



# CLINIGENE CURRENT GENE THERAPY WEEKLY

From June 28<sup>th</sup> to July 5<sup>th</sup> 2010

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PMID:  
20596091

Cancer Gene Ther. 2010 Jul 2. [Epub ahead of print]

**A fully replication-competent adenovirus vector with enhanced oncolytic properties.**

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We have studied the oncolytic efficacy of two adenovirus vectors named KD3 and INGN 007, which differ from each other only in that whereas KD3 has two small deletions in its e1a gene that restrict its replication to rapidly cycling cells, INGN 007 has wild-type e1a gene. Both vectors overexpress the adenovirus death protein (ADP). Both KD3 and INGN 007 effectively suppressed the growth of subcutaneous human A549 and Hep3B tumors in nude mice upon intratumoral injection, and contained the growth of subcutaneous LNCaP tumors after intravenous injection, making some tumors shrink or disappear. However, in a more demanding model, intravenous injections of neither KD3 nor wild-type Ad5 were effective against subcutaneous A549 tumors, whereas INGN 007 increased the mean survival time by 35%. INGN 007 was also effective in suppressing tumor growth in a challenging A549 orthotopic lung cancer model. INGN 007 was superior to dl1520 (ONYX-015) in repressing subcutaneous A549 tumors. Our results suggest that vectors such as INGN 007 might provide better antitumor efficacy in the clinic as well.

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20596090

Cancer Gene Ther. 2010 Jul 2. [Epub ahead of print]

**Enhanced target gene knockdown by a bifunctional shRNA: a novel approach of RNA interference.**

Rao DD, Maples PB, Senzer N, Kumar P, Wang Z, Pappen BO, Yu Y, Haddock C, Jay C, Phadke AP, Chen S, Kuhn J, Dylewski D, Scott S, Monsma D, Webb C, Tong A, Shanahan J, Nemunaitis J.

Gradalis, Inc., Dallas, TX, USA.

RNA interference (RNAi) is a natural cellular regulatory process that inhibits gene expression by transcriptional, post-transcriptional and translational mechanisms. Synthetic approaches that emulate this process (small interfering RNA (siRNA), short hairpin RNA (shRNA)) have been shown to be similarly effective in this regard. We developed a novel 'bifunctional' RNAi strategy, which further optimizes target gene knockdown outcome. A bifunctional construct (bi-sh-STMN1) was generated against Stathmin1, a critical tubulin modulator that is overexpressed in human cancers. The bifunctional construct is postulated to concurrently repress the translation of the target mRNA (cleavage-independent, mRNA sequestration and degradation) and degrade (through RNase H-like cleavage) post-transcriptional mRNA through cleavage-dependent activities. Bi-sh-STMN1 showed enhanced potency and durability in parallel comparisons with conventional shRNA and siRNAs targeting the same sequence. Enhanced STMN1 protein knockdown by bi-sh-STMN1 was accompanied by target site cleavage at the mRNA level showed by the rapid amplification of complementary DNA ends (RACE) assay. Bi-sh-STMN1 also showed knockdown kinetics at the mRNA level consistent with its multieffector silencing mechanisms. The bifunctional shRNA is a highly effective and advantageous approach mediating RNAi at concentrations significantly lower than conventional shRNA or siRNA. These results support further evaluations.

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20596089**

Cancer Gene Ther. 2010 Jul 2. [Epub ahead of print]

**Recombinant adenovirus IL-24-Bax promotes apoptosis of hepatocellular carcinoma cells in vitro and in vivo.**

Li J, Li L, Zhang X, Kang X, Wen Y, Qian H, Zhou Y, Xu W, Zhang Y, Wu M, Yin Z.  
Molecular Oncology Laboratory, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China.

Gene therapy promises to become an alternative choice for the treatment of hepatic cancer. In many cancers, the delivery of chimeric proteins by adenovirus vector has been reported to induce apoptosis. This study was performed to evaluate whether the recombinant adenovirus interleukin (IL)-24-Bax can induce apoptosis in hepatocellular carcinoma cells in vitro and in vivo. Several recombinant adenoviruses were constructed, and the expression of their encoded proteins was measured. The effects of the recombinant adenovirus on hepatocellular carcinoma cells and the normal hepatocyte cell line were investigated through cell viability and apoptosis assays after the cells were treated with Ad.Luc, Ad.IL-24, Ad.Bax or Ad.IL-24-Bax. The mechanism involved was also explored. A tumor-bearing mouse model was used to evaluate the effects of the adenovirus on tumor volume and cell apoptosis in vivo. Ad.IL-24-Bax selectively suppressed growth of hepatocellular carcinoma cells and induced apoptosis, but it had little influence on the normal hepatocytes. The mechanism of this response may include the effect of the 10HRE/VEGF385 promoter and the synergistic effect of IL-24 and Bax. Ad.IL-24-Bax also suppressed tumor growth in nude mice and induced apoptosis. Ad.IL-24-Bax may be a useful tool for gene therapy of hepatic cancer.

**PMID:  
20596059**

Gene Ther. 2010 Jul 1. [Epub ahead of print]

**AAVrh.10-mediated genetic delivery of bevacizumab to the pleura to provide local anti-VEGF to suppress growth of metastatic lung tumors.**

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Vascular endothelial growth factor (VEGF) produced by tumor cells has a central role in stimulating angiogenesis required for tumor growth. Humanized monoclonal anti-VEGF antibody (bevacizumab, Avastin), approved as a treatment for non-squamous, non-small cell lung cancer, requires administration every 3 weeks. We hypothesized that an intrapleural administration of an adeno-associated virus (AAV) vector expressing an anti-VEGF-A antibody equivalent of bevacizumab would result in sustained anti-VEGF-A localized expression within the lung and suppress metastatic tumor growth. The AAV vector AAVrh.10alphaVEGF encodes the light chain and heavy chain complementary DNAs of monoclonal antibody A.4.6.1, a murine antibody that specifically recognizes human VEGF-A with the same antigen-binding site as bevacizumab. A metastatic lung tumor model was established in severe combined immunodeficient mice by intravenous administration of human DU145 prostate carcinoma cells. Intrapleural administration of AAVrh.10alphaVEGF directed long-term expression of the anti-human VEGF-A antibody in lung, as shown by sustained, high-level anti-human VEGF titers in lung epithelial lining fluid for 40 weeks, which was the duration of the study. In the AAVrh.10alphaVEGF-treated animals, tumor growth was significantly suppressed ( $P<0.05$ ), the numbers of blood vessels and mitotic nuclei in the tumor was decreased ( $P<0.05$ ) and there was increased survival ( $P<0.05$ ). Thus, intrapleural administration of an AAVrh.10 vector, encoding the murine monoclonal antibody equivalent of bevacizumab, effectively suppresses the growth of metastatic lung tumors, suggesting AAV-mediated gene transfer to the pleura to deliver bevacizumab locally to the lung as a novel alternative platform to conventional monoclonal antibody therapy.

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20596058

Gene Ther. 2010 Jul 1. [Epub ahead of print]

**Soluble TNF-alpha receptor secretion from healthy or dystrophic mice after AAV6-mediated muscle gene transfer.**

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Muscle is an attractive target because it is easily accessible; it also offers a permissive environment for adeno-associated virus (AAV)-mediated gene transfer and has an abundant blood vascular supply providing an efficient transport system for the secretion of proteins. However, gene therapy of dystrophic muscle may be more difficult than that of healthy tissue because of degenerative-regenerative processes, and also because of the inflammatory context. In this study we followed the expression levels of secreted inhibitors of the proinflammatory tumor necrosis factor (TNF) cytokine after intramuscular (i.m.) injection of AAV6 into dystrophic mdx and healthy C57BL/10 mice. We used two chimeric proteins, namely, the human or murine TNF-soluble receptor I fused with the murine heavy immunoglobulin chain. We conducted an AAV6 dose-response study and determined the kinetics of transgene expression. In addition, we followed the antibody response against the transgenes and studied their expression pattern in the muscle. Our results show that transduction efficiency is reduced in dystrophic muscles as compared with healthy ones. Furthermore, we found that the immune response against the secreted protein is stronger in mdx mice. Together, our results underscore that the pathological state of the muscle has to be taken into consideration when designing gene therapy approaches.

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20596057

Gene Ther. 2010 Jul 1. [Epub ahead of print]

**Addition of adenoviral vector targeting of chemotherapy to the MUC-1/ecdCD40L VPPP vector prime protein boost vaccine prolongs survival of mice carrying growing subcutaneous deposits of Lewis lung cancer cells.**

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We studied the effect of adding chemotherapy or vector targeted chemotherapy to the administration of an Ad-sig-hMUC-1/ecdCD40L adenoviral vector prime-hMUC-1/ecdCD40L protein boost cancer vaccine (designated hMUC-1/ecdCD40L VPPP vaccine), which were administered to test mice 10 days following subcutaneous (s.c.) inoculation of 500 000 Lewis Lung Carcinoma cells, at a time when the average volume of the s.c. tumors was 50 cu mm. The survival of hMUC-1/ecdCD40L VPPP vaccine-treated mice was twice as long as untreated mice. Addition of vector-targeted chemotherapy (AdCMVCDIRESE1A/5FC) to the hMUC-1/ecdCD40L VPPP vaccine 10 days after tumor inoculation significantly ( $P=0.0062$ ) prolonged the survival of the test mice over administration of the hMUC-1/ecdCD40L VPPP vaccine alone or the control mice ( $P<0.00001$ ). Interestingly, the combination of the AdCMVCDIRESE1A/5FC vector-targeted chemotherapy to the hMUC-1/ecdCD40L VPPP vaccine decreased the levels of CD44(+)CD24(-) cells in s.c. deposits of the human MUC-1-positive Lewis Lung Cancer cell line (LL2/LL1hMUC-1) by 20 fold. These results suggest that the addition of vector-targeted chemotherapy to an adenoviral-based cancer vaccine is a strategy that deserves further testing.

**PMID:  
20595824**

Blood Coagul Fibrinolysis. 2010 Jul;21(5):464-73.

**Highly efficient lentiviral transduction of phenotypically and genotypically characterized endothelial progenitor cells from adult peripheral blood.**

Stockschlaeder M, Shardakova O, Weber K, Stoldt VR, Fehse B, Giers G, Scharf RE.  
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Postnatal vasculogenesis has been implicated as an important mechanism for neovascularization via bone marrow-derived endothelial progenitor cells (EPCs) circulating in peripheral blood. In preparation of the utilization of EPCs in clinical protocols, we have generated blood-derived EPCs according to two established protocols by culturing either nonadherent mononuclear cells on fibronectin or adherent mononuclear cells on collagen. To explore the feasibility of these EPCs for their potential clinical use as target cells for genetic transduction to enhance their thromboresistance, newly designed retroviral and lentiviral gene ontology expression vectors were tested. Whereas cell clusters derived from the nonadherent cells demonstrated an only limited proliferative potential, cell colonies derived from collagen-adherent cells expanded more than a million-fold. Characterization of the exponentially growing cells by surface antigen and gene expression profiling revealed a consistently strong expression of characteristic endothelial markers, whereas expression of leukocyte markers was gradually lost. Using a single-step transduction protocol, we were able to achieve gene transfer efficiency of up to 99%. Our results suggest that the generated blood-derived EPC population might be attractive target cells for tissue engineering and gene therapy protocols due to their well defined phenotype, extensive proliferative potential, and efficient genetic transducibility, three important qualities that need to be defined prior to any clinical use.

**PMID:  
20594165**

Curr Diabetes Rev. 2010 Jul 1. [Epub ahead of print]

**Gene Therapy to Improve Pancreatic Islet Transplantation for Type 1 Diabetes Mellitus.**

Hughes A, Jessup C, Drogemuller C, Mohanasundaram D, Milner C, Rojas D, Russ GR, Coates PT.

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Pancreatic islet transplantation is a promising treatment option for Type 1 Diabetics, offering improved glycaemic control through restoration of insulin production and freedom from life-threatening hypoglycaemic episodes. Implementation of the Edmonton protocol in 2000, a glucocorticoid-free immunosuppressive regimen has led to improved islet transplantation success. >50% of islets are lost post-transplantation primarily through cytokine-mediated apoptosis, ischemia and hypoxia. Gene therapy presents a novel strategy to modify islets for improved survival post-transplantation. Current islet gene therapy approaches aim to improve islet function, block apoptosis and inhibit rejection. Gene transfer vectors include adenoviral, adeno-associated virus, herpes simplex virus vectors, retroviral vectors (including lentiviral vectors) and non-viral vectors. Adeno-associated virus is currently the best islet gene therapy vector, due to the vectors minimal immunogenicity and high safety profile. In animal models, using viral vectors to deliver genes conferring local immunoregulation, anti-apoptotic genes or angiogenic genes to islets can significantly improve islet survival in the early post-transplant period and influence long term engraftment. With recent improvements in gene delivery and increased understanding of the mechanisms underlying graft failure, gene therapy for islet transplantation has the potential to move closer to the clinic as a treatment for patients with Type 1 Diabetes.

PMID:  
20593011

PLoS One. 2010 Jun 25;5(6):e11306.

**Functional and behavioral restoration of vision by gene therapy in the guanylate cyclase-1 (GC1) knockout mouse.**

Boye SE, Boye SL, Pang J, Ryals R, Everhart D, Umino Y, Neeley AW, Besharse J, Barlow R, Hauswirth WW.

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**BACKGROUND:** Recessive mutations in guanylate cyclase-1 (Gucy2d) are associated with severe, early onset Leber congenital amaurosis-1(LCA1). Gucy2d encodes guanylate cyclase (GC1) is expressed in photoreceptor outer segment membranes and produces cGMP in these cells. LCA1 patients present in infancy with severely impaired vision and extinguished electroretinogram (ERG) but retain some photoreceptors in both their macular and peripheral retina for years. Like LCA1 patients, loss of cone function in the GC1 knockout (GC1KO) mouse precedes cone degeneration. The purpose of this study was to test whether delivery of functional GC1 to cone cells of the postnatal GC1KO mouse could restore function to these cells. **METHODOLOGY/PRINCIPAL FINDINGS:** Serotype 5 AAV vectors containing either a photoreceptor-specific, rhodopsin kinase (hGRK1) or ubiquitous (smCBA) promoter driving expression of wild type murine GC1 were subretinally delivered to one eye of P14 GC1KO mice. Visual function (ERG) was analyzed in treated and untreated eyes until 3 months post injection. AAV-treated, isogenic wild type and uninjected control mice were evaluated for restoration of visual behavior using optomotor testing. At 3 months post injection, all animals were sacrificed, and their treated and untreated retinas assayed for expression of GC1 and localization of cone arrestin. Cone-mediated function was restored to treated eyes of GC1KO mice (ERG amplitudes were approximately 45% of normal). Treatment effect was stable for at least 3 months. Robust improvements in cone-mediated visual behavior were also observed, with responses of treated mice being similar or identical to that of wild type mice. AAV-vectored GC1 expression was found in photoreceptors and cone cells were preserved in treated retinas. **CONCLUSIONS/SIGNIFICANCE:** This is the first demonstration of gene-based restoration of both visual function/vision-elicited behavior and cone preservation in a mammalian model of GC1 deficiency. Importantly, results were obtained using a well characterized, clinically relevant AAV vector. These results lay the ground work for the development of an AAV-based gene therapy vector for the treatment of LCA1.

PMID:  
20592487

Cancer Biol Ther. 2010 Aug 21;10(3). [Epub ahead of print]

**Targeted therapy for malignant cancer mediated by adenovirus vector expressing diphtheria toxin A based on loss of IGF2 imprinting.**

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Loss of genomic imprinting of the insulin-like growth factor 2 gene (IGF2) maybe associated with the loss of activity of CTCF, an insulator of transcription, in many human neoplasms. This is because the inactive CTCF cannot bind to the differentially methylated domains (DMD). This results in an interaction between the IGF2 promoters and enhancers and IGF2 is produced. In this study we investigated the feasibility of using a combination of expression vectors AdDC312-EGFP and AdDC312-DT-A driven by H19 enhancer DMD-H19 promoter complex. HCT-8, HT-29 and H-522 (LOI) infected with AdDC312-EGFP produced the EGFP protein. However, in the MCF-7 and GES-1 cells (MOI) no EGFP protein was produced. The AdDC312-DT-A significantly decreased cell viability and induced cell apoptosis only in LOI cells in vitro and therefore effectively suppressed tumor development in HCT-8 xenograft in nude mice. To conclude, the toxin gene therapy vector proved effective in inhibiting LOI cells growth in vitro and in vivo and provides an additional option for gene therapy based on loss of IGF2 imprinting.

PMID:  
20592278

J Immunol. 2010 Jun 30. [Epub ahead of print]

**Implications for Gene Therapy-Limiting Expression of IL-2R $\gamma$ c Delineates Differences in Signaling Thresholds Required for Lymphocyte Development and Maintenance.**

Orr SJ, Roessler S, Quigley L, Chan T, Ford JW, O'Connor GM, McVicar DW.  
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X-linked SCID patients are deficient in functional IL-2R $\gamma$ (c) leading to the loss of IL-2/IL-4/IL-7/IL-9/IL-15/IL-21 signaling and a lack of NK and mature T cells. Patients treated with IL-2R $\gamma$ (c) gene therapy have T cells develop; however, their NK cell numbers remain low, suggesting antiviral responses may be compromised. Similarly, IL-2R $\gamma$ (c)(-/-) mice reconstituted with IL-2R $\gamma$ (c) developed few NK cells, and reconstituted T cells exhibited defective proliferative responses suggesting incomplete recovery of IL-2R $\gamma$ (c) signaling. Given the shift toward self-inactivating long terminal repeats with weaker promoters to control the risk of leukemia, we assessed NK and T cell numbers and function in IL-2R $\gamma$ (c)(-/-) mice reconstituted with limiting amounts of IL-2R $\gamma$ (c). Reconstitution resulted in lower IL-2/-15-mediated STAT5 phosphorylation and proliferation in NK and T cells. However, TCR costimulation restored cytokine-driven T cell proliferation to wild-type levels. Vector modifications that improved IL-2R $\gamma$ (c) levels increased cytokine-induced STAT5 phosphorylation in both populations and increased NK cell proliferation demonstrating that IL-2R $\gamma$ (c) levels are limiting. In addition, although the half-lives of both NK and T cells expressing intermediate levels of IL-2R $\gamma$ (c) are reduced compared with wild-type cells, the reduction in NK cell half-life is much more severe than in T cells. Collectively, these data indicate different IL-2R $\gamma$ (c) signaling thresholds for lymphocyte development and proliferation making functional monitoring imperative during gene therapy. Further, our findings suggest that IL-2R $\gamma$ (c) reconstituted T cells may persist more efficiently than NK cells due to compensation for suboptimal IL-2R $\gamma$ (c) signaling by the TCR.

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20588260

Mol Ther. 2010 Jun 29. [Epub ahead of print]

**Therapeutic Effect of Sodium Iodide Symporter Gene Therapy Combined With External Beam Radiotherapy and Targeted Drugs That Inhibit DNA Repair.**

Hingorani M, White CL, Zaidi S, Pandha HS, Melcher AA, Bhide SA, Nutting CM, Syrigos KN, Vile RG, Vassaux G, Harrington KJ.  
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Adenoviral (AdV) transfer of sodium iodide symporter (NIS) gene has translational potential, but relatively low levels of transduction and subsequent radioisotope uptake limit the efficacy of the approach. In previous studies, we showed that combining NIS gene delivery with external beam radiotherapy (EBRT) and DNA damage repair inhibitors increased viral gene expression and radioiodide uptake. Here, we report the therapeutic efficacy of this strategy. An adenovirus expressing NIS from a telomerase promoter (Ad-hTR-NIS) was cytotoxic combined with relatively high-dose (50 microCi) ( $^{131}$ I) therapy and enhanced the efficacy of EBRT combined with low-dose (10 and 25 microCi) ( $^{131}$ I) therapy in colorectal and head and neck cancer cells. Combining this approach with ataxia-telangiectasia mutated (ATM) or DNA-dependent protein kinase (DNA-PK) inhibition caused maintenance of double-stranded DNA breaks (DSBs) at 24 hours and increased cytotoxicity on clonogenic assay. When the triplet of NIS-mediated ( $^{131}$ I) therapy, EBRT, and DNA-PKi was used in vivo, 90% of mice were tumor-free at 5 weeks. Acute radiation toxicity in the EBRT field was not exacerbated. In contrast, DNA-PKi did not enhance the therapeutic efficacy of EBRT plus adenovirus-mediated HSVtk/ganciclovir (GCV). Therefore, combining NIS gene therapy and EBRT represents an ideal strategy to exploit the therapeutic benefits of novel radiosensitizers.

PMID:  
20588258

Mol Ther. 2010 Jun 29. [Epub ahead of print]

**A Ubiquitous Chromatin Opening Element (UCOE) Confers Resistance to DNA Methylation-mediated Silencing of Lentiviral Vectors.**

Zhang F, Frost AR, Blundell MP, Bales O, Antoniou MN, Thrasher AJ.

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DNA methylation may restrict the activity of gene transfer vectors due to inadvertent silencing. In P19 embryonic carcinoma cells in vitro, we found that transgene expression regulated by the SFFV LTR and EF1 $\alpha$  promoter declined rapidly within 16 days, but for A2UCOE derived from the human HNRPA2B1-CBX3 housekeeping gene locus, remained completely stable. Silencing correlated with extensive epigenetic methylation of CpG sites, whereas the A2UCOE was almost completely resistant. Linking of the A2UCOE upstream of the SFFV LTR protected this element from both DNA methylation and silencing. Analysis of engrafted hematopoietic cells in vivo transduced with the same vectors revealed a similar pattern. The A2UCOE displayed little or no methylation in either primary or secondary graft recipients, and gene expression profiles were highly conserved between the two groups. These studies provide convincing evidence that DNA methylation plays a direct role in regulating self-inactivating (SIN) lentiviral transgene expression, and that the stability of expression from the A2UCOE is, at least in part, due to methylation resistance. The A2UCOE therefore has considerable utility for gene therapy applications where reliable and sustained gene expression is desirable.

PMID:  
20588256

Mol Ther. 2010 Jun 29. [Epub ahead of print]

**Statistical Issues in Longitudinal Data Analysis for Treatment Efficacy Studies in the Biomedical Sciences.**

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Longitudinally collected outcomes are increasingly common in cell biology and gene therapy research. In this article, we review the current practice of statistical analysis of longitudinal data in these fields, and recommend the "best performing" statistical method among those available in most statistical packages. A survey of papers published in Molecular Therapy indicates that longitudinal data are only properly analyzed in a small fraction of articles, and the most popular approach was analyzing each measurement time point data separately using an analysis of variance (ANOVA) model with Tukey's post hoc tests. We show that first, such cross-sectional ANOVA approach does not utilize all the power that the longitudinal design of a study provides, and second, Tukey's post hoc tests applied at each measurement time separately could result in a false positivity rate as high as 30% using a simulation study. We recommend mixed effects model analysis instead. We also discuss the complexities of multiple comparison adjustment in the post hoc testing that result from within experimental unit correlation existing in longitudinal data. We recommend resampling as a method that readily adjusts the post hoc testing to be limited to only interesting comparisons and thereby avoids unduly sacrificing the power.

**PMID:**  
**20587341**

Discov Med. 2010 Jun;9(49):519-27.

**Liver-directed gene expression using recombinant AAV 2/8 vectors--a tolerogenic strategy for gene delivery?**

Sharland A, Logan GJ, Bishop A, Alexander IE.

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Vectors based on recombinant adeno-associated virus (AAV) 2/8 hold considerable promise for use in human gene therapy. These vectors are safe, and have minimal immunostimulatory properties. Their combination with efficient, liver-specific promoters allows high-level transgene expression in the hepatocytes of small and large animals. In small animal models, this high level of liver expression results in tolerance to the transgene products. Tolerance to transgene products may also be achievable using these vectors for human gene therapy, but the HLA diversity (and thus variability in T cell recognition of transgene products) and high frequency of prior natural exposure to AAV in human populations impose additional challenges that must be overcome in order for this strategy to succeed.

**PMID:**  
**20586119**

J Gene Med. 2010 Jun 29. [Epub ahead of print]

**Modeling of congenital erythropoietic porphyria by RNA interference: a new tool for preclinical gene therapy evaluation.**

Robert-Richard E, Lalanne M, Lamrissi-Garcia I, Guyonnet-Duperat V, Richard E, Pitard V, Mazurier F, Moreau-Gaudry F, Ged C, de Verneuil H.  
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**BACKGROUND:** Congenital erythropoietic porphyria (CEP) is a severe autosomal recessive disorder characterized by a deficiency in uroporphyrinogen III synthase (UROS), the fourth enzyme of the heme biosynthetic pathway. We recently demonstrated the definitive cure of a murine model of CEP by lentiviral vector-mediated hematopoietic stem cell (HSC) gene therapy. In the perspective of a gene therapy clinical trial, human cellular models are required to evaluate the therapeutic potential of lentiviral vectors in UROS-deficient cells. However, the rare incidence of the disease makes difficult the availability of HSCs derived from patients. **METHODS:** RNA interference (RNAi) has been used to develop a new human model of the disease from normal cord blood HSCs. Lentivectors were developed for this purpose. **RESULTS:** We were able to down-regulate the level of human UROS in human cell lines and primary hematopoietic cells. A 97% reduction of UROS activity led to spontaneous uroporphyrin accumulation in human erythroid bone marrow cells of transplanted immune-deficient mice, recapitulating the phenotype of cells derived from patients. A strong RNAi-induced UROS inhibition allowed us to test the efficiency of different lentiviral vectors with the aim of selecting a safer vector. Restoration of UROS activity in these small hairpin RNA-transduced CD34(+) cord blood cells by therapeutic lentivectors led to a partial correction of the phenotype in vivo. **CONCLUSIONS:** The RNAi strategy is an interesting new tool for preclinical gene therapy evaluation. Copyright (c) 2010 John Wiley & Sons, Ltd.

PMID:  
20585193

Magn Reson Med Sci. 2010;9(2):37-47.

**Molecular MR imaging of cancer gene therapy: ferritin transgene reporter takes the stage.**

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Molecular imaging using magnetic resonance (MR) imaging has been actively investigated and made rapid progress in the past decade. Applied to cancer gene therapy, the technique's high spatial resolution allows evaluation of gene delivery into target tissues. Because noninvasive monitoring of the duration, location, and magnitude of transgene expression in tumor tissues or cells provides useful information for assessing therapeutic efficacy and optimizing protocols, molecular imaging is expected to become a critical step in the success of cancer gene therapy in the near future. We present a brief overview of the current status of molecular MR imaging, especially in vivo reporter gene imaging using ferritin and other reporters, discuss its application to cancer gene therapy, and present our research of MR imaging detection of electroporation-mediated cancer gene therapy using the ferritin reporter gene.

PMID:  
20583868

Hum Gene Ther. 2010 Jun 28. [Epub ahead of print]

**MicroRNA-20a Overexpression Inhibited Proliferation and Metastasis of Pancreatic Carcinoma Cells.**

Yan HJ, Wu JX, Liu WS, Zuo YF, Chen SP, Zhang SN, Zeng MS, Huang WL.

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The aim of this study was to investigate the effect of microRNA-20a on pancreatic carcinoma cell proliferation and invasion and to find a new effective treatment strategy for pancreatic carcinoma. MicroRNA-20a expression was determined in 10 matched normal pancreatic tissues and pancreatic carcinoma by in situ hybridization. Quantitative real-time RT-PCR was used to evaluate the expression of microRNA-20a in two pancreatic carcinoma cell lines (BxPC-3, Panc-1) and immortal human pancreatic duct epithelial cell line H6C7. Proliferation and invasion capacity were analyzed for the cells with lentivirus-mediated overexpression of microRNA-20a both in vitro and in vivo. In addition, the regulation of Stat3 by microRNA-20a was determined to elucidate the underlying mechanisms. The pancreatic cancer cell lines (Panc-1 and BxPC-3) stably overexpressed microRNA-20a showed reduced proliferation and invasion capacity in vitro and in vivo, compared to parental cells or cells transfected with a control vector. Furthermore, we found that microRNA-20a negatively regulated Stat3 protein expression in a dose dependent manner without changing the Stat3 mRNA level and decreased the activity of a luciferase reporter construct containing the Stat3-3'untranslated region. These results show that microRNA-20a regulates Stat3 at the post-transcriptional level result in inhibiting of cell proliferation and invasion of pancreatic carcinoma. It may open a new perspective for the development of effective gene therapy for pancreatic carcinoma.

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**Application of dynamic diffractive optics for enhanced femtosecond laser based cell transfection.**

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We demonstrate the advantages of a dynamic diffractive optical element, namely a spatial light modulator (SLM) for the controlled and enhanced optoinjection and phototransfection of mammalian cells with a femtosecond light source. The SLM provides full control over the lateral and axial positioning of the beam with sub-micron precision. Fast beam translation enables time-sequenced irradiation, which is shown to enhance the optoinjection efficiency and alleviate the problem of exact beam positioning on the cell membrane. We show that irradiation in three axial positions doubles the number of viably optoinjected cells when compared with a single dose. The presented system also enables untargeted raster scan irradiation which provides a higher throughput transfection of adherent cells at the rate of 1 cell per second. Additionally, fluorescent imaging is used to demonstrate cell selective two-step gene therapy.

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**American Society of Gene & Cell Therapy--13th Annual Meeting.**

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The American Society of Gene & Cell Therapy's 13th Annual Meeting, held in Washington, DC, included topics covering new developments in the field of gene therapy. This conference report highlights selected presentations on adenoviral therapies for the treatment of cancer, HIV immunotherapies and gene/cell therapies for the treatment of genetic disorders. Investigational drugs discussed include the TAG vaccine and INGN-007 (both Gradalis Inc), AdCD40L (Uppsala University), Ad5-SSTR/TK-RGD (University of Alabama at Birmingham), CGTG-102 (Oncos Therapeutics Ltd) and lexgenleucel-T (VIRxSYS Corp).

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2058215

HIV Ther. 2010 May 1;4(3):361-369.

### **Possible applications for replicating HIV 1 vectors.**

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Since its discovery some 25 years ago, much has been learned about HIV type 1 and the molecular details of its replication cycle. This insight has been used to develop lentiviral vector systems that have advantages over conventional retroviral vector systems. For safety reasons, the lentiviral vector systems are replication incompetent and the risk of generating a replication competent virus has been minimized. Nevertheless, there may be certain applications for replication competent HIV based vector systems, and we will review our activities in this particular field. This includes the generation of a conditionally replicating HIV 1 variant as a safe live attenuated virus vaccine, the construction of mini HIV variants as cancer selective viruses for virotherapy against leukemia, and the use of a conditionally live anti HIV gene therapy vector. Although safety concerns will undoubtedly remain for the use of replication competent HIV based vector systems, some of the results in cell culture systems are very promising and warrant further testing in appropriate animal models.

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20580914

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### **Improvement of the monitoring and biosafety of encapsulated cells using the SFG(NES)TGL triple reporter system.**

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Cell microencapsulation may represent a breakthrough to overcome problems associated with cell therapy. Advances in material biocompatibility and production protocols have put this field close to its clinical application. However, issues such as the possibility of tracking cell-containing microcapsules, monitoring cell viability, and discontinuation of the therapeutic activity when necessary, still remain unsolved. We demonstrate here simultaneous monitoring and pharmacological control of myoblasts-containing alginate microcapsules, injected in immunocompetent mice after transduction with the SFG(NES)TGL triple reporter retroviral vector, which contains green fluorescence protein (GFP), firefly luciferase and herpes simplex virus type 1 thymidine-kinase (HSV1-TK). Naked (as controls) or microencapsulated cells were subcutaneously injected in C57BL/6J mice and followed up by luminometry. Signal for naked cells disappeared 2weeks after cell injection, whereas signal for microencapsulated cells remained strong for 8months, thus demonstrating the presence of living cells. Treatment of mice with the thymidine-kinase substrate ganciclovir caused death of microencapsulated myoblasts, as seen by a drastic decay in the light emission and histological analysis. Hence, we conclude that incorporation of the SFG(NES)TGL vector into microencapsulated cells represents an accurate tool for controlling cell location and viability in a non-invasive way. Moreover, cell death can be induced by administration of ganciclovir, in case therapy needs to be interrupted. This system may represent a step forward in the control and biosafety of cell- and gene- therapy-based microencapsulation protocols.

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20580766**

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**Folate mediated in vitro targeting of depolymerised trimethylated chitosan having arginine functionality.**

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Delivery vectors having targeting ligands provide an impending impact on cancer gene therapy. Our work focuses on folate mediated targeting induced by conjugating poly(ethylene glycol)-folate (PEG-FA) with arginine modified chitosan polymer having low molecular weight of 15 kDa and high degree of quaternization (ATFP15H). The ATFP15H derivative on condensation with plasmid DNA formed nanoparticles with core shell nanostructure. It also affirmed good buffering capacity. The derivative was found to protect DNA from DNase I degradation and also from disassembly in presence of negatively charged plasma proteins. It exhibited blood compatibility in terms of percentage hemolysis, erythrocyte aggregation and also by platelet activation. At a concentration of 10 microg, the capability of the derivative to enhance cell growth at normal cell growing conditions was observed. The transfection efficiency was also found to be comparable to PEI when transfected in KB cell line, which over expressed the folate receptor (FR) in presence of 10% fetal bovine serum (FBS). On comparison with native chitosan and trimethylated chitosan, ATFP15H derivative exhibited high cellular uptake and nuclear localization due to the superior colloidal stability attained on conjugation with polyethylene glycol. This has been ascertained by flow cytometry and YOYO labeling of plasmid DNA. Copyright 2010 Elsevier Inc. All rights reserved.

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**Gene therapy for muscle disease.**

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The molecular mechanisms of Duchenne muscular dystrophy (DMD) have been extensively investigated since the discovery of the dystrophin gene in 1986. Nonetheless, there is currently no effective treatment for DMD. Recent reports, however, indicate that adeno-associated viral (AAV) vector-mediated transfer of a normal dystrophin cDNA into the affected muscle is a promising strategy. In addition, antisense-mediated exon skipping technology has been emerging as another promising approach to restore dystrophin expression in DMD muscle. Ongoing clinical trials show restoration of dystrophin in DMD patients without serious side effects. Here, we summarize the recent progress in gene therapy, with an emphasis on exon skipping for DMD. Copyright © 2010. Published by Elsevier Inc.

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**Mitochondrial matrix delivery using MITO-Porter, a liposome-based carrier that specifies fusion with mitochondrial membranes.**

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Mitochondria are the principal producers of energy in cells of higher organisms. It was recently reported that mutations and defects in mitochondrial DNA (mtDNA) are associated with various mitochondrial diseases including a variety of neurodegenerative and neuromuscular diseases. Therefore, an effective mitochondrial gene therapy and diagnosis would be expected to have great medical benefits. To achieve this, therapeutic agents need to be delivered into the innermost mitochondrial space (mitochondrial matrix), which contains the mtDNA pool. We previously reported on the development of MITO-Porter, a liposome-based carrier that introduces macromolecular cargos into mitochondria via membrane fusion. In this study, we provide a demonstration of mitochondrial matrix delivery and the visualization of mitochondrial genes (mtDNA) in living cells using the MITO-Porter. We first prepared MITO-Porter containing encapsulated propidium iodide (PI), a fluorescent dye used to stain nucleic acids to detect mtDNA. We then confirmed the emission of red-fluorescence from PI by conjugation with mtDNA, when the carriers were incubated in the presence of isolated rat liver mitochondria. Finally, intracellular observation by confocal laser scanning microscopy clearly verified that the MITO-Porter delivered PI to the mitochondrial matrix. Copyright (c) 2010 Elsevier Inc. All rights reserved.

**Cardiomyocyte-targeted HIF-1alpha gene therapy inhibits cardiomyocyte apoptosis and cardiac allograft vasculopathy in the rat.**

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**BACKGROUND:** Hypoxia-inducible factor-1 (HIF-1), a key transcription factor in hypoxia, affects a wide range of adaptive cell functions. We examined the kinetics of endogenous HIF-1alpha during acute and chronic rejection, and the effect of exogenous HIF-1alpha in chronically rejecting rat cardiac allografts. **METHODS:** Heterotopic cardiac transplantations were performed between major MHC-mismatched Dark Agouti and Wistar-Furth rats. Cyclosporine A (CsA) was used to prevent acute rejection in the chronic rejection model. The effect of HIF-1alpha overexpression was investigated by adeno-associated virus 2 (AAV2)-mediated gene transfer of a constitutively stabilized form of mouse HIF-1alpha (AAV-HIF-1alpha). The analysis of allografts was based on histology, immunohistochemistry and quantitative reverse transcript-polymerase chain reaction (RT-PCR). **RESULTS:** Acute and chronic rejection significantly induced HIF-1alpha mRNA in rat cardiac allografts when compared with syngeneic controls. Immunohistochemistry localized significantly increased HIF-1alpha immunoreactivity to vascular smooth muscle cells, vascular endothelial cells, post-capillary venules and graft-infiltrating mononuclear inflammatory cells of the allograft, whereas expression in cardiomyocytes remained unchanged. Regression analysis revealed a linear correlation between the progression of cardiac allograft vasculopathy (CAV) and HIF-1alpha immunoreactivity in post-capillary venules and graft-infiltrating mononuclear inflammatory cells in chronically rejecting rat cardiac allografts. AAV-HIF-1alpha enhanced cardiomyocyte HIF-1alpha production and significantly reduced cardiomyocyte apoptosis and the development of CAV in chronically rejecting rat cardiac allografts. **CONCLUSIONS:** We found that acute and chronic rejection increased HIF-1alpha mRNA and protein levels in rat cardiac allografts. On the other hand, cardiomyocyte-targeted HIF-1alpha gene transfer inhibited cardiomyocyte apoptosis and the development of CAV, suggesting a novel therapeutic strategy for HIF-1alpha in cardiac allografts. Copyright © 2010 International Society for Heart and Lung Transplantation. Published by Elsevier Inc. All rights reserved.

**B-cell delivered gene therapy for tolerance induction: Role of autoantigen-specific B cells.**

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Antigen-specific tolerance induction using autologous B-cell gene therapy is a potential treatment to eliminate undesirable immune responses. For example, we have shown that experimental autoimmune encephalomyelitis (EAE) and type 1 diabetes in NOD mice can be ameliorated using antigen-Ig fusion protein transduced B cells. However, it is well established that auto-reactive antigen-specific B cells are activated in many autoimmune diseases and can contribute to pathogenesis. While syngeneic B cells from immunized or autoimmune mice can serve as tolerogenic antigen-presenting cells (APC), this observation begs the question of whether the antigen-specific B cells per se can be transduced as tolerogenic APC. To test this, we employed two model systems employing B cell receptor (BCR) transgenic or wild type (wt) mice as B-cell donors. While adoptively transferred MOG-Ig transduced wt C57Bl/6 B cells were highly tolerogenic and ameliorated EAE, MOG-Ig transduced anti-MOG B cells from BCR transgenic mice were not. This phenomenon was reproduced in the NOD diabetes model in which pro-insulin-Ig transduced polyclonal wt NOD B cells were protective, whereas similarly transduced anti-insulin BCR B cells were not. Since the frequency of antigen-specific B cells in an immunized animal is quite low, we wished to determine the threshold numbers of BCR transgenic B cells that could be present in an effective transduced population. Therefore, we "spiked" polyclonal wt C57Bl/6 B cells with different numbers of anti-MOG BCR transgenic B cells. In the EAE model, we found protection when BCR B cells were present at 1%, but they prevented tolerance induction at 10%. Antigen-specific B cells expressed normal levels of co-stimulatory molecules and were tolerogenic when transduced with an irrelevant antigen (OVA). Thus, the presence of a BCR specific for the target autoantigen may interfere with the tolerogenic process to that antigen, but BCR-specific B cells are not intrinsically defective as tolerogenic APC. Taken together, these data suggest that antigen-specific tolerance induction can be achieved in the presence of a limited number of antigen-specific B cells, but higher numbers of pathogenic B cells may mask this induction. This observation should guide future development of therapies using autologous B cells to treat patients with autoimmune diseases.