



FRIDAY, JUNE 3, 2005

SS300 ETHICS: ETHICAL ISSUES IN CLINICAL TRIAL DESIGN: INFORMED CONSENT AND CHILDREN

Gene Transfer Trials in Children: What's Ethical? What's Allowable?

Robert M. Nelson, MD, PhD

The pediatric research regulations are based on the deliberations of The National Commission (1977) and reflect the proper role of parents as “caretakers” in balancing a child’s protection from and exposure to risk. Simply, a child should not be disadvantaged by enrollment in a research study. As such, a parent’s decision to enroll a child in research should be similar to a decision to permit exposure to the risks and benefits of any non-research alternative. The regulations thus restrict the risk exposure for interventions not offering the prospect of direct benefit. Interventions that offer the prospect of direct benefit are restricted to those whose risks and benefits are comparable to any available alternatives. Even if a proposed research intervention remains within these restrictions, there is debate about the ability of parents to make a voluntary and informed decision under duress when faced with a critically ill and perhaps dying child.

This presentation will review the pediatric research regulations (i.e., Subpart D), arguing that the regulatory restrictions on allowable risk offer greater protection for children than the requirement for parental permission (and child assent). The discussion will be illustrated with two examples: gene transfer interventions for (1) ornithine transcarbamylase deficiency (OTC), and (2) severe combined immunodeficiency (X-SCID). The OTC trials were conducted in adults based on the ethical (and regulatory) argument that such trials could not be conducted in critically ill neonates as the intervention offered insufficient benefit and parents were not capable of an informed and voluntary decision under these circumstances. The X-SCID trials were conducted in children (of necessity), yet were suspended recently due to the development of leukemia in recipients of the intervention. Reports suggest that the X-SCID trials will be modified to include only the most critically ill infants, precisely the population that were excluded from the OTC trial.

The presentation will also cover parental permission (and to a minimal extent, child assent) in the context of gene transfer trials, focusing on the ability to assure informed and voluntary consent. An argument will be made for “focused” assessment of key items that should be communicated as part of the consent process, rather than a complete inventory of the elements of informed

consent. The overall goal of the presentation is to impart practical advice about (1) issues in study design based on the regulatory and ethical framework of Subpart D, and (2) approaches to informed and voluntary parental permission (i.e., consent) to enroll critically ill infants and children in gene transfer research.

Children in Gene Transfer Trials: Design, Benefit, and Consent Issues

Nancy M. P. King, JD

Designing and conducting gene transfer trials with child subjects presents important ethical considerations for investigators. Constraints on research decisionmaking for the protection of child subjects may frustrate investigators and families, and may promote “data spinning” and “benefit creep” in order to justify enrolling children. Clear and candid discussion of these challenges is needed.

When is it safe, reasonable, and fair to enroll children in gene transfer research? How should the risks of harm and the potential benefits from the intervention be balanced? What information should be provided to parents and children, and how should it be presented to promote autonomous decisions about participation?

The goal of clinical research is to maximize knowledge gained while minimizing harms to subjects. This balance is challenging, especially when the potential subjects are sick children. Because most gene transfer trials are early-phase studies with relatively small enrollments, maximizing knowledge may be difficult. The potential for benefit from the gene transfer intervention in such trials is very low, which poses a problem for child subjects. Gene transfer in children may also pose heightened risks of harm. Who should be the first subjects – the sickest children, or the healthiest? How should monitoring and long-term follow-up in children be addressed?

Decisionmaking about research participation can be very difficult for parents of sick children. Informed consent in gene transfer raises some special issues – e.g., making complex scientific information accessible – but also provides helpful models. Consent monitoring has been useful in pediatric gene transfer research; analysis of gene transfer research consent forms offers ideas for improvement; and the RAC has produced a comprehensive web-based informed consent guidance document for investigators and IRBs.

A key concern is whether it is possible – and if so, how – to make clear to parents that their child’s participation in early-phase gene transfer research is not treatment, and is not likely to be beneficial, even though nothing else has worked or is available for their child’s disease (except perhaps palliative or supportive care). This reality may also be difficult for investigators to accept. It is sometimes assumed that achievement of a study goal expressed as a

surrogate endpoint is directly beneficial to subjects. It may also be assumed that the risks of harm to very sick subjects may matter less to their parents, that a very small chance of benefit might mean more to them, or even that the sickest subjects have “nothing to lose.” Each of those assumptions is understandable under the circumstances, and all are unwarranted.

To guard against these assumptions, investigators should make information disclosure clear and thorough, and should focus on the goals of minimizing harms to subjects and contributing to knowledge for the future. Parents will always have hopes for their sick children, but participation in gene transfer research should not be offered in ways that exploit those hopes. Parents’ decisions should be built on reasonable understanding of and reasonable expectations from sound pediatric research.

Resources for investigators:

NIH Guidance on Informed Consent in Gene Transfer Research: <http://www4.od.nih.gov/oba/rac/ic>

Social Construction of Benefit in Gene Transfer Research Project web site: <http://socialmedicine.med.unc.edu/scob/>

SS301: GENE-BASED VACCINES: CANCER VACCINES

GVAX® Vaccines in Hematologic Malignancies

Kristen Hege, MD

GVAX® cancer vaccines are composed of whole tumor cells genetically modified to secrete the immunostimulatory cytokine, GM-CSF. Local secretion of GM-CSF at the vaccine injection site serves as an adjuvant whose major role is recruitment and activation of dendritic cells. These antigen presenting cells can then present tumor-associated antigens from the tumor cell vaccine to the immune system, thereby inducing a systemic anti-tumor immune response. GM-CSF gene-modified tumor cell vaccines (GVAX® vaccines) have been explored in hematologic malignancies including multiple myeloma (MM) and acute myeloid leukemia (AML). In both settings vaccines were administered in association with autologous stem cell transplant (ASCT) as a form of pre and posttransplant immunotherapy based on preclinical data demonstrating enhanced vaccine potency in the post transplant setting. Immunologic responses including induction of autologous tumor-reactive antibodies and delayed-type hypersensitivity (DTH) reactions as well as vaccine-associated reductions in minimal residual disease were seen. In the myeloma trial several patients with rising m-protein levels following ASCT demonstrated m-protein declines after receipt of post transplant vaccines. In the AML trial all patients were vaccinated while in a complete hematologic remission and were monitored for minimal residual disease by quantitative assessment of WT-1, a leukemia

associated marker, by RT-PCR. Sixty-nine percent of patients showed a decrease in WT-1 following the first (pre transplant) vaccination and this was associated with longer relapse-free survival. Achievement of an undetectable level of WT-1 in peripheral blood post transplant was also associated with improved relapse-free survival suggesting that WT-1 is a valid marker of minimal residual disease. A review of the preclinical and clinical experience with GM-CSF modified cancer vaccines development in hematologic malignancies will be presented.

SS302: GENETIC DISEASES: PROTEIN MODIFICATIONS TO ENHANCE THERAPY OF GENETIC DISEASE

Seven Years in Tibet: Optimizing Therapeutic Proteins by DNA Shuffling

Nay Wei Soong, PhD

The global market for protein therapeutics is huge – amounting to \$32 billion in 2003 and predicted to double by 2010. This reflects a tremendous potential for the expanded use of approved proteins and the entry of new therapeutics. With the eventual advent of gene-based delivery of protein therapeutics, this market can only grow further. Yet in their native forms, most proteins are not optimal for therapeutic applications. They may suffer from limited efficacy and stability *in vivo*, or cause unacceptable side effects. Thus many proteins are modified to enhance their therapeutic profiles or to improve their production properties.

Many methods exist to optimize proteins molecularly; they fall into the broad categories of rational design and directed molecular evolution. Rational design relies on detailed knowledge of structure and mechanism to engineer improvements. Directed molecular evolution employs screening strategies to selectively sieve through libraries created by diversity generating processes for improved variants; these include random mutagenesis, phage display and DNA shuffling.

DNA shuffling has been successfully applied to improve or alter proteins with diverse functions, such as antibodies, cytokines, enzymes and viral functions. There is now a practical, mature framework for the application of the shuffling platform, coupled with screening strategies, to improve a variety of protein traits. This talk will focus on key examples and insights obtained in the engineering of therapeutically relevant proteins and viruses using this platform.



SS303: INFECTIOUS DISEASE: GENE THERAPY FOR HIV: LESSONS LEARNED, PARADIGMS TO APPLY TO OTHER AREAS OF GENE THERAPY

Treatment of HIV Infection with Gene-Protected Autologous T cells

Dorothee von Laer, MD

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C. H. June, M. Bonyhadi, and A. Alexandrov.

Gene therapy and other alternative strategies for the treatment of HIV infection are receiving growing interest, as limitations of HAART, such as drug resistance and toxicity, have become more and more evident. The approach developed here involves the infusion of T lymphocytes protected from HIV infection by an antiviral gene.

Mathematical modeling and computer simulations predicted that antiviral genes, which inhibit prior to proviral integration, would be more effective than late inhibitory genes. Entry of HIV into the target cell is a complex, multi-step process. Ultimately, the HIV-1 transmembrane envelope glycoprotein gp41 mediates fusion of the viral and cellular membranes. This process can be inhibited very effectively by C-peptides derived from the C-terminal heptad repeat of gp41. These peptides interact with the trimeric coiled coil structure formed by the gp41 N-terminal heptad repeat and thereby lock gp41 in a fusion incompetent state. We engineered the inhibitory C46 peptide for expression on the cell membrane of CD4+ T cells, leading to a high local concentration of peptide at the site of its antiviral action. This membrane-anchored peptide was expressed from a retroviral vector (M87o) and has a strong antiviral activity, with a more than 4 log inhibition of virus entry. Cells expressing M87o are selected out in mixed (M87o-positive and -negative cells) HIV-1-infected primary T cell cultures and the T helper cells are completely preserved while they die rapidly in the infected control cultures. M87o inhibits replication of diverse HIV isolates, including T-20 resistant strains in cell lines and primary T cell cultures.

After preclinical safety and toxicity testing, a clinical phase I trial was initiated. Autologous T cells were obtained by lymphapheresis from 10 HIV-1-infected patients with advanced disease (CD4 count <200/ μ l) and failing HAART (VL >5000 copies/ml, median CD4 count 93/ μ l, VL 4.96 log₁₀ copies/ml). After CD8-depletion, cells were expanded *ex vivo* using anti-CD3/anti-CD28-coated beads and transduced with M87o. A median of 1.26 x 10⁹ gene-modified T cells (corresponding to a median of 7x10⁹ total T cells) was reinfused. At a median follow up of 4 months, no severe side effects or grade 3-4 AE's

occurred. Gene-marked cells were followed *in vivo* by qPCR in lymph nodes and peripheral blood. Transduced T cells were enriched in the peripheral blood immediately after reinfusion and peaked at 5-15 minutes reaching a gene marking level of 20% of the peripheral blood CD4+ T cells. A rapid redistribution to lymphoid organs was observed thereafter, where transduced T cells are still detectable 12 months after reinfusion. A significant increase of CD4 counts was observed despite unchanged HAART. For the whole group, the mean relative increase of CD4 counts at week 12 was 43% compared to baseline (110 CD4+ cells/ μ l to 153 CD4+ cells/ μ l, p < 0.01). No significant changes of viral load were detected. A positive correlation between the absolute number of infused gene-modified CD4 T cells, the level of gene marking and the increase of CD4 counts was observed. In conclusion, the clinical development of this first gene therapeutic approach for HIV infection based on entry inhibition shows promising results and warrants further trials preferably in less immunocompromised cohorts.

RNAi Gene Therapy for HIV-1

John Rossi, PhD

M. Li, H. Li, J. Ji, M. Amarzguoui, and H. Unwalla

The control of HIV infection is a major challenge given the genetic variability of the virus. Drug resistant variants arise readily under selective pressure. Our lab has been exploring various RNA based therapeutics for the treatment of HIV infection in a gene therapy setting. The most recent addition to the RNA based therapeutics is RNA interference, or RNAi. RNAi is a potent innate immune mechanism that directs both transcriptional and post transcriptional gene silencing. It is very potent against HIV, and can under the appropriate conditions completely block viral replication. Nevertheless, the genetic variability of HIV in a patient setting will undoubtedly result in RNAi resistant variants of HIV. One of our goals is to determine how to circumvent these resistant variants by using RNAi in combinatorial fashion or in combination with other RNA and protein inhibitors. A summary of recent results of combinatorial gene expression on the emergence of HIV resistant variants as well as the impact that expression of small interfering RNAs has on endogenous micro RNA populations in hematopoietic cells will also be discussed.

SS304: MUSCULO-SKELETAL: REGENERATIVE APPROACHES FOR SKELETAL DISORDERS

Genetically Engineered Mesenchymal Stem Cells as a Platform for Skeletal Tissue Engineering

Dan Gazit, DMD, PhD

Adult Mesenchymal Stem Cells (MSCs) are multipotent cells that give rise to chondroblasts, tenocytes and osteoblasts, which in turn are responsible for the formation of cartilage, ligament, tendon, and bone tissues. Individuals maintain a reserve of MSCs throughout their lives. These adult stem cells play a key role in our bodies' natural ability to regenerate tissues. Therefore, MSCs are excellent candidates for tissue engineering of skeletal tissues. We aimed to investigate stem cell-based platforms towards skeletal tissue regeneration. In order to engineer functional tissues, a convergence of several fields of expertise must take place. Addressing this goal, our studies integrate stem cell biology, gene therapy, bioinformatics and nanotechnology. We have genetically engineered MSCs with different genes in order to drive MSCs to various skeletal differentiation pathways. The osteogenic genes, rhBMP-2 and -9 were utilized to induce bone regeneration, demonstrated in spinal fusion and critical bone defect models using quantitative uCT imaging. Cartilage tissue formation was achieved by engineering MSCs to express the transcription factor, Brachyury. The cells exhibited chondrogenic differentiation *in vitro*, and formed proliferative cartilage when implanted *in vivo*. The engineered cell survival in a mouse knee joints was monitored using *in vivo* fibered confocal fluorescence microscopy system. We have also discovered that the over expression of SMAD8 molecule in MSCs induced tenocytic differentiation *in vitro* and form tendon/ligament tissue in injury sites *in vivo*, as demonstrated by uMRI. The engineered MSCs demonstrated an increase in proliferation and survival when implanted on specially designed nanostructured biodegradable scaffolds, which mimic the natural fibers in the extracellular matrix. Moreover, we have evaluated the biomechanical properties of the engineered bone tissue using a nanoindentation technique. The hardness and reduced modulus of the engineered bone were similar to those of a native one. Ongoing efforts are being made to identify novel candidate genes, which contribute to the "stemness" of MSCs or enhance bone regeneration. Such a quest requires the use of high throughput gene arrays and clustering analysis and has already yielded the discovery of an inhibitor of osteogenesis, a member of the wnt pathway, DKK3. We conclude, that in order to promote and enhance skeletal tissue engineering, the synergistic integration of stem cell biology, gene therapy, nanotechnology and informatics should be achieved.

Revitalization of Structural Allografts via Immobilized rAAV Gene Therapy

Edward Schwarz, PhD

Large defects in bone that occur from traumatic injury or tumors must be replaced by a structural graft or prosthesis in order to save the limb. Due to its biocompatibility, allograft bone from a human cadaver remains the graft of choice. However, since the dead bone is incapable of revascularizing, forming new bone, or remodeling, up to 50% of these large allografts fracture within 10 years, requiring a complicated autograft surgery. In order to study the molecular mechanisms that govern structural auto and allograft incorporation, we have developed a murine femur model that faithfully recapitulates the salient features of human bone grafting. This model was used for microarray studies on RNA isolated from auto and allograft tissues to identify angiogenic, osteogenic and osteoclastic candidates for gene therapy. To the end of developing a revitalizing allograft, we have developed an approach in which recombinant adeno-associated virus (rAAV) can be freeze-dried onto any implant surface without losing infectivity *in vitro* and *in vivo*. We then demonstrated the efficacy of this approach using two different strategies. The first induces a new bone collar around the cortical surface of the allograft through bone morphogenetic protein (BMP) signals transduced by a rAAV-caAlk2 coating. The second induces vascular ingrowth and osteoclastic resorption through transduction of a combination of rAAV-VEGF and rAAV-RANKL. Although detectable transgene expression only persists for 2 to 3-weeks, the treatment triggers the natural bone remodeling process that perpetuates until healing is complete. Outcome measures to evaluate the efficacy of a rAAV-coated, revitalizing structural allograft in a canine study and clinical trial will be discussed.

SS310: EMBRYONIC STEM CELLS AND TISSUE ENGINEERING

Using RNA Interference Approaches to Modulate Gene Expression in ES Cells

Mervin C. Yoder, MD

Embryonic stem (ES) cells hold great promise as a means to understand basic biologic and molecular concepts in developmental biology, as well as, to generate differentiated cellular elements that may one day be useful as a cell therapy for human diseases. Murine ES cell differentiation into various progenitors of the hematopoietic system has been effectively used to examine fundamental steps in the molecular regulation of blood cell development. Murine ES cells have been particularly useful as a means to modulate gene expression via homologous recombination and then to generate transgenic mice carrying the targeted gene



mutation. Recently, silencing of gene expression has been accomplished in eukaryotic cells via introduction of double-stranded RNA (dsRNA) and has been referred to as RNA interference (RNAi). This presentation will review the basic mechanisms of RNAi via short interfering RNA (siRNA) and discuss several approaches to using this approach to modulate gene expression in murine and human ES cells. Examples will include use of dsRNA to diminish the expression of Shp-2 a tyrosine phosphatase in murine ES-derived EB and then examine the consequences of decreased Shp-2 expression on hemangioblast, primitive, and definitive progenitor cell emergence. Other examples from the published literature include use of RNAi to silence expression of Oct4 in human ES cells resulting in significant changes in ES cell differentiation. These examples will discuss the use of several strategies to introduce the dsRNA including duplex siRNA, retroviral, and lentiviral vectors.

SS313: NONVIRAL: NONVIRAL GENE DELIVERY: TOWARDS CLINICAL APPLICATIONS

Electric Pulses-based Gene Transfer: Safety and Efficacy of Combinations of High Voltage and Low Voltage Pulses

Luis M. Mir, DSc

As a potential alternative approach to the use of viral vectors in gene therapy, the delivery of electric pulses for DNA transfer to tissues *in vivo* is regarded as an increasingly interesting method. Moreover, electropermeabilizing electric pulses are already delivered for clinical purposes in the frame of the solid tumors treatment by electrochemotherapy.

High efficacy of gene transfer with simultaneous good preservation of the treated tissue have already been reported when DNA electrotransfer is performed using trains of identical square pulses, particularly in the case of the skeletal muscle. Typically, 4, 6 or 8 pulses of 20 ms (usual pulse duration ranges from 5 to 50 ms) are delivered to the tissues (muscle, tumors, liver) by transcutaneous plate electrodes or by needles inserted in the tissue. A still higher efficacy with no tissue damage according to histological evaluation can be achieved in the skeletal muscle using combinations of (i) high voltage short pulses, that electropermeabilize the target tissue, and (ii) low voltage long pulses, that electrophoretically push the DNA in the tissue towards the electropermeabilized cells. These combinations of pulses allow an easy adaptation of the electrical parameters to the various target tissues. Indeed, there are striking differences between the tested tissues, that rely not only on individual cells size and tissue organization, but also on the electrical connectivity between the cells of the tissue. These results highlight the interest of DNA

electrotransfer (electrogenotherapy) as an efficient non viral approach for gene therapy.

A Novel Non-viral Episomal Vector as Expression System for Gene Therapy

Hans J. Lipps, PhD

Currently used viral vectors for gene therapy suffer from a number of limitations including integration into the host genome which may lead to insertional mutagenesis and silencing of the transgene, expression of viral proteins leading to immunological reactions of the recipient organism or only transient expression of the transgene. There is increasing agreement that the ideal vectors for gene therapy should exclusively contain chromosomal elements and replicate episomally in the recipient cell. Based on the observation that the binding of an origin of replication to the nuclear matrix precedes the onset of S-phase in mammalian cells, we constructed a vector containing a scaffold/matrix attachment sequence (S/MAR). This vector replicates at low copy number episomally in a variety of mammalian cells, including primary cells. It is mitotically stable in the absence of selection by binding to the nuclear matrix through an interaction with the matrix protein SAF-A. We show that its function depends exclusively on a transcription unit linked to the S/MAR. This allowed us to construct a synthetic episomally replicating vector. Possible application of this vector system will be discussed and to overcome the problem of low transfection efficiency results to deliver it as a pseudovirus are presented.

Intravenous, Non-Viral Gene Therapy of Brain in Rodents and Primates

William M. Pardridge, MD

Therapeutic genes, in the form of plasmid DNA, can be targeted to brain, and other organs *in vivo*, without viruses, following intravenous administration of low volumes, providing gene-targeting technology is used. The plasmid DNA is delivered to tissues *in vivo* with Trojan horse liposomes (THLs), also called pegylated immunoliposomes (PILs). In this formulation, a single plasmid DNA is encapsulated in the interior of 100 nm liposomes, which have a net anionic charge. The surface of the liposome is conjugated with several thousand strands of polymers, such as 2000 Dalton polyethyleneglycol (PEG). The tips of 1-2% of the PEG strands are conjugated with one, or more, receptor-specific targeting ligands or molecular Trojan horses, such as a peptidomimetic monoclonal antibody (MAb). The MAb binds an exofacial epitope on the cell membrane receptor, and this triggers receptor-mediated transcytosis across the brain capillary endothelium, which forms the blood-brain barrier (BBB), followed by receptor-mediated endocytosis into brain cells. MAb's have been

developed that deliver genes to mice, rats, and old world primates, such as Rhesus monkeys.

THLs deliver genes across the vascular barrier in brain. Since every neuron is perfused by its own blood vessel, the THL gene transfer technology results in gene transfection of all regions of the brain. Ectopic expression of the transgene is eliminated with the combination of the THL delivery technology with tissue-specific gene promoters. Characteristics of gene transfer with this technology include:

- Intravenous, non-viral, non-toxic
- Tissue specific gene expression with tissue specific promoters
- Gene expression in the Rhesus monkey is 50-fold higher than in the rat
- 100% increase in survival time in mice with intracranial brain cancer using antisense gene therapy directed at the epidermal growth factor receptor (EGFR)
- 100% normalization of striatal tyrosine hydroxylase (TH) activity in experimental Parkinson's disease with intravenous TH gene therapy
- Enables intravenous RNA interference (RNAi), and provides first demonstration of increase in cancer survival in animals with experimental cancer with weekly intravenous RNAi gene therapy directed against the EGFR
- Genetic engineering of molecular Trojan horses to enable use in humans has been completed

SS314: RESPIRATORY TRACT: RESPIRATORY EPITHELIUM AS A MODEL FOR MUCOSAL GENE DELIVERY

Title: Non-Viral Vectors for Airway Gene Epithelium: The Pros and Cons

Stephen Hyde, PhD

The lung is an important target organ for gene therapy of many acute and chronic diseases, including cancer, asthma, cystic fibrosis (CF), alpha-1-antitrypsin deficiency and respiratory distress syndrome, among others. The lung is a particularly attractive target organ due to relatively non-invasive accessibility through the airways and vasculature, and the availability of well-developed technologies for the delivery of aerosols. While acute respiratory diseases may be amenable to treatment with viral based gene delivery systems, chronic airway diseases that require multiple rounds of administration (to achieve gene expression for months or years) necessitate the use of non-viral gene transfer agents (GTAs). A review of the activity in the lungs of a range of non-viral GTAs will be presented with particular emphasis on duration of gene expression following aerosol mediated GTA delivery.

While the majority of non-viral GTA formulation development effort has historically been focused upon the synthesis of improved GTAs, equally important is the optimisation of the plasmid (pDNA) vector employed. The discovery that unmethylated CpG sequences within pDNA is recognized as foreign in mammalian hosts, activating several immune cells types including B cells, macrophages, dendritic cells, and natural killer cells has profound implications for gene therapy approaches employing pDNA. These effects have ramifications for use in humans, as highlighted by the transient but not insignificant inflammatory response, which included fever, myalgia, and a reduction in pulmonary function in CF subjects that received an aerosolized pDNA-cationic liposome complex (Alton *et al.*, 1999 Lancet 353:947). We, and others, have reduced the frequency of CpG motifs in pDNAs by eliminating nonessential regions within the plasmid backbone and by redesigning regulatory elements and open reading frames. The impact of CpG reduction on inflammatory responses to non-viral gene transfer will be discussed.

One of the major limitations of non-viral gene transfer formulations is the short duration of transgene expression that is typically observed *in vivo*. We have previously demonstrated that promoter inactivation rather than loss of vector DNA is the primary factor limiting long-term gene expression in the airways. The transcriptional activity of the widely used CMV immediate early promoter is robust, but prone to inactivation over time. We, and others, have evaluated alternative promoters, including cellular promoters, tissue-specific promoters, and CpG-free variants. During our search for promoters that would provide greater longevity of expression *in vivo*, we observed that promoters from the cellular ubiquitin genes showed promise. The activity of a range of promoter elements with emphasis on the human UbC promoter will be discussed.

Viral Vectors for Airway Gene Delivery: Pros and Cons

Jim Hu, PhD

The difficulty of gene medicine was initially underestimated. In fact, the term "gene medicine" may be misleading since most conventional drugs can be efficiently delivered orally, intravenously or subcutaneously. However, genes delivered in viral vectors or complexed with liposomes have difficulty reaching the target cells, for the following reasons. First, viral vectors or DNA/liposome complexes are much larger in size than conventional drugs and therefore cannot move freely inside the body. For example, most viral vectors after entering a cell cannot leave. Wild-type viruses can spread inside a body because they can propagate in infected cells and cause the cells to burst allowing the progeny to infect other cells or travel to other organs if they can enter the



blood circulation. For safety reasons, however, most viral vectors are replication-incompetent. Regarding *in vivo* migration, recombinant AAV is the only known viral vector that can cross the blood vessel barrier. Second, “gene medicine” is biologically labile and vulnerable to the host’s defense systems. Viral vectors can be inactivated by pre-existing antibodies, the innate immune response, and plasmids are quickly degraded inside and outside cells when they are separated from liposomes.

The lung represents an attractive target for gene transfer because materials can be easily delivered to the airway surface. The lung epithelium is a continuous layer of cells lining the airways from the trachea to alveoli, and is composed of at least eight different cell types that have a range of functions. In addition to its function to provide gas exchange for the body, the lung has developed potent defenses for the elimination of foreign particles and infectious agents.

To achieve safe and effective airway gene therapy, there are three types of obstacles that need to be overcome: physical barriers, immunological barriers, and maintaining transgene expression. The physical barriers include mucosal trapping and mucociliary action, a lack of viral receptors on the apical surface and loss of vectors to non-target cells, such as macrophages. The immunological barriers consist of innate immune response initiated by macrophages that leads to inflammation, and adaptive immune response that prevents vector readministration by host antibodies and eliminates transduced cells by cytotoxic T lymphocytes. In addition, it is still a challenge to achieve long term transgene expression in airway epithelium due to the cell turnover as well as host immune responses. I will discuss these problems in the context of viral vector-mediated gene transfer and propose possible solutions. I will also show examples of some strategies to efficiently deliver viral vectors to the airways of small and large animals to achieve robust transgene expression.

Microarray Analysis of Gene Expression in the Human Small and Large Airways to Identify Targets for Pulmonary Gene Therapy

Adriana Heguy, PhD

Gene transfer by the pulmonary route requires a detailed understanding of the biology of the airways. As a tool for understanding the airway biology, we have an ongoing program of expression profiling of epithelium from large and small airways of normal human subjects. The data will be significant to the gene therapy field in two ways: (1) gene transfer viral vectors require binding to receptors on the apical surface of airway epithelium and the epithelial cell subtypes transduced depend on receptor distribution; and (2) novel gene therapy vectors could be engineered to bind to specific membrane proteins in different airway epithelial cell types. In addition, new

targets related to airway epithelial disease pathogenesis can be identified for therapeutic intervention by gene transfer.

The rationale for analyzing the transcriptomes of both small and large airway epithelium is that the population of cells differ along the respiratory tract. Further, different diseases are associated with the two locations: for example, the acute manifestations of chronic bronchitis occur in the small airways. Based on these differences, we hypothesized that gene expression patterns of the small and large airways will partially overlap, but also will reveal significant differences of importance for treatment of pulmonary diseases by gene transfer. We used fiberoptic bronchoscopy to sample pure epithelium of small (10th 12th order) and large airways (3rd order) from non smokers and phenotypically normal 20 pack yr smokers. Consistent with histologic studies, we found differences in the cell populations in the two sites, e.g. more ciliated cells, fewer basal cells, and presence of Clara cells in the small airways. Surprisingly, despite these differences, microarray analysis using the Affymetrix HG133A chip showed that of >10,000 genes expressed in the small and large airway epithelium, >98% were common to both locations, in both smokers and non smokers. However, some genes are expressed exclusively in one or the other location, and some genes are predominantly expressed in small or large airways. The only small airway specific genes are the surfactant protein genes, and the small airway predominant genes include transcription factors relevant to lung morphogenesis, and signal transduction mediators including membrane receptors. Several membrane proteins involved in signal transduction, cell communication, and tumor suppression were exclusive to the large airway epithelium. These may offer routes for selective targeting of gene transfer vectors to these cell types. Genes with preferential expression in large airways (1.3% of expressed genes) included transcription and signal transduction factors involved in cell proliferation, serpins, and basal cell markers.

With respect to the identification of molecular targets for intervention, we characterized the response of small and large airway epithelium to cigarette smoke. The response to smoking, including induction of genes encoding antioxidant related, xenobiotic metabolizing enzymes and tissue remodeling factors, was similar in large and small airways in spite of the differences in the cell types that predominated in each locations. This type of analysis can be used to compare the expression profile in smokers with lung disease and in healthy subjects, to identify susceptibility genes that may represent good candidates for intervention by gene transfer.

MI320: Meet-the-Investigator: Integrating Vectors: From Production to Clinic*Kenneth Cornetta, MD*

Integrating vectors continue to have advantages for clinical applications where the target cells are expected to undergo many rounds of replication or persistence over an individual's lifetime. Vectors based on the murine leukemia viruses were the first to enter clinical gene therapy trials, and to date, Indiana University has certified over 20 vectors for use in clinical trials. The majority of these vectors were produced as part of the NIH sponsored National Gene Vector Laboratory. In this session we will review two aspects of this experience. After a brief overview of the information gained by this large experience in vector production, challenges faced by investigators seeking to utilize the material in clinical trial will be discussed. After this review, the majority of the session will focus on the challenges in bringing lentiviral vectors to trial. While retroviral vectors have traditionally utilized stable vector producer cell lines, certain toxicity issues inherent in lentiviral vectors have limited this approach. Many investigators have utilized transient transfection methods to generate the small scale vector required for most research purposes. Transient methods have the potential advantage of decreasing the time to vector production, as stable producer cell lines often take months to generate and certify. Nevertheless, production of membrane bound viruses using transient production methods presents unique technical challenges. In addition, there are safety issues inherent in generating vectors based on HIV-1, and our experience with addressing these issues will be discussed. Included in the discussion will be the current Guidance documents available from the FDA and a highlight of areas where no such guidelines are available. The goal of this session is not to provide specific expertise in vector production per se, but to familiarize the participants with the key issues that will be required to be addressed by investigators wishing to conduct clinical trials with integrating vectors. It is hoped that a better understanding of the production and testing issues will assist participants in designing in vivo experiments in small and large animal models and in understanding the important regulatory requirements when utilizing these vectors in clinical trials.

SATURDAY, JUNE 4, 2005**SS400: CANCER: CLINICAL TRIALS****Suicide Gene Therapy in Stem Cell Transplantation.***Chiara Bonini, MD**F. Ciceri and C. Bordignon*

Since the hypothesis that the immune system can target malignant cells has been formulated, approximately a century ago, its exploitation has become a major area of medical interest. Although preclinical data in rodents and clinical observations strongly suggest that this hypothesis is correct, it is now accepted that efficient cancer immunotherapy approaches cannot be limited to the exploitation of specificity, plasticity and power of the immune system, but instead need to rely on strategies aimed at safely increase anti-tumor responses. A compelling example of this concept is represented by allogeneic hematopoietic stem cell transplantation (allo-HSCT), the treatment of choice for hematologic malignancies and the first example of wide clinical application of cancer immunotherapy. The anti-tumor potential of allo-HSCT strongly relies upon the immune advantage conferred by donor T-lymphocytes (graft-vs-leukemia effect-GvL). The power of the allogeneic immune advantage is well documented by the effect of the delayed infusions of donor lymphocytes (DLI), resulting, in the absence of additional therapy, in the complete and persistent remission of malignancies relapsed after transplant. Despite undeniable efficacy, the extensive exploitation of DLI after allo-HSCT is limited by the risk of a severe, and potentially life-threatening complication: Graft-versus-host disease (GvHD). To overcome this limitation, we investigated the therapeutic potential of donor lymphocytes engineered with the suicide gene thymidine kinase of Herpes Simplex virus (TK) in high-risk patients experiencing recurrence of hematologic malignancies after allo-HSCT. In our pilot study as well as in similar studies performed in Europe and in the US, the expression of the suicide phenotype proved effective in providing a selective tool for the elimination of TK-cells and the resolution of GvHD. However, difficulties in the standardization of the gene transfer procedure in terms of culture conditions and degree of in vitro expansion of transduced lymphocytes, resulted in variable modifications of the immune competence of transduced cells, potentially responsible of the different clinical outcomes observed in different studies. We show the feasibility, safety, and efficacy of donor TK-cells in 23 patients who relapsed after HLA-identical allo-HSCT. A standardized preparation of engineered donor T cells in GMP conditions resulted highly feasible (almost 80% of patients enrolled