



heterogeneous population makes this difficult as many factors such as sex, age, body weight, diet, life style, ethnicity and geographical location and underlying disease states have profound effects on pharmacokinetic parameters. Several pharmacokinetic models and algorithms have been developed to address these differences and translate data obtained from individuals to provide an estimate of pharmacokinetic parameters for the population. These models will be discussed in detail as well as:

- The application and interpretation of basic pharmacokinetic concepts in the clinical trial setting, including single dose versus multiple dose pharmacokinetics, determination of sampling scheme, definition/determination of dose and therapeutic window, and introduction of population pharmacokinetics.
- The utility/feasibility of allometric scaling to predict various clinical PK parameters such as half-life, clearance, and volume of distribution from in vitro and preclinical data in the context of gene transfer.
- Pharmacokinetic/pharmacodynamic modeling, which relates the concentrations provided by kinetic model to the observed pharmacological effects by establishing the exposure-response relationship, will be briefly introduced. An example of how pharmacokinetic/pharmacodynamic modeling is applied in gene therapy will be discussed, as well as how this approach may help in optimizing gene delivery systems.

THURSDAY, JUNE 2, 2005

WS210: CANCER: OPTIMIZATION OF T-CELL GENE THERAPY

Redirected Specificity of T Cells - Improving Their Potential

Laurence JN Cooper, MD, PhD

Clinical trials are currently underway using genetically modified T cells that are re-directed in their specificity for tumor antigens. These "first in human" trials are important proofs of principle that generating genetically modified T cells from patients with cancer is feasible and that adoptive transfer of these cells is safe. While these investigations will establish platforms for adoptive immunotherapy, further pre-clinical development is likely to be needed to improve the potency of these gene therapy T-cell products. While the order of importance may differ for adoptive immunotherapy for a given tumor system, it is predicted that therapeutic efficacy of tumor-specific genetically modified T cells will depend on, (i) the number of adoptively transferred cells, (ii) the ability to traffick to sites of disease, (iii) the ability to achieve fully competent activation within the tumor microenvironment, (iv) and the ability to persist after infusion and encounter with tumor. The laboratories in the Division of Cancer Immunotherapeutics and Tumor Immunology (CITI) at the City of Hope are developing approaches to the manufacture of genetically modified T cells that address each of these four areas. (i) Rapid expansion methods using allogeneic feeder cells are available to propagate large numbers of T cells and these technologies are being refined in the context of automated culture-systems to rapidly achieve clinically-meaningful doses of both CD4⁺ and CD8⁺ T cells. (ii) T cells which can traffick to desired sites of minimal residual disease are being identified *a priori* and then genetically modified and expanded. (iii) Tumor-specific T cells are being combined with interleukin-2-directed therapy to provide localized help in the tumor microenvironment. (iv) T cells are being genetically modified to receive a fully-competent activation signal either by vaccination or through binding tumor antigen. The success of these pre-clinical experiments to improve the *in vivo* potential of genetically modified T cells will be influenced by the *ex vivo* growth conditions, such as the use of exogenous cytokine or cytokines, the immunogenicity of the transgenes, and the development of pre-adoptive immunotherapy patient preparative regimens. Given the expense and time needed to prepare patient-specific T-cell products and evaluate their immunobiology in clinical trials, it is likely that investigators will develop protocols that incorporate many of these changes in second generation

immunotherapy protocols with the expectation of achieving clinical responses.

Augmenting T-cell Trafficking to Tumors

Steven M. Albelda, M.D.

The requirements for a successful anti-tumor immune reaction include: 1) generation of CD4 and/or CD⁺ T-cells with appropriate specificity, 2) amplification/activation of these cells, 3) trafficking of T-cells into tumors, and 4) tumor cell killing. Although tremendous progress has been made in our ability to generate T-cells capable of killing tumor cells, very little attention has been paid to the crucial process of enhancing the trafficking of these cells into tumors.

T-cell trafficking is a multi-step process that involves rolling, activation, firm adhesion, and diapedesis through tumor endothelium. These steps require **both** the presence of appropriate chemokine receptors and cell adhesion molecules on the lymphocytes, as well as the appropriate corresponding chemokines and adhesion molecules on the tumor endothelium and tumor cells.

Naïve T-cells do not traffic to peripheral tissues efficiently. The transition from a naïve T-cell to an activated Memory/effector T-cell is characterized by changes in specific chemokine receptors (i.e., loss of CCR7, upregulation of CCR5 and CXCR3) and cell adhesion molecules (i.e. loss of expression of CD62L, upregulation of CD44, selectin ligands, LFA-1, and α 1 integrins). Reciprocally, effective recruitment of lymphocytes into tumors requires endothelial “activation” characterized by upregulation of appropriate adhesion molecules, including: selectins (CD62E or CD62P), immunoglobulin superfamily adhesion receptors (ICAM-1, ICAM-2, VCAM-1) or other EC adhesion receptors (vascular adhesion protein-1, VAP-1). It also requires production of chemokines by the endothelial cells, themselves, or by the underlying tumor or stromal cells. The chemokines most likely to be most important in the recruitment of activated T-cells and NK cells, are CCL5 (RANTES), CCL3 (MIP-1 β), CXCL9 (Mig), CXCL10 (IP-10), CCL21 (6Ckine/SLC), and CCL20 (MIP-3 β).

Leukocytes commonly fail to infiltrate tumors, but remain localized within the periphery or fibrous septa of the tumor. This is likely due to the immunosuppressive environment within the tumor that tends to down regulate production of immunoactivating cytokines (such as TNF, IL-1, INF- γ) and chemokines (such as Mig, RANTES, IP-10).

Given this defect in endothelial cell activation, we hypothesized that selective EC stimulation in tumor beds could augment immune responses. In our first approach, we inhibited the immune suppressive agent PGE₂ made by tumor cells and macrophages. We established a cancer vaccine model that had relatively little efficacy by itself on

established tumors. However, when combined with inhibition of COX-2 using an oral inhibitor, there was marked augmentation of anti-tumor effects. Mechanisms for this enhanced activity included upregulation of immunostimulatory intratumoral cytokines leading to enhanced numbers of T-cells within the tumor. Our second approach involved the flavanoid derivative, 5,6 Di-methylxanthenone-4-acetic Acid (DMXAA). DMXAA has the ability to selectively activate tumor-associated macrophages. After systemic treatment with DMXAA, mRNA and protein levels of immunostimulatory cytokine/chemokines were markedly increased. Combining a tumor vaccine with DMXAA therapy also led to markedly enhanced anti-tumor effects.

These studies highlight the fact that generating highly active T-cells for adoptive transfer may not be enough. It may be equally important to combine adoptive transfer with agents that will enhance trafficking of these T-cells into disseminated tumors to achieve maximum efficacy.

T-cell Gene Therapy: Regulatory Issues

Carolyn A. Wilson, PhD

Gene transfer products that rely on ex vivo modification of T-cells must comply with regulatory requirements and recommendations relevant to cellular therapies as well as those relevant to the gene transfer vector used. Of particular note, during ex vivo modification of T cells, the product manufacture may result in exposure of the product to infectious agents, compromising the safety of the final product. Potential sources of infectious agents may be donor-derived, even if autologous, adventitious agents introduced from other products used in the manufacturing process (antibodies or cytokines, for example), or intentionally as part of the product manufacture (exposure to EBV as a stimulant for producing EBV-specific cytolytic T cells, for example). Adequate methods should be used to qualify the reagents and the process used during product manufacture to ensure production of a safe final product.

At this time, retroviral vectors are still the predominant vector used in clinical protocols that use ex vivo modified T cells. Therefore, the presentation will also review regulatory issues unique to the use of retroviral vector-mediated clinical protocols.



WS211: GENE-BASED VACCINES: NEW DEVELOPMENTS FOR GENETIC VACCINES

Means to Induce Mucosal Immunity by HIV Genetic Vaccination

Britta Wahren, MD, PhD

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DNA vaccination is a strategy involving direct inoculation of genetic material that is capable of producing antigens intracellularly. Such DNA based immunization induces both cellular and humoral immunity as well as protection from infectious challenge. The mode of delivery is important for induction of mucosal immunity. We aimed to assess the efficacy of DNA vaccination in induction of mucosal immune responses in experimental animals and in HIV-1 infected human beings. Routes of delivery to induce the strongest mucosal responses were by intranasal or skin delivery by jet inoculations of a mixture of DNA plasmids. Boosting with peptides revealed long term memory.

Symptom-free HIV-1 infected patients were immunized with DNA constructs encoding the nef, rev, or tat regulatory genes of HIV-1. Cellular immune reactivities against the HIV-1 regulatory proteins were absent or low before DNA immunization by jet inoculation in the human oral mucosa (Syrjet) or by intradermal jet inoculation (Biojector in mice and macaques).

Cytotoxicity and memory cells were induced in all animals and patients. Both oral and intramuscular routes induced specific systemic T cell immunity.

Immunohistochemical analyses of oral biopsies revealed an increase of T-cells with markers of the Th1 pathway, but not of the B-cell compartment.

Immunization in conjunction with antiretroviral therapy was also effective in patients, which strongly suggests that a combination with highly active antiretroviral therapy would improve

Developing Multiple Viral Protein Genes and Virus-like Particles as Gene-based Vaccines

Ning-Sun Yang, PhD

P-W. Hsiao

Comparative studies of the capsid precursor polypeptide P1 and the capsid protein VP1 cDNA vectors revealed that the multiple viral gene approach for DNA vaccination provided a good vaccine strategy against foot-and-mouth disease virus. Skin immunization using gene gun with a cDNA vector expressing the major viral antigen (VP1) alone routinely failed to induce the production of anti-VP1 or neutralizing antibodies in test mice. As a second approach, the plasmid L-VP1 that produces a transgenic membrane-anchored VP1 protein elicited a strong antibody response, but all test mice failed

in the FMDV challenge experiment. In contrast, for mice immunized with the viral capsid precursor protein (P1) cDNA expression vector, both neutralizing antibodies and 80-100% protection in test mice were detected. This strategy of using the whole capsid precursor protein P1 cDNA for vaccination, intentionally without the use of virus-specific protease or other encoding genes for safety reasons, may thus be employed as a relevant experimental system for induction or upgrading of effective neutralizing antibody response. As a separate approach, virus-like particles (VLP) of SARS CoV were generated in Vero E6 cells by assembly of the transgenic S.E.M.N viral proteins of the SARS corona virus and the design of gene-based vaccines. Efficacy and advantage of using the VLP strategy for vaccination against life-threatening viral infections are being evaluated using the SARS CoV VLP system.

WS212: HEMOPOIETIC: HEMOPOIETIC STEM CELL GENE THERAPY

Addressing Safety Concerns for Hemopoietic Stem Cell Gene Therapy – An FDA Perspective

Carolyn A. Wilson, PhD

The therapeutic potential for using genetically modified hematopoietic stem cells was highlighted by the clinical results from two exploratory clinical studies using retroviral vector modified hematopoietic stem cells in children with X-linked Severe Combined Immunodeficiency Disease [1] [2]. Unfortunately, the clinical results also indicated that the use of a retroviral vector to perform the gene transfer caused leukemia in 3 out of 11 children treated in one of the clinical trials, and death in one of these three [3] (as well as data presented at the March 4, 2005, meeting of the Cellular, Tissues, and Gene Therapies Advisory Committee). The FDA responded to these serious adverse events with a series of open, public discussions held with an FDA Advisory Committee (October, 2002; February, 2003, and March, 2005, transcripts available, <http://www.fda.gov/cber/advisory/advisory.html>). The outcome of these discussions and current FDA recommendations for the use of retroviral vectors for ex vivo modified hematopoietic stem cells will be presented.

In Vivo Stem Cell Selection without Myeloablation

Stanton L. Gerson, MD

One of the most promising developments *in vivo* stem cell collection is the use of the drug resistant gene MGMT. MGMT encodes a DNA repair protein for O⁶-alkoguanine lesions formed by nitrosourea and methylating agents such as temozolomide. Mutant forms of MGMT G156 A and P140 K are resistant to the clinically used inhibitor benzylguanine. Retroviral and lentiviral gene transfer of MGMT into hematopoietic

stem cells followed by transplantation has been used to show a very high degree of stem cell selection and reconstitution under both ablative and myeloablative conditions in mouse and dog models. Using a colony-based oncoretroviruses, MFG carrying a mutant MGMT, a clinical trial has been initiated CD34 purified cells mobilized by G-CSF and GM-CSF have been collected from patients with advanced malignancies. Following transduction 8-26% of the cells show evidence of gene expression and up to 40% of the cells show gene transfer. Low copy number/CFU has been noted. In this ongoing trial, adverse events have not been noted and the most recent data regarding gene transfer, gene expression, and selection *in vivo* will be presented. Use of a mutant MGMT in conjunction with gene transfer, transplantation, and post infusion drug selection after non-myeloablative conditioning appears a promising approach to stem cell selection technology in patients with malignancies and other disorders.

Correction of Chronic Granulomatous Disease by Gene Therapy

Manuel Grez, PhD

Chronic Granulomatous Disease (CGD) is a rare inherited immunodeficiency caused by a functional defect in the microbial killing activity of phagocytes.

Accordingly, CGD patients suffer from recurrent, often life threatening bacterial and fungal infections. CGD is caused by mutations or deletions in any of four genes encoding for essential subunits of the phagocytic nicotinamide dinucleotide phosphate (NADPH) oxidase complex (gp91^{phox}, p22^{phox}, p47^{phox} and p67^{phox}). Almost 60% of CGD patients contain defects in the X-linked gene encoding for gp91^{phox}. Although the disease can be cured by bone marrow transplantation, this treatment is recommended only to patients with HLA-identical sibling or matched unrelated donors, since haploidentical bone marrow transplantation is still associated with high morbidity and mortality rates due to graft failure and slow immunoreconstitution.

One therapeutic option for CGD patients is the genetic modification of autologous bone marrow stem cells. CGD is an ideal candidate for a gene replacement therapy. The molecular bases of the disease are well known and the genes involved have been cloned. Moreover, animal models and female carriers of the X-linked form of the disease with >10% residual oxidase activity are mostly healthy, suggesting that the functional correction of a few phagocytes could be sufficient to ameliorate the course of the disease. Although CGD has been successfully corrected in animal models by gene transfer into HSC in combination with nonmyeloablative conditioning or bone marrow ablation, similar successes have been difficult to achieve in unconditioned CGD patients.

Based on our preclinical work, two X-CGD patients, 26 and 25 years old, were treated with gene modified cells. G-CSF mobilized peripheral blood CD34+ cells were collected (1.6×10^8 for P1 and 3.5×10^8 for P2), transduced at a density of 1×10^6 cells/ml with a monocistronic gammaretroviral vector expressing gp91^{phox} (SF71gp91^{phox}) and reinfused 5 days later. Transduction efficiency was 45% for P1 and 39.5% for P2 as estimated by gp91^{phox} expression by FACS. The proviral copy number per cell as estimated by quantitative PCR was 1.2 and 0.9 for P1 and P2, respectively. P1 received a total of 5.3×10^6 CD34+/gp91+ cells per kg while P2 received 3.7×10^6 CD34+/gp91+ cells per kg. Previous to reinfusion, liposomal busulfan (L-Bu) was administered intravenously on days -3 and -2 at a dose of 4 mg/kg/day. Both patients experienced no acute toxicity from infusion of transduced cells or from chemotherapy. Both patients experienced a period of myelosuppression (neutrophil nadir for P1: day +14 and for P2: day +15) with absolute neutrophils counts below 500 cells per μ l between days +12 and +21 for P1 and between days +13 and +18 for P2. A significant fraction of gene marked cells (>20%) has been detected in peripheral blood of both patients since day +21. Similarly, therapeutic relevant levels of NADPH oxidase activity have been observed since day +21. Both patients are well and have been free of severe bacterial and fungal infections since transplantation. Our data suggests that gene therapy is an option for the treatment of CGD.

Gene Therapy of ADA-deficient SCID

Alessandro Aiuti, MD, PhD

C. Bordignon, M.G. Roncarolo

Severe combined immunodeficiencies (SCID) are genetic diseases caused by mutations in crucial genes controlling the development and functions of immune cells. Adenosine deaminase (ADA)- SCID is characterized by a purine defect which leads to impaired immune functions, recurrent infections, and systemic metabolic abnormalities. In the absence of an HLA-identical sibling donor, bone marrow (BM) transplantation results in high morbidity and mortality. Enzyme replacement therapy (PEG-ADA) corrects the metabolic alterations but is limited by the variable degree of immune recovery, the high costs, and the occurrence of neutralizing antibodies and autoimmunity.

The first clinical gene therapy trials provided the proof of principle for the safety and feasibility of retroviral mediated gene transfer in peripheral blood lymphocytes (PBL) or hematopoietic stem/progenitor cells (HSC). However, it is only recently that the efficacy of ADA gene transfer has been investigated in the absence of PEG-ADA. Our results showed that infusions of engineered PBL allowed to correct the T-cell defect, but provided insufficient systemic detoxification. In contrast, HSC-



based gene therapy, combined with non-myeloablative conditioning, allowed full correction of both the immune and metabolic defects of ADA-deficiency.

We have now enrolled six ADA-SCID children in the HSC gene therapy protocol. Previous therapy included haploidentical BM transplant or PEG-ADA associated with insufficient immune reconstitution or side effects. Autologous BM CD34⁺ cells were transduced with an MLV-based retroviral vector encoding ADA cDNA and Neo^R, and reinfused in the patients following a low intensity conditioning with busulfan, in the absence of PEG-ADA. After gene therapy, multilineage, stable engraftment of gene corrected HSC was achieved in the BM of all patients, at higher levels (5-10%), in the patients who received the highest CD34⁺ cell dose and experienced a relatively stronger myelosuppression. In the five children with a follow-up >6 months, vector-ADA⁺ cells became over time the large majority of T, B and NK lymphocytes. This led to the progressive increase of lymphocyte counts, development of polyclonal thymopoiesis, and normalization of proliferative responses to mitogens and antigens. Serum Ig levels improved in all patients and production of specific antibodies after IVIG discontinuation and antigen vaccination was observed in two patients. Inverse-PCR and LM-PCR analysis showed a profile of polyclonal integrations in T cells, oligoclonal integrations in myeloid cells, and no evidence of clonal expansion. ADA activity persisted in lymphocytes and RBC, resulting in correction of purine metabolism and amelioration of systemic toxicity. All the children are healthy and thriving, in the absence of PEG-ADA. None of the patients experienced severe infections or adverse events after gene therapy, with the longest follow up being of 54 months. In summary, these results show that gene therapy is safe and efficacious in correcting both the immune and metabolic defect of ADA-deficiency, with proven clinical benefit. In addition, this study represents an important platform for the development of other HSC gene transfer protocols for the treatment of acquired or genetic disorders.

WS213: IMMUNOLOGY OF GENE THERAPY: ORGAN SPECIFIC IMMUNITY

In Vivo Gene Transfer to Muscle and Liver: Two Organs, Two Different Stories

Roland Herzog, PhD

In vivo viral gene transfer is a wildly pursued approach toward systemic delivery of therapeutic proteins. Examples include gene replacement therapy for treatment of hemophilia, lysosomal storage disorders, α_1 -antitrypsin deficiency, treatment of obesity, β -thalassemia, metabolic disorders, and others. Skeletal muscle and liver have been popular target tissues because of easy accessibility or

efficient secretion of proteins into the blood stream, respectively. Phase I clinical trials on muscle- and liver-directed AAV-mediated transfer of a functional coagulation factor IX (F.IX) gene to subjects with severe hemophilia B have been carried out. A major concern in gene replacement therapies is the risk of immune responses to the therapeutic transgene product. Among other factors, the target organ plays an important role in immune responses. In AAV-F.IX gene transfer, we found that the combination of target organ and level of transgene expression as determined by vector dose, serotype, and promoter, is critical for the risk of immune responses. Moreover, data from several labs have demonstrated that expression of a non-self transgene product (e.g. human F.IX in a mouse or canine F.IX in a dog with a null mutation) typically results in neutralizing antibody formation, while hepatic gene transfer often does not cause an immune response. Using ovalbumin as a model antigen for secreted proteins that can be expressed in a defined system (T cell receptor transgenic mice), we found that systemic muscle-derived protein delivery is limited by a local immune response that results in T cell activation in the draining lymph node of the transduced muscle. Similar data were obtained with F.IX. Th1 and Th2 subsets of CD4⁺ T helper cells are activated. Depending on the transgene product, MHC class II restricted, i.e. antibody-mediated, or MHC class I-restricted, i.e. CTL-mediated, responses dominate the ensuing response. In contrast, hepatic gene transfer may result in induction of T cell anergy and deletion of T cells specific to the transgene product. Moreover, CD4⁺CD25⁺ regulatory T cells are generated that actively suppress immune responses to the transgene product. These mechanisms prevent antibody formation, CTL responses, and lymphocytic infiltration of the liver. The remarkable difference in T cell activation between the two organs may be explained by differences in location of antigen presentation or subsets of antigen presenting cells. These possibilities are under active investigation in our laboratory.

WS215: PROGRAMS OF EXCELLENCE IN GENE THERAPY

Preclinical Gene Therapy of Hemophilia A with New AAV Serotypes

Haig H. Kazazian, Jr, MD

R Sarkar, JM Wilson, G Gao, TC Nichols, DA Bellinger, M Mucci, and E Toorens.

A major recent development in gene therapy efforts has been the discovery of alternative AAV capsid serotypes from primate and human tissues. Efficacy studies have previously suggested that the most effective serotypes for delivery of genes to liver cells are AAV8 and AAV9. We have previously shown the complete correction of

hemophilia A mice with B domain-deleted canine FVIII cDNA delivered in either in a single vector or in two vectors, one containing FVIII heavy chain cDNA and the other containing FVIII light chain cDNA. Correction was accomplished by $3\text{-}10 \times 10^{10}$ gc/mouse using either intraportal delivery or tail vein delivery of vectors. Now we have shown similar results with two-vector delivery of canine FVIII cDNA using AAV9 serotype. We also find that immunosuppression using cytoxin is unnecessary to achieve correction in the hemophilia A mouse. These results have led us to test the efficacy of AAV8 and AAV9 in two-vector delivery of canine FVIII cDNA to hemophilia A dogs. One male dog given 1.25×10^{13} gc/v/kg of each AAV8 vector intraportally and followed over 800 days had 4-8% FVIII activity in plasma and no adverse bleeding events. Two other female dogs given higher doses of FVIII cDNA in two AAV8 vectors had FVIII activity in the 2% range over 400 to 600 days. One male dog given only 6×10^{12} gc/v/kg in two AAV9 vectors has had consistent FVIII plasma levels of 2-2.5%, no bleeding events, and whole blood clotting times close to the normal range during 200 days of followup. Although the dogs have not responded with FVIII levels similar to those observed in mice, the results are encouraging and suggest that both AAV8 and AAV9 serotypes are promising vectors for delivery of FVIII cDNA in liver gene therapy.

WS216: RESPIRATORY TRACT: WHAT'S NEW IN ANIMAL MODELS FOR LUNG GENE THERAPY

FABp-CF Knockout Mice as Models for CFTR Gene Transfer

Uta Griesenbach, PhD

Screening of non-viral gene transfer agents (GTAs) in the nasal epithelium of cystic fibrosis (CF) knock-out mice is an important aspect of the UK Cystic Fibrosis Gene Therapy Consortium research strategy. We have chosen the FABp-CF knockout mouse (Zhou 1994) as the standard model in our core facility. Fatty acid binding promoter (FABp)-mediated expression of the cystic fibrosis transmembrane regulator (*CFTR*) gene in the gut, but not the airways, prevents the intestinal disease of the CF knockout mouse, thus easing work in a core facility setting. In addition, homozygote FABp-CF mice can breed, which significantly reduces costs.

The pathophysiology of CF is well described. Mutation in the *CFTR* gene cause alterations in ion and water transport across the apical membrane of airway epithelial cells, leading to a reduction in airway surface liquid (ASL) height, which in turn leads to impaired mucociliary clearance (MCC). Impaired MCC contributes to accumulation of sticky sputum, which provides an ideal

environment for bacterial colonisation and ultimately leads to chronic bacterial infection.

Over the last two years the UK CF Gene Therapy Consortium has devoted a significant amount of time and resources to refine further existing assays and set-up and validate a panel of new pre-clinical assays to screen non-viral gene transfer agents in FABp-CF mice. When choosing assays we aimed to cover several aspects of defective CFTR level and function [1. CFTR protein (vector- and epithelial cell-specific detection of CFTR mRNA and protein). 2. Ion transport (potential difference and short circuit current measurements). 3. Airway surface liquid (ASL) height, 4. Host bacterial interaction (Bacterial adherence to airway epithelial cells and goblet cell quantification). During assay validation we compared FABp-CF mice and wild-type littermates and used the results to carry out power calculations that informed cohort size when assessing efficiency of gene transfer agents. Importantly, we learnt that n numbers have to be large to detect small, but potentially clinically relevant, changes in these assays and that care has to be taken not to underpower assays for assumed treatment effects. We have recently used these assays in a large pre-clinical study comparing 3 non-viral GTAs and results will be presented.

Evaluation of Non-viral Gene Transfer in the Ovine Lung

Gerry McLachlan, PhD

A number of promising candidate gene transfer agents (GTAs) for airway gene transfer have failed to live up to the expectations raised by encouraging preclinical mouse data. This has called into question the ability to predict the safety and efficacy of airway gene transfer protocols in human clinical trials purely based on studies in small animals. A realistic assessment of the scale of the task involved in developing improved lung-directed gene therapy strategies for Cystic Fibrosis (CF) has focussed our attention on the relevance of model systems to the clinical setting. Such model systems should ideally demonstrate similar size and dynamic characteristics to the human lung such that the modelled response to gene delivery, whether in terms of biological efficacy or toxicity, bears relevance to what might be anticipated in the clinical setting. With this in mind, we have recently directed efforts towards developing a novel large animal lung model for gene delivery. The animal we chose for our model was the sheep. Although no CF sheep model exists to date, the similarities to humans in lung physiology and architecture are proving to be invaluable in the assessment of gene delivery and efficacy, the localisation of transgene expression and the safety of the protocol, all crucially relevant endpoint measurements. We have previously described studies based on instillation of gene therapy agents to individual segments of the sheep lung and a small preliminary study of whole lung aerosol



delivery of GTAs. In our model, a negative pressure respiration system is used to expose anaesthetised sheep to sinusoidal variations in extrathoracic pressure thereby inducing respiratory flow. Importantly, the system facilitates both inspiratory-gated aerosol delivery and bronchoscopic access with the airway under atmospheric pressure. This allows the delivery of relevant quantities of DNA in a relatively short time interval; an important factor if these studies are to be extrapolated to human clinical trials. Importantly we have also used the sheep model to develop and validate airway sampling strategies that could be directly translated to the clinic.

We have now completed two larger scale studies of airway gene transfer. The first study demonstrated that using reporter genes, we could distinguish between different doses of DNA/Polyethyleneimine (PEI) and in addition we could demonstrate positive effects of adjunct therapies on the efficiency of DNA/PEI-mediated aerosol gene transfer. The second study was a product evaluation study to compare the efficiency of three different GTAs using plasmid expressing human CFTR cDNA. For all three of the GTAs studied we have evidence of airway epithelial gene transfer and expression at the mRNA and/or protein level. The data obtained from these studies will be an important factor in assessing which (if any) of these GTAs show enough promise to be taken forward into the clinic.

WS217: VIRAL: KEY DESIGN ISSUES FOR ONCOLYTIC VECTORS

Oncolytic Adenovirus Vectors that Overexpress ADP

William S. M. Wold, Ph.D.

We have developed a number of oncolytic adenovirus (Ad) vectors for potential use in cancer gene therapy. These vectors destroy infected cancer cells as part of the Ad life cycle. Our vectors "overexpress" ADP (E3-11.6K), an Ad5-coded protein that is synthesized late in infection and is required for efficient release of progeny virions at the culmination of the Ad infection cycle. The rationale behind these vectors is that elevated ADP levels facilitate vector-mediated cell lysis and spread of the vector through a tumor. Three of our vectors are named VRX-007, KD3, and VRX-011. VRX-007 has most of the E3 genes deleted and the *adp* gene inserted such that ADP is overexpressed as part of the Ad major late transcription unit. KD3 is identical to VRX-007 except it has two small deletions in the Ad *E1A* gene that allow replication in cancer cells but preclude efficient replication in normal cells. VRX-011 is identical to VRX-007 except it has the Ad E4 promoter replaced by the hTERT promoter; as such, VRX-011 replicates well in cancer cells but not in normal cells. All three vectors have a broad host range, replicating efficiently in (and killing) many different cancer cell lines. They effectively suppress the growth of

various types of human tumors in nude mice following intratumoral or intravenous injection of the vectors. They also suppress the growth of human lung tumors in a lung tumor metastasis model.

Because mice (and most other rodents) do not support Ad replication, researchers in the oncolytic Ad field usually employ the human xenograft/nude (or SCID) mouse model for *in vivo* efficacy studies. Safety studies are usually performed in C57BL/6 mice. We have developed a golden Syrian hamster model that is both immunocompetent and permissive for Ad replication, with the idea that the efficacy and safety of oncolytic Ad vectors could thus be more accurately assessed prior to their use in clinical trials. We and others have shown that Ad replicates in the lungs of Syrian hamsters. We have also established that several tumor-forming Syrian hamster cell lines are permissive for replication of (and killing by) Ad5, VRX-011, KD3, and VRX-007 in cell culture. We prove that ADP exerts its cell lysis-promoting effect in these hamster cell lines. These cell lines form tumors in Syrian hamsters following subcutaneous injection of the cells, and VRX-007 and Ad5 injected directly into these tumors effectively suppress the growth of the tumors. There is an antibody response to these viruses as shown in indirect immunofluorescence and virus neutralization assays. Further, we show that high doses of VRX-007 and Ad5 can be safely administered to Syrian hamsters not only by intratumoral injection but also by intravenous injection. Our studies should help advance our vectors and possibly oncolytic Ad vectors from other research groups towards clinical trials.

WS222: GENETIC DISEASES: FOCUS ON THE METABOLIC DISEASES OF THE LIVER

SV40-based Vectors for Metabolic Diseases of the Liver and Beyond

Jayanta Roy-Chowdhury, MD

Methodological advances in gene transfer to bone marrow cells, stem/progenitor cells and hepatocytes using recombinant viruses and non-viral gene delivery vehicles should permit gene therapy for a large number of inherited metabolic diseases. Both cell-transplantation based gene therapy and direct *in vivo* gene transfer are being pursued vigorously for the treatment of inherited metabolic disorders. For the purpose of this discussion, inherited metabolic disorders may be categorized into four groups. In the first category are very rare disorders, such as tyrosinemia [fumarylacetoacetate hydrolase (FAH) deficiency], in which the life-span of hepatocytes is markedly shortened. Phenotypic correction of a very small number of hepatocytes or transplantation of FAH-competent cells leads to extensive repopulation of the liver by the phenotypically normal cells. The second category includes the largest number of inherited

metabolic disorders, where the life-span of the mutant hepatocytes are normal, and the expression of a therapeutic gene in a small percentage of cells should make a major therapeutic impact. Crigler-Najjar syndrome type 1 (bilirubin glucuronidation deficiency), ornithine transcarbamylase deficiency, phenylketonuria and familial hypercholesterolemia (LDL receptor deficiency) fall in this group. Both cell-based (ex vivo) gene therapy and in vivo gene transfer are expected to be effective in these cases. The third category consists of inherited liver-based metabolic diseases in which the hepatocytes have normal or nearly normal life-span, but a great majority of hepatocytes need to be replaced by wildtype hepatocytes or phenotypically corrected autologous hepatocytes for therapeutic benefit. For example in primary hyperoxaluria-1 (PH-1), hepatocytes generate excessive amounts of oxalate and, therefore, at least three quarters of the mutant hepatocytes must be replaced with wild-type cells or transduced with therapeutic genes. Previously, such extensive gene replacement would be possible only with adenoviral vectors. Newer vectors, such as pseudotyped adenoassociated viral vectors, recombinant SV40 vectors and, possibly, lentiviral vectors provide potential alternatives. For cell-based therapy, preparative irradiation has been used in murine and rodent models to achieve massive hepatic repopulation by transplanted hepatocytes, leading to the reversal of hyperoxaluria in a murine model of PH-1. It should be noted that although life-long correction of a metabolic disease in murine or rodent models may be possible using episomal vectors, for longer-lived species, including humans, integration of the transgene into the host genome will be essential, unless the vector can be administered repeatedly without immunological complications. The fourth category comprises systemic disorders in which multiple organs, including the brain need to be transduced. Lysosomal storage diseases (mucopolysaccharidoses) are prototypes of this category. Many lysosomal enzymes, including β -glucuronidase, are secreted into plasma and are endocytosed via ubiquitous cell surface mannose-6-phosphate receptors. Thus, expression of these enzymes following gene transfer to the liver results in cross-correction of all abdominal viscera and the skeletal system. However, since these enzymes do not cross the blood-brain barrier, brain cells must be transduced also. This problem could be addressed by injecting the vectors into the brain, or by using vectors that not only transduce visceral parenchymal cells, but also pass the blood-brain barrier to transduce brain cells. Recently, we have observed that recombinant SV40 vectors can transduce hepatocytes and bone marrow cells, as well as brain cells after intravenous injection. Such vectors could be particularly useful in the treatment of lysosomal storage disease.

WS223: INFECTIOUS DISEASE: INNATE IMMUNITY AND VECTOR FUNCTION

Stat3 Targeting and Immunology

Hua Yu, PhD

Immune response is generally down-regulated in patients with cancer and chronic infectious disease. Restoring and/or increasing immunity in patients should help their ability to combat their illness and enhance the efficacy of various immunotherapeutic approaches. Our recent studies have unexpectedly revealed a critical role of Signal Transducers and Activator of Transcription 3 (Stat3) in mediating immune impairment in tumor-bearing hosts. Gene therapy to block Stat3 signaling in tumors *in vivo* is associated with potent “bystander” antitumor effects and heavy infiltration of immune cells. Detailed mechanistic studies show that Stat3 activation in both tumor and immune cells is an effective negative regulator of immune responses. Stat3 activity not only inhibits expression of pro-inflammatory cytokines and chemokines, but also up-regulates expression of factors that inhibit dendritic cell maturation and activation. Thus, blocking Stat3 signaling in either tumor cells or immune cells activates both innate and adaptive immune responses, leading to therapeutic antitumor immunity. Based on these findings in the tumor setting, it might also be possible to target Stat3 to restore immune responses in hosts with chronic infection, thereby enhancing the efficacy of various immunotherapeutic interventions.

WS224: MUSCULO-SKELETAL: MYOGENIC STEM CELLS AND REGENERATION

Human Muscle Stem Cell Quiescence Maintained by Elevated BMP4 Expression.

Emanuela Gussoni, PhD

N. Y. Frank, A. T. Kho, M. Molloy, M. Ramoni, M. H. Frank, and I. S. Kohane

Muscle side population (SP) cells are thought to be primitive, ‘stem’-like cells, within skeletal muscle, capable of generating more highly committed myogenic precursors, known as satellite cells. However, the molecular mechanisms that regulate this progression remain vastly unknown. In the current study, gene expression analyses of human fetal skeletal muscle demonstrate that high levels of Bone Morphogenic Protein 4 (BMP4) maintains the undifferentiated ‘niche’ and quiescence of muscle SP cells while inducing proliferation of a more committed population of myogenic cells expressing BMP4 receptor 1A via upregulation of Myf5. Further, the BMP4 antagonist gremlin appears highly upregulated in committed myogenic cells and counteracts the proliferative effects of BMP4 in BMP4 positive cells. Thus, developmentally conserved morphogen gradients of BMP4 and its



antagonist gremlin may regulate quiescence, proliferation and differentiation of immature and mature myogenic precursors in human fetal skeletal muscle.

WS225: NEURAL DISORDERS: GENE THERAPY AND BEHAVIOR

Functional Assessment of Gene-based A β Immunotherapy in a Mouse Model of Alzheimer's Disease

William J. Bowers, Ph.D.

Alzheimer's disease (AD) is an age-related neurodegenerative disorder associated with progressive functional decline, dementia and neuronal loss. Demographics make evident that the prevalence of AD will increase substantially. The enveloping societal burden created by this debilitating disease should provide sufficient incentive for the development of novel therapeutic approaches. However, because the mechanistic origins of AD are not completely understood, the clinical disease spectrum broad, and the neuropathological features of its initiation and progression limited, the development of such potential disease modifying therapies has been hindered. Given the amyloid beta (A β) contribution to mechanisms of AD pathogenesis the derivation of experimental therapeutics targeted toward prevention of A β fibrillogenesis and/or removal of extant amyloid deposits represent potential disease natural history modifying approaches. The implementation of active A β -based immunotherapies for treatment of AD has shown tremendous promise in mouse AD models, but has been hampered in human trials by development of moderate brain inflammation in a subset of patients following A β peptide vaccination. The immunologic mechanisms underlying the benefits (prevention of A β deposition; behavioral improvement) or negative side effects (brain inflammation) have yet to be firmly established, but suggest that A β -based vaccination has clinical merit if optimal immune responses can be engendered. Our approach involves use of Herpes Simplex virus (HSV)-derived amplicon vectors to elicit optimized immune responses toward A β . The HSV-1 amplicon possesses a number of advantages over other gene delivery platforms. First, the amplicon is not a live virus (as are vaccinia, canarypox, etc.) and therefore, has an inherently safer *in vivo* profile. Second, compared to DNA delivery systems or most virus-based vectors, expression is directed from multiple episomal copies within each transduced cell, and the genome is maintained in non-dividing cells such as antigen presenting cells (APCs). Third, the transgene size limit is larger (d \approx 130 kb) than many other viral vectors affording an opportunity to co-express factors with known immunomodulating activity. Lastly, the lack of encoded viral genes avoids the effects that wild-type herpesviruses typically use to evade the immune system, such as

downregulation of MHC expression and antigen processing, and inhibition of dendritic cell maturation.

Our ongoing A β -directed immunotherapeutic studies seek to determine the relationship between A β antigen structure/context and the elicitation of protective immune responses that attenuate A β toxicity, prevent amyloid plaque deposition and/or lead to dissolution of pre-existing amyloid, ultimately leading to preservation of learning and memory functioning. This presentation will illustrate our progress to date regarding the use of HSV amplicons to reduce AD-like pathology and to prevent concomitant degradation of memory retention. Our approach will not only enable the development of novel AD immunotherapeutics, but will contribute to the mechanistic dissection of AD pathogenesis and the immune responses required to mediate protection.

Gene Therapy to Alter Sexual Behavior

Michael G. Kaplitt, MD, PhD

One of the most well-characterized behavioral circuits is the lordosis reflex. Lordosis is the primary sexual behavior of female rodents and other quadrupeds in response to stimulation by a male or by manual stimulation. This behavior is dependent upon the presence of estrogen followed by progesterone in specific brain regions, particularly the ventromedial nucleus (VMN) of the hypothalamus. The mechanism of estrogen action is clouded, however, by the fact that these regions possess two receptors for estrogen (ERa and ERb) and that receptor-independent estrogen functions have also been identified. To define the mechanism of estrogen action on female sexual behavior, we generated adeno-associate virus (AAV) vectors capable of overexpressing ERa and ERb. Introduction of AAV-ERa into the VMN of ERa knockout mice not only restored receptor expression focally to this region, but this was physiologically functional since this restored estrogen-mediated induction of progesterone receptor (PR) expression, which remained absent in controls. Despite this confirmation that our ERa was functional, no improvement in the sexual behavior deficit was noted. Since estrogen signaling is necessary for normal sexual development, it is possible that this reflected an insurmountable developmental defect. In addition, one flaw in the knockout mice was the continued expression of an aberrant ERa transcript capable of expressing a protein which could bind estrogens. Therefore, we generated an AAV vector expressing a small double-stranded RNA designed to completely inhibit ERa expression in normal, non-transgenic mice. Local infusion of this vector completely blocked ERa expression in the VMN but not elsewhere, while expression levels in controls matched wild-type mice. This site-specific blockade of ERa completely abolished estrogen-induced sexual behaviors in female mice,

including the lordosis reflex. This physiologically correlated failure to induce PR expression by estrogen. These studies highlight the potential for viral vectors to alter complex behaviors by manipulating gene expression within specific brain regions of otherwise normal mammals.

WS226: NONVIRAL: NEW APPROACHES IN NONVIRAL GENE DELIVERY

Tumor-targeting Nanodelivery Systems: Expanding the Potential for Cancer Therapy and Diagnosis

Esther H. Chang, PhD

Many issues need to be addressed before the promise of tumor-targeting diagnosis and therapy for cancer can be realized. Foremost among these is the efficient and selective delivery of diagnostic or therapeutic molecules to the site(s) in the body where the target tumor cells reside. Of particular relevance to cancer is the ability to target cells that have migrated via metastases from the site of the primary tumor. Our laboratory has demonstrated that a systemically administered, cationic liposomal nanocomplex bearing molecules that home to the surface of tumor cells can efficiently and selectively deliver diagnostic contrast agents and nucleic acid-based therapeutics to primary tumors and metastases in animal models of a variety of human cancers. The nanodelivery of imaging agents results in a significant improvement in the sensitivity and resolution in detecting metastatic lesions. Moreover, certain of the nucleic acid-based therapeutics have been shown to dramatically synergize with conventional radio- and chemotherapies.

DNA Nanoparticles for Non-viral Ocular Gene Delivery

Muna I. Naash, PhD

Blindness due to genetic abnormalities in photoreceptor-specific proteins represents a significant and largely intractable health problem worldwide. Our objective is to develop an effective and robust therapeutic vector system which can be utilized in the treatment of genetically-based blinding diseases. Our laboratory has generated transgenic mice carrying point mutations in the photoreceptor-specific protein, peripherin/*rds* (*P/rds*), to model human hereditary retinal diseases. In the first set of experiments, we tested the ability of genetic supplementation of normal mouse *P/rds* (NMP) to override the retinal degenerative phenotype and to promote normal visual function in these transgenic lines. Electroretinography, histology at the light and electron microscopy levels, immuno-gold cytochemistry (using anti-rod and -cone opsin antibodies), Western blot analysis, and limited tryptic digestion were used to assess rescue of the retinal disease phenotypes. Herein, we

provide the first evidence for *P/rds* supplementation-mediated rescue of both rod and cone-dominant disease-causing mutations.

In the second set of experiments, we evaluated the ability of compacted DNA nanoparticles to transfect mouse ocular tissues *in vivo*. EGFP expression plasmids transcriptionally-controlled by the CMV promoter were compacted into neutral-charged DNA nanoparticles (having a diameter < 15 nm) using polyethylene glycol-substituted lysine peptides and injected subretinally or intravitreally into the eyes of adult BALB/c mice. Both delivery routes were examined in order to target photoreceptors, RPE, and optic nerve cells (subretinal) or inner retinal cells (intravitreal). In a set of control animals, naked plasmid DNA was injected at the same concentration as the nanoparticles or a mock injection was performed. After 2 days post-injection, mice were euthanized and the retinas were assayed for EGFP expression by quantitative real-time RT-PCR (qRT-PCR) and immunohistochemistry. Significant levels of EGFP expression were obtained by either subretinal or intravitreal delivery of the compacted DNA nanoparticles. Immunohistochemistry demonstrated the ability of these nanoparticles to transfect and express EGFP *in vivo* at extraordinarily high efficiencies. After subretinal injection, EGFP was detected in almost 100% of the photoreceptors and expression was also observed in the inner nuclear layer, RPE, and optic nerve. By qRT-PCR, EGFP mRNA levels were comparable in abundance to rhodopsin mRNA. After either subretinal or intravitreal delivery, minimal or no expression of EGFP was detected with naked plasmid or mock-injected controls. Ocular delivery of the DNA nanoparticles did not induce any apparent toxicity. Our results demonstrate the efficiency of compacted DNA nanoparticles in targeting post-mitotic cells of the retina and resulting in high levels of transgene expression. Transfection of different retinal cell layers can be achieved by the route of delivery. This non-viral system is a safe and very effective tool for ocular gene therapy. Further studies are underway utilizing this technology to rescue several well characterized animal models of retinal disease.

Controlled Release Systems for Non-viral Vectors

Lonnie D. Shea, PhD

Adapting controlled release technologies to the delivery of DNA has the potential to enhance gene delivery relative to more traditional delivery methods (e.g., injection), which limits the clinical application of gene therapy. Controlled release systems can overcome extracellular barriers that limit gene transfer by delivering vectors locally, which can avoid distribution to distant tissues, decrease toxicity to non-target cells, and reduce the immune response to the vector. Delivery vehicles for controlled release are fabricated from natural and



synthetic polymers, and the mechanisms of delivery can be generally categorized as i) polymeric release or ii) substrate-mediated delivery. For polymeric release systems, the vector is often continually released into the local tissue environment, which results in persistent levels extracellularly that extends the opportunities for internalization. Alternatively, substrate-mediated delivery involves immobilization of the non-viral vector to a biomaterial that supports cell adhesion, thereby placing the vector directly in the cell microenvironment. Vector release or binding is regulated by the effective affinity of the vector for the polymer, which depends upon the strength of molecular interactions. In this workshop, I will present examples of controlled release systems for delivery of non-viral vectors.

WS227: SPECIAL: THE CLINICAL TRIAL DATA ON ADP53 GENE THERAPY OF CANCER

Adp53 Clinical Trials

Jack A. Roth, MD

Absence of tumor suppressor gene (TSG) expression is the most common of all genetic abnormalities associated with cancer identified to date. Restoration of functional TSG expression is the most direct therapeutic approach. The most frequent TSG abnormalities identified in human cancers to date interact in a complex network that regulates cell cycle progression, DNA repair, and apoptosis. The p53 tumor suppressor gene plays a central role in this network and is the most commonly mutated gene yet identified in human cancers. Studies have shown that restoration of p53 function in the cancer cell results in cancer cell death, and that corrective genes could be delivered by viral vectors. We evaluated the safety and gene transfer efficacy of monthly intratumoral injections of a recombinant replication-defective adenovirus containing wildtype p53 (Adp53) in patients with advanced NSCLC who failed conventional treatments. Adp53 doses were escalated from 10^6 plaque-forming units (pfu) in log increments to 10^{10} pfu with the final level being 10^{11} . Patients were treated with (n=28) or without (n=24) cisplatin (CDDP) 80 mg/m² IV given three days prior to Adp53 injection because of previous animal studies showing synergistic cancer cell kill with the combination of p53 gene replacement and DNA damaging agents. Adp53 vector was injected monthly into the same primary or metastatic tumor under computed tomography (CT) guidance. No significant vector related toxicity has been seen with up to six monthly injections. Clinical responses were evaluated with monthly abdominal and chest CT scans. Prolonged (up to 18+ months) disease stabilization (63%) and regression of tumors >50% (8%) has been observed in some patients including those previously treated with CDDP. Of 12 patients with a major airway obstruction, six showed

opening of the airway. Evaluable posttreatment tumor biopsies in patients receiving Ad-p53 alone showed adenoviral vector sequences by DNA polymerase chain reaction (DNA-PCR) in 18/21 (86%) patients and vector specific p53 mRNA sequences by reverse transcription polymerase chain reaction (RT-PCR) in 12/26 (46%) patients. Apoptosis was demonstrated by increased TUNEL staining in posttreatment biopsies from 11 patients. Vector related toxicity was minimal. Transgene expression occurred in post-treatment tumor biopsies in patents with circulating antibodies to adenovirus. Actuarial survival at one year was 40% for those patients treated with Ad-p53 alone, which compares favorably to chemotherapy naive patients treated only with chemotherapy. Phase II clinical trials of adenoviral-mediated p53 gene transfer in conjunction with radiation therapy were carried out in 17 patients with localized NSCLC. The overall response rate was 5/17 (29%); response rate at the local injected site was 9/17 (53%). The survival rate at one year was 56%. Posttreatment biopsies of the original tumor site were obtained 3 months following completion of treatment. In 12 cases, the biopsy showed no evidence of tumor. This biopsy negative rate of 70% compares favorably to that of 17% reported in studies of chemotherapy combined with radiation therapy, suggesting that the interaction of the Adp53 and radiation therapy can potentially improve local tumor control. Safety data indicated that this combination had an acceptable safety profile. 13 patients underwent 61 CT-guided biopsies or drug administrations. 13 (21%) resulted in pneumothoraces, one of which required hospital admission. Six of the 17 patients experienced a grade 3 or 4 adverse event. A phase I study of 33 patients with head and neck squamous cell carcinoma (HNSCC) also concluded that transfer of the *Adp53* construct caused little toxicity and, once again, significant clinical response was observed in 9 out of 18 clinically evaluable patients. A subsequent phase II clinical trial of *Adp53* in over 200 recurrent or refractory HNSCC patients resulted in complete or partial responses in approximately 10% of patients with some evidence of anti-tumor activity observed in 60% of patients. Thus intratumor injection of Adp53 can mediate anti-tumor effects including complete responses, has low toxicity, shows high levels of gene expression, can mediate apoptosis, and can regulate the expression of other genes in the p53 pathway.

WS228: SPECIAL: SKIN GENE THERAPY

Bioresponsive Deshielding of Targeted DNA Polyplexes

Ernst Wagner, PhD

For the development of novel nonviral vectors, polyplexes were designed to employ delivery pathways similarly as natural viruses do. For this purpose plasmid DNA was complexed with polyethylenimine (PEI)

conjugates. The conjugates contain transferrin or epidermal growth factor as cell-binding ligands and polyethylene glycol for masking the polyplex surface from unspecific interactions with the environment. Applying such polyplexes systemically, targeted gene transfer has been demonstrated in several mouse models.

Key steps in further optimization of polyplexes towards artificial viruses include better purification protocols for polyplexes inclusion of more efficient endosomal release functions use of bioresponsive polymers for presentation of virus-like nucleic acid packaging and entry functions.

Membrane-active peptides derived from the melittin sequence were evaluated as endosomolytic function. Melittin which was C-terminally conjugated to PEI displayed high membrane-destabilizing activity. Polyplexes containing this conjugate displayed enhanced gene transfer efficiency, however also high toxicity due to lysis of the cell surface membrane. To reduce the lytic activity at neutral pH, acidic peptide analogs were generated which should focus the lytic activity to the acidic environment of endosomes. As expected, the modified peptide-PEI conjugates with the highest erythrocyte lysis activity at pH 5 were most effective in gene delivery.

pH-triggered removal of the PEG shield from the polyplex at endosomal pH can be realized by linkage of PEG to the DNA-binding polycation via pyridylhydrazines. The reversible shielded polyplexes exhibited up to two log orders of magnitude higher gene expression *in vitro* and one log magnitude higher gene expression in an *in vivo* mouse model compared to the control polyplexes. Engineering of nanoparticle structures with such dynamic virus-like domains is an encouraging direction in vector design.

MI233: MEET-THE-INVESTIGATOR: CAN ANIMAL MODELS PREDICT CLINICAL EFFICACY OF RESPIRATORY GENE TRANSFER

Gerry McLachlan, PhD

One of the main challenges in the field of airway gene transfer is developing an ability to predict the clinical efficacy of gene transfer from animal models. It has become apparent that data obtained in the mouse lung is not always a reliable guide to how gene transfer agents (GTAs) will perform in the clinic. Important confounding factors in this species include limitations with respect to available methods of delivery, the difficulties in determining which cells are being targeted in mouse lung, particularly when using reporter genes and the fine interface between demonstrating efficacy and causing toxicity. Our main interest is in gene therapy for Cystic Fibrosis. An additional reason for an inability to predict how GTAs will work in CF patients may be the fact that there is no animal model for CF lung disease.

Although a number of CF mouse lines have been developed which include nulls and specific CF mutations, these mice still fail to develop the characteristic CF lung disease where chronic bacterial infection, neutrophilia and excess mucus undoubtedly form additional barriers to successful gene transfer. We felt strongly that evaluation in a large animal model should be included in the preclinical assessment of GTAs and have developed the sheep lung as a model system. At present there are no CF sheep therefore the sheep model suffers from the same problem of having no CF lung disease. However, the similarities in size, architecture and physiology to the human lung make it a valuable tool to investigate issues such as gene delivery, efficacy and safety using protocols which are directly relevant to the patient. In particular it has allowed us to develop and validate a number of bronchoscopy-based assays which are allowing us to address the important issue of the localisation of transgene expression. The challenge of predicting clinical benefit in CF patients is still a considerable one. Without a model of CF lung disease itself it will be impossible to judge how successful gene transfer needs to be to demonstrate clinical benefit in patients using measures currently used to monitor progress of disease in CF patients. Clinical benefit may be easier to predict for respiratory conditions where the disease phenotype can be modelled in the animal or where the transgene is a secreted protein being delivered to overcome a deficiency. The question remains as to whether animal models can predict clinical efficacy of respiratory gene transfer. If by this we mean can we predict safety and efficiency of gene transfer and expression and where that expression may be localised then we feel that the ovine model will play a useful role in predicting outcomes.