



WEDNESDAY, JUNE 1, 2005

ES100: RETROVIRUS AND LENTIVIRUS VECTORS

Retrovirus Vectors: We are Family!

Christopher Baum, MD

Retroviral vectors created on the basis of simple gammaretroviruses, complex lentiviruses, or potentially non-pathogenic spumaviruses represent well characterized but still surprising tools for stable gene transfer. Detailed studies of retroviral elements are ongoing to improve the performance of current vectors, and to derive mechanisms that are of principal interest for the design of future recombinant vectors. A key task of this session is to understand to what extent different types of retroviral vectors, just as different members of a human family, educate each other to develop distinct and potentially useful features by sharing certain common motifs.

In general terms, efficiency and safety of retroviral vectors depend on

- the pool size and specific targeting of the relevant cell type,
- the multiplicity of insertions per host genome, and
- the choice of appropriate *trans*-acting vector components and *cis*-acting elements of the retroviral transgene.

Trans-acting components are derived from the retroviral genes *gag*, *pol*, and *env*. These need to be expressed in safety-modified producer cells to package the retroviral transcripts in viral particles (*gag*), to determine the host range at the level of receptor recognition (*env*), and to allow intracellular transport (mainly *gag*), reverse transcription (*pol*), and chromosomal insertion (integrase encoded by *pol*) of the vector genome. Research regarding *env* functions has significantly improved vector design, resulting in satisfactory transduction rates of several clinically relevant target cell types. Manipulations of *gag* or *pol* functions are far less advanced. Modifications of *gag* may overcome post-entry defense mechanisms affecting cytoplasmic fate and nuclear translocation. Alterations of *pol* would be required to improve the fidelity of reverse transcription and to target transgenes to specific sites within the genome.

Distinct retroviral *cis*-acting sequences regulate packaging of proviral RNA, reverse transcription, genomic insertion and transgene expression. A major advantage of simple gammaretroviruses such as murine leukemia virus (MLV) is that these elements do not overlap with retroviral coding regions (except the rather small splice acceptor). Insights into the molecular regulation of retroviral enhancer-promoters and RNA processing have resulted in a series of promising vectors that mediate sufficient

levels of constitutive transgene expression from one or two transgene copies. Moreover, non-retroviral *cis*-elements can be incorporated to achieve differentiation-specific or inducible expression, efficient coexpression of two or more genes from a single vector, and to further increase transcriptional autonomy. Recent advances in the construction and biosafety analyses of retroviral vectors with self-inactivating (SIN) long terminal repeats will also be addressed.

With increasing reports of malignant complications following retroviral vector-mediated gene transfer into somatic cells, understanding and preventing the pathomechanisms of insertional mutagenesis has become a major task. Probably both *cis*- and *trans*-acting components need to be modified to solve this problem. Recent insights from murine studies will be presented.

Finally, it should be noted that retroviral vectors can be modified to achieve transient delivery of mRNA or circular episomal DNA into target cells. Exploiting these intermediate steps of the retroviral life cycle greatly enhances the potential utility of this fascinating vector system.

Foamy Virus Vectors

David W. Russell, MD, PhD

Foamy viruses (FVs) comprise the third class of retroviruses, in addition to oncogenic viruses and lentiviruses. Significant progress has been made in the production and use of FV vectors. The advantages of FV vectors include a lack of pathogenicity, large packaging capacity, broad host range, stability in quiescent cells, and efficient transduction of stem cells from many species. I will review FV vector design, methods for production, transduction mechanisms including integration site distributions, and applications with a focus on stem cell gene transfer.

ES101: NONVIRAL VECTORS

In vivo Electrotransfer of Plasmids and Synthetic Nucleotides : Basic Concept and Potential Therapeutic Applications

Daniel Scherman, PhD

The lecture will review results obtained on the use of electrotransfer to enhance plasmid DNA tissue and cell penetration by in vivo electrotransfer. In vivo electrotransfer is a physical method of gene delivery in various tissues and organs such as muscle and tumors, relying on the injection of a plasmid DNA followed by electric pulse delivery. The importance of the association between cell permeabilization and DNA electrophoresis for electrotransfer efficiency has been highlighted.

In vivo electrotransfer is of special interest since it is the most efficient non-viral strategy of gene delivery and also

because of its low cost, easiness of realization and safety. The potentiality of this technique can be further improved by optimizing plasmid biodistribution in the targeted organ, plasmid structure, and the design of the encoded protein. In particular, plasmids of smaller size are electrotransferred more efficiently than large plasmids.

It is also of importance to study and understand kinetic expression of the transgene, which can be very variable, depending on many factors including cellular localization of the protein, physiological activity and regulation. One of the most widely targeted tissue is skeletal muscle, because this strategy is not only promising for the treatment of muscle disorders, but also for the systemic secretion of therapeutic proteins. Vaccination and oncology gene therapy are also major fields of application of electrotransfer, whereas application to other organs such as liver, brain and cornea are expanding.

Many published studies have shown that plasmid electrotransfer can lead to long-lasting therapeutic effects in various pathologies such as cancer, blood disorders, rheumatoid arthritis or muscle ischemia. DNA

electrotransfer is also a powerful laboratory tool to study gene function in a given tissue.

ES103: REGULATORY HURDLES TO PHASE III CLINICAL TRIALS

Common Challenges in the Development of Gene Therapy Products

Andrew P. Byrnes, PhD

Biological products are frequently complicated to manufacture and characterize. Gene therapy products (including ex-vivo transduced cells) raise special issues because of their complexity and novelty. Manufacturers must be able to demonstrate that they can produce a consistent product, and lack of this can be a barrier to pivotal phase trials. In the worst case, an inconsistent product can yield uninterpretable clinical results.

Fortunately, these issues can often be resolved with some planning ahead, and experienced manufacturers have found that the best approach is to start thinking about characterization during early product development. This presentation will emphasize areas of product characterization that, in the FDA's experience, provide particular value or present special challenges. We will discuss assays for potency, identity, and stability, with a focus on some unique issues that these assays raise for gene therapy products. We will discuss how to use product characterization assays during manufacturing changes such as scale-up. We will emphasize the value that these assays add to the development of products, and the importance of early communication with the FDA.

ES104: EXPRESSION CASSETTE DESIGN

Expression Cassette Design - 20 Years in 20 Minutes

Mitchell H. Finer PhD

All Gene Therapy projects, by definition, require expression of an exogenously introduced therapeutic gene. Although many viral vectors or physical methods can efficiently enable cellular uptake and delivery of the therapeutic gene to the nucleus, maintaining sufficient expression to provide therapeutic correction for a meaningful period of time has been difficult to achieve in relevant animal models and human clinical studies. In 1995, NIH director Harold Varmus appointed an ad hoc committee to assess the status of gene therapy, identify areas for improvement and provide recommendations regarding research direction. One of the major conclusions of that committee was that there is inadequate knowledge of vector components necessary to maintain adequate expression of the therapeutic gene to lead to clinical success and the NIH funding should invest in basic research on control of gene expression¹.

Over the last 10 years, a significant amount of effort has been invested in development of optimized expression cassettes that provide sustained, high – level expression of therapeutic proteins in relevant disease models with viral and non – viral vectors. The purpose of this session will be to provide guidance for new investigators in the field concerning approaches for high level, sustained expression of their desired therapeutic gene. The session will feature three presentations and an interactive question and answer session. The first presentation, “Expression Vectors – 20 years in 20 minutes” presented by Dr. Finer, will provide an overview to expression cassette design for viral and non – viral vectors, with a focus on using viral and house – keeping promoters for highest level of gene expression initially following gene transfer. The second presentation by Dr. Miao will focus on strategies that have been successfully used to generate expression cassettes that provide sustained, high level tissue – specific expression in the liver and muscle, as well as other targets. The third and final presentation by Dr. Hope will focus on vector strategies that optimize protein expression through the use of optimal intron configuration and mRNA splicing, mRNA stability elements and translation initiation site configuration. A question and answer session will follow.

¹Orkin, S.H. and Motulsky, A.G. (1995) Report and recommendations of the panel to assess the NIH investment in research on gene therapy. *NIH Report December 1995*.



Establishment of High-Level and Tissue-Specific Gene Expression Cassettes

Carol H. Miao, PhD

It is highly desirable to generate tissue-specific and persistently high-level transgene expression per genomic copy from gene therapy vectors. Such vectors can reduce the cost and preparation of the vectors, and reduce possible host immune responses to the vector and potential toxicity. Many gene therapy vectors have failed to produce therapeutic levels of transgene because of inefficient promoters, loss of vector or gene expression from episomal vectors, or a silencing effect of integration sites on integrating vectors. In this session, it will be described that by *in vivo* screening of vectors incorporating many different combination of gene regulatory sequences, liver-specific, high-expressing vectors to accommodate factor IX, factor VIII, and other genes for effective gene transfer were established. Persistent and high-levels of factor IX and factor VIII gene expression for treating hemophilia B and A respectively were achieved in the mouse livers by using hydrodynamics-based gene transfer of naked plasmid DNA incorporating these novel gene expression systems. Some other systems to prolong or stabilize the gene expression following gene transfer will also be discussed.

ES105: CANCER GENE THERAPY

HSV Oncolytic Viruses

E. Antonio Chiocca, MD, PhD

Oncolytic viruses based on HSV1 have been used in phase I clinical trials for malignant glioma. We have been studying in animal models the early phases of the host response against viral-mediated oncolysis. Generalized inhibition of such phases leads to enhanced oncolysis, but there is a need for further dissection of the processes that impeded effective viral infection of tumor cells and subsequent replication in tumors. The role of innate immune and other host responses will be discussed and experimental results analyzed.

VSV as an Oncolytic Vector: Mechanisms of Action

Glen Barber, PhD

The ability of viruses to efficiently reproduce in transformed cells, in many instances to induce cytolysis, was first recognized nearly 100 years ago in experiments using rhabdoviruses and paramyxoviruses. However, it was not until the 1990's that interest in re-evaluating viruses as a cancer therapy agents occurred in studies involving the DNA viruses, herpes simplex virus and adenovirus. Subsequently, similar investigations re-emphasized that RNA viruses such as Reovirus, Newcastle Disease Virus (NDV), Vesicular Stomatitis

Virus (VSV) and Measles Virus (MV) exerted similar oncolytic properties. The mechanisms underlining how certain viruses preferentially replicate in malignant cells and less so in normal cells are now the focus of intense study, with evidence indicating that these agents exploit defects in cellular host defense including those involving the p53 and interferon pathways. Such flaws in antiviral defense may be prevalent in many types of tumor cells making this approach to cancer therapy an attractive option. Indeed a growing number of clinical trials have now been initiated to evaluate the use of different 'viral oncolytic' regimes for the treatment of malignant disease. This presentation will focus on evaluating the use of the rhabdovirus, vesicular stomatitis virus (VSV), as oncolytic gene therapy agent for the treatment of cancer. Data on the mechanisms by which VSV and perhaps other RNA viruses achieve their anti-tumor effectiveness will be presented. In addition, future prospects involving the genetic engineering of VSV, which are designed to increase the viruses' specificity, safety and efficacy of action will be discussed.

ES111: HSV VECTORS

HSV Vectors for Chemotherapy Delivery

E. Antonio Chiocca, MD, PhD

Oncolytic viruses based on HSV1 can be employed for delivery of variety of chemotherapy-sensitizing genes. Over the years, we and others have worked with several types of genes. Broadly, these can be divided into two categories: genes whose activated metabolites possess anticancer action that enhances viral oncolysis and genes whose activated metabolites possess anticancer action but can also inhibit oncolytic viral replication and may thus antagonize the oncolytic effect. Experimental results in *in vitro* and *in vivo* models will be discussed with both types as well how to evaluate pharmacologic synergy in this type of paradigm.

ES114: GENE TRANSFER TO MUSCLE

Cell Therapy for Muscular Dystrophy

Giuliana Ferrari, PhD

Muscular dystrophies are caused by progressive degeneration of skeletal muscle fibers. Lack of one of several proteins either at the plasma membrane or, less frequently, within internal membranes increases the probability of damage during contraction and eventually leads to fiber degeneration. Fiber degeneration is counterbalanced by regeneration of new fibers at the expense of resident myogenic cells, located underneath the basal lamina and termed satellite cells. In the most severe forms, such as Duchenne muscular dystrophy (DMD), caused by the absence of dystrophin, regeneration is exhausted and skeletal muscle is progressively replaced by fat and fibrous tissue. In DMD

patients the mechanical stress associated with contraction progressively leads to degeneration of skeletal muscle fibers, muscle wasting, progressive impairment of movements, and eventually paralysis and death. Current therapeutic approaches involve steroids and result in modest beneficial effects. Novel experimental approaches are in the area of gene therapy to efficiently transduce muscle fibers or myogenic progenitors and cell therapy, based initially on myoblast transplantation and more recently on the transplantation of stem-progenitor cells. Different labs have shown that myogenic progenitors are present in the bone marrow or in stem cell-containing fractions, and are recruited to myogenic differentiation restoring dystrophin gene expression in a murine model of DMD. More recently, blood-vessel associated, multipotent progenitor cells, the mesoangioblasts, have been shown to home and differentiate into muscle fibers when delivered to a muscle through the arterial circulation. Intra-arterial delivery of genetically modified mesoangioblasts led to repopulation and massive replacement of sarcoglycan-deficient fibers in a murine model of limb-girdle muscular dystrophy.

Gene therapy of DMD is based on the concept of providing a copy of a normal dystrophin cDNA to muscle fibers or myogenic progenitors, *in vivo* or *ex vivo*. The recent identification of novel types of stem cells opens new perspectives for combined gene/cell therapy tool to treat muscular dystrophy. However, the limited knowledge of stem cell biology is an obstacle that must be overcome to devise protocols with a significant prospect of clinical improvement in patients affected by severe forms of muscular dystrophy. A better definition of the cells capable of changing fate in response to these signals, as well as a better knowledge of the signals themselves, is therefore needed. Expansion and active recruitment to myogenic differentiation of transplantable genetically corrected stem-progenitor cells are in fact critical factors for a possible use in cell/gene therapy of muscular dystrophy. The potential use and the limitations of different stem cells endowed with myogenic activity will be discussed.

ES115: BUILDING A STRATEGY FOR TRANSLATIONAL MEDICINE: FOCUS ON IMMUNODEFICIENCIES

Development of Gene Therapy for Wiskott-Aldrich Syndrome

Arthus W. Nienhuis, MD

The ability to reliably transfer a gene into stem cells capable of regenerating bone marrow and lymphoid organs and to achieve its regulated expression in differentiated cell populations would provide an effective way to treat inherited disorders that affect the blood forming tissues. One such disorder, Wiskott-Aldrich

syndrome (WAS), is an X-linked disease in which boys are variably affected with a triad of symptoms. Defects in the immune system render them susceptible to infections, a low platelet count creates risk for serious bleeding and recurrent skin rashes may be problematic. Older boys are susceptible to the development of lymphoma and paradoxically, autoimmune disorders despite the deficiency in the immune system. Currently life expectancy is less than 20 years. Although bone marrow transplantation may be curative, many boys lack a matched donor and older boys are particularly susceptible to transplant complications.

The gene which is mutated in WAS patients encodes a 54 Kd protein called the WAS protein (WASP) which is expressed predominantly in hematopoietic cells. WASP transmits and integrates signals arising at the cell membrane that result in shape change or cell movement through reorganization of the actin cytoskeleton. WASP is also involved in formation of the immunological synapse between T-cells and antigen presenting cells. T-cell dysfunction as reflected by poor proliferation and low cytokine secretion in response to T-cell receptor stimulation is thought to be a key defective component in the pathophysiology of WAS. Mouse strains having WASP deficiency, created by gene knockout technology, exhibit many of the defects characteristic of WAS patients. Such mice have been shown to exhibit defective immune responses to several pathogens including influenza A-virus, Streptococcus pneumonia, and Mycobacterium bovis. These defects can be partially corrected by retroviral vector-mediated gene transfer of the WASP gene into WASP^{-/-} stem cells.

Extension of these findings into a clinical gene therapy protocol for WAS is limited by two factors: 1) the relatively low efficiency of gene transfer into human hematopoietic stem cells achieved with oncoretroviral vectors; and 2) the propensity of oncoretroviral vectors to integrate within genes and near promoters. Such integrated vectors have the capability of increasing expression of proto-oncogenes as occurred in three patients in a clinical gene therapy trial for treatment of severe combined immunodeficiency (SCID). Lentiviral vectors based on HIV are more efficient at transducing primate hematopoietic stem cells. They have been adapted for use in a self-inactivating design and thus are more amenable to expressing gene from internal regulatory elements. Although lentiviral vectors integrate into genes, they do not show a predilection for promoter region. Accordingly, current efforts are focused on developing appropriate lentiviral vectors for WAS gene therapy.

Remaining challenges include the following: 1) derivation of an appropriate vector design that insures appropriate expression in all hematopoietic cells in which WASP has an important role; 2) evaluation of the effects of various enhancer blocking and/or boundary elements



in the vector to reduce the risk of proto-oncogene activation by an integrated vector genome; 3) demonstration that a WASP transgene function can fully correct the defects in a murine models and/or human cells from WAS patients; and 4) developments of methods for producing clinical grade vector preparations suitable for use in early stage, exploratory trials. Experience in the SCID trial has unequivocally demonstrated that retroviral insertion can contribute to neoplastic transformation by proto-oncogene activation. Therefore, clinical trials for WAS should not be initiated until there is a reasonable probability of a successful therapeutic outcome and the risk of insertional mutagenesis by the vector to be used has been evaluated in the context of assessing its potential to contribute to cell transformation.

ES120: NOVEL VECTOR SYSTEMS

Targetable/Injectable Adenoviral Vectors for Cell-specific Gene Transfer In Vivo

David T. Curiel, MD, PhD

Adenoviral (Ad) vectors are of utility for many therapeutic applications. Strategies have been developed to alter adenoviral tropism to achieve a cell-specific gene delivery capacity employing fiber modifications allowing genetic incorporation of complex targeting motifs. In this regard, single chain antibodies (scFv) represent potentially useful agents to achieve targeted gene transfer. However, the distinct biosynthetic pathways that scFv and Ad capsid proteins are normally routed through have thus far been problematic with respect to scFv incorporation into the Ad capsid. Utilization of genetically engineered "stabilized" scFv would overcome this restriction. We genetically incorporated a stabilized scFv into a de-knobbed, fibrin-foldon trimerized Ad fiber and demonstrated targeting to the cognate epitope expressed on the membrane surface of cells. We have shown that the scFv employed in this study retains functionality and it is clear that stabilizing the targeting molecule, *per se*, is critical to allow retention of antigen recognition in the adenovirus capsid-incorporated context.

ES122: RNAi

The Long and Short of RNAi

John J. Rossi, PhD

RNA interference (RNAi) is emerging as perhaps the most powerful tool available for targeted gene knockdown in mammalian cells. The triggers for RNAi are small double stranded RNA duplexes of 21-23 nucleotides in length that have 2 base 3' overhangs. These have been termed small interfering RNAs or siRNAs. One of the two strands of the siRNA is selectively incorporated into the RNA Induced Silencing Complex

(RISC) and is ultimately bound by the Argonaute 2 protein (Ago2) which cleaves the target RNA in the middle of the duplex generated by the siRNA pairing. We have demonstrated that the process of siRNA triggered RNAi is independent of translation-and in fact is enhanced about 3 fold when translation is blocked. The implications of these results for the localization of the RNAi process will be discussed. We have also determined that synthetic RNAs which are processed intracellularly by the RNaseIII family member Dicer can potentiate target knockdown by up to 100 fold. This observation has led to a careful examination of how to design the most potent RNAi triggers. Understanding the mechanism of Ago 2 strand selection is paramount to achieving this. First, we have observed that Dicer enters substrate duplexes preferentially via single stranded overhangs. The site of Dicer cleavage in a duplex appears to dictate to a marked extent which strand of the duplex will be selected as the antisense by Ago2. We have also observed strong biases in the binding of siRNAs by Ago2, and have correlated the Ago2 binding with intracellular efficacy of the siRNAs. Given these new findings, we can predictably generate good target knockdowns using Dicer substrate RNAs that generate a siRNA that is a preferred Ago2 substrate. These results will be discussed in the context of the RNAi mechanism.

ES123: GENE TRANSFER TO THE NERVOUS SYSTEM

Gene Transfer to DRG for Treatment of Neuropathy or Chronic Pain

David J. Fink, MD

The peripheral nervous system (PNS) presents distinct challenges and special opportunities for gene therapy. The challenge resides in the distributed anatomy of the PNS, which has widespread projections peripherally and converging of central projections in the spinal cord, and the relative inaccessibility of the neuronal cell bodies in the dorsal root ganglion. The enormous opportunity exists because many peptide growth factors and neurotransmitters that have demonstrated potential utility and well-understood mechanisms of action could be harnessed for therapeutic purposes if they could be delivered to the PNS in adequate concentrations at the required location.

Viral vectors may be injected directly into dorsal root ganglion to transduce sensory neurons and vector-mediated transduction of muscle may be used to produce and release peptide factors that will subsequently be taken up by sensory nerve terminals. Vectors based on herpes simplex virus (HSV) are uniquely suited for transduction of DRG neurons from peripheral inoculation. HSV is a neurotropic virus that is taken up with high affinity by the peripheral sensory terminals in the skin, and naturally

establishes a lifelong latent state as an intranuclear episomal element. Nonreplicating HSV recombinants created by the deletion of essential immediate early genes can be propagated to high titers without wild-type recombinants.

Two classes of condition affecting the peripheral nervous system have proven amenable to gene transfer; sensory polyneuropathies and chronic regional pain.

Subcutaneous inoculation of HSV-based vectors expressing neurotrophin-3 (NT-3) or nerve growth factor (NGF) can prevent the progression of the pure sensory neuropathy caused by high dose of pyridoxine in a subacute model, and also prevents the neuropathy induced by the chemotherapeutic drug cisplatin in a chronic model, and with appropriate choice of promoter element is effective at least 6 months after vector inoculation. Similar results have been demonstrated with HSV vectors expressing NGF or the vascular endothelial growth factor in a model of diabetic neuropathy.

Transduction of DRG neurons by an HSV vector expressing proenkephalin effectively reduces pain in rodent models of inflammatory pain, neuropathic pain, pain from cancer in bone, and craniofacial pain. Both central and peripheral effects of the transgene mediated opioid peptide have been demonstrated in the various models. Transduction of DRG neurons with an HSV vector expressing glutamic acid decarboxylase results of the release of gamma-aminobutyric acid from the transduced neurons and provides a remarkable analgesic effect in models of central and peripheral neuropathic pain. HSV vector expressing an antisense oligonucleotide for a voltage gated sodium channel reduces inflammatory pain.

The redundant use of a limited repertoire of neurotransmitters and growth factors for diverse functions in the nervous system constrains the use of these molecules as drugs for therapeutic purposes. Targeted expression achieved by gene transfer will allow the investigators to discern regional effects of the substances in the PNS and spinal cord, and shows promise for unlocking the therapeutic potential of these molecules.

ES124: BIOAVAILABILITY AND PHARMACOKINETICS

Pharmacokinetics Made Easy

Maria A. Croyle, PhD

Pharmacokinetics is the quantitative analysis of the fate of a drug in the body over time. In the context of gene therapy, this includes the fate of the vector as well as that of the transgene product. Basic pharmacokinetics requires a thorough understanding of biology, physiology and knowledge of the concepts and limitations of mathematical models. Processes included in this analysis,

absorption, distribution, metabolism, and elimination are dynamic and can be profoundly influenced by physiology and the biological and physicochemical properties of the medicinal agent. Historically, the plasma concentration of a drug remains the best measurable correlate of biological activity, assuming that the drug at the site of action is in equilibrium with the drug in the blood. In the context of gene delivery, one must also consider the extensive processing and transport of each vector at the cellular level. Thus, it is not only essential to have a solid understanding of conventional pharmacokinetics and pharmacodynamics, but also knowledge of intracellular pharmacokinetics.

The bioavailability of a drug product is defined in terms of the amount of active drug that reaches the circulation and considers the rate and extent to which an active medicinal agent is absorbed from the site of administration. Medicines administered by intravenous injection are most often considered to have 100% bioavailability and serve as the standard by which to compare bioavailability of a compound administered by an extravascular route. Unlike traditional medications, many gene delivery vectors and the transgene product are inactivated by complement and neutralizing antibodies in the circulation. The rate at which this occurs can have a significant impact on the bioavailability of the vector.

In this presentation, the following topics will be discussed:

- General terminology (AUC, volume of distribution, half-life, clearance)
- Compartmental modeling of pharmacokinetic data
- The effect of protein binding and other physiological factors on pharmacokinetic profiles
- Selection of the appropriate pharmacokinetic model for a given dataset
- Guidelines for the design of pre-clinical pharmacokinetic studies

Bridging Preclinical and Clinical Pharmacokinetics in Gene Therapy

Sally Choe, PhD

The success of gene therapy will be highly dependent upon the design of specific dosing regimens. The goal of a properly designed dosing regimen is to achieve an optimal concentration of therapeutic transgene to induce the desired therapeutic response with minimum adverse effects. Individual variation in pharmacokinetics makes the design of dosage regimens difficult. Therefore, the application of pharmacokinetics to dosing regimen design must be coordinated with proper clinical evaluation and monitoring.

Traditional pharmacokinetic study involves taking multiple blood samples over time in healthy volunteers or patients and characterizing basic pharmacokinetic parameters such as the clearance and half-life. A



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 principal 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