



# CLINIGENE CURRENT GENE THERAPY WEEKLY

From April 26<sup>th</sup> to May 03<sup>rd</sup> 2010

## Table of contents:

Silk-elastinlike protein polymers for matrix-mediated cancer gene therapy.....	3
Interactions between DNA and Poly(amido amine) Dendrimers on Silica Surfaces. ....	3
TOXICITY PATHWAY FOCUSED GENE EXPRESSION PROFILING OF PEI-BASED POLYMERS FOR PULMONARY APPLICATIONS. ....	4
Chemokines mediate mesenchymal stem cell migration toward gliomas in vitro.....	4
Noteworthy clinical case studies in cancer gene therapy: tumor-targeted Rexin-G advances as an efficacious anti-cancer agent. ....	5
Ex vivo culture of chimeric antigen receptor T cells generates functional CD8(+) T cells with effector and central memory-like phenotype. ....	5
rAAV2/5 gene-targeting to rods:dose-dependent efficiency and complications associated with different promoters.....	6
A probasin promoter, conditionally replicating adenovirus that expresses the sodium iodide symporter (NIS) for radiovirotherapy of prostate cancer. ....	6
Mass spectrometry measurement of a therapeutic peptide for use in multiple sclerosis.....	7
Electroporation- and mechanical ventilation-mediated gene transfer to the lung.....	7
Ets-1 promotes the progression of cerebral aneurysm by inducing the expression of MCP- 1 in vascular smooth muscle cells.....	8
Cell-based osteoprotegerin therapy for debris-induced aseptic prosthetic loosening on a murine model. ....	8
Large animal models of hematopoietic stem cell gene therapy.....	9
Both CD4 and CD8 T Cells Mediate Equally Effective In Vivo Tumor Treatment When Engineered with a Highly Avid TCR Targeting Tyrosinase. ....	9
Review of the clinical development of alipogene tiparovec gene therapy for lipoprotein lipase deficiency. ....	10
SNAI2 as a novel radioprotector of normal tissue by gene transfer using a lentiviral bicistronic SIN vector.....	10
Immuno- and gene-therapeutic strategies targeted against cancer (mainly focusing on pancreatic cancer).....	11
Hypocretin ligand deficiency in narcolepsy: recent basic and clinical insights. ....	11
The influence of skeletal muscle anisotropy on electroporation: in vivo study and numerical modeling. ....	12
Fiber-modified adenovirus can mediate human adipose tissue-derived mesenchymal stem cell-based anti-angiogenic gene therapy. ....	12
Biosafety Assessment of Site-directed Transgene Integration in Human Umbilical Cord- lining Cells.....	13
Combined Paracrine and Endocrine AAV9 mediated Expression of Hepatocyte Growth Factor for the Treatment of Renal Fibrosis. ....	13
Suppression of CDK2 expression by siRNA induces cell cycle arrest and cell proliferation inhibition in human cancer cells. ....	14

Bax expression remains unchanged following antisense treatment directed against BCL-2. ....	14
Genetically engineered T-cells expressing a ganciclovir-sensitive HSV-tk suicide gene for the prevention of GvHD. ....	15
Genetic correction of sickle cell anemia and beta-thalassemia: progress and new perspective. ....	15
Combining bio-electrospraying with gene therapy: a novel biotechnique for the delivery of genetic material via living cells. ....	16
Neighbor effects of neurons bearing protective transgenes. ....	16
Inorganic nanomedicine - Part 2. ....	17
Intracellular FRET analysis of lipid/DNA complexes using flow cytometry and fluorescence imaging techniques. ....	17

PMID:  
20430059

Adv Drug Deliv Rev. 2010 Apr 26. [Epub ahead of print]

### **Silk-elastinlike protein polymers for matrix-mediated cancer gene therapy.**

Gustafson JA, Ghandehari H.

Department of Bioengineering, Nano Institute of Utah, University of Utah, Salt Lake City, UT, USA; Utah Center for Nanomedicine, Nano Institute of Utah, University of Utah, Salt Lake City, UT, USA.

Silk-elastinlike protein polymers (SELPs) are recombinant polymers designed from silk fibroin and mammalian elastin amino acid repeats. These are versatile materials that have been examined as controlled release systems for intratumoral gene delivery. SELP hydrogels comprise monodisperse and tunable polymers that have the capability to control and localize the release and expression of plasmid DNA and viruses. This article reviews recent developments in the synthesis and characterization of SELP hydrogels and their use for matrix-mediated gene delivery.

PMID:  
20429604

Langmuir. 2010 Apr 29. [Epub ahead of print]

### **Interactions between DNA and Poly(amido amine) Dendrimers on Silica Surfaces.**

Ainalem ML, Campbell RA, Nylander T.

Physical Chemistry, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden.

This study increases the understanding at a molecular level of the interactions between DNA and poly(amido amine) (PAMAM) dendrimers on solid surfaces, which is a subject of potential interest in applications such as gene therapy. We have used in situ null ellipsometry and neutron reflectometry to study the structure of multilayer arrangements formed by PAMAM dendrimers of generation 2 (G2), 4 (G4), and 6 (G6) and DNA on silica surfaces. Specifically, we adsorbed cationic dendrimer layers, then we condensed DNA to form dendrimer-DNA bilayers, and last we exposed further dendrimer molecules to the interface to encapsulate DNA in dendrimer-DNA-dendrimer trilayers. The dendrimer monolayers formed initially result in the deformation of the cationic adsorbates as a result of their strong electrostatic attraction to the hydrophilic silica surface. The highest surface excess and most pronounced deformation occurs for the G6 molecules due to their relatively large size and high surface charge density. G6-functionalized surfaces give rise to the highest surface excess of DNA during the bilayer formation process. This result is explained in terms of the high number of charged binding sites in the G6 monolayer and the low electrostatic repulsion between DNA and exposed patches of silica surface due to the relatively thick G6 monolayer. The binding strengths of the silica-dendrimer and dendrimer-DNA interactions are demonstrated by the high stability of the interfacial bilayers during rinsing. For the formation of trilayers of dendrimers, DNA, and dendrimers, G2 adsorbs as a smooth layer while G4 and G6 induce the formation of less well-defined structures due to more complex DNA layer morphologies.

PMID:  
20429563

Mol Pharm. 2010 Apr 29. [Epub ahead of print]

### **TOXICITY PATHWAY FOCUSED GENE EXPRESSION PROFILING OF PEI-BASED POLYMERS FOR PULMONARY APPLICATIONS.**

Beyerle A, Irmeler M, Beckers J, Kissel TH, Stoeger T.

Polyethylene imine (PEI) based polycations, successfully used for gene therapy or RNA interference in vitro as well as in vivo have been shown to cause well known adverse side effects, especially high cytotoxicity. Therefore, various modifications have been developed to improve safety and efficiency of these non-viral vector systems, but profound knowledge about the underlying mechanisms responsible for the high cytotoxicity of PEI is still missing. In this in vitro study, we focused on stress and toxicity pathways triggered by PEI-based vector systems to be used for pulmonary application in comparison to two well known lung toxic particles: fine crystalline silica (CS) and nano-sized ZnO (NZO). The cytotoxicity profiles of all stressors were investigated in alveolar epithelial like type II cells (LA4) to define concentrations with matching toxicity levels (cell viability >60% and LDH release <10%) for subsequent qRT-PCR based gene array analysis. Within the first 6h pathway analysis revealed for CS an extrinsic apoptotic signaling (TNF pathway) in contrast to the intrinsic apoptotic pathway (mitochondrial signaling) which was induced by PEI25kDa after 24h treatment. The following causative chain of events seems conceivable: reactive oxygen species derived from particle surface toxicity triggers TNF signaling in the case of CS, whereby endosomal swelling and rupture upon endocytotic PEI 25kDa uptake causes intracellular stress and mitochondrial alterations, finally leading to apoptotic cell death at higher doses. PEG modification most notably reduced the cytotoxicity of PEI 25kDa but increased proinflammatory signaling on mRNA and even protein level. Hence in view of the lung as a sensitive target organ this inflammatory stimulation might cause unwanted side-effects related to respiratory and cardiovascular disorders. Thus further optimization of the PEI-based vector systems is still needed for pulmonary application.

PMID:  
20428810

Oncol Rep. 2010 Jun;23(6):1561-7.

### **Chemokines mediate mesenchymal stem cell migration toward gliomas in vitro.**

Xu F, Shi J, Yu B, Ni W, Wu X, Gu Z.

Department of Neurosurgery, Shanghai Neurosurgical Center, Huashan Hospital, Fudan University, Shanghai 200040, PR China. fengxu.dr@gmail.com

Previous studies have demonstrated the tremendous tropism of mesenchymal stem cells (MSCs) for malignant gliomas, making these cells a potential vehicle for delivery of therapeutic genes to disseminated glioma cells. However, the mechanisms underlying the tropism of MSCs for gliomas remain poorly defined. It has been suggested that malignant gliomas secrete a variety of chemokines, including macrophage chemoattractant protein-1 (MCP-1) and stromal cell-derived factor-1 alpha (SDF-1 alpha). We isolated and cultured MSCs from rat bone marrow and found that these cells express CCR2 and CXCR4, the respective receptors for MCP-1 and SDF-1 alpha. In vitro analysis revealed that MCP-1 and SDF-1 alpha induce the migration of MSCs. Furthermore, neutralization data suggest that MCP-1 and SDF-1 alpha play a role in the mediation of MSC migration toward gliomas. These results highlight the potential of these cells as a tumor targeting strategy for glioma gene therapy.

PMID:  
20428757

Int J Oncol. 2010 Jun;36(6):1341-53.

**Noteworthy clinical case studies in cancer gene therapy: tumor-targeted Rexin-G advances as an efficacious anti-cancer agent.**

Gordon EM, Hall FL.

Epeius Biotechnologies Corporation, San Marino, CA 91108, USA.  
egordon@epeiusbiotech.com

The advent of pathotropic (disease-seeking) targeting technology has ushered cancer gene therapy across the threshold of history, marking the beginning of a new epoch of medical praxis. For the first time, clinical oncologists can reach beyond the finest of catheters, beyond the reach of the most gifted surgeons, to the very fabric of metastatic disease in an effort to halt the progression and turn the tide of otherwise intractable cancers. The enabling molecular biotechnologies embodied in the leading tumor-targeted agent, Rexin-G, and its timely development as a safe and effective anti-cancer drug - from oncogene discovery and target validation, to molecular engineering of the core nanotechnologies, to the first clinical proofs-of principle, confirmatory trials, expanded access programs, and accelerated regulatory approvals - have been extensively documented in the medical literature. Therefore, this paper represents a final chapter, highlighting a series of noteworthy cases studies in the emergent field of targeted genetic medicine: case studies which, in and of themselves, reveal vital and important aspects of the molecular-genetic bio-pharmacology, advanced clinical protocols, refinement of patient monitoring, expanding treatment options, and strategic medical approaches to patient care that exemplify and thereby extend the established principles of pathotropic targeting and cancer gene therapy to a new generation of clinical practitioners.

PMID:  
20428216

Gene Ther. 2010 Apr 29. [Epub ahead of print]

**Ex vivo culture of chimeric antigen receptor T cells generates functional CD8(+) T cells with effector and central memory-like phenotype.**

Neeson P, Shin A, Tainton KM, Guru P, Prince HM, Harrison SJ, Peinert S, Smyth MJ, Trapani JA, Kershaw MH, Darcy PK, Ritchie DS.

[1] Hematology and Immunology Translational Research Lab, Peter MacCallum Cancer Center, Melbourne, Australia [2] Department of Pathology, University of Melbourne, Melbourne, Australia.

The anti-tumor efficacy of adoptively transferred T cells requires their in vivo persistence and memory polarization. It is unknown if human chimeric antigen receptor (CAR)-expressing T cells can also undergo memory polarization. We examined the functional status of CAR CD8(+) T cells, re-directed to Lewis Y antigen (LeY-T), throughout a period of ex vivo expansion. Immediately before culture CD8(+) T cells comprised a mixture of phenotypes including naive (CD45RA(+)/CCR7(+)/CD27(+)/CD28(+)/perforin-), central memory (CM, CD45RA(-)/CCR7(lo)/CD27(+)/CD28(+)/perforin(lo)), effector memory (EM, CD45RA(-)/CCR7(-)/CD27(+)/CD28(+)/perforin(mod)) and effector (Eff, CD45RA(+)/CCR7(-)/CD27(-)/CD28(-)/perforin(hi)) cells. After transduction and expansion culture of peripheral blood mononuclear cells from normal donors or multiple myeloma patients, CD8(+) LeY-T cells polarized to EM- and CM-like phenotype. CD8(+) LeY-T cells differed from starting CD8(+) CM and EM T cells in that CD27, but not CD28, was downregulated. In addition, CD8(+) LeY-T cells expressed high levels of perforin, similar to starting CD8(+) Eff. CD8(+) LeY-T cells also showed hallmarks of both memory and Eff function, underwent homeostatic proliferation in response to interleukin (IL)-15, and showed interferon (IFN)-gamma production and cytotoxicity in response to Le-Y antigen on OVCAR-3 (human ovarian adenocarcinoma) cells. This study confirms CD8(+) LeY-T cells have a CM- and EM-like phenotype and heterogeneous function consistent with potential to persist in vivo after adoptive transfer.

PMID:  
20428215

Gene Ther. 2010 Apr 29. [Epub ahead of print]

**rAAV2/5 gene-targeting to rods:dose-dependent efficiency and complications associated with different promoters.**

Beltran WA, Boye SL, Boye SE, Chiodo VA, Lewin AS, Hauswirth WW, Aguirre GD.  
Section of Ophthalmology, Department of Clinical Studies School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA.

A prerequisite for using corrective gene therapy to treat humans with inherited retinal degenerative diseases that primarily affect rods is to develop viral vectors that target specifically this population of photoreceptors. The delivery of a viral vector with photoreceptor tropism coupled with a rod-specific promoter is likely to be the safest and most efficient approach to target expression of the therapeutic gene to rods. Three promoters that included a fragment of the proximal mouse opsin promoter (mOP), the human G-protein-coupled receptor protein kinase 1 promoter (hGRK1), or the cytomegalovirus immediate early enhancer combined with the chicken beta actin proximal promoter CBA were evaluated for their specificity and robustness in driving GFP reporter gene expression in rods, when packaged in a recombinant adeno-associated viral vector of serotype 2/5 (AAV2/5), and delivered via subretinal injection to the normal canine retina. Photoreceptor-specific promoters (mOP, hGRK1) targeted robust GFP expression to rods, whereas the ubiquitously expressed CBA promoter led to transgene expression in the retinal pigment epithelium, rods, cones and rare Müller, horizontal and ganglion cells. Late onset inflammation was frequently observed both clinically and histologically with all three constructs when the highest viral titers were injected. Cone loss in the injected regions of the retinas that received the highest titers occurred with both the hGRK1 and CBA promoters. Efficient and specific rod transduction, together with preservation of retinal structure was achieved with both mOP and hGRK1 promoters when viral titers in the order of  $10^{11}$  vg ml<sup>-1</sup> were used.

PMID:  
20428214

Gene Ther. 2010 Apr 29. [Epub ahead of print]

**A probasin promoter, conditionally replicating adenovirus that expresses the sodium iodide symporter (NIS) for radiovirotherapy of prostate cancer.**

Trujillo MA, Oneal MJ, McDonough S, Qin R, Morris JC.  
Department of Internal Medicine, Division of Endocrinology, Diabetes, Metabolism, Nutrition, Mayo Clinic, Rochester, MN, USA.

The sodium iodide symporter (NIS) directs the uptake and concentration of iodide in thyroid cells. We have extended the use of NIS-mediated radioiodine therapy to other types of cancer, we transferred and expressed the NIS gene into prostate, colon and breast cancer cells using adenoviral vectors. To improve vector efficiency we have developed a conditionally replicating adenovirus (CRAd) in which the E1a gene is driven by the prostate-specific promoter, Probasin and the cassette RSV promoter human NIScDNA-bGH polyA replaces the E3 region (CRAd Ad5PB\_RSV-NIS). In vitro infection of the prostate cancer cell line LnCaP resulted in virus replication, cytolysis and release of infective viral particles. Conversely, the prostate cancer cell line PC-3 (androgen receptor negative) and the pancreatic cancer cell line Panc-1 were refractory to the viral cytopathic effect and did not support viral replication. Radioiodine uptake was readily measurable in LnCaP cells infected with Ad5PB\_RSV-NIS 24 h post-infection, confirming NIS expression. In vivo, LnCaP tumor xenografts in nude-mice injected intratumorally with Ad5PB\_RSV\_NIS CRAd expressed NIS actively as evidenced by (99)Tc uptake and imaging. Administration of therapeutic (131)I after virus injection significantly increased survival probability in mice carrying xenografted LnCaP tumors compared with virotherapy alone. These data indicate that Ad5PB\_RSV\_NIS replication is stringently restricted to androgen-positive prostate cancer cells and results in effective NIS expression and uptake of radioiodine. This construct may allow multimodal therapy, combining cytolytic virotherapy with radioiodine treatment, to be developed as a novel treatment for prostate cancer.

PMID:  
20428213

Gene Ther. 2010 Apr 29. [Epub ahead of print]

**Mass spectrometry measurement of a therapeutic peptide for use in multiple sclerosis.**

Dadgari JM, Moore RE, Louie KA, Lee TD, McMillan M.

[1] Department of Neurology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA [2] Department of Microbiology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA.

Multiple sclerosis is an autoimmune disease of the central nervous system believed to be mediated by pathogenic T lymphocytes. We have developed a next-generation therapy in which cells secrete specific therapeutic molecules to silence these aberrant T cells. We have shown that fibroblasts, transduced to secrete a myelin basic protein-derived peptide, abrogate disease in the murine experimental autoimmune encephalomyelitis model of multiple sclerosis, which we hypothesized using a low-zone tolerance mechanism. To determine the efficacy (or not) of this therapy in humans, we must ensure that patients receive comparable doses of therapeutic peptide. To this end, we have used liquid chromatography coupled to tandem mass spectrometry to detect a tryptic peptide, derived from the secreted therapeutic product, at nanomolar concentrations. Success depended on growing the transduced fibroblasts in defined PC-1 medium in the presence of a cocktail of protease inhibitors.

PMID:  
20428212

Gene Ther. 2010 Apr 29. [Epub ahead of print]

**Electroporation- and mechanical ventilation-mediated gene transfer to the lung.**

Kaufman CD, Geiger RC, Dean DA.

[1] Department of Pediatrics, School of Medicine and Dentistry, University of Rochester, Rochester, NY, USA [2] Division of Pulmonary and Critical Care Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA.

Our laboratory has previously demonstrated that cytoplasmic trafficking and subsequent nuclear entry of nonviral plasmid DNA can be significantly enhanced through the application of cyclic stretch after transfection *in vitro*. In this study, we show that cyclic stretching of the murine lung using ventilation immediately after endotracheal administration and transthoracic electroporation of plasmid DNA increases exogenous gene expression up to fourfold in mice that were not ventilated after plasmid administration and transfection by electroporation *in vivo*. This increase is both time and sequence specific (that is, the ventilation must occur immediately after the transfection event). The ventilation-enhanced gene transfer is also amplitude dependent, confirming similar studies completed *in vitro*, and is mediated, at least in part, through the cytoplasmic tubulin deacetylase, HDAC6. Using immunohistochemistry, we show that this increase in expression is due to an increase in the number of cells expressing the exogenous protein rather than an increase in the amount of protein produced per cell. These studies show the potential mechanical stimulation has *in vivo* in significantly increasing nonviral DNA gene expression, and may ultimately pave the way for more successful clinical trials using this type of therapy in the future.

PMID:  
20428211

Gene Ther. 2010 Apr 29. [Epub ahead of print]

**Ets-1 promotes the progression of cerebral aneurysm by inducing the expression of MCP-1 in vascular smooth muscle cells.**

Aoki T, Kataoka H, Nishimura M, Ishibashi R, Morishita R, Miyamoto S.

Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Cerebral aneurysm (CA) rupture is one of the leading causes of stroke death. Recent experimental studies suggest that the pathophysiology of CA is closely associated with inflammation. A transcription factor, Ets-1, has been shown to regulate vascular inflammation and remodeling in a physiological and pathological condition. The expression and role of Ets-1 in CA development has been investigated in this study. Ets-1 was expressed and activated mainly in vascular smooth muscle cells (VSMCs) in both experimentally induced rat CAs and human CA walls by immunohistochemistry, western blotting and enzyme-linked mobility shift assay. The downstream target of Ets-1 in CA development was identified by chromatin immunoprecipitation (CHIP) analysis. CHIP analysis revealed that Ets-1 transactivated monocyte chemoattractant protein-1 (MCP-1) expression in CA walls. Treatment with ets decoy oligodeoxynucleotides resulted in the prevention of CA enlargement, upregulation of MCP-1 expression and increase in macrophage accumulation in CA walls. In conclusion, Ets-1 mediates MCP-1 expression in VSMCs in CA walls, thus promoting the progression of CAs. Inhibition of DNA-binding activity of Ets-1 may lead to the prevention of human CA enlargement and rupture. Results of this study will provide us a clue to a novel therapeutic strategy for CAs.

PMID:  
20428210

Gene Ther. 2010 Apr 29. [Epub ahead of print]

**Cell-based osteoprotegerin therapy for debris-induced aseptic prosthetic loosening on a murine model.**

Zhang L, Jia TH, Chong AC, Bai L, Yu H, Gong W, Wooley PH, Yang SY.

[1] Orthopaedic Research Institute, Via Christi Regional Medical Center, Wichita, KS, USA

[2] Department of Orthopaedic Surgery, Jinan Central Hospital, Shandong University School of Medicine, Jinan, China.

Exogenous osteoprotegerin (OPG) gene modification appears a therapeutic strategy for osteolytic aseptic loosening. The feasibility and efficacy of a cell-based OPG gene delivery approach were investigated using a murine model of knee prosthesis failure. A titanium pin was implanted into mouse proximal tibia to mimic a weight-bearing knee arthroplasty, followed by titanium particles challenge to induce periprosthetic osteolysis. Mouse fibroblast-like synoviocytes were transduced in vitro with either AAV-OPG or AAV-LacZ before transfused into the osteolytic prosthetic joint 3 weeks post surgery. Successful transgene expression at the local site was confirmed 4 weeks later after killing. Biomechanical pullout test indicated a significant restoration of implant stability after the cell-based OPG gene therapy. Histology revealed that inflammatory pseudo-membranes existed ubiquitously at bone-implant interface in control groups, whereas only observed sporadically in OPG gene-modified groups. Tartrate-resistant acid phosphatase+osteoclasts and tumor necrosis factor alpha, interleukin-1beta, CD68+ expressing cells were significantly reduced in periprosthetic tissues of OPG gene-modified mice. No transgene dissemination or tumorigenesis was detected in remote organs and tissues. Data suggest that cell-based ex vivo OPG gene therapy was comparable in efficacy with in vivo local gene transfer technique to deliver functional therapeutic OPG activities, effectively halted the debris-induced osteolysis and regained the implant stability in this model.

PMID:  
20428209

Gene Ther. 2010 Apr 29. [Epub ahead of print]

### **Large animal models of hematopoietic stem cell gene therapy.**

Trobridge GD, Kiem HP.

[1] Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

[2] Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA.

Large animal models have been instrumental in advancing hematopoietic stem cell (HSC) gene therapy. Here we review the advantages of large animal models, their contributions to the field of HSC gene therapy and recent progress in this field. Several properties of human HSCs including their purification, their cell-cycle characteristics, their response to cytokines and the proliferative demands placed on them after transplantation are more similar in large animal models than in mice. Progress in the development and use of retroviral vectors and ex vivo transduction protocols over the last decade has led to efficient gene transfer in both dogs and nonhuman primates. Importantly, the approaches developed in these models have translated well to the clinic. Large animals continue to be useful to evaluate the efficacy and safety of gene therapy, and dogs with hematopoietic diseases have now been cured by HSC gene therapy. Nonhuman primates allow evaluation of aspects of transplantation as well as disease-specific approaches such as AIDS (acquired immunodeficiency syndrome) gene therapy that can not be modeled well in the dog. Finally, large animal models have been used to evaluate the genotoxicity of viral vectors by comparing integration sites in hematopoietic repopulating cells and monitoring clonality after transplantation.

PMID:  
20427771

J Immunol. 2010 Apr 28. [Epub ahead of print]

### **Both CD4 and CD8 T Cells Mediate Equally Effective In Vivo Tumor Treatment When Engineered with a Highly Avid TCR Targeting Tyrosinase.**

Frankel TL, Burns WR, Peng PD, Yu Z, Chinnasamy D, Wargo JA, Zheng Z, Restifo NP, Rosenberg SA, Morgan RA.

Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Tyrosinase, an enzyme involved in melanin synthesis, is expressed in nearly all primary and metastatic melanoma lesions and thus is an attractive target for TCR-based gene therapy using adoptive cell transfer. The TCR alpha- and beta-chain genes from a tumor-infiltrating lymphocyte, which recognized the tyrosinase 368-376 peptide in the context of HLA-A2, were cloned into a gamma-retroviral vector. Following transduction of PBL, specific reactivity was confirmed by cytokine production following coculture with tumor targets. Experiments using Ab blockade and CD4/CD8 sorting of the transduced PBLs demonstrated that this antityrosinase TCR was CD4/CD8 independent. The introduction of a second disulfide bond between the TCR constant regions and/or creation of a chimeric protein in which the human constant regions were replaced by murine homologs resulted in enhanced TCR expression as demonstrated by tetramer staining and improved tumor reactivity that was comparable to PBL transduced with either anti-melanoma Ag recognized by T cells-1 or anti-gp100 TCR vectors currently used in clinical trials. The chimeric TCR also allowed us to test antitumor function of in HLA-A2/K(b)-transgenic mice. Transfer of the antityrosinase TCR into mouse splenocytes conferred CD4/CD8-independent, HLA-A2-restricted Ag reactivity against B16/A2K(b) murine melanoma in vitro. Furthermore, adoptive transfer of transduced splenocytes mediated B16/A2K(b) melanoma tumor regression in lymphodepleted mice, and, surprisingly, both CD8 and CD4 T cells were equally effective in mediating tumor regression. These results suggest that this highly active tyrosinase-specific TCR could be of value in adoptive cell transfer for melanoma.

PMID:  
20427244

Atheroscler Suppl. 2010 Apr 26. [Epub ahead of print]

**Review of the clinical development of alipogene tiparvovec gene therapy for lipoprotein lipase deficiency.**

Gaudet D, de Wal J, Tremblay K, Déry S, van Deventer S, Freidig A, Brisson D, Méthot J. Department of Medicine, Université de Montréal, ECOGENE-21 Clinical Research Center, Chicoutimi Hospital, Canada.

Alipogene tiparvovec (AAV1-LPL(S447X)) gene therapy is developed to prevent complications and decrease the clinical morbidity of lipoprotein lipase deficiency (LPLD). LPLD is an autosomal recessive disease associated with severe hypertriglyceridemia (hyperTG), severe chylomicronaemia, and low HDL. Acute pancreatitis, the most frequent serious clinical LPLD complication, is a complex and heterogeneous inflammatory condition having many causes including hyperTG and chylomicronaemia. In many patients, low fat diet and currently available lipid lowering drugs are ineffective to prevent hyperTG or pancreatitis in LPLD. The clinical development program of alipogene tiparvovec includes observational studies as well as phase I/II and II/III clinical trials. Pooled data are collected on safety and efficacy issues, including the incidence of pancreatitis.

PMID:  
20426660

Radiat Res. 2010 May;173(5):612-9.

**SNAI2 as a novel radioprotector of normal tissue by gene transfer using a lentiviral bicistronic SIN vector.**

Maier P, Herskind C, Barzan D, Zeller WJ, Wenz F. Department of Radiation Oncology, Mannheim Medical Center, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany. patrick.maier@medma.uni-heidelberg.de

Tumor radiotherapy with large-field irradiation results in an increase of p53-dependent apoptosis of the radiosensitive hematopoietic stem cells. Proapoptotic PUMA is a transcriptional target of p53. Thus suppression of PUMA expression by gene therapy with the transcription repressor SNAI2 as transgene might be a potential approach for normal tissue protection during radiotherapy. SNAI2 cDNA was cloned in a lentiviral SIN vector in a bicistronic expression cassette followed by a floxed IRES-EMCV linker and EGFP as selection gene. Wild-type p53 TK6 cells were used as the cellular model system. We could demonstrate the significant radioprotective effect of SNAI2 overexpression in a cytotoxicity assay after irradiation with 0-5 Gy compared with untransduced or control vector (inverse oriented SNAI2 cDNA)-transduced cells. Additionally, TK6-SNAI2 compared to TK6-SNAI2<sup>inv</sup> cells showed a survival advantage in a clonogenic assay after irradiation with 0-3 Gy. Determination of the proportion of sub-G(1) cells in TK6-SNAI2 cells revealed an approximately 50% reduction in apoptosis compared with both control entities. In this study using a bicistronic lentiviral vector, we were able to provide proof of principle that lentiviral overexpression of SNAI2 might be used for radioprotective gene therapy to widen the therapeutic range in radiotherapy.

**PMID:**  
**20425541**

Surg Today. 2010 May;40(5):404-10. Epub 2010 Apr 28.

**Immuno- and gene-therapeutic strategies targeted against cancer (mainly focusing on pancreatic cancer).**

Yoshimura K, Oline K, Edil BH, Schulick RD, Oka M.

Department of Surgery II, Yamaguchi University School of Medicine, Ube, Yamaguchi, Japan.

Current treatment modalities of surgical resection and chemotherapy against cancers have improved survival. However, mortality from tumor recurrence remains high. Immunotherapy and gene therapy are potential additions to the treatment arsenal in the care of cancer patients. These novel therapeutic approaches need further investigation in in vitro and in vivo models as they are developed for potential use in humans. Here we reviewed immunotherapies and gene therapies that included clinical trials against cancers (mainly focusing on pancreatic cancer) suggesting the strong possibility of using these novel approaches.

**PMID:**  
**20425033**

Curr Neurol Neurosci Rep. 2010 May;10(3):180-9.

**Hypocretin ligand deficiency in narcolepsy: recent basic and clinical insights.**

Ritchie C, Okuro M, Kanbayashi T, Nishino S.

Center for Narcolepsy, Stanford School of Medicine, Stanford University Sleep and Circadian Neurobiology Laboratory, 1201 Welch Road, MSLS, P213, Palo Alto, CA 94304, USA.

Narcolepsy is a chronic sleep disorder characterized by excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, and sleep paralysis. Both sporadic and familial forms exist in humans. Recently, the major pathophysiology of human narcolepsy was indicated, based on discovery, through animal study, of narcolepsy genes involved in the pathology of hypocretin/orexin ligand and its receptor. Hypocretin ligand deficiency is found in most patients with narcolepsy with cataplexy. This deficiency likely is the result of postnatal cell death of hypocretin neurons, and involvement of autoimmune mechanisms is suggested. Hypocretin deficiency also is found in symptomatic narcolepsy and excessive daytime sleepiness with neurologic conditions, including immune-mediated neurologic disorders. These findings have significant clinical relevance and promote understanding of hypocretin cell death mechanisms. Already, discoveries in humans have led to a new diagnostic test for narcolepsy. Currently, hypocretin replacement therapy has emerged as a promising therapeutic option, and experiments using gene therapy and cell transplantation are in progress.

**PMID:**  
**20424926**

Med Biol Eng Comput. 2010 Apr 28. [Epub ahead of print]

**The influence of skeletal muscle anisotropy on electroporation: in vivo study and numerical modeling.**

Corović S, Zupanič A, Kranjc S, Al Sakere B, Leroy-Willig A, Mir LM, Miklavčič D.

Faculty of Electrical Engineering, University of Ljubljana, Trzaska 25, SI-1000, Ljubljana, Slovenia.

The aim of this study was to theoretically and experimentally investigate electroporation of mouse tibialis cranialis and to determine the reversible electroporation threshold values needed for parallel and perpendicular orientation of the applied electric field with respect to the muscle fibers. Our study was based on local electric field calculated with three-dimensional realistic numerical models, that we built, and in vivo visualization of electroporated muscle tissue. We established that electroporation of muscle cells in tissue depends on the orientation of the applied electric field; the local electric field threshold values were determined (pulse parameters: 8 x 100  $\mu$ s, 1 Hz) to be 80 V/cm and 200 V/cm for parallel and perpendicular orientation, respectively. Our results could be useful electric field parameters in the control of skeletal muscle electroporation, which can be used in treatment planning of electroporation based therapies such as gene therapy, genetic vaccination, and electrochemotherapy.

**PMID:**  
**20424891**

Biotechnol Lett. 2010 Apr 28. [Epub ahead of print]

**Fiber-modified adenovirus can mediate human adipose tissue-derived mesenchymal stem cell-based anti-angiogenic gene therapy.**

Liu H, Chu Y, Lou G.

Department of Gynecological Oncology, Affiliated Tumor Hospital of Harbin Medical University, 150081, Harbin, China.

A fiber-modified adenovirus (rAd5F11B), loaded with the Kringle1-5 gene (rAd-K1-5) was used to infect human adipose tissue-derived mesenchymal stem cells (HAMSCs). At a multiplicity of infection of 20, the transfection efficiency in HAMSCs was 90% and the cell expansion and differentiation of infected HAMSCs were not significantly suppressed. HAMSCs infected with rAd-K1-5 expressed the exogenous Kringle1-5 protein, an angiogenic inhibitor, and conditioned media from HAMSCs expressing the Kringle1-5 protein blocked VEGF-induced neovascularization both in vitro and in vivo. rAd5F11B may therefore be a promising gene transfer vector in HAMSCs-based anti-angiogenic gene therapy because of its low toxicity and high transfection efficiency.

PMID:  
20424600

Mol Ther. 2010 Apr 27. [Epub ahead of print]

### **Biosafety Assessment of Site-directed Transgene Integration in Human Umbilical Cord-lining Cells.**

Sivalingam J, Krishnan S, Ng WH, Lee SS, Phan TT, Kon OL.

[1] Division of Medical Sciences, Laboratory of Applied Human Genetics, Humphrey Oei Institute of Cancer Research, National Cancer Centre, Singapore, Republic of Singapore [2] Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Republic of Singapore.

Biosafety and efficacy considerations that impede clinical application of gene therapy could be addressed by nonviral ex vivo cell therapy, utilizing transgenic cells that have been comprehensively pre-evaluated for genotoxic potential and transgene expression. We evaluated the genotoxic potential of phiC31 bacteriophage integrase-mediated transgene integration in cord-lining epithelial cells (CLECs) readily cultured from the outer membrane of human umbilical cords, by sequencing and mapping integration sites, spectral karyotyping, high-resolution genome copy number, transcriptome, and transgene copy number analyses and in vivo tumorigenicity. Of 44 independent integration events, <5% were exonic and 85% of modified cells had integrated  $\leq 2$  transgene(s). Expression of 95.6% of genes was unaltered in modified cells. Only three small regions showed genome copy number changes that did not correlate with altered gene expression or integration sites. Spectral karyotyping revealed rare nonrecurrent occurrence of three different translocations. Integrase-modified cells were not tumorigenic in immunocompromised mice for at least 4 months. Stable integration of a human factor VIII (FVIII) construct conferred durable FVIII secretion in vitro. Xenotransplantation of FVIII-secreting CLECs in immunocompetent hemophilic mice achieved significant phenotypic correction. Pre-evaluated clonal populations of phiC31 integrase-modified CLECs could be useful as bioimplants for monogenic diseases such as hemophilia.

PMID:  
20424598

Mol Ther. 2010 Apr 27. [Epub ahead of print]

### **Combined Paracrine and Endocrine AAV9 mediated Expression of Hepatocyte Growth Factor for the Treatment of Renal Fibrosis.**

Schievenbusch S, Strack I, Scheffler M, Nischt R, Coutelle O, Hösel M, Hallek M, Fries JW, Dienes HP, Odenthal M, Büning H.

Institute for Pathology, University of Cologne, Cologne, Germany.

In chronic renal disease, tubulointerstitial fibrosis is a leading cause of renal failure. Here, we made use of one of the most promising gene therapy vector platforms, the adeno-associated viral (AAV) vector system, and the COL4A3-deficient mice, a genetic mouse model of renal tubulointerstitial fibrosis, to develop a novel bidirectional treatment strategy to prevent renal fibrosis. By comparing different AAV serotypes in reporter studies, we identified AAV9 as the most suitable delivery vector to simultaneously target liver parenchyma for endocrine and renal tubular epithelium for paracrine therapeutic expression of the antifibrogenic cytokine human hepatocyte growth factor (hHGF). We used transcriptional targeting to drive hHGF expression from the newly developed CMV-enhancer-Ksp-cadherin-promoter (CMV-Ksp) in renal and hepatic tissue following tail vein injection of rAAV9-CMV-Ksp-hHGF into COL4A3-deficient mice. The therapeutic efficiency of our approach was demonstrated by a remarkable attenuation of tubulointerstitial fibrosis and repression of fibrotic markers such as collagen1alpha1 (Col1A1), platelet-derived growth factor receptor-beta (PDGFR-beta), and alpha-smooth muscle actin (SMA). Taken together, our results show the great potential of rAAV9 as an intravenously applicable vector for the combined paracrine and endocrine expression of antifibrogenic factors in the treatment of renal failure caused by tubulointerstitial fibrosis.

PMID:  
20423616

BMB Rep. 2010 Apr;43(4):291-6.

**Suppression of CDK2 expression by siRNA induces cell cycle arrest and cell proliferation inhibition in human cancer cells.**

Long XE, Gong ZH, Pan L, Zhong ZW, Le YP, Liu Q, Guo JM, Zhong JC.

School of Medicine, Ningbo University, 818 Fenghua Road, Ningbo 315211, People's Republic of China.

Cyclin-dependent kinase 2 (CDK2) is a member of serine/ threonine protein kinases, which initiates the principal transitions of the eukaryotic cell cycle and is a promising target for cancer therapy. The present study was designed to inhibit cdk2 gene expression to induce cell cycle arrest and cell proliferation suppression. Here, we constructed a series of RNA interference (RNAi) plasmids which can successfully express small interference RNA (siRNA) in the transfected human cells. The results showed that the RNAi plasmids containing the coding sequences for siRNAs down-regulated the cdk2 gene expression in human cancer cells at the mRNA and the protein levels. Furthermore, we found that the cell cycle was arrested at G0G1 phases and the cell proliferation was inhibited by different siRNAs. These results demonstrate that suppression of CDK2 activity by RNAi may be an effective strategy for gene therapy in human cancers.

PMID:  
2042317

Med Oncol. 2010 Apr 27. [Epub ahead of print]

**Bax expression remains unchanged following antisense treatment directed against BCL-2.**

Rubenstein M, Hollowell CM, Guinan P.

Division of Cellular Biology, Hektoen Institute for Medical Research, 2240 West Ogden Avenue, 2<sup>nd</sup> floor, Chicago, IL, 60612, USA, DrMarv@Prodigy.net.

Antisense oligonucleotides (oligos) have been evaluated in both in vivo and in vitro prostate cancer models. Although most contain a single mRNA binding site, our laboratory has also evaluated bispecific types directed toward two proteins. This study evaluates the inhibition of in vitro propagating LNCaP cells employing mono- and bispecific oligos directed against bcl-2 [the second binding site was directed against the epidermal growth factor receptor (EGFR)]. Employing RT-PCR, the expression of two apoptosis regulating proteins, bcl-2 and non-targeted bax, was then evaluated. LNCaP prostate tumor cells were initially incubated for 24 h in the presence of oligos (6.25 μM) directed against bcl-2 and compared to lipofectin containing controls. Comparable and significant growth inhibition was produced by both mono- and bispecific forms. Employing RT-PCR to determine the expression of bcl-2, we found that the greatest amount of mRNA suppression approached 100% for each oligo type: monospecific MR(4) (directed only against bcl-2), 100%; and bispecifics MR(24) and MR(42), 86 and 100%, respectively. We conclude, based upon both inhibition of in vitro growth and bcl-2 expression, that bispecific antisense oligos directed against EGFR and bcl-2 mRNAs are at least as effective as a monospecific directed solely toward bcl-2. In an effort to determine a compensatory response by cells evading apoptosis in the presence of bcl-2 suppression, the levels of mRNA encoding the non-targeted apoptosis activating protein bax were evaluated. Non-targeted protein suppression by these bispecifics has previously been demonstrated against prostate-specific membrane antigen (PSMA). However, in contrast to effects against bcl-2 and PSMA, no significant alteration in bax expression was produced by either oligo type. In LNCaP cells, bcl-2 suppression does not influence bax expression and, at least for this protein, there is no compensatory change in bax expression regulating apoptosis at this level. Identifying changes in the expression of proteins which regulate apoptosis is important if gene therapy targets bcl-2.

PMID:  
20419602

Curr Opin Investig Drugs. 2010 May;11(5):559-70.

**Genetically engineered T-cells expressing a ganciclovir-sensitive HSV-tk suicide gene for the prevention of GvHD.**

Mailly L, Leboeuf C, Tiberghien P, Baumert T, Robinet E.

INSERM U748, Institut de Virologie, 3 rue Koeberlé, 67000 Strasbourg, France.  
e.robinet@unistra.fr

In vitro and in vivo preclinical studies and phase I/II clinical trials have demonstrated that the retroviral-mediated transfer of the suicide gene HSV-thymidine kinase into donor T-cells prior to infusion (ie, a 2-week ex vivo process including activation, retroviral transduction and selection of transduced cells), at the time of T-cell-depleted hematopoietic stem cell transplantation (HSCT) or as donor lymphocyte infusion after relapse, allows for the efficient control of donor T-cell alloreactivity. These donor suicide gene-modified T-cells (SGMTCs) can provide beneficial anti-leukemic, antiviral and immune reconstitution-facilitating effects to the recipient of an allogeneic HSCT. However, if the infused SGMTCs lead to GvHD, a severe complication of HSCT, these cells can be specifically depleted in vivo by the administration of the prodrug ganciclovir (GCV), without any associated immunosuppression. Limitations to this approach include a gene transfer-induced decrease in alloreactivity and antiviral reactivity, the immunogenicity of SGMTCs, and the development of GCV-resistant SGMTCs. However, major improvements that can prevent these limitations, such as introducing CD3/CD28 costimulation and immunomagnetic selection, have been applied to this approach, but further improvements are still required. The efficacy of suicide gene therapy as a safety control system allows the development of this strategy for gene therapy or immunotherapy approaches.

PMID:  
20419277

ScientificWorldJournal. 2010 Apr 13;10:644-54.

**Genetic correction of sickle cell anemia and beta-thalassemia: progress and new perspective.**

Perumbeti A, Malik P.

Divisions of Hematology-Oncology and Experimental Hematology/Cancer Biology, Cancer and Blood Institute, Cincinnati Children's Research Foundation, Cincinnati Children's Hospital Medical Center (CCHMC), Cincinnati, OH, USA.

Gene therapy for beta-globinopathies, particularly Beta-thalassemia and sickle cell anemia, holds promise for the future as a definitive corrective approach for these common and debilitating disorders. Correction of the beta-globinopathies using lentivirus vectors carrying the beta- or  $\gamma$ -globin genes and elements of the locus control region has now been well established in murine models, and an understanding of "what is required to cure these diseases" has been developed in the first decade of the 21st century. A clinical trial using one such vector has been initiated in France with intriguing results, while other trials are under development. Vector improvements to enhance the safety and efficiency of lentivirus vectors are being explored, while new strategies, including homologous recombination in induced pluripotent cells, for correction of sickle cell anemia have shown proof-of-concept in vitro. Here, a review is provided of the current substantial progress in genetic correction of beta-globin disorders.

PMID:  
20419255

Analyst. 2010 May 26;135(5):1042-9. Epub 2010 Jan 27.

**Combining bio-electrospraying with gene therapy: a novel biotechnique for the delivery of genetic material via living cells.**

Ward E, Chan E, Gustafsson K, Jayasinghe SN.

Molecular Immunology Unit, Institute of Child Health, University College London, 30 Guilford Street, London, UK WC1N 1EH.

The investigations reported in this article demonstrate the ability of bio-electrosprays and cell electrospinning to deliver a genetic construct in association with living cells. Previous studies on both bio-electrosprays and cell electrospinning demonstrated great promise for tissue engineering and regenerative biology/medicine. The investigations described herein widen the applicability of these biotechniques by combining gene therapy protocols, resulting in a novel drug delivery methodology previously unexplored. In these studies a human cell line was transduced with recombinant self-inactivating lentiviral particles. These particles incorporated a green fluorescent protein fused to an endosomal targeting construct. This construct encodes a peptide, which can subsequently be detected on the surface of cells by specific T-cells. The transduced cell line was subsequently manipulated in association with either bio-electrospraying or cell electrospinning. Hence this demonstrates (i) the ability to safely handle genetically modified living cells and (ii) the ability to directly form pre-determined architectures bearing living therapeutic cells. This merged technology demonstrates a unique approach for directly forming living therapeutic architectures for controlled and targeted release of experimental cells/genes, as well as medical cell/gene therapeutics for a plethora of biological and medical applications. Hence, such developments could be applied to personalised medicine.

PMID:  
20417625

Brain Res. 2010 Apr 22. [Epub ahead of print]

**Neighbor effects of neurons bearing protective transgenes.**

Lee AL, Campbell LB, Sapolsky RM.

Department of Biology, Stanford University, Stanford, CA 94305-5020.

Viral vectors bearing protective transgenes can decrease neurotoxicity after varied necrotic insults. A neuron that dies necrotically releases glutamate, calcium and reactive oxygen species, thereby potentially damaging neighboring neurons. This raises the possibility that preventing such neuron death via gene therapy can secondarily protect neighboring neurons that, themselves, do not express a protective transgene. We determined whether such "good neighbor" effects occur, by characterizing neurons that, while uninfected themselves, are in close proximity to a transgene-bearing neuron. We tested two genes whose overexpression protects against excitotoxicity: anti-apoptotic Bcl-2, and a calcium-activated K(+) channel, SK2. Using herpes simplex virus type 2-mediated transgene delivery to hippocampal cultures, we observed "good neighbor" effects on neuronal survival following an excitotoxic insult. However, in the absence of insult, "bad neighbor effects" could also occur (i.e., where being in proximity to a neuron constitutively expressing one of those transgenes is deleterious). We also characterized the necessity for cell-cell contact for these effects. These phenomena may have broad implications for the efficacy of gene overexpression strategies in the CNS.

PMID:  
20417314

Nanomedicine. 2010 Apr 21. [Epub ahead of print]

### **Inorganic nanomedicine - Part 2.**

Sekhon BS, Kamboj SR.

PCTE Institute of Pharmacy, Jhande, Near Baddowal Cantt. Ludhiana-142021, India.

Inorganic nanomaterials/nanoparticles are important in our life due to their use as drugs/imaging agents/antiseptics. Amongst the most promising inorganic nanomaterials being developed are metal, silica, dendrimers, organic-inorganic hybrids, and bioinorganic hybrids. Gold nanoparticles are important in imaging, as drug carriers, and for thermotherapy of biological targets. Gold-nanoparticles/-nanoshells/-nanorods/-nanowires have the extensive potential to be an integral part of our imaging toolbox and useful in the fight against cancer. Metal nanoparticle contrast agents enhance magnetic resonance imaging and ultrasound results in biomedical applications of in vivo imaging. Hollow and porous inorganic nanomaterials have been exploited for drug/gene delivery, for imaging/diagnosis and photothermal therapy. Silver nanoparticles improved antimicrobial activity. Silica nanoparticles have been used in drug delivery and gene therapy. Biomolecular-inorganic nanohybrids/nanostructured biomaterials have been exploited for targeted imaging and therapy, drug and gene delivery, and regenerative medicine. Dendrimers find use as drug or gene carriers, contrast agents and sensors for different metal ions.

PMID:  
20417238

J Control Release. 2010 Apr 22. [Epub ahead of print]

### **Intracellular FRET analysis of lipid/DNA complexes using flow cytometry and fluorescence imaging techniques.**

Schneider S, Lenz D, Holzer M, Palme K, Süss R.

Department of Pharmaceutical Technology and Biopharmacy, Albert-Ludwigs University, D-79104 Freiburg, Germany.

Gene therapy is a promising therapeutic concept for a large number of incurable diseases. Lipid/DNA complexes (lipoplexes) are used to deliver genes into cells. However, while large efforts have been made to investigate the fate of lipoplexes once inside the cell, the rate of intracellular dissociation is still largely unknown. Analysis of the dissociation rates of DNA from lipid/DNA complexes is crucial for the evaluation of a gene delivery system's efficiency. This study introduces a new fluorescence resonance energy transfer (FRET) approach for the intracellular dissociation analysis of lipid/DNA complexes. Here, the labeling of both complex components, DNA as well as lipid, reveals whether DNA is still associated with the lipid or has dissociated. In this study the kinetic properties of complex dissociation were consistently measured with flow cytometry and fluorescence microscopy, and indicated that most complexes were dissociated after 24h in A-10 cells.