline (Tet) activator in rats injected intracranially with Tet-off rAAV vectors.**

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The ability to safely control transgene expression from viral vectors is a long-term goal in the gene therapy field. We have previously reported tight regulation of GFP expression in rat brain using a self-regulating tet-off rAAV vector. The immune responses against tet regulatory elements observed by other groups in nonhuman primates after intramuscular injection of tet-on encoding vectors raise concerns about the clinical value of tet-regulated vectors. However, previous studies have not examined immune responses following injection of AAV vectors into brain. Therefore, rat striatum was injected with tet-off rAAV harboring a therapeutic gene for Parkinson's disease, either hAADC or hGDNF. The expression of each gene was tightly controlled by the tet-off regulatory system. Using an ELISA developed with purified GST-tTA protein, no detectable immunogenicity against tTA was observed in sera of rats that received an intrastriatal injection of either vector. In contrast, sera from rats intradermally injected with an adenovirus containing either tTA or rtTA, as positive controls, had readily detectable antibodies. These observations suggest that tet-off rAAV vectors do not elicit an immune response when injected into rat brain and that these may offer safer vectors for Parkinson's disease than vectors with constitutive expression.

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**20164858**

**Rhadinovirus vector-derived human telomerase reverse transcriptase expression in primary T cells.**

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The rhadinovirus herpesvirus saimiri (HVS) as a gene delivery vector allows large DNA insertions and long-termed gene expression. In the case of T-cell transduction, such vectors use the viral transformation-associated genes of HVS C488 for T-cell amplification. In this report, we investigated whether the gene for the catalytic telomerase subunit human telomerase reverse transcriptase (hTERT) can substitute for the transformation-associated genes in rhadinoviral T-cell transduction and amplification. By using virus mutants generated by en passant mutagenesis from bacterial artificial chromosomes, we observed a very early and functional transgene expression even by virus mutants without transformation-associated genes. The markers of T-cell transformation by HVS, namely CD2 hyperreactivity, overexpression of interleukin-26, and of the tyrosine kinase Lyn could neither be induced nor enhanced by ectopic hTERT expression. When the viral transformation-associated genes were replaced by the hTERT gene, it was not sufficient for growth transformation, although hTERT was efficiently transduced and functionally expressed by the rhadinovirus vector. Thus, the transformation-associated proteins StpC and Tip are responsible for the T-cell phenotype after transduction by HVS and, additionally, modulate telomerase activity independently of hTERT expression.

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20164857

Gene Ther. 2010 Feb 18. [Epub ahead of print]

**Lentiviral vectors incorporating a human elongation factor 1alpha promoter for the treatment of canine leukocyte adhesion deficiency.**

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Canine leukocyte adhesion deficiency (CLAD) provides a unique large animal model for testing new therapeutic approaches for the treatment of children with leukocyte adhesion deficiency (LAD). In our CLAD model, we examined two different fragments of the human elongation factor 1alpha (EF1alpha) promoter (EF1alphaL, 1189 bp and EF1alphaS, 233 bp) driving the expression of canine CD18 in a self-inactivating (SIN) lentiviral vector. The EF1alphaS vector resulted in the highest levels of canine CD18 expression in CLAD CD34(+) cells in vitro. Subsequently, autologous CD34(+) bone marrow cells from four CLAD pups were transduced with the EF1alphaS vector and infused following a non-myeloablative dose of 200 cGy total-body irradiation. None of the CLAD pups achieved levels of circulating CD18(+) neutrophils sufficient to reverse the CLAD phenotype, and all four animals were euthanized because of infections within 9 weeks of treatment. These results indicate that the EF1alphaS promoter-driven CD18 expression in the context of a RRLSIN lentiviral vector does not lead to sufficient numbers of CD18(+) neutrophils in vivo to reverse the CLAD phenotype when used in a non-myeloablative transplant regimen in dogs.

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20164856

Gene Ther. 2010 Feb 18. [Epub ahead of print]

**Evaluation of cross-reactive cell-mediated immune responses among human, bovine and porcine adenoviruses.**

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The absence of preexisting immunity against porcine adenovirus (Ad) serotype 3 (PAd3) and bovine Ad serotype 3 (BAd3) in humans makes them attractive alternatives to human Ad serotype 5 (HAd5) vectors. To determine whether there is significant cross-reactivity among HAd5, BAd3 and PAd3 at the level of cell-mediated immune responses, BALB/c mice were inoculated intraperitoneally with wild-type (WT) or replication-defective (RD) HAd5, BAd3 or PAd3. After 35 days of the first inoculation, cross-reactive CD8+ cytotoxic T cells, as well as CD4+ Th1- and Th2-helper T cells, in the spleen were analyzed by enzyme-linked-immunospot, flow cytometry and cytotoxic T lymphocyte assays. Virus-neutralization assays were used to evaluate humoral cross-reactivity. CD8+ or CD4+ T cells primed with WT or RD HAd5, PAd3 or BAd3 showed significant ( $P < 0.005$ ) reactivity with homologous Ad antigens, whereas only minimal cross-reactivity was observed on stimulation with heterologous Ad antigens. Ad-neutralizing antibodies were found to be homologous Ad specific. Overall, these results suggest that there is no significant immunological cross-reactivity among HAd5, BAd3 and PAd3, thereby supporting the rationale for the use of BAd3 and PAd3 as alternative HAd vectors to circumvent anti-HAd immunity in humans.

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20164855

Gene Ther. 2010 Feb 18. [Epub ahead of print]

**Generation of multi-functional antigen-specific human T-cells by lentiviral TCR gene transfer.**

Perro M, Tsang J, Xue SA, Escors D, Cesco-Gaspere M, Pospori C, Gao L, Hart D, Collins M, Stauss H, Morris EC.

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T-cell receptor (TCR) gene transfer is an attractive strategy to generate antigen-specific T-cells for adoptive immunotherapy of cancer and chronic viral infection. However, current TCR gene transfer protocols trigger T-cell differentiation into terminally differentiated effector cells, which likely have reduced ability to mediate disease protection in vivo. We have developed a lentiviral gene transfer strategy to generate TCR-transduced human T-cells without promoting T-cell differentiation. We found that a combination of interleukin-15 (IL15) and IL21 facilitated lentiviral TCR gene transfer into non-proliferating T-cells. The transduced T-cells showed redirection of antigen specificity and produced IL2, IFN $\gamma$  and TNF $\alpha$  in a peptide-dependent manner. A significantly higher proportion of the IL15/IL21-stimulated T-cells were multi-functional and able to simultaneously produce all three cytokines ( $P < 0.01$ ), compared with TCR-transduced T-cells generated by conventional anti-CD3 plus IL2 stimulation, which primarily secreted only one cytokine. Similarly, IL15/IL21 maintained high levels of CD62L and CD28 expression in transduced T-cells, whereas anti-CD3 plus IL2 accelerated the loss of CD62L/CD28 expression. The data demonstrate that the combination of lentiviral TCR gene transfer together with IL15/IL21 stimulation can efficiently redirect the antigen specificity of resting primary human T-cells and generate multi-functional T-cells.

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20164833

Clin Pharmacol Ther. 2010 Feb 17. [Epub ahead of print]

**Study of the Efficacy, Biodistribution, and Safety Profile of Therapeutic Gutless Adenovirus Vectors as a Prelude to a Phase I Clinical Trial for Glioblastoma.**

Muhammad AK, Puntel M, Candolfi M, Salem A, Yagiz K, Farrokhi C, Kroeger KM, Xiong W, Curtin JF, Liu C, Lawrence K, Bondale NS, Lerner J, Baker GJ, Foulad D, Pechnick RN, Palmer D, Ng P, Lowenstein PR, Castro MG.

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Glioblastoma multiforme (GBM) is the most common and most aggressive primary brain tumor in humans. Systemic immunity against gene therapy vectors has been shown to hamper therapeutic efficacy; however, helper-dependent high-capacity adenovirus (HC-Ad) vectors elicit sustained transgene expression, even in the presence of systemic anti-adenoviral immunity. We engineered HC-Ads encoding the conditional cytotoxic herpes simplex type 1 thymidine kinase (TK) and the immunostimulatory cytokine fms-like tyrosine kinase ligand 3 (Flt3L). Flt3L expression is under the control of the regulatable Tet-ON system. In anticipation of a phase I clinical trial for GBM, we assessed the therapeutic efficacy, biodistribution, and clinical and neurotoxicity with escalating doses of HC-Ad-TetOn-Flt3L + HC-Ad-TK in rats. Intratumoral administration of these therapeutic HC-Ads in rats bearing large intracranial GBMs led to long-term survival in ~70% of the animals and development of antiglioma immunological memory without signs of neuropathology or systemic toxicity. Systemic anti-adenoviral immunity did not affect therapeutic efficacy. These data support the idea that it would be useful to develop HC-Ad vectors further as a therapeutic gene-delivery platform to implement GBM phase I clinical trials.

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**20163988**

**New aspects in vascular gene therapy.**

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Advances in clinical gene therapy have been modest although significant progress has been made during the past few years. New viruses have been introduced and new results have been collected from preclinical and clinical studies. This review will focus on cardiovascular and especially proangiogenic gene therapy. Recent results from preclinical developments and clinical trials will be discussed.

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**20163742**

**Characterization of transgene expression in adenoviral vector-based HIV-1 vaccine candidates.**

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**ABSTRACT:** Recombinant adenovirus vectors have been extensively used in gene therapy clinical studies. More recently, the capability of inducing potent cell-mediated and humoral immunity has made these vectors equally attractive candidates for prophylactic or therapeutic vaccine applications. Merck and Co., Inc., developed HIV-1 vaccine candidates based on adenovirus serotype 5 (Ad5) vectors in which the E1 gene, a critical component for adenovirus replication, was replaced by the cytomegalovirus immediate/early promoter, followed by mutated versions of the HIV-1 gag, pol or nef genes (constructs referred to as MRKAd5gag, MRKAd5pol and MRKAd5nef, respectively). Vaccine performance was evaluated in vitro in a novel assay that measures the level of transgene expression in non-permissive A549 cells. Various combinations of vectors were studied. The results indicate that the vaccine induces a dose-dependent expression of the HIV-1 transgenes in vitro. Furthermore, the gag, pol, and nef transgenes are expressed differentially in A549 cells in an MOI-dependent and formulation-dependent manner, yielding an unexpected enhancement of protein expression in trivalent vs. monovalent formulations. Our data suggest that the presence of additional virus in multivalent formulations increases individual transgene expression in A549 cells, even when the amount of DNA encoding the gene of interest remains constant. This enhancement appears to be controlled at the transcriptional level and related to both the total amount of virus and the combination of transgenes present in the formulation.

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**20163248**

Hum Gene Ther. 2010 Feb 17. [Epub ahead of print]

**Evaluation of Hydrodynamic Limb Vein Injections in Non-human Primates.**

Hegge JO, Wooddell CI, Zhang G, Hagstrom JE, Braun S, Huss T, Sebestyén MG, Emborg ME, Wolff JA.

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The administration route is emerging as a critical aspect of non-viral and viral vector delivery to muscle so as to enable gene therapy for disorders such as muscular dystrophy. While direct intramuscular routes were used initially, intravascular routes are garnering interest due to their ability to target multiple muscles at once and their ability to increase the efficiency of delivery and expression. For the delivery of naked plasmid DNA (pDNA), our group has developed a hydrodynamic, limb vein (HLV) procedure that entails placing a tourniquet over the proximal part of the target limb to block all blood flow and injecting the gene vector rapidly in a large volume so as to enable the gene vector to be extravasated and access the myofibers. The present study was conducted in part to optimize the procedure in preparation for a human clinical study. A variety of injection parameters such as the effect of papaverine pre-injection, tourniquet inflation pressure and duration, and rate of injection were evaluated in rats and non-human primates. In addition, the safety of the procedure was further established by determining the effect of the procedure on the neuromuscular and vascular systems. The results from these studies provide additional evidence that the procedure is well tolerated and they provide a foundation on which to formulate the procedure for a human clinical study.

**PMID:**  
**20163246**

Hum Gene Ther. 2010 Feb 17. [Epub ahead of print]

**Future prospects and challenges of anti-angiogenic cancer gene therapy.**

Samaranayake H, Määttä AM, Pikkarainen J, Yla-Herttuala S.

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In 1971 Judah Folkman proposed the concept of anti-angiogenesis as a therapeutic target for cancer. More than 30 years later, concept became a reality with the approval of anti-vascular endothelial growth factor (VEGF) monoclonal antibody bevacizumab as a first-line treatment for metastatic colorectal cancer. Monoclonal antibodies and small molecular drugs are the most widely applied methods for inhibition of angiogenesis. Efficacy of these anti-angiogenic modalities has been proven, in both pre-clinical and clinical settings. Although angiogenesis plays a major role in wound healing, hypoxia and in the female reproductive cycle, inhibition of angiogenesis seems to be a relatively safe therapeutic option against cancers, and has therefore become a logical and widely experimented area. The 20th century has shown the boom of gene therapy and thus it has been applied also in anti-angiogenic setting. This review summarises methods to induce anti-angiogenic responses with gene therapy and discusses the obstacles and future prospects of anti-angiogenic cancer gene therapy.

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**20162780**

Chest. 2009 Nov;136(5 Suppl):e30.

**Gene therapy for cystic fibrosis. 1996.**

Rosenfeld MA, Collins FS.

No Abstract available

**PMID:** J Inherit Metab Dis. 2010 Feb 17. [Epub ahead of print]  
**20162365**

**Serum MIP-1 alpha level: a biomarker for the follow-up of lentiviral therapy in mucopolysaccharidosis IIIB mice.**

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Mucopolysaccharidosis (MPS) IIIB is an inherited lysosomal storage disorder caused by deficiency of alpha-N-acetylglucosaminidase (NAGLU). The disease is characterized by mild somatic features and severe neurological involvement, with high mortality rates. Although some therapeutic approaches have been applied to the murine model of the disease, no effective therapy is available. Moreover, assessing therapeutic efficacy is challenged by the lack of markers to for progression and severity. In this study, we examined the effect of brain-directed lentiviral (LV) gene therapy on serum levels of macrophage inflammatory protein 1 alpha (MIP-1alpha) and brain-derived neurotrophic factor (BDNF) proteins in the murine model of MPS IIIB to identify novel serum biomarkers. The cytokine MIP-1alpha was elevated in MPS IIIB mouse serum, and following gene therapy, it was reduced to normal levels. For neurotrophin BDNF, the difference in serum levels between MPS IIIB and normal mice was not statistically significant; after LV gene therapy, an increase in protein was found in treated mice, although the values were not statistically significant. Our studies suggest MIP-1alpha as the first serum biomarker that could be used to monitor disease progression and treatment for MPS IIIB disease.

**PMID:** Adv Funct Mater. 2009 Jul 24;19(14):2244-2251.  
**20160995**

**PEI-PEG-Chitosan Copolymer Coated Iron Oxide Nanoparticles for Safe Gene Delivery: synthesis, complexation, and transfection.**

Kievit FM, Veiseh O, Bhattarai N, Fang C, Gunn JW, Lee D, Ellenbogen RG, Olson JM, Zhang M.

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Gene therapy offers the potential of mediating disease through modification of specific cellular functions of target cells. However, effective transport of nucleic acids to target cells with minimal side effects remains a challenge despite the use of unique viral and non-viral delivery approaches. Here we present a non-viral nanoparticle gene carrier that demonstrates effective gene delivery and transfection both in vitro and in vivo. The nanoparticle system (NP-CP-PEI) is made of a superparamagnetic iron oxide nanoparticle (NP), which enables magnetic resonance imaging, coated with a novel copolymer (CP-PEI) comprised of short chain polyethylenimine (PEI) and poly(ethylene glycol) (PEG) grafted to the natural polysaccharide, chitosan (CP), which allows efficient loading and protection of the nucleic acids. The function of each component material in this nanoparticle system is illustrated by comparative studies of three nanoparticle systems of different surface chemistries, through material property characterization, DNA loading and transfection analyses, and toxicity assessment. Significantly, NP-CP-PEI demonstrates an innocuous toxic profile and a high level of expression of the delivered plasmid DNA in a C6 xenograft mouse model, making it a potential candidate for safe in vivo delivery of DNA for gene therapy.

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**Adenoviral vector-based strategies for cancer therapy.**

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Definitive treatment of cancer has eluded scientists for decades. Current therapeutic modalities like surgery, chemotherapy, radiotherapy and receptor-targeted antibodies have varied degree of success and generally have moderate to severe side effects. Gene therapy is one of the novel and promising approaches for therapeutic intervention of cancer. Viral vectors in general and adenoviral (Ad) vectors in particular are efficient natural gene delivery systems and are one of the obvious choices for cancer gene therapy. Clinical and preclinical findings with a wide variety of approaches like tumor suppressor and suicide gene therapy, oncolysis, immunotherapy, anti-angiogenesis and RNA interference using Ad vectors have been quite promising, but there are still many hurdles to overcome. Shortcomings like increased immunogenicity, prevalence of preexisting anti-Ad immunity in human population and lack of specific targeting limit the clinical usefulness of Ad vectors. In recent years, extensive research efforts have been made to overcome these limitations through a variety of approaches including the use of conditionally-replicating Ad and specific targeting of tumor cells. In this review, we discuss the potential strengths and limitations of Ad vectors for cancer therapy.

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**20160042**

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**Gene Therapy for Mesothelioma and Lung Cancer.**

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Both malignant pleural mesothelioma and advanced stage lung cancer are associated with a poor prognosis. Unfortunately, current treatment regimens have had only a modest effect on their progressive course. Gene therapy for thoracic malignancies represents a novel therapeutic approach and has been evaluated in a number of clinical trials over the last two decades. Using viral vectors or anti-sense RNA, strategies have included induction of apoptosis, tumor suppressor gene replacement, suicide gene expression, cytokine-based therapy, various vaccination approaches, and adoptive transfer of modified immune cells. This review will consider the clinical results, limitations, and future directions of gene therapy trials for thoracic malignancies.

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**Generation and characterization of a Tet-On (rtTA-M2) transgenic rat.**

Sheng Y, Lin CC, Yue J, Sukhwani M, Shuttleworth JJ, Chu T, Orwig KE.

**ABSTRACT: BACKGROUND:** The tetracycline-inducible gene regulation system is a powerful tool that allows temporal and dose-dependent regulation of target transgene expression in vitro and in vivo. Several tetracycline-inducible transgenic mouse models have been described with ubiquitous or tissue-specific expression of tetracycline-transactivator (tTA), reverse tetracycline-transactivator (rtTA) or Tet repressor (TetR). Here we describe a Tet-On transgenic rat that ubiquitously expresses rtTA-M2 driven by the murine ROSA 26 promoter. **RESULTS:** The homozygous rat line (ROSA-rtTA-M2) generated by lentiviral vector injection, has a single integration site and was derived from the offspring of a genetic mosaic founder with multiple transgene integrations. The rtTA-M2 transgene integrated into an intron of a putative gene on chromosome 2 and does not appear to affect the tissue-specificity or expression of that gene. Fibroblasts from the ROSA-rtTA-M2 rats were transduced with a TetO7/CMV-EGFP lentivirus and exhibited doxycycline dose-dependent expression of the EGFP reporter transgene, in vitro. In addition, doxycycline-inducible EGFP expression was observed, in vivo, when the TetO7/CMV-EGFP lentivirus was injected into testis, kidney and muscle tissues of ROSA-rtTA-M2 rats. **CONCLUSIONS:** This conditional expression rat model may have application for transgenic overexpression or knockdown studies of gene function in development, disease and gene therapy.

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**Gene therapy in Alzheimer's disease -potential for disease modification.**

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Alzheimer's disease (AD) is the major cause of dementia in the elderly, leading to memory loss and cognitive decline. The mechanism underlying onset of the disease has not been fully elucidated. However, characteristic pathological manifestations include extracellular accumulation and aggregation of the amyloid beta-peptide (Abeta) into plaques and intracellular accumulation and aggregation of hyperphosphorylated tau, forming neurofibrillary tangles. Despite extensive research worldwide, no disease modifying treatment is yet available. In this review, we focus on gene therapy as a potential treatment for AD, and summarize recent work in the field, ranging from proof-of concept studies in animal models to clinical trials. The multifactorial causes of AD offer a variety of possible targets for gene therapy, including two neurotrophic growth factors, nerve growth factor and brain-derived neurotrophic factor, Abeta-degrading enzymes, such as neprilysin, endothelin-converting enzyme and cathepsin B, and AD associated apolipoprotein E. This review also discusses advantages and drawbacks of various rapidly developing virus-mediated gene delivery techniques for gene therapy. Finally, approaches aiming at downregulating amyloid precursor protein and beta-site APP-cleaving enzyme 1 levels by means of siRNA-mediated knockdown are briefly summarized. Overall, the prospects appear hopeful that gene therapy has the potential to be a disease modifying treatment for AD.

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### **Listeria Monocytogenes as a Vector for Anti-Cancer Therapies.**

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The intracellular pathogen *Listeria monocytogenes* represents a promising therapeutic vector for the delivery of DNA, RNA or protein to cancer cells or to prime immune responses against tumour-specific antigens. A number of biological properties make *L. monocytogenes* a promising platform for development as a vector for either gene therapy or as an anti-cancer vaccine vector. *L. monocytogenes* is particularly efficient in mediating internalization into host cells. Once inside cells, the bacterium produces specific virulence factors which lyse the vacuolar membrane and allow escape into the cytoplasm. Once in the cytosol, *L. monocytogenes* is capable of actin-based motility and cell-to-cell spread without an extracellular phase. The cytoplasmic location of *L. monocytogenes* is significant as this potentiates entry of antigens into the MHC Class I antigen processing pathway leading to priming of specific CD8(+) T cell responses. The cytoplasmic location is also beneficial for the delivery of DNA (bactofection) by *L. monocytogenes* whilst cell-to-cell spread may facilitate access of the vector to cells throughout the tumour. Several preclinical studies have demonstrated the ability of *L. monocytogenes* for intracellular gene or protein delivery in vitro and in vivo, and this vector has also displayed safety and efficacy in clinical trial. Here, we review the features of the *L. monocytogenes* host-pathogen interaction that make this bacterium such an attractive candidate with which to induce appropriate therapeutic responses. We focus primarily upon work that has led to attenuation of the pathogen, demonstrated DNA, RNA or protein delivery to tumour cells as well as research that shows the efficacy of *L. monocytogenes* as a vector for tumour-specific vaccine delivery.

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20157303

Nat Rev Urol. 2010 Feb 16. [Epub ahead of print]

### **Emerging gene and stem cell therapies for the treatment of erectile dysfunction.**

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Erectile dysfunction is a prevalent condition that leads to significant morbidity and distress, not just for affected men but also for their partners. Very few currently available treatments ameliorate the underlying causes of the disorder and 'cure' the disease state. Much recent effort has been focused on the development of gene and cell-based approaches to rectify the molecular and tissue defects responsible for ED. Gene therapy has been investigated in animal models as a means to restore normal function to the penis; at this time, however, only one human trial has been published in the peer-reviewed literature. Recent gene therapy studies have focused on the modulation of enzymes associated with the NOS/cGMP pathway, and supplementation of trophic factors, peptides and potassium channels. Stem cell therapy has been a topic of interest in more recent years but there are currently very few published reports in animal models and none in human men. Although stem cell therapy offers the potential for restoration of functional tissues, legitimate concerns remain regarding the long-term fate of stem cells. The long-term safety of both gene and stem cell therapy must be thoroughly investigated before large-scale human studies can be considered.

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**Engineered E. coli as Vehicles for Targeted Therapeutics.**

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Improving the means of drug delivery has become an important field of pharmaceutical research. The development of safe and advanced vectors for gene therapy and other novel therapies will allow for targeted delivery of pharmaceutically active agents and carries promise to improve therapies both through increased efficiency (e.g. improved cellular uptake of the active drug) as well as lower toxicity (e.g. through targeted delivery only to the cells requiring treatment) for a large number of pharmaceutical agents. Here we are reviewing the nascent field using live bacteria as vectors for therapeutic and preventive agents in a wide range of areas, from vaccine purposes to gene therapy and delivery of therapeutic RNA interference. This review focuses particularly on the use of E. coli derived strains for therapeutic delivery.

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**Salmonella as Live Trojan Horse for Vaccine Development and Cancer Gene Therapy.**

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The design of efficient vectors for vaccine development and cancer gene therapy is an area of intensive research. Bacteria-based vectors are being investigated as optimal vehicles for antigen and therapeutic gene delivery to immune and tumour cells. Attenuated Salmonella strains have shown great potential as live vectors with broad applications in human and veterinary medicine. An impressively high, and still growing, number of reports published over the last two decades have demonstrated the effectiveness in animal models of Salmonella-based therapies for the prevention and treatment of infectious and non-infectious diseases, as well as cancer. Further, the recent dramatic expansion in knowledge of genetics, biology and pathogenesis of the bacteria allows more rational design of Salmonella constructs tailored for specific applications. However, only few clinical trials have been conducted so far, and although they have conclusively demonstrated the safety of this system, the results on immunogenicity are less than optimal. Thus, more research particularly in target species is required to bring this system closer to human and veterinary use. In this review we first describe some particularities of the bacteria and its relationship with the host that could be on the basis of its success as vector, and then summarize the different strategies used so far to develop Salmonella-based vaccines for infectious diseases as well as for non-traditional indications such as prion and Alzheimer disease vaccination. Finally, we review the many different approaches that employ Salmonella for the design of new therapies for cancer.

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**Gene therapy in Parkinson's disease: rationale and current status.**

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Parkinson's disease is the second most common age-related neurodegenerative disorder, typified by the progressive loss of substantia nigra pars compacta dopamine neurons and the consequent decrease in the neurotransmitter dopamine. Patients exhibit a range of clinical symptoms, with the most common affecting motor function and including resting tremor, rigidity, akinesia, bradykinesia and postural instability. Current pharmacological interventions are palliative and largely aimed at increasing dopamine levels through increased production and/or inhibition of metabolism of this key neurotransmitter. The gold standard for treatment of both familial and sporadic Parkinson's disease is the peripheral administration of the dopamine precursor, levodopa. However, many patients gradually develop levodopa-induced dyskinesias and motor fluctuations. In addition, dopamine enhancement therapies are most useful when a portion of the nigrostriatal pathway is intact. Consequently, as the number of substantia nigra dopamine neurons and striatal projections decrease, these treatments become less efficacious. Current translational research is focused on the development of novel disease-modifying therapies, including those utilizing gene therapeutic approaches. Herein we present an overview of current gene therapy clinical trials for Parkinson's disease. Employing either recombinant adeno-associated virus type 2 (rAAV2) or lentivirus vectors, these clinical trials are focused on three overarching approaches: augmentation of dopamine levels via increased neurotransmitter production; modulation of the neuronal phenotype; and neuroprotection. The first two therapies discussed in this article focus on increasing dopamine production via direct delivery of genes involved in neurotransmitter synthesis (amino acid decarboxylase, tyrosine hydroxylase and GTP [guanosine triphosphate] cyclohydrolase 1). In an attempt to bypass the degenerating nigrostriatal pathway, a third clinical trial utilizes rAAV2 to deliver glutamic acid decarboxylase to the subthalamic nucleus, converting a subset of excitatory neurons to GABA-producing cells. In contrast, the final clinical trial is aimed at protecting the degenerating nigrostriatum by striatal delivery of rAAV2 harbouring the neuroprotective gene, neurturin. Based on preclinical studies, this gene therapeutic approach is posited to slow disease progression by enhancing neuronal survival. In addition, we discuss the outcome of each clinical trial and discuss the potential rationale for the marginal yet incremental clinical advancements that have thus far been realized for Parkinson's disease gene therapy.

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**Experimental study of antiangiogenic gene therapy targeting VEGF in oral cancer.**

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It is well known that tumor angiogenesis plays an important role in local growth and metastasis of oral cancer; therefore, inhibiting angiogenesis is considered to be effective for treating oral cancer. This study aimed to investigate the effectiveness of systemically available antiangiogenic gene therapy targeting vascular endothelial growth factor (VEGF), which is one of the most important angiogenesis accelerators. We administered a soluble form of VEGF receptor-expressing gene incorporated into adenovirus (AdVEGF-ExR) intraperitoneally to nude mice to which oral cancer cell lines (SAS, HSC-3, and Ca9-22) had been transplanted subcutaneously in vivo to inhibit angiogenesis and tumor proliferation. Then, we measured tumor volumes over time, and tumors were enucleated and examined histopathologically and immunohistologically at 28 days after AdVEGF-ExR administration. Compared to the controls to which we administered AdLacZ or saline, significant antiproliferative effects were observed ( $P < 0.05$ ) in the AdVEGF-ExR administration group, and extensive tumor necrosis was found histopathologically. Immunohistochemical analysis with CD34 (NU-4A1) revealed tumor angiogenesis was suppressed significantly ( $P < 0.05$ ), and that with ssDNA revealed apoptosis induction was significantly high ( $P < 0.05$ ) in the AdVEGF-ExR group. However, analysis with Ki-67 (MIB-1) revealed tumor proliferative capacity was not significantly different between the groups. Consequently, we consider that AdVEGF-ExR administration achieved tumor growth suppression by inhibiting angiogenesis and inducing apoptosis, but not by inhibiting the proliferative capacity of tumor cells. Neither topical administration of a soluble form of VEGF receptor (sVEGFR) to the tumor nor a megadose was needed to achieve this inhibition effect. These results suggest gene therapy via sVEGFR would be an effective oral cancer therapy and benefit future clinical applications.

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**20154380**

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**Combination of adenovirus and cross-linked low molecular weight PEI improves efficiency of gene transduction.**

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Recombinant adenovirus (Ad)-mediated gene therapy is an exciting novel strategy in cancer treatment. However, poor infection efficiency with coxsackievirus and adenovirus receptor (CAR) down-regulated cancer cell lines is one of the major challenges for its practical and extensive application. As an alternative method of viral gene delivery, a non-viral carrier using cationic materials could compensate for the limitation of adenovirus. In our study, adenovectors were complexed with a new synthetic polymer PEI-DEG-bis-NPC (PDN) based on polyethylenimine (PEI), and then the properties of the vehicle were characterized by measurement of size distribution, zeta potential and transmission electron microscopy (TEM). Enhancement of gene transduction by Ad/PDN complexes was observed in both CAR-overexpressing cell lines (A549) and CAR-lacking cell lines (MDCK, CHO, LLC), as a result of facilitating binding and cell uptake of adenoviral particles by the cationic component. Ad/PDN complexes also promoted the inhibition of tumor growth in vivo and prolonged the survival time of tumor-bearing mice. These data suggest that a combination of viral and non-viral gene delivery methods may offer a new approach to successful cancer gene therapy.

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20154339

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**Plant thymidine kinase 1: a novel efficient suicide gene for malignant glioma therapy.**

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The prognosis for malignant gliomas remains poor, and new treatments are urgently needed. Targeted suicide gene therapy exploits the enzymatic conversion of a prodrug, such as a nucleoside analog, into a cytotoxic compound. Although this therapeutic strategy has been considered a promising regimen for central nervous system (CNS) tumors, several obstacles have been encountered such as inefficient gene transfer to the tumor cells, limited prodrug penetration into the CNS, and inefficient enzymatic activity of the suicide gene. We report here the cloning and successful application of a novel thymidine kinase 1 (TK1) from the tomato plant, with favorable characteristics *in vitro* and *in vivo*. This enzyme (toTK1) is highly specific for the nucleoside analog prodrug zidovudine (azidothymidine, AZT), which is known to penetrate the blood-brain barrier. An important feature of toTK1 is that it efficiently phosphorylates its substrate AZT not only to AZT monophosphate, but also to AZT diphosphate, with excellent kinetics. The efficiency of the toTK1/AZT system was confirmed when toTK1-transduced human glioblastoma (GBM) cells displayed a 500-fold increased sensitivity to AZT compared with wild-type cells. In addition, when neural progenitor cells were used as delivery vectors for toTK1 in intracranial GBM xenografts in nude rats, substantial attenuation of tumor growth was achieved in animals exposed to AZT, and survival of the animals was significantly improved compared with controls. The novel toTK1/AZT suicide gene therapy system in combination with stem cell-mediated gene delivery promises new treatment of malignant gliomas.

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20151849

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**Advances with the use of bio-inspired vectors towards creation of artificial viruses.**

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Importance of the field: In recent years, there has been a great deal of interest in the development of recombinant vectors based on biological motifs with potential applications in gene therapy. Several such vectors have been genetically engineered, resulting in biomacromolecules with new properties that are not present in nature. Areas covered in this review: This review briefly discusses the advantages and disadvantages of the current state-of-the-art gene delivery systems (viral and non-viral) and then provides an overview on the application of various biological motifs in vector development for gene delivery. Finally, it highlights some of the most advanced bio-inspired vectors that are designed to perform several self-guided functions. What the reader will gain: This review helps the readers get a better understanding about the history and evolution of bio-inspired fusion vectors with the potential to merge the strengths of both viral and non-viral vectors in order to create efficient, safe and cost-effective gene delivery systems. Take home message: With the emergence of new technologies such as recombinant bio-inspired vectors, it may not take long before non-viral vectors are observed that are not just safe and tissue-specific, but even more efficient than viral vectors.