to critically regulate the vasculature and mass of the pancreas. Here we examined the feasibility of using lentiviral vector-expressed human PEDF gene to treat human pancreatic tumor cell line-derived tumors in a murine model. Mono-cistronic FIV vectors encoding human PEDF or a control protein (eGFP) with each gene linked to neoR via an IRES, were used. PEDF protein produced from transduced cells and direct application of PEDF vector significantly reduced angiogenesis (63% inhibition compared to control) in a capillary formation assay using human umbilical vascular endothelial cells (HUVECs) and in a chick chorioallantoic membrane assay (14% angiogenesis index compared to control index of 64%). Enhanced HUVEC apoptosis was also observed. We examined the therapeutic efficacy of injected PEDF and control vectors on established human pancreatic tumor xenografts in nude mice. Significant anti-tumor effects and prolonged overall survival benefit was observed in PEDF vector-treated mice (> 80% tumor reduction after 1- 2 x 10^7 TU at day 30 post injection). PEDF vector-treated animals survived 52 ±2 days compared to 30±3 days with control vector, n = 16 in each group. Tumors treated with PEDF vector had enhanced apoptosis and vascular endothelial growth factor receptor-1 expression. We also studied tumor formation by stable pancreatic cell lines derived by transduction with PEDF and control vectors and G418 selection. PEDF secreted from these stable tumor cell lines was shown to be biologically active in the in vitro assays and a significant antitumor effect and prolonged overall survival benefit was seen. However, injection of vectors into established tumors had a better therapeutic effect than pre-transducing and selecting cells prior to establishing tumors, and this correlated with up-regulation of VEGFR-1 in the stable tumor cell lines. PEDF is a promising transgene for pancreatic cancer gene therapy.

810. Comparison of Anti-HIV Activity from Various Lentiviral Vectors Carrying Anti-HIV shRNA *In Vitro*

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Eight pHIV-7-GFP lentiviral vectors encoded anti-HIV-1 small hairpin RNA (shRNA) were constructed. Three target sites in the HIV genome were selected to construct the shRNAs. The first site (SI) resides in a region of *rev* that overlaps with the adjacent *tat* gene, while the second (SII) and third sites (SIII) are selected in conserved regions that reside exclusively in *rev* or *tat*. The expression and functionality were compared using snRNA Pol III U6 versus Pol II U1 promoters in the following constructs:

U1-S1: shRNA targets SI controlled by a U1 promoter

U1-S2: shRNA targets SII controlled by a U1 promoter

U6-S2: shRNA targets SII controlled by a U6 promoter

U1-3S: Three shRNAs individually target SI, SII and SIII controlled by one U1 promoter

U6-3S: Three shRNAs individually target SI, SII and SIII controlled by one U6 promoter driving from 5' to 3'

U6-3SF: Three shRNAs individually target SI, SII and SIII controlled by one U6 promoter driving from 3' to 5'

3U6-3S: Three shRNAs individually target SI, SII and SIII controlled by separate U6 promoters for each shRNA

U1-S2-U6-S2: Two shRNAs target SII controlled by either U1 or U6 promoters

Control: Lentiviral vector encoding GFP only

All vectors were transducted into CEM cells, sorted for eGFP positive cells, challenged with HIV-1 strains IIIB and NL4-3, observed for HIV RT activities in culture supernatant, and evaluated for anti-HIV activity by comparison with control.

All constructs demonstrated inhibition of HIV-1, but U1-S1 showed the most potent HIV-1 inhibition of both strains of virus with 2-3 log reduction of supernatant RT activity. In comparison, the other constructs produced 1-2 logs of inhibition. The comparison of U1-S2 vs U6-S2; three shRNAs controlled by either U1 or the U6 promoters (U1-3S vs U6-3S) and the single or multiple shRNAs controlled by the same promoter (U1-S2 vs U1-3S or U1-S2-U6-S2; and U6-S2 vs U6-3S or U6-3SF or 3U6-3S) all showed no further significant differences in inhibition. Subsequent investigation attributed this to lack of proper processing of the hairpins from the single promoter, whereas the three independent promoters showed toxicity when challenged with HIV-1. The three independent promoters were shown to be producing large amounts of the shRNAs, perhaps contributing to the toxicity. Thus, with shRNAs, an increase in RNAi does not necessarily contribute to an improved antiviral effect.

Current studies are investigating the differences in the Pol III and Pol II expression systems that might affect the efficacy of the shRNAs, including the possibility that the U1-transcribed shRNAs use different export pathways alleviating the competition for export with the endogenous microRNAs. Finally, these results demonstrate that the anti-HIV-1 *tat* and *rev* specific shRNAs expressed from U1 pol II promoter in lentiviral vectors provide an important potential advantage for AIDS gene therapy and will be used in primary cells for preclinical study necessary for further clinical application.

811. Correction of Laminin-5 β3 Chain Deficiency in Human Epidermal Stem Cells by Transcriptionally Targeted Lentiviral Vectors

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Mutations in any of the genes encoding the laminin 5 heterotrimer (α3, β3 and γ2) cause junctional epidermolysis bullosa (JEB), a severe and often fatal skin adhesion defect. We and others have shown that expression of a retrovirally transferred β3-chain cDNA in keratinocytes from affected patients reconstitutes normal synthesis, assembly and secretion of laminin 5, and corrects the adhesion defect in vitro and in vivo. We have recently started a phase-I clinical trial of gene therapy of JEB based on transplantation of cultured skin derived from autologous epidermal stem cells transduced with a MLV-derived retroviral vector. Since gamma-retroviral vectors have raised safety concerns for the genotoxic risk associated with the insertion of LTR elements into the human genome, we developed an alternative gene transfer strategy based on LTR-modified, HIV-derived lentiviral vectors. Two self-inactivating (SIN) lentiviral vectors were built, in which expression of either GFP or a LAMB3 cDNA is under the control of either a constitutive promoter (PGK) or the keratinocyte-specific, 2.2-kb promoter-enhancer of keratin 14 (K14). In a third construct, expression of the transgene is under the control of the viral LTR, modified by replacing the U3 region with two K14 enhancer elements. Analysis in human keratinocyte cultures and in full-thickness human skin equivalents reconstituted onto immunodeficient mice showed that GFP expression directed by the K14 elements is tissue-specific and restricted to the basal layer of the epidermis. Expression of laminin5 from the three alternative vectors was evaluated in keratinocyte cultures derived from skin biopsies of JEB patients. Biochemical and cell kinetics assays demonstrated transduction of epidermal clonogenic stem/progenitor cells and full phenotypic correction of JEB keratinocytes with all vectors. Southern blot analysis of

individual cell clones showed that LTR-modified lentiviral vectors are genetically stable and integrate in multiple copies in the human genome. This study shows that the use of lentiviral vectors transcriptionally targeted to the basal keratinocytes by the insertion of restricted enhancer elements is an effective, and potentially safer, alternative for gene therapy of JEB.

812. Cellular Delivery of Gene Therapy Vectors

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To achieve targeted, truly systemic therapeutic gene delivery to tumors, we exploited the observation that infection by a retrovirus proceeds initially through a non-specific adsorption stage prior to envelope-receptor recognition/internalization. Antigen specific, primary murine OT-1 T cells can be incubated *in vitro* with retroviral stocks which adhere to the cell surface. *In vitro*, virus can be handed off to target cells upon co-culture with the T cells, dependent upon cell contact, envelope/receptor interactions and local heparanase expression as provided by T cell activation upon encounter of antigen (ovalbumin) by the OT-1 T cells. Retroviral particles, pre-adsorbed to the surface of OT-1 T cells *in vitro*, were available for hand off *in vivo* to B16ova tumors distant from the site of T cell injection at high enough concentrations for therapeutic levels of gene transfer to occur. We have now delivered a variety of therapeutic genes to tumors, including HSVtk, IL-12 and the chemokine CCL-21. Pre-adsorption of vectors encoding each of these genes has led to therapeutic benefits in terms of direct local cell killing (HSVtk in combination with lymphodepletion), generation of potent immunizing activity (IL-12) and significant enhancement of the efficacy of subsequent doses of adoptively transferred, unmodified T cells (CCL-21).

We have also characterized a second pathway by which viral particles expressing no envelope (BALD particles) can be handed off from T cells dependent upon T cell activation. Release of viral particles from the T cell surface coincides with the penetration of perforin-containing cytotoxic vesicles from the antigen activated T cells. The endosomolytic properties of perforin promote release of these viral particles from the endosome and, in situations where direct T cell killing does not occur, the virus goes onto to generate a productive infection. Thus, T cell mediated delivery of MLV core particles can be used to deliver therapeutic retroviral vectors *in vivo* using targeting at the level of T cell activation, thereby dispensing with the need to target viral envelopes.

We have also investigated the possibilities of using antigen specific T cells to deliver replication competent viruses to tumors both *in vitro* and *in vivo*. In particular, we have demonstrated that replication competent Vesicular Stomatitis virus expressing GFP can be used to load OT-1 T cells. The virus infects these T cells only at very low levels and does not kill them. However, the T cells produce VSV particles at appreciable levels 48-72 hours post loading suggesting that they may be able to carry the viruses to tumors *in vivo* and lead to productive infection following intratumoral T cell accumulation.

Therefore, the protection, concentration, and targeted delivery of viruses by T cell carriers offers great potential for the delivery of vector stocks into the circulation of patients. By careful selection of the virus, and therapeutic gene that is used, significant synergy can be achieved over the use of virotherapy, or adoptive T cell therapy, alone.

813. Targeted Delivery of Biotinylated Compounds by Lodavin™, LDL-Receptor – Avidin Fusion Protein, Expressing Lentivirus

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Targeted drug delivery is a fascinating option to maximize the beneficial effects of the used drug molecule in the correct target tissue or to diminish the possible side effects of toxic drug compounds in erroneous tissues. Conventional approaches for targeting in gene therapy are vector pseudotyping and the use of tissue specific promoters. An alternative approach would be to modify the target cells/tissues so that they contain an artificial targeting receptor. For this aim our group has recently constructed Lodavin™, a fusion protein consisting of cytoplasmic and transmembrane domains of LDL receptor and avidin, a specific high affinity biotin-binding protein from chicken. The fact that humans and other mammals do not contain a counterpart for avidin makes Lodavin™ a promising artificial receptor for the targeted drug delivery. In addition, biotinylation of drug molecules is considerably easy. In the present study we constructed HIV-1 based Lodavin™ vector: Lodavin™-lenti. The Lodavin™-lenti transduced cells show stable expression of Lodavin™ on their cell membranes.

Lodavin™ expression was detected from transduced cells by western blotting and confocal microscopy. Lodavin™-lenti was studied *in vivo* by direct injection of mouse subcutaneous tumors wherefrom it was detected by immuno-histochemistry.

Our results demonstrate that Lodavin™-lenti presents a viable alternative for the targeted drug delivery. It drives a long term expression of the Lodavin™ on the membranes of transduced cells, which allows development of several *ex vivo* and *in vivo* targeting approaches. Besides targeted drug delivery, imaging of the transduced cells with Lodavin™-lenti should be possible from intact animals using several non-invasive methods like SPECT and MRI.

814. Biomaterial-Mediated Retroviral Gene Delivery

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Introduction Inefficient and uncontrolled gene delivery has hampered the widespread efficacy of gene therapy. Biomaterial-mediated gene transfer represents a promising strategy to address these limitations by immobilizing the gene carrier onto a biocompatible substrate. This approach permits control of gene transfer by co-localizing cell adhesion and the gene delivery vehicle. These interactions must be carefully balanced to adequately immobilize the gene carrier to the biomaterial but also permit cellular uptake. Self-assembled monolayers (SAMs) of alkanethiols on gold are well-characterized biomaterials used to regulate adsorption of proteins and cell adhesion. We hypothesized that functionally-terminated SAMs presenting diverse surface chemistries can be used to control retroviral-mediated gene transfer to cells seeded onto these materials.

Methods Tissue culture plastic (TC) was sequentially coated with titanium and gold by electron beam evaporation before overnight incubation with the indicated alkanethiol (CH_3, COOH, or NH_2).