

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



succeeded in receiving DLI with genetically modified cells). Long-term follow-up of infused patients included analysis of engraftment of genetically engineered lymphocytes, in vivo assessment of anti-tumor effect and control of GvHD by ganciclovir. All 17 patients evaluable for engraftment and graft-versus-leukemia (GvL) had circulating TK-cells detectable beginning at a median time of 18 days. Eleven patients (65%) experienced a substantial clinical benefit resulting in 6 complete remissions (35%) and 5 (29%) partial responses. The anti-tumor effect strongly correlated with the in vivo expansion of TK-cells. Seven patients received ganciclovir that resulted in elimination of TK-cells and effective and selective treatment of GvHD. Immunization against HSV-TK was observed in 7 patients, but did not preclude an effective GvL. These data validate the use of TK-cells in the context of allografting, and represents the basis for a broader application of this technology.

GVAX® Cancer Vaccines – Clinical Development

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. Sources of tumor cells for vaccine manufacturing have included both non patient-specific established allogeneic tumor cell lines as well as autologous tumor cells derived from individual patients via a tumor harvest procedure. Non patient specific approaches have been pursued in prostate cancer, pancreatic cancer, and hematologic malignancies, whereas a patient specific approach was evaluated in lung cancer. Immunologic and clinical activity of GVAX® cancer vaccines has been demonstrated in multiple tumor types with both approaches. Clinical development of the non patient-specific GVAX® vaccine platform will be reviewed with a focus on clinical development of a prostate cancer vaccine that is currently undergoing phase 3 testing in hormone-refractory prostate cancer.

SS401: CARDIOVASCULAR: ADVANCES IN CARDIOVASCULAR CELL AND GENE THERAPY

Novel Therapy for Obesity and Diabetes: Implications for Cardiovascular Disease

Lawrence Chan, MD

Diabetes mellitus is a major cause of accelerated atherosclerosis. Two-thirds of patients with diabetes die of cardiovascular disease. The association between

diabetes and cardiovascular pathology holds true for both type 1 and type 2 diabetes. Type 1 diabetes is caused by absolute insulin deficiency due to a diseased pancreatic, whereas type 2 diabetes is caused by a combination of defects in insulin production and insulin action, i.e., development of insulin resistance. The prevalence of type 2 diabetes has been increasing rapidly in the last few years among both adults and children due primarily to an increase in the prevalence of obesity. We have developed effective experimental protocols for molecular therapies that correct the two underlying pathologies in type 2 diabetes, viz. beta cell dysfunction and insulin resistance.

Our approach to reverse the insulin production defect is the induction of islet neogenesis in the liver by gene transfer of pancreatic islet-specific transcription factors to the liver. Like normal pancreatic islets, the newly formed islets in the liver are regulated by the level of plasma glucose. When we used this strategy to treat streptozotocin-induced diabetic mice, the maneuver reversed the hyperglycemia as it normalized the blood glucose and insulin response to a glucose tolerance test. Studies are underway to examine the utility of this approach to correct the insulin production defect in type 2 disease models.

With respect to the treatment of insulin resistance, we devised a treatment strategy to reverse obesity, because (over)weight-induced insulin resistance is the cause of insulin resistance in the majority of patients with type 2 diabetes. In collaboration with R. Pasqualini and W. Arap at MD Anderson Cancer Center, we targeted adipose tissue vasculature as a means to ablate fat. Adipose vasculature-binding peptides were identified by in vivo phage display. Using a 7-amino acid peptide motif as the homing peptide, we targeted a proapoptotic peptide to the vessel wall of adipose tissue. Administration of this chimeric peptide by subcutaneous injection into mice with diet-induced obesity led to efficient ablation of fat. Reversal of obesity was accompanied by an increased metabolic rate and the disappearance of excess fat in the liver and muscle. The treatment also reversed insulin resistance and type 2 diabetes in these animals.

We believe that a combination of islet neogenesis and fat ablation holds promise for the treatment of diabetes; conceptually, both approaches can be applied to human patients.

Adenylyl Cyclase Gene Transfer for Heart Failure

H. Kirk Hammond, MD

Heart failure affects 6 million patients in the United States, and is the most common diagnosis for urgent admission to the hospital in patients over 60 years old. It is the only cardiovascular disease that is increasing in prevalence, in part because of the growing numbers of older people in our population. Once symptoms (breathlessness, fatigue, edema) become severe enough

that they occur at rest (Class IV) or with minimal activity (Class III), the 4-year survival is less than 50% — a mortality rate that is worse than most cancers. This mortality rate pertains to patients on optimal pharmacological and device therapy. Cardiac transplantation provides help to only 3000 patients per year, a small minority of patients with this problem. Despite these measures, prognosis remains dreadful for patients with severe heart failure. Clearly we need new treatments.

Patients with heart failure have abnormally low levels of functioning adenylyl cyclase (AC) and low levels of cardiac cAMP. This is a major contributing factor in poor pump function. We reasoned that if we could increase the amount of AC in the failing heart, it would increase heart function. We set about to explore this hypothesis by conducting a series of experiments first in cultured cardiac myocytes, then in transgenic mice, and finally in pigs with heart failure. These studies, which will be discussed in detail during the presentation, confirmed that increased levels of cardiac AC_{VI} were associated with salutary effects. These results contrast with those obtained from cardiac-directed expression of β -adrenergic receptors or G \pm s, which cause heart failure.

Based on the efficacy and safety profile of this strategy in preclinical studies, we will soon initiate a gene transfer trial in patients with compensated Class III and Class IV heart failure. This study is funded by the NHLBI through their Centers of Excellence in Gene Therapy program. The clinical trial will be conducted at UCSD Medical Center and at the VA San Diego Healthcare System. Patients will be brought to the cardiac catheterization laboratory and receive intracoronary infusion of a replication-incompetent recombinant adenovirus (E1/E3-deleted) encoding human AC type VI (AC_{VI}). In preclinical studies this same strategy resulted in increased heart function within six days, an effect that lasts for at least eighteen weeks and which appears to be safe. The design of the clinical trial will be discussed in detail during the presentation.

SS402: HEMOPOIETIC: ADVANCES IN STEM CELL SELF RENEWAL

On-demand Expansion of Genetically Corrected Blood Cells

Akihiro Kume, MD, PhD

With self-renewal capacity and multipotentiality, hematopoietic stem cells (HSCs) are promising targets for gene therapy to treat a number of blood and metabolic diseases. Unlike studies with mouse disease models, however, success has been limited in clinical trials targeting human HSCs with oncoretroviral vectors. Conventional retroviral vectors are relatively inefficient to transduce human HSCs, partly because they are unable

to integrate into nondividing cells, and human HSCs poorly express the receptors for these vectors. In addition, accessible HSC number is limited at a time of transduction, and genetically corrected cells are diluted out with a large number of untransduced cells unless these competitors are ablated. Recent gene therapy trials for adenosine deaminase deficiency and chronic granulomatous disease (CGD) have underscored the latter aspect, where preconditioning with busulfan appeared to enhance the engraftment of transduced HSCs, thereby lead to unequivocal efficacy. Another example is gene therapy for X-linked severe combined immunodeficiency, where the therapeutic gene confers very strong and exclusive proliferative advantage on lymphoid progenitors, but unlimited growth stimulation may be harmful.

Based on these considerations, we have developed a conditional system to boost transduced hematopoietic cells in vivo. Our strategy is to confer a reversible growth advantage on the target cells with a chimeric receptor and a specific ligand. The cytoplasmic domain of a homodimerizing cytokine receptor is linked to a molecular switch; ligand binding to the switch turns on the receptor moiety to generate a growth signal. We designated the genes encoding these chimeric receptors ‘selective amplifier genes (SAGs)’, and demonstrated that at least two classes of molecular switch can be used in this system. In the first generation SAGs, the hormone-binding domain of the estrogen receptor turns on/off the machinery. In the second generation SAGs, the extracellular domain of the erythropoietin (Epo) receptor does the job. Both classes of SAGs worked in the X-linked CGD mouse model, where functionally corrected neutrophils were increased in response to estrogen or Epo. Furthermore, the efficacy of the second generation SAGs was demonstrated in a nonhuman primate autotransplant model, where transduced progenitors were expanded following drug administration. More strikingly, the SAG-marked blood cells were expanded up to 10% of the peripheral blood in cynomolgus monkeys without any preconditioning, when transduced cells were directly injected into the bone marrow cavity to enhance homing.

These results demonstrate that the present SAG system can expand (mainly myeloid) progenitors, and that the expansion is reversible depending upon drug administration. Nevertheless, the on-demand cell expansion would be beneficial in some clinical settings such as treating CGD patients in acute infection. In terms of safety, the drug-dependent, reversible expansion may be more desirable than constitutive growth stimulation, because it is less likely to induce secondary mutations in HSCs which may eventually lead to malignant transformation.



Emerging Strategies for High Level Expansion of Hematopoietic Stem Cells

R. Keith Humphries MD, PhD

Engineered overexpression of the homeobox transcription factor HOXB4 has emerged as a powerful stimulator of hematopoietic stem cell (HSC) expansion *in vitro* (>40-fold). In this talk I will review 1) recent findings revealing the advantage of ex-vivo HSC expansion in the non-myeloblated transplant setting; 2) development of novel Hox based molecules with even more potent HSC expansion activity; and 3) further advances in applying these strategies to human HSC.

Reduced conditioning regimens hold great interest for many therapies involving HSC transplants because of their minimal associated toxicity and hence broader applicability. However, this approach requires the transplantation of very high doses of HSCs to outcompete the large surviving endogenous HSC reservoir. For example, to achieve 20% chimerism in mice given 200 cGy requires the transplantation of 1.5×10^6 day-4 5-FU marrow cells (i.e., ~500 HSCs). Strikingly, HOXB4 transduced day-4 5-FU cells after expansion for 7 days in culture, could produce 30-50% chimerism in mice given 200-250 cGy starting from ~20-fold fewer cells (i.e., ~25-50 initial HSCs). High level chimerism is sustained (53, 35 and 14% in mice sacrificed 12, 19 and 19 months post-transplant) and is polyclonal in nature. HOXB4-transduced HSCs regenerated to near normal numbers (22,000) entirely from the initial transplant of HOXB4-transduced HSCs. These results highlight the potential of HOXB4 to expand HSC *in vitro* sufficient to achieve a high-level, permanent and polyclonal reconstitution of non-myeloablated recipients.

Novel variant Hox genes consisting of the N-terminus of NUP98, a gene that fuses with multiple partners in human AML, and the 2nd exon of specific Hox genes have shown even more potent HSC expansion activity. Notably an engineered fusion between NUP98 and the second exon of HOXA10 yields ex-vivo HSC expansion > 1,000 fold. Intriguingly, further deletion analyses show that the 60 amino acid homeodomain alone in a NUP98-fusion retains full HSC expansion capacity. These findings demonstrate the extreme potency of NUP98-HOX fusions as novel agents for HSC expansion, reveal the sufficient contribution of the DNA-binding homeodomain to achieve this effect and set the stage for the design of minimal Hox-based molecules for HSC expansion.

We previously reported an enhanced recovery (~4-fold) of HOXB4-transduced human cord blood cells with lympho-myeloid repopulating activity after 1-2 days of culture. More dramatic effects are now apparent after a more extended (10-day) period of culture. Highly enriched CD34⁺ cord blood cells were prestimulated for

3 days, exposed to MSCV-HOXB4/GFP and then maintained for another 5 days in serum-free cultures. Short and longterm repopulating activities (STRC and LTRC) before and after culture were quantitated by limiting dilution transplantation assays in sublethally irradiated NOD/SCID mice. Calculated outputs showed a > 10-fold difference between HOXB4-transduced and control LTRC yields. In the same experiments, the effect of HOXB4 overexpression on STRC output was even more pronounced (12 ± 5 -fold net increase over input, range = 4-27-fold vs up to a 3-fold reduction in control cultures). These findings underscore the promise of HOXB4 as an agent for stimulating a selective and important amplification in culture of human cells with rapid as well as sustained repopulating activities.

SS403: OLIGONUCLEOTIDE BASED THERAPIES: EMERGIING APPLICATIONS OF OLIGONUCLEOTIDE THERAPEUTICS

Targeted Gene Correction via Triplex Formation

Peter Glazer, MD, PhD

Triple helix-forming oligonucleotides (TFOs) can bind to polypurine/polypyrimidine regions in DNA in a sequence-specific manner. The resulting triplexes have been shown to constitute a DNA structure sufficiently abnormal to provoke binding by the damage recognition factors, XPA and RPA, and to stimulate DNA repair via the nucleotide excision repair (NER) pathway. Based on these observations, experiments were undertaken to determine the extent to which triplex structures can provoke recombination events within genomic DNA. Studies in which TFOs were targeted to episomal SV40 genomes in COS cells suggested that intermolecular triplexes could stimulate recombination between tandemly repeated reporter gene sequences in a pathway dependent on XPA and other NER factors. Experiments in mouse Ltk- cells containing a dual TK gene substrate demonstrated that intrachromosomal gene conversion could be provoked by high affinity triplex formation, at frequencies as high as 1-2%, if the TFOs were delivered into the cells by microinjection. Work in both COS cells using an episomal target and in human cell free extracts using plasmid substrates has further demonstrated that triplex formation can promote intermolecular recombination. TFOs were shown to induce recombination between a target gene and short DNA fragment, in cases where the fragment is either covalently linked to or is completely separate from the TFO. In the extracts, immunodepletion and complementation with purified proteins demonstrated a requirement for XPA and for the human recombinase, Rad51, in the reaction. *In vitro* studies also highlighted the potential utility of TFO analogs, such as peptide nucleic acids (PNAs), for inducing site-specific recombination. Recent work has also shown that TFOs can stimulate recombination and

gene correction at chromosomal sites in mammalian cells. We have also extended this work to demonstrate that TFOs administered systemically to mice via intraperitoneal injection can mediate gene targeting within the somatic tissues of the animals. Overall, this work raises the possibility that DNA binding ligands such as TFOs and related molecules may be useful in strategies designed to mediate genome modification and gene correction in site-specific manner.

SS404: VIRAL: VECTOR CELL INTERACTIONS

Adenovirus Cell Entry Mechanisms

Glen R. Nemerow, PhD

The mechanisms by which human adenovirus crosses the barrier of the host cell membrane remains unresolved despite the fact that Ad vectors are well known for their ability to accomplish this feat. In the studies to be presented here, we determined the relationship between Ad capsid disassembly and the generation of membrane lytic activity. Exposure to low pH or heating induced conformational changes in wild-type Ad but not in temperature-sensitive Ad (*ts1*) particles that lack the ability to escape the endosome. Wild-type Ad but not *ts1* particles permeabilized model membranes (liposomes) and facilitated the cytosolic delivery of a ribotoxin (a-sarcin). Alterations in wild-type Ad capsids were associated with the exposure of a pH-independent membrane lytic factor. Unexpectedly, this factor was identified as protein VI, a 22 kDa cement protein located beneath the peripentonal hexons in the viral capsid. Recombinant protein VI and preprotein VI, but not a deletion mutant lacking an N-terminal amphipathic α -helix, possessed membrane lytic activity similar to partially disassembled virions. These findings have prompted us to consider a revised model of Ad entry in which acidification of the endosome initiates viral capsid disassembly and subsequent exposure of a highly membranolytic capsid protein. Further biochemical and functional analyses are underway to test this model of Ad entry.

Targeting AAV Vectors

Nicholas Muzyczka, PhD

Our laboratory has focused on two approaches for changing the tropism of AAV vectors. The first is to use mutational analysis, cryo-electron microscopy and X-Ray crystallography to determine the structure and function of different regions of the AAV capsid. The second is to find regions of the AAV capsid that will accommodate the insertion of large peptide ligands. Progress in both of these areas will be discussed.

SS500: YOUNG INVESTIGATORS SYMPOSIUM

Gene Transfer into Peripheral Blood T Lymphocytes: Clinical Benefits and Safety Profile

Chiara Bonini, MD

Since the first gene therapy trials documented, in the early 90', that human peripheral blood lymphocytes can be efficiently transduced by retroviral vectors and infused to treat patients affected by severe combined immunodeficiencies, several gene transfer approaches aimed at correcting genetic defects or augmenting immune responses to microbes and tumors, were translated into clinical trials. As the field progressed, several insights on T cell biology were revealed and translated into new transduction protocols, with the purpose of increasing the overall therapeutic index of transduced T-cells. A general observation is that the transduction procedure associated to viral vectors invariably produces modifications of T-cells in terms of repertoire, maturation, cytokine secretion profile, effector functions and life-span. In an attempt of controlling transduced lymphocytes our group pioneered the retroviral mediated transfer of two genes: 1. The low affinity receptor for nerve growth factor, truncated of the intracytoplasmic domain ("LNGFR). "LNGFR is a non-immunogenic human surface marker that allows to avoid toxic and time-consuming drug selection to isolate transduced cells, resulting in preservation of T-cell function. 2. The thymidine kinase (TK) gene from Herpes Simplex virus. TK is the prototype of suicide genes, genes able to confer an inducible suicide phenotype to permit the *in vivo* selective elimination of transduced cells in case of unwanted effects. TK expression confers selective ganciclovir sensitivity to transduced cells. "LNGFR has been the first cell surface marker utilized in a gene therapy clinical trial. In a recent multicentric study, the safety of the "LNGFR cell marking molecule was supported by cumulative results on >900 animals transplanted with "LNGFR⁺ hematolymphopoietic cells.

T-cells transduced to express "LNGFR and TK (TK-cells) have been extensively used in the context of allogeneic hematopoietic stem cell transplantation (HSCT) performed to treat hematologic malignancies. In this context, TK-cells are utilized to promote immune-reconstitution, mediate anti-tumor activity, and selectively control graft-versus-host disease (GvHD). In 45 patients treated with donor TK-cells in the context of HSCT, we showed selective control of GvHD in 100% of cases, preservation of antiviral activity (in all patients with sustained TK-cell engraftment) and anti-tumor activity (in 65% of patients). The direct role of TK cells in mediating clinical events was documented by consistent expansion and long-term persistence of transduced cells,