AMERICAN SOCIETY of GENE & CELL THERAPY

Late Breaking Abstracts

Addendum

Late Breaking Abstracts: Presented at the American Society of Gene & Cell Therapy's 16th Annual Meeting, May 15-18, 2013, Salt Lake City, Utah

The following abstracts were late breaking abstract submissions that are being presented at the American Society of Gene & Cell Therapy's 16th Annual Meeting in Salt Lake City, Utah. These abstracts are scheduled in one Oral Abstract Session and three Poster Sessions as noted below.

Thursday, May 16, 2013

Oral Abstract Session 230

2:00 pm – 4:00 pm

Room: Ballroom A

Clinical Gene & Cell Therapy

676. Update on Toca 511 - A Retroviral Replicating Vector in Clinical Trials for High Grade Glioma

Douglas J Jolly[10], Joan M. Robbins[10], Derek Ostertag[10], Amy Lin[10], Carlos Ibanez[10], Harry E. Gruber[10], Noriyuki Kasahara[1], Timothy F. Cloughesy[2*], Manish K. Aghi[3*], Susan M. Chang[3*], Michael A. Vogelbaum[4*], Santosh Kesari[5*], Tom Mikkelsen[6*], Joseph Landolfi[7*], E. Antonio Chiocca[8*^], James Elder[8*], Greg Foltz[9*], Dan Pertschuk[10]

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Retroviral replicating vectors (RRV) based on a Moloney murine simple retrovirus with an amphotropic envelope have a marked native preference for replication in tumors in vivo in immunocompetent animal models (Wang et al Neurosurg Focus 20 (4):E25, 2006; Ostertag et al. Neuro-oncology 14:145-149,2012) The tumor-selectivity is due to their intrinsic inability to infect quiescent post-mitotic cells and, presumptively, their susceptibility to innate antiviral defences that exist in normal cellular environments but are attenuated in many cancers. Toca 511 (vocimagene amiretrorepvec), is based on an improved RRV platform that utilizes a modified virus backbone for delivery of an optimized yeast cytosine deaminase (CD) gene. The CD enzyme converts the prodrug 5-fluorocytosine (5-FC) into the cytotoxic drug, 5-fluorouracil (5-FU) directly within the infected cancer cells. Toca 511 is currently being investigated in 'first-in-human' Phase I investigational clinical trials for patients with recurrent high-grade glioma (rHGG) (www.clinicaltrials.gov: NCT01156584 and NCT01470794) in combination with Toca FC (an extended-release formulation of 5-FC). In these clinical studies, Toca 511 is administered by stereotactic injection under real-time MRI guidance or by multiple injections into the walls of the resection cavity at the time of resection. To date, Toca 511 has been administered to 44 patients at ascending vector doses and has been well-tolerated. Proof of mechanism and preliminary evidence of therapeutic benefit has been observed in patients with rHGG, including symptomatic improvement, radiographic evidence of tumor stabilization or shrinkage, and pathological evidence of tumor necrosis. Toca 511 proteins, genes, including CD, have been detected in resected tumors, even after multiple courses of Toca FC. Potentially therapeutic concentrations of the anti-cancer agent 5-FU have also been detected in tumor. These encouraging data support continued investigation of Toca 511 and Toca FC in patients with rHGG and potentially other indications.

Poster Session I
4:00 pm – 6:00 pm
Room: Exhibit Hall C/D
Late Breaking Abstracts I

677. RNA-guided Genome Editing Using CRISPR-Cas Systems

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The ability to introduce targeted modifications into genomes and engineer model organisms holds enormous promise for biomedical and biotechnological applications. The development of tools such as zinc fingers, transcription activator-like effectors, and homing meganucleases has greatly facilitated efficient and precise editing of genomes. However, these approaches still require engineering of proteins de novo for each target, and there remains a deficit for technologies that are easily customizable, multiplexable, and affordable. To achieve this, we have adapted RNA-guided nucleases from the Streptococcus pyogenes SF370 and S. thermophilus LMD-9 bacterial adaptive immune systems to mediate multiplexed genome editing in mammalian cells.

In the type II CRISPR (clustered regularly interspaced short palindromic repeats) system, a pair of non-coding RNAs – crRNA and tracrRNA – directs the Cas9 nuclease to introduce double stranded breaks (DSBs) at loci complementary to the crRNA sequence. Through heterologous expression of these three components in mouse and human cells, we show that Cas9 can be programmed by custom RNAs to induce DSB at endogenous mammalian loci with up to 59% cutting efficiency.
Cas9 can be further converted into a nicking enzyme to facilitate template-directed homologous recombination while minimizing mutagenic indel formations. Finally, using a single crRNA array to encode a pair of guide sequences, we show that CRISPR can be used to direct simultaneous efficient cleavage of multiple sites within the human genome. The tractability and multiplex capability of this system presents unique possibilities for practical and therapeutic applications.

678. Rescue of T-cell deficiency in Prkdc SCID mice by transplantation of gene-repaired haematopoietic stem cells

Hayder H Abdul-Razak [1], Céline Rocca [1], Steven J Howe [2], María Eugenia Alonso-Ferrero [2], Richard Gabriel [3], Cynthia C Bartholomae [3], Chih Hao V Gan [2], Marina I Garín [4], Francisco Javier Molina Estévez [1,4], Alison Roberts [5], Michael Blundell [2], Guillermo Güenechea [4], Jianbin Wang [6], Michael C Holmes [6], Philip D Gregory [6], Christine Kinnon [2], Christof von Kalle [3], Manfred Schmidt [3], Juan Antonio Bueren [4], Adrian J Thrasher [2] and Rafael J Yáñez-Muñoz [1]

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The classical severe combined immunodeficiency (SCID) mouse is a model of human DNA-dependent protein kinase catalytic subunit (DNA-PKcs, PRKDC) deficiency. In this disease model, corrected cells are expected to have a selective advantage, a likely ideal scenario to assess the feasibility of therapeutic gene repair. We have developed an ex vivo system to correct Prkdc SCID by such genome surgery. We have produced donor templates to correct the mutation, a zinc finger nuclease (ZFN) that targets Prkdc close to the SCID mutation, and incorporated ZFN genes into integration-deficient lentiviral vectors (IDLVs) or standard integrating lentivectors, and donor templates into IDLVs. Using these tools we have demonstrated specific ZFN cutting in SCID fibroblasts and haematopoietic progenitors using a Cel-I assay

and deep sequencing. We have unequivocally observed ZFN-mediated repair of the SCID mutation via the incorporation of a diagnostic restriction site from the donor template into the targeted locus, alongside the corrected nucleotide. In fibroblasts, we have shown rescue of DNA-PKcs activity and increased resistance to DNA damage upon gene correction. In haematopoietic progenitors we have demonstrated in vitro gene correction in short-term cell culture through the incorporation of the diagnostic restriction site. Upon primary transplantation of gene-corrected SCID HSCs into sublethally irradiated SCID mice, we have observed double-positive CD4/CD8 cells in the thymus, and single-positive CD3, CD4 and CD8 cells in peripheral blood. Correction of the SCID mutation and concurrent incorporation of the diagnostic restriction site have been confirmed by deep sequencing of thymic DNA. Upon secondary transplantation (currently a year from the beginning of the primary transplant) we have observed single-positive CD3, CD4 and CD8 cells in blood. Our observations suggest that we have been able to correct the T-cell deficiency of Prkdc SCID mice by transplantation of ex vivo gene corrected SCID HSCs, indicating that gene repair-based rescue of SCID disease is a feasible approach.

Acknowledgments: The authors acknowledge financial support from the 7th EU Framework Programme (PERSIST project, grant agreement no. 222878) and the Primary Immunodeficiency Association.

679. A microRNA-based system for selective gene expression in tumor-associated macrophages after adenovirus mediated gene transfer

Michelle Giovani [1]; Kamola Saydaminova [1]; Hongjie Wang [1]; Jonas Persson [1]; Hua Cao [1]; Erini Papapetrou [2]; Il-Kyu Choi [3]; Chae-Ok Yun [3]; Andre Lieber [1]

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All of the current treatment options for cancer patients are focused on destroying the malignant cells. These genetically unstable and heterogeneous cells are a moving target for therapies. The tumor stroma contains genetically stable tumor-infiltrating myeloid cells, of which tumor-associated macrophages (TAMs) are the dominant cell type. TAMs are phenotypically distinct subsets of macrophages and critically contribute to tumor growth, angiogenesis, immunosuppression, and resistance to chemotherapy. Due to their functional significance, TAMs act as an “Achilles’ heel” that can be used for cancer therapy. Our long-term goal is to develop approaches to specifically kill TAMs. In studies of human cancer biopsies, we found that TAMs are positive for desmoglein 2 (DSG2). Furthermore, ~30% of human peripheral blood mononuclear cells (PBMC) and ~100% of human hematopoietic (CD34+) stem cells are positive for DSG2. Recently, we generated human DSG2 transgenic mice with a DSG2 expression pattern similar to humans. We also reported that DSG2 is used as receptor for a number of human adenoviruses (Ad3, Ad7, Ad11, Ad14) and fiber-chimeric vectors (for example Ad5/11). In our studies, after intravenous injection of Ad5/11 vectors into human DSG2 transgenic mice with syngeneic huDSG2-high tumors, transgene expression was seen in TAMs, but also in ~10% of PBMC. To restrict transgene expression to TAMs, we used a microRNA-regulated gene expression system. To generate such a system, we isolated (F4/80+) macrophages from mouse bone marrow, PBMCs, and tumors and delineated their microRNA expression profile using microarrays with >1,000 known microRNAs. We selected a microRNA with a high expression level in bone marrow and PBMC and a low level in TAMs (mmu-miR-106a). As a negative control, we chose a microRNA that was high in TAMs (mmu-miR-210). We then inserted 4 target sites with 100% homology to the selected microRNAs into the 3’UTR of a GFP gene, which was under the control of the ubiquitously active EF1alpha promoter. The GFP expression cassettes were inserted into Ad5/11 vectors. The vectors also contained a PGK promoter-driven mCherry expression cassette that was not regulated by the selected microRNAs. We demonstrated in vitro and in vivo, after intravenous injection of Ad5/11 vectors into huDSG2 transgenic mice that, based on mCherry expression, the efficacy of transduction of PBMC and TAMs was comparable for the Ad5/11-miR-106a and Ad5/11-miR-210 vectors. In contrast, GFP expression from Ad5/11-miR106 was high in TAMs but absent in PBMCs. In situ, TAMs are localized in the tumor stroma and are therefore only poorly accessible to intravenously injected Ads. To address this problem, we injected into tumor-bearing mice a tumor-cell transducing Ad vector expressing relaxin two

days before Ad5/11-miR-106 injection. Intratumoral relaxin expression resulted in transient degradation or downregulation of tumor stroma proteins. This significantly increased the percentage of transduced GFP-positive TAMs in mice injected with Ad5/11-miR106. We are currently generating Ad5/11 vectors expressing suicide genes under miR106 regulation.

680. Overcoming problems in the generation of adenovirus vectors for genome editing in human hematopoietic stem cells

Kamola Saydaminova [1]; Xun Ye [2]; Hongjie Wang [1]; Akseli Hemminki [3]; Eirini Papapetrou [4]; Andre Lieber [1]

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Replication-deficient, E1/E3-deleted Ad5/35 vectors allow for efficient gene transfer into human hematopoietic CD34+ stem cells (HSCs) with transgene expression in the majority of cells within 2 days after infection. However, because of leaky viral gene expression, transduced CD34+ cells either die or lose the ability to differentiate in semisolid cultures or engraft in NOG mice. These problems can be circumvented by the use of helper-dependent (HD) Ad5/35 vectors that lack all viral genes. Our goal is to employ HD-Ad5/35 for the transient expression of Zinc-Finger Nucleases (ZFNs) or Transcription Activator-Like Effector Nucleases (TALENs) in order to achieve genome editing or tansgene addition in HSCs. While ZFNs and TALENs preferentially cleave genomic DNA in selected target sites, off-target cleavage has also been reported. In the past all, all of our attempts to produced HD-Ad5/35-ZFN vectors failed due to cytotoxic effects of ZFNs in adenovirus producer 293-Cre cells. To address this problem, we used a micro-RNA-regulated gene expression system that would suppress transgene expression from HD-Ad5/35 vectors in 293-Cre cells, but allow it in human CD34+ cells. Using microarrays, we established the miRNA expression profiles in HD-Ad5/35-infected 293-Cre and CD34+ cells. We selected two miRNAs (has-miR183-5p and has-miR218-5p), which had the highest expression levels in 293-Cre cells and the lowest expression levels in CD34+ cells. We then inserted 4 target sites with 100% homology to the selected miRNA into the 3'UTR of a GFP gene, which was under the control of an EF1alpha promoter. The GFP expression cassettes were inserted into a first-generation Ad5/35 vectors. The vectors also contained a PGK promoter-driven mCherry expression cassette that was not regulated by the selected microRNAs. A control vector did not contain miR183-5p and miR218-5p target sites (Ad5/35-control). We then transduced human CD34+ cells and 293-Cre cells with the vectors at an MOI of 50pfu/cell and analyzed GFP and mCherry expression 48 hours later by flow cytometry. Both GFP and mCherry expression were comparably high in CD34+ cells for Ad5/35-miR183-5p, Ad5/35-miR218-5p, and Ad5/35 control vectors. In contrast, in 293-Cre cells, GFP expression from the Ad5/35-miR183-5p and Ad5/35-miR218-5p vectors was at the background level of non-transduced cells, while transduction with the Ad5/35-control vector resulted in efficient GFP expression. mCherry expression levels in 293-Cre cells were high for all three vectors. The efficient miRNA-mediated suppression of transgene expression in 293-Cre cells is remarkable considering that first-generation Ad5/35 vectors replicate in these cells. We are currently testing the miR183-5p- and miR218-5p-based systems in combination with ZFN and TALEN in the context of HD-Ad5/35 vectors. The miRNA systems have implications for the generation of Ad vectors expressing proteins with genotoxic or cytotoxic activities.

681. Scalable contaminant-free production of adenoassociated virus vectors utilizing cytoplasmic helper carrier

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Scalable and efficient production of high-quality recombinant adeno-associated virus (rAAV) for gene therapy remains a challenge despite recent clinical successes. We developed a new strategy for scalable and efficient rAAV production by sequestering the AAV helper genes and the rAAV vector DNA in two different sub-cellular compartments, made possible by utilizing cytoplasmic vaccinia virus as a carrier for the AAV helper genes. For the first time, the contamination of replication-competent particles (rcAAV) can be completely eliminated by avoiding ubiquitous non-homologous recombination. Vector DNA can be integrated into the host genomes or delivered by a nuclear targeting vector such as adenovirus. In suspension HeLa cells, the achieved vector yield per cell is similar to that from traditional triple-plasmid transfection method. The rcAAV contamination was undetectable at the limit of our assay. Using this novel system, canine factor VIII was successfully expressed in HA mice using a dual chain strategy. Furthermore, this new concept can be used not only for production of rAAV, but also for other DNA vectors.

682. Abstract Withdrawn from Presentation

683. Pre-T cell receptor for improved expansion of T cells in the absence of TCR alpha

Roman Galetto, Céline Lebuhotel, Laurent Poirot, Cécile Schiffer Mannioui, Julianne Smith and Andrew Scharenberg

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Recent data have emerged from adoptive T-cell therapies where exogenous expression of a chimeric antigen receptor (CAR) has been shown to confer cancer recognition on autologous T cells. However, the ability to apply this technology in an allogeneic setting would permit the generation of universal “off the shelf” T cells that would overcome many current technical and logistic hurdles to the practical application of adoptive immunotherapies. Transcription Activator-Like Effector Nucleases (TALENTM) can be used to inactivate the T cell receptor (TCR) alpha gene, eliminating the TCR and thus the potential of graft versus host disease (GVHD), one of the major hurdles towards an allogeneic approach. However, TCR disruption also results in the elimination of the CD3 signaling complex from the T-cell surface, and thus may alter the cells’ capacity for expansion and/or survival.

The pre-T cell receptor (pre-TCR) is expressed by immature thymocytes and is crucial for T cell development. Pre-TCR consists of the invariant pre-T alpha chain, variable rearranged TCR beta chains and CD3 signaling components. In contrast to the TCR, that requires interaction with peptide-loaded major histocompatibility complexes to initiate T cell signaling, the pre-TCR is thought to signal through a ligand-independent mechanism that occludes TCR surfaces required for MHC interaction. Here we demonstrate that the expression of the invariant pre-T alpha chain, in the absence of TCR alpha, results in the restoration of CD3 at the cell surface in association with a pre-TCR. Cells with preTCR/CD3 complexes have an improved life span, and can be expanded ex vivo through standard CD3/CD28-based bead methods. Application of this technology in association with allogeneic CAR modified T cells will also be presented.

684. An inducible Caspase-9 delivery-system using Mesenchymal Stromal Cells for lung cancer treatment

Miki Ando*, Valentina Hoyos*, Shigeki Yagyu, Carlos Ramos, Antonio Di Stasi, Gianpietro Dotti, Lisa Bouchier-Hayes and Malcolm Brenner

*Contributed equally to the work.

Center for Cell and Gene Therapy. Baylor College of Medicine. Department of Medicine and Pediatrics

We have previously shown that an inducible version of caspase-9 (iC9) can be used as a suicide gene for adoptively transferred T cells. Our objective is to adapt iC9 therapy to induce apoptosis of tumor cells in

vivo using mesenchymal stromal cells (MSCs) as vehicles. MSCs are promising carriers of gene therapy for lung cancer given their tendency to accumulate in lung microvasculature after infusion and their homing towards tumor sites. MSCs were transduced with a retroviral vector encoding the Adenovirus (Ad) gene E1A, which is required for active Ad replication and missing from our vectors. E1A expression was verified by immunofluorescence staining and seen in 20-60% of MSC (n=6). To test the ability of these cells to produce Ad vectors that can infect tumor cells, we cocultured lung cancer cell line H1299 and E1A-MSC transduced with Ad encoding GFP (Ad.GFP). Fluorescent microscopy video showed that within 24-48hr, E1A-MSC die due to the cytopathic effect of Ad replication and that H1299 cells are infected with Ad.GFP. E1A-MSC were then infected with an Ad carrying the iC9 gene and truncated CD19 as a marker (iC9-E1A-MSC). 5 days later, supernatant from MSCs was transferred to H1299 cells. CD19-positivity of H1299 was 56% (E1A-MSC) vs 6% (non-E1A MSC). Next, AP20187 (CID) 200nM was added to the H1299 cells and this treatment induced 74% apoptosis of CD19+ cells measured by AnnexinV-7AAD FACS. Other lung cancer cell lines, however, were partially resistant to iC9 induced death (25-40% versus 70%-80% in sensitive cells). The sensitivity to iC9 was independent of the level of transgene expression. To overcome this resistance, we combined iC9-CID therapy with the Proteasome Inhibitor, Bortezomib (Bor) 80nM. This combination therapy increased apoptosis of resistant cell lines to 70-80%. The combination index of resistant cell line A549 was 2.05e-011 (ED90), indicating potent synergy. To understand the mechanism of this effect, we added the pan-caspase inhibitor qVD to the culture. In presence of qVD the apoptotic effect of CID, Bor and combination therapy was completely inhibited, indicating that the mechanism of action is caspase dependent. Western Blot analysis showed the combination treatment increased cleaved caspase 9 and caspase 3, suggesting accumulation of these proteins induced by Bor is responsible for the increased apoptosis. We next tested this effect using our MSC delivery-system to deliver Ad-iC9 to resistant A549 cells. CID alone induced only 43% apoptosis but the combination of both induced 82% apoptosis. Finally, SCID-Beige mice were injected IV with FFLuc labeled A549 cells (n=5/group). Mice were then treated with iC9-E1A-MSC IV weekly x 2. The mice that received CID and Bor consistently showed better tumor control (2.13E+9 signal change) compared to mice receiving Bor (1.28E+10) or CID alone (7.03E+9) (p=0.0003). In summary, MSCs can be used as carriers of iC9 suicide gene therapy for the treatment of lung cancer and sensitivity to this therapy can be increased by concomitant Bor treatment.

685. Human Erythropoietin Gene Delivery for Cardiac Remodeling of Myocardial Infarction in Rats

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Background—Considerable efforts have been made to exploit cardioprotective drugs and gene delivery systems for myocardial infarction. The promising cardioprotective effects of recombinant human erythropoietin (rHuEPO) protein in animal experiments have not been consistently reproduced in clinical human trials of acute myocardial infarction; however, the molecular mechanisms underlying the inconsistent discrepancies are not yet fully understood. We hypothesized that the plasmid human erythropoietin gene (phEPO) delivered by our bioreducible polymer might produce a cardioprotective effect on post-infarct cardiac remodeling through the suppression of angiotensin II and TGF-β. Methods and Results—We demonstrated that intramyocardial delivery of phEPO by an arginine-grafted poly(cystaminebisacrylamide-diaminohexane) (ABP) polymer in infarcted rats preserves cardiac geometry and systolic function. The reduced infarct size due to phEPO/ABP delivery was followed by a decrease in fibrosis, protection from cardiomyocyte loss, and down-regulation of apoptotic activity. In addition, the increased angiogenesis and decreased myofibroblast density in the border zone of the infarct support the beneficial effects of phEPO/ABP administration. Furthermore, phEPO/ABP delivery induced prominent

suppression on Ang II and TGF-β activity in the border zone of the infarct, interventricular septum, right ventricle, and atria. Conclusions—phEPO gene therapy delivered by a bioreducible ABP polymer for acute myocardial infarction protected against the expansion of the infarct and functional impairment, thereby attenuating adverse cardiac remodeling. These results provide insight into the lack of phEPO gene therapy translation in the treatment of heart disease to human trials.

686. MGN1703 – structure of agonist determines cellular responses to TLR9 activation

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Introduction: DNA-based TLR9 agonists are potent activators of immune cell populations and the immune system. MGN1703 is a covalently closed dumbbell-like DNA molecule consisting entirely of natural DNA with two single-stranded CG-containing loops separated by a double-stranded stem (dSLIM®). In a recent phase II clinical IMPACT trial MGN1703 as maintenance treatment of patients with metastatic colorectal carcinoma improved progression-free survival compared to placebo. Doses of 60 mg of MGN1703, twice a week for several months, were very well tolerated and safe. The archetypes of TLR9 agonists - e.g. ProMune® - are instead single-stranded DNA oligonucleotides containing CG-motifs (CpG-ODN) and usually are modified by phosphorothioates (PTO) to enhance stability. However, ProMune® failed in previous advanced oncology trials, most likely due to narrow therapeutic windows. Even at doses lower than 10x those used with MGN1703, PTO-protected CpG-ODN caused toxicities. Here we compare MGN1703 to ProMune® with respect to cytokine secretion patterns and activation of immune cells. We also explore structure-function relationship through exchange and comparison of the CG-motif environments of ProMune® and MGN1703. Results: In human peripheral blood mononuclear cells (PBMC) MGN1703 showed a superior cytokine secretion and cellular activation pattern: Compared to ProMune®, MGN1703 evoked a 82-fold secretion of IFN-alpha, the central anti-cancer cytokine; a 69-fold better secretion of IP-10, a potent angiostatic factor; and a 2-fold higher stimulation of IFN-gamma, the key activator of NK-, NKT-, and cytotoxic T-cell responses. MGN1703 stimulation leads to better activation of monocytes, for the processing of tumor-associated antigens; and NK / NKT cells as anti-tumor effector cells of innate immunity. However, ProMune® leads to 4.7-fold higher secretion of IL-8, what may promote tumor growth through angiogenesis, and to a 2-3-fold better maturation of plasmacytoid dendritic cells (pDC) and activation of B-cells. We could ascribe these striking biological differences to the unique structure of the MGN1703: If the single stranded loops of MGN1703 were converted into a PTO-protected CpG-ODN (LMLS-PTO), both the cytokine and activation patterns in almost all aspects shifted to those of ProMune®. Additionally, if the CG-motifs in LMLS-PTO were changed into GC, a TLR9 neutral motif, the secretion of IL-8, maturation pDC and activation of B-cells remained more-or-less identical to the LMLS-PTO and ProMune®. Conclusions: The superior clinical efficacy and toxicity profiles of MGN1703 are most likely due to its covalently closed dumbbell-shaped conformation and lack of PTO-modification. Systematic comparison of MGN1703 with the single-stranded, PTO-modified ProMune® in human PBMC cultures shows that – besides CG-motifs – also the structure of the agonist determines it`s function in TLR9-dependent immune cell activation. However, MGN1703, representing a new class of TLR9 agonists in principle, may likely be detected also by other cellular DNA-sensors.

687. Abstract Withdrawn from Presentation

688. Mini-LCC DNA Vector: The Gold Standard of Gene Delivery in Molecular Medicine

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Background: Plasmid DNA (pDNA) vectors are the fundamental of modern molecular medicine involving in all forms of gene transfer approaches in human cells including mal/non functional gene replacement, DNA vaccination, and production of therapeutic proteins. The conventional pDNA vector suffers from several safety and efficiency limitations: 1) it imparts adverse immune responses to bacterial sequences required for maintenance and amplification in prokaryotes; 2) its bioavailability can be compromised due to size; 3) it may be genotoxic due to its potential to integrate into the host chromosome and yield an oncogenic event. We have constructed an in vivo platform for the production of mini linear DNA vectors with covalently closed ends (mini LCC DNA) that are devoid of unwanted bacterial sequences, encoding only the gene(s) of interest and necessary complementary eukaryotic expression/enhancement genetic elements. Results: Transfection of mini LCC DNA vectors encoding the enhanced Green Fluorescent Protein gene (eGFP) into rapidly dividing and slow dividing human cells resulted in significantly higher transfection efficiency, bioavailability, and cytoplasmic kinetics compared to the parental plasmid precursor and isogenic covalently closed â€œminicircle DNAâ€ counterparts. As we previously noted in prokaryotes, we showed that integration of LCC DNA into the human host genome results in chromosomal disruption and subsequent apoptotic elimination of potentially oncogenic vector integrants from the cell population, thus improving the safety profile of mini LCC DNA vectors. Conclusion: Mini LCC DNA vectors are the gold standard in gene delivery that significantly improve the bioavailability, immunocompatibility, safety, and efficiency obstacles encountered by conventional pDNA and other circular DNA vectors.

689. Overcoming physical barriers in oncolytic virotherapy using a serotype 3-based oncolytic adenovirus expressing relaxin

Joung-Woo Choi[1], Il-Kyu Choi[2], Andre Lieber[3], and Chae-Ok Yun[2]

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The potency of oncolytic virus used to treat solid tumors is limited by uneven penetration and distribution of viruses within tumor due to both tumor extracellular matrix (ECM) and intercellular junctions between epithelial cancer cells. With the aim of enhancing viral spreading and penetration in vivo, we have generated a serotype 3-based oncolytic adenovirus (Ad) expressing relaxin which replicates under the control of hybrid promoter of hTERT and E2F promoter (hTE-Ad3/RLX). In both pancreatic cancer cells in vitro and established pancreatic tumors in vivo, junction opening efficacy of hTE-Ad3 was greatly improved than that of untreated group, demonstrating that Ad3 can induce the intercellular junction opening by binding to desmoglein 2. Infection with hTE-Ad3/RLX also substantially reduced the ECM components (collagen, fibronectin, and elastin) of tumor spheroids as well as established pancreatic tumors tissues in vivo compared with hTE-Ad3-treated tissue tissues, showing that expression of relaxin can degrade ECM components. Furthermore, intratumoral administration of hTE-Ad3/RLX elicited strong antitumor effects and increased survival in pancreatic cancer xenograft model that create a complex tumor ECM, in comparison to control hTE-Ad3. Consistent with these results, more abundant Ad particles and necrosis were detected across wider areas of tumor tissues treated with hTE-Ad3/RLX. Taken together, these data indicate that a serotype 3-based oncolytic Ad expressing relaxin exhibits a high potential in overcoming physical barriers in oncolytic virotherapy.

690. Design of oncolytic HSV1 that express cellular gene moieties that improve antiglioma efficacy and normal cell safety

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For clinical use of oncolytic HSV-1 (oHSV1), safety and therapeutic efficacy are essential. We have been employing an engineered oHSV1 (rQNestin34.5, Kambara et al. , Cancer Res., 2005) where the nestin promoter, highly expressed in glioblastoma stem-like cells (GSCs), drives expression of the viral ICP34.5

gene to enhance viral replication in GSCs with less toxicity to normal human cells. The c-terminus moiety of the viral ICP34.5 gene is necessary to initiate protein translation through dephosphorylation of the translation factor eIF2alpha during viral infection but other moieties of ICP34other moieties of ICP34.5 lead to neurovirulence in the brain because they bind to beclin-1, leading to autophagy. To circumvent the possibility of neurovirulence by low-level expression of ICP34.5, we have now engineered a novel oHSV1 vector, in which the nestin-promoter drives the cellular GADD34 (NG34) or a truncated GADD34 gene (NG34C). The rationale for this is that the C terminus of GADD34 dephosphorylates eiF2alpha, like ICP34.5, but does not produce neurovirulence since it does not possess the beclin-1 binding moiety. We found that those ΔICP34.5 mutant HSV1 viruses, NG34 and NG34C are as efficacious as rQNestin34.5 against a panel of glioma cell lines and primary GBM cells. However, normal human primary cells did not support NG34C replication. We are currently evaluating neurovirulency of NG34 and NG34C in mouse brains. In summary, this newest generation of oHSV1s utilizes moieties from cellular genes that mimic those of viral genes required for efficient replication and lysis of gliomas without expressing viral gene moieties that are toxic to normal cells.

691. Polymeric Carriers for the Delivery of Neurotrophins to the Brain

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Neurotrophins like brain-derived neurotrophic factor (BDNF) will sustain neuronal cell survival, facilitate synaptic function and improve the overall neuroplasticity upon brain injury. Lack of effective carriers limits delivery of BDNF across the blood-brain barrier. Polyelectrolyte complexes of plasmid DNA (pDNA) and cationic block copolymers have been widely investigated as an effective strategy to deliver nucleic acid drugs. We have identified 2 polymer candidates (poly(ethylene glycol)x-block-polyaspartamide with diethylenetriaminey side chains (PEG5k-b-DET50) and poly(2-methyl-2-oxazoline)n-block-poly[2-(methylamino)methyl-2-oxazoline]m; P(MeOx50-b-MAOx20)) to evaluate their suitability to deliver BDNF pDNA to the mouse brain. We will determine the effect of the structure of the non-ionic block (PEG vs. POx) on serum protein binding properties of the resulting pDNA polyplexes which is expected to be a critical determinant of its in vivo disposition. In vitro cytotoxicity and transfection activity of selected pDNA polyplexes will be studied using an immortalized human brain microvessel endothelial cell model. Safe and efficient formulations of pDNA polyplexes will be advanced to in vivo transfection studies in a mouse. Results of the above studies will be presented.

692. A Lentiviral System for Analysis of Signaling Pathways Involved in Reprogramming of Induced Pluripotent Stem Cells

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Induced pluripotent stem (iPS) cells are generated by reprogramming fully-differentiated adult cells into pluripotent, embryonic-like stem cells via the overexpression of combinations of factors, such as the four "Yamanaka factors," Oct4, Sox2, Klf4, and c-Myc. As the resulting cells have the capacity to form any cell type in the adult body, they have immense biomedical potential. In particular, patient-derived iPS cells can be used for cell-replacement therapies or for in vitro models of human disease.

Fully reprogramming cells to an embryonic state, however, is relatively inefficient, and most effective methods still involve the use of integrating viruses harboring oncogenes such as Klf4, c-Myc, and potentially Oct4. Basic advances in understanding cellular mechanisms that are involved during reprogramming could improve the overall efficiency or reduce the need for overexpression of oncogenes.

To date the field has largely focused its mechanistic investigation on nuclear factors and epigenetic effects in reprogramming, and the potential roles of cellular signaling networks are not well known. To study the impact of key signal transduction pathways on iPS cell reprogramming, we created a lentiviral vector library encoding 38 constitutively-active and dominant-negative variants of proteins involved in canonical signaling pathways. Murine embryonic fibroblast (MEF) infection with Oct4, Sox2, Klf4, and c-Myc – as well as a lentiviral vector encoding one signaling protein – enabled analysis of whether individual signaling pathways exerted an effect on iPS cell reprogramming. High-throughput imaging was used to determine reprogramming efficiency by measuring both colony area and colony number. To further investigate the importance of signaling effectors on reprogramming, we used vectors containing three of the four transcription factors to determine whether Oct4, Sox2, or Klf4 could be replaced with a signal transduction protein. This work has revealed a signal transduction protein and a small molecule that can replace Oct4 in iPS cell reprogramming of mouse embryonic fibroblasts. The resulting colonies and cell lines expressed pluripotency markers and differentiated into three germ layers through in vitro differentiation. Additionally, the small molecule was able to replace Oct4 with similar reprogramming efficiency (0.71% of cells reprogram compared to 1.11% with Oct4 overexpression).

This approach can identify signaling pathways that can be harnessed, with gene delivery or small molecular perturbation, to enable efficient approaches to reprogram cells to pluripotency. Additionally, this lentiviral signal transducer library is not limited to stem cell reprogramming but has broader uses in understanding the roles of key pathways in mammalian cell and stem cell behavior.

693. Pluronic block copolymers for gene delivery

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Introduction: Pluronic block copolymers or poloxamers are recognized pharmaceutical excipients listed in british and US pharmacopoeia and have been used extensively for the delivery of low molecular mass drugs and polypeptides. These non-ionic block copolymers have recently gained interest for in vivo non-viral gene delivery. When co-delivered with plasmid DNA, Pluronics increase transgene expression in local (injected muscle) and distal tissues (draining lymph node and spleen) of healthy mice. We studied Pluronic formulated DNA delivery to and through antigen presenting cells in healthy and inflammation animal models.

Methodology: Distal inflammation (unilateral hind limb ischemia, peritonitis) and DNA injection were simultaneously performed at separate sites of same mouse. Distal inflammation was induced by excision of femoral artery or i.p. injection of 1mg CGN/200ul PBS. Local inflammation was induced by injecting relatively high concentrations of Pluronics (1%, 3%, 10% wt/wt) before doing DNA/DNA+P85 injections. In vitro DNA uptake studies were performed by exposing cells to YoYo1-DNA alone or Pluronic/YoYo1-DNA mix for 2hrs. Similarly for in vitro gene expression studies, cells were exposed to DNA alone or Pluronic/DNA mix for 2-4 hours. Coculture experiments were performed by culturing 50,000 RAW 264.7 cells/well with 50,000 C2C12 myoblasts or C2C12 derived myotubes in 96 well plate.

Results and Conclusion: Pluronic mediated increase in transgene expression (healthy animals) further increased upon inducing distal and local inflammation only in mice injected with Pluronic formulated DNA and not in DNA alone. Our in vitro studies with macrophages, myoblasts and myotubes showed that non cytotoxic Pluronic concentrations increased plasmid DNA uptake and gene expression in a dose dependent manner. Moreover, gene expression further increased upon coculture of transfected macrophages with myoblasts/myotubes in presence of pluronics. Bottleneck of plasmid DNA gene therapy is the limited transfection efficiency and we propose to increase it by co-delivering block copolymer (Pluronics) with plasmid DNA.

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694. Gold nanoparticles assisted laser optoporation of cells: a novel physical method for efficient, safe and versatile gene delivery

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Context The choice of an appropriate gene delivery method is often a determinant factor for successful gene therapy. Although many gene delivery methods have demonstrated effectiveness in some pre-clinical and clinical studies, their further applications in other contexts are often constrained by their intrinsic limitations, such as confined cargo size, cytotoxicity, lack of specificity and efficiency, etc. Indeed, these limitations impeded the development of new therapeutic options for gene and molecular therapy, and many potential gene therapy solutions were undermined due to the lack of an efficient, safe and versatile gene delivery technique. Methodology We present a novel gene delivery technique that could surmount the obstacles encountered with some existing gene delivery methods. We use an ultrafast laser to irradiate biocompatible gold nanoparticles (AuNPs) dispersed on cell membrane. Upon irradiation, these AuNPs locally amplify the laser energy and create transient pores on the cell membrane, allowing the penetration of exogenous genes into the cells by fluid exchange. This AuNPs assisted optoporation technique presents several advantages. First, the technique is safe: the laser beam is emitted at an extremely weak energy level and is harmless to the cells. The transient membrane disruption is only made by light-triggered FDA-approved AuNPs at submicron scale. Second, it is highly efficient: thousands of cells can be perforated simultaneously in one second with the help of AuNPs. Third, AuNPs can be functionalized to target specific cell populations, avoiding undesired cell transfection and opening the door for more versatile in vitro and in vivo applications. Results Our studies on melanoma cells showed that AuNPs assisted laser optoporation allowed high cell viability (>99%) and high perforation rate (70~80%). The transfection rate was about 23% using plasmids expressing fluorescence, and was about three times higher than the standard transfection method with lipofectamine. We also tested the method on other cell lines such as breast cancer cells and neurons. Preliminary results indicated similar performance in these cell lines. Conclusion Until now, all our in vitro studies suggested remarkable potential of the AuNPs assisted laser optoporation as a novel physical method enabling efficient, safe and versatile gene delivery in a variety of cells or tissues. We believe it would be an appropriate tool for the development and improvement of new gene therapies for some important diseases such as cancers or neural degenerative diseases.

695. A potential therapeutic role for survivin in mitigating the harmful effects of ionizing radiation

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Survivin, often only thought to aid in tumorgenesis, can be used to benefit patients with malignancies requiring the use of radiation therapy. Elevated levels of surviving have been shown to coincide with the

growth and proliferation of cancer cells in humans. The role of survivin in decreasing the cell's susceptibility to apoptosis is thought to help malignant cells avoid the body's natural defense against mutations that would normally result in programmed cell death via apoptosis. Nonmalignant human tissue is void of survivin, however, with gene modification therapy, it is now possible to confer the same ability to inhibit apoptosis to healthy tissue that is already seen in malignant cells. The therapeutic potential of survivin is vast, particularly when it comes to treating the adverse effects of radiation therapy where unwanted apoptosis of healthy cells is a common side effect. Radiation therapy is a common form of adjuvant care used in many oncological treatment protocols. Ionizing radiation can be very effective in disrupting the growth of any remaining malignant cells following surgical excision; however nonmalignant neighboring cells are inevitably affected as well. Radiation exposure in the range associated with oncological therapy has been shown to cause both acute and chronic damage to healthy, human cells. Most acute effects stem from DNA damage, increased inflammation, and cell apoptosis that compromise the body's ability to heal. By selectively decreasing the radiation induced apoptosis of healthy cells, the harmful effects can be mitigated while the desired destruction of malignant cells can still be achieved. To this end, C57Bl/6 mice were injected intramuscular with either rAAV.Survivin or rAAV.YFP to an area on the leg that was then irradiated with 50 Gy total over the course of 10 days. No significant differences were apparent between the YFP and survivin groups throughout the 10 day radiation protocol. Following the last day of radiation both groups showed erythema and desquamation, however, by day 14 the YFP group had experienced significant ulceration of the irradiated area while survivin treated mice showed only slight hair loss. Moist, open wounds were present on the YFP group up through the six-week time period after radiation therapy, whereas the survivin treated mice never showed any significant ulceration of the skin. Histological analysis showed that at eight weeks post irradiation, following the acute response phase, YFP treated mice showed a significant thickening of the dermal tissue as well as an increase in collagen that was not present in the survivin treated mice. Molecular analysis shows that YFP mice had an increase in several fibrotic markers as well as the apoptotic marker p53. Gait analysis showed that both mice performed at a similar level prior to the radiation protocol, however, at four weeks post irradiation there was a significant difference in the instant run speed and overall run speed between the two groups, with YFP mice averaging a significantly lower speed in both cases. Therefore, our data suggests that tissues gene modified to express survivin show the potential to overcome many of the negative side effects normally associated with ionizing radiation and could be seen as a potential agent for increasing the effectiveness of many oncological treatment protocols.

696. HIV Latency: Activating Virus Expression with Combined Genetic and Chemical Tools

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One difficulty in curing HIV/AIDS is the tendency of the virus to enter into latency, where minimal or no viral gene expression occurs. The sequestered virus thus is immune to chemotherapy. One way to subject the virus to therapy again would be to activate viral gene expression. Latency likely involves block both of transcriptional initiation and transcript elongation. We have tested this idea by combination genetic and chemical approaches. We used a cell culture model of latency where lymphocytic Jurkat cells harbor latent provirus [env-/GFP+]. We subjected these cells to treatment, singly and in combination, with prototype agents that induce transcriptional initiation, [NFkB activation with prostratin (5 uM)]; agents of chromatin remodeling [hydroxamic acid (SAHA) (5uM)]; and agents that cause promoter demethylation [Aza-deoxycytidine (AzaCdR) (1 uM)], along with deblockers of elongation by transactivation with Tat-1 by way of lentiviral vector transduction (50 ul). The results (see Table 1) support the idea that latent virus expression can be activated and it is advantageous to activate both transcriptional initiation and elongation.

The opinion expressed in this abstract are those of the authors and do not necessarily represent views of the National Cancer Institute.

Table 1. Reactivation of HIV in latently infected cells by NFkB induction, tat transactivation,histone deacetylation, and promoter demethylation

Agent	% GFP+ cells	Agent	% GFP+ cells	Agent	% GFP+ cells
None	0.3 ± 0.2	None	0.8 ± 0.6	None	1.2 ± 2.0
Prostratin	7.1 ± 2.1	Prostratin	8.4 ± 7.6	Prostratin	7.8 ± 6.0
Tat-1 vector	0.6 ±0.2	Tat-1 vector	1.0 ± 0.8	Prostratin	10.0 ± 5.7
Prost.+Tat-1	34.2 ±7.2	Saha+Prost	28.6 ±15.1	Tat-1(vector)	1.2 ± 0.9
Saha+Tat-1	12.0 ± 2.7	AzaCdR+Prost.	31.8 ± 10.1		
Saha+Prost. +Tat-1	40.0 ± 8.2	AzaCdR+Tat-1	11.2 ± 9.7		

697. Sensitisation of neuroblastoma cells for TRAIL-induced apoptosis by downregulated XIAP

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Neuroblastoma (NB) is the most common solid cancer in childhood and recently the usage of stem cells brings a new aspect for the effective therapy. The stem cells are present in almost every tissue and are useful vehicle for cancer therapy since they can be loaded with antitumor agents. Stem cells have the potential of migrating to tumor tissues.A new therapeutic strategy has been developed that uses mesenchymal stem cells (MSC) for the targeted delivery.

Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) which is a member of the tumor necrosis factor superfamily, is a type 2 transmembrane protein that causes apoptosis of target cells through extrinsic pathway. TRAIL can selectively induce apoptosis in tumorogenic or transformed cells, but not in normal cells. Studies have shown that a number of cancer cells are resistant to TRAIL. In this direction, TRAIL can be inhibited by X-linked inhibitor of apoptosis protein (XIAP).

In this study, human mesenchymal stem cells (hMSCs) were isolated from human adipose tissue and characterized. The TRAIL gene vector were transfected to hMSCs and shXIAP plasmid were transfected to SK-N-AS cells (which is an aggressive NB cell line) by Lipofectamine 2000 reagent and Amaxa Nuclefactor 4D. A GFP transfected group and fibroblast cells were used as control. These genetically modified hMSCs and fibroblasts were co-cultured with SK-N-AS cells for 24 hours. Thereafter cell survival of neuroblastoma cells were determined with flow cytometry analysis. For the evaluation of the NF-κB, MEK1, JNK 1/3 and Akt 1/2/3 signal pathways related to SK-N-AS cells by AlphaScreen Technology (Perkin Elmer).

The results showed that hMSCs induced apoptosis of the NB cells. Also, TRAIL secreting hMSCs increased apoptosis in tumor cells as compared to control cells and TRAIL secreting fibroblast cells. In this study, we observed TRAIL resistance in SK-N-AS cells. Inhibition of XIAP by shRNA significantly enhanced TRAIL-induced apoptosis in tumor cells.

NFκB plays a key role in regulating the cellular activity. Observed that in control cells, I-κB level was the highest level. And this level was decreased significantly in tumor cells co-cultured with hMSCs. Moreover, in neuroblastoma cells which inhibiton of XIAP by shRNA co-cultured with TRAIL plasmid transfected hMSCs was the lowest level of phosphorilated I-κB level. Observed that the MEK1, JNK 1/3 and Akt 1/2/3 levels were inversely correlated with the apoptotic cell number. All groups were significant versus control group.

In conclution, we have shown that genetically modified hMSCs secreting TRAIL reduce the cell survival of cancer cells by inducing apoptosis and inhibiting the proliferation. By demonstrated that XIAP inhibition

sensitizes neuroblastoma cells for TRAIL-induced apoptosis and the signal pathways as NF-κB and others plays a critical role in this process.

Use of the MSCs as "tumor cell killing ligand carrying vehicles like a Trojan horse" is provides a novel treatment option for metastatic tumors. We believe that this therapy as delivery vehicles of therapeutic genes will be of great interest for the clinical application of stem cell based cancer therapy.

698. Abstract Withdrawn from Presentation

699. Culture and cryopreservation of human epidermal melanocytes (MC) for preclinical trials

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One of the most effective and safe therapeutic methods for treating vitiligo, mixed keratinocytes and melanocytes culture have been used for autologous cell transplantation. However, this method have problem that may require taking an amount of skin tissue, moreover keratinocytes have limited culture potency. However, the adipose-derived stem cell (ASC) was easly obtained and cultured in vitro from small amount of aspirated fat tissue. The present study was examined for in-vitro effect of ASC on in vitro expension of MC and optimal condition of cryopreservation. MC was co-cultured with ASCs with or without growth factors for improves in vitro expansion, and also frozen-thawed with or without several non-permiable cryoprotactants. The growth rate of MC colony co-culture with ASC was 6.85 folds elevated compared with MC only group. And the MC was expressed stem cell marker(c-Kit and CD133) and immature melanocyte protein (Trp-2). Twenty percent FBS was more protective effect than SSS on MC cryopreservation, and morphological and immunophenotypical changes were not observed in this group. As a result, ASCs could be used in place of keratinocytes for MC culture and growth. With further culture and animal studies, co-culture of MC with ASC could be applied to the vitiligo patients.

700. Universal Stem Cell Gene Therapy Platform: Upgrading the Long-Term Gene Therapy Arm by shifting from Analogic to Breakthrough Digital Endonuclease- Boosted Gene Targeting and Focussing on Next Generation Ex Vivo Protocols

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As anticipated, the discovery of the unique property of bacterial Clustered Regularly Interspaced Short

Palindromic Repeats (CRISPR)-associated protein Cas9 to act as a programmable dual-RNA-guided DNA endonuclease in vitro (Doudna, Charpentier & co-workers, 2012) has been readily converted into a breakthrough gene targeting tool for human cells (Church, Zhang, Doudna & co-workers, 2013). The target DNA sequence recognition of such a site-specific endonuclease is mediated by a short guide RNA, thereby substituting digital Watson-Crick base-pairing for analogic protein-DNA binding of well-established chimeric zinc-finger (ZF)/transcription activator-like effector (TALE) nucleases. Indeed, chimeric zinc-finger nucleases (ZFNs) and emerging TALE nucleases (TALENs) are the very drives of endonuclease-boosted gene targeting, a key feature of the long-term gene therapy arm of our proposed Universal Stem Cell Gene Therapy Platform (Bertolotti, 2006). The strategic location of a double-strand break (DSB) into target genomic DNA is essential to the gene targeting efficiency and was up-to-now depending on the DNA-binding specificity of custom assembly of ZF or TALE proteic modules. Although simple in principle, modular assembly of ZF units is fairly complex and was the bottleneck of our endonuclease-boosted gene targeting approach (Bertolotti, 2006). Although this former bottleneck of custom site-specific DNA endonuclease genesis has been recently eliminated by cost- and time-effective FLASH assembly of TALENs (Joung and co-workers, 2012), digital RNA-guidance is simpler and eventually

more efficient than analogic protein-DNA binding, and stands thus as an ultimate upgrade of the gene targeting facet of our proposed universal platform. This upgrade is discussed 1) in light of the target specificity of current human-codon-optimized Cas protein (14 to 16 bp) and derived nickase mutant, thereby focussing on true gene targeting efficiency and off-target genotoxicity, and 2) in terms of gene repair/genomic editing for genetic cardiomyopathies and other inherited diseases using next generation ex vivo stem cell gene therapy (Bertolotti, 2013) relying on the genesis of hazard-free patient-specific iPS cells and combining it to transient local regenerative gene therapy and injectable tissue/organ-specific extracellular matrix (ECM) hydrogel carriers.

Friday, May 17, 2013

Poster Session II

5:30 pm – 7:30 pm

Room: Exhibit Hall C/D

Late Breaking Abstracts II

701. Abstract Withdrawn from Presentation

702. Preclinical study of a scAAV2/8-Lp1-huPPCA vector in the treatment of the galactosialidosis mouse model

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Galactosialidosis (GS) is a lysosomal storage disease caused by a primary defect of the protective protein/cathepsin A (PPCA) and severe secondary deficiency of the sialidase NEU1. Phenotypic changes include widespread vacuolization of cells in most systemic organs and excretion of sialyloligosaccharides in the urine. These features are closely recapitulated in Ppca-/- mice, a model of GS. Treatment for GS is currently not available. We have initiated a series of preclinical therapeutic studies in Ppca-/- mice, using a scAAV2/8-Lp1-huPPCA vector with restricted expression of the transgene in the liver. In the initial dose finding studies, we demonstrated that injection of 4-week-old mice with a dose of rAAV as low as 1x10e11 vg/kg corrected extensively their systemic phenotype. In the current studies we intended to identify potential differences in the treatment efficacy between mice injected at 4 weeks or 12 weeks of age with a single dose of vector. In addition, we wanted to precisely determine the minimal dose of rAAV that would no longer afford correction of the phenotype.

For the time of injection study, two cohorts of 8 Ppca-/- mice (4 males and 4 females, age 4 and 12 weeks) were injected with a dose of 1x10e11 vg/kg and sacrificed 12 weeks later. Histological examination indicated that the mice treated at 4 weeks had complete reversal of the disease phenotype in all the tissues examined, whereas mice treated at 12 weeks showed less efficient clearance of storage in some cells of the visceral organs, but particularly the testis and the epididymis This correlated with the sialic acid contents in the urine and kidney of both cohorts of treated mice. Urinary sialic acid levels were normalized as early as 25 days after injection in mice treated at 4 weeks of age. Cathepsin A and Neu1 enzyme activities were higher in the liver, spleen and kidney of the mice treated at 4 weeks than those treated at 12 weeks. Mice injected at 4 weeks did not have any neutralizing antibodies against huPPCA, but some were detected in the mice injected at 12 weeks, which explained why the levels of huPPCA were reduced in the mice treated at older age.

For the minimal dose study, 6 groups of mice (3 male mice/group) were treated at 4 weeks of age with 6 different doses respectively, i.e. 3.3 x10e10, 6.8 x10e10, 1.4 x10e11, 2.7 x10e11, 5.4 x10e11, 1x10e12

vg/kg. Histological examination showed that the mice treated with the highest doses achieved full correction in all tissues. However, in mice injected even with the lowest dose of 3.3 x10e10 vg/kg storage material was still cleared efficiently in all tissues except the epididymis. The huPPCA levels varied with the doses and correlated well with the sialic acid content in urine and kidneys.

In conclusions, a dose of rAAV as low as 3.3 x10e10/vg/kg can still revert the pathology in most affected organs and early treatment affords an overall better reversal of the disease phenotype. These studies strongly support the use of this therapeutic approach for the treatment of non-neuropathic GS patients. (This work was supported in part by the NIH grant (DK52025), the Assisi Foundation of Memphis and ALSAC).

703. Improved Retroviral Replicating Vectors for Prodrug-Activator Gene Therapy of Cancer

Noriyuki Kasahara[1], Christopher Logg[1], Omar Perez[2], Oscar Diago[2], Ryan Burnett[2], Aki Inagaki[1], Amy Lin[2], Cindy Burrascano[2], Mitchel Berger[3], Krystof Bankiewicz[3], Weijun Wang[4], Hyun Kim[5], Walter Wolf[5], Thomas Chen[4], Kenneth Cornetta[6], Carlos Ibañez[2], Joan Robbins[2], Harry Gruber[2], Douglas J. Jolly[2].

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Retroviral replicating vectors (RRV) can deliver therapeutic genes throughout a tumor mass by preferentially infecting and spreading through dividing cancer cells, without causing cell lysis. In previous reports, an earlier-generation RRV encoding wild-type yeast cytosine deaminase (CD), followed by 5-FC treatment, led to significantly increased animal survival compared to controls in intracranial glioma models. We have now developed Toca 511 (vocimagene amiretrorepvec), based on an improved RRV platform that utilizes a modified virus backbone for delivery of a codon-optimized heat stabilized yeast cytosine deaminase (CD) gene. The CD enzyme converts the prodrug 5-FC (5-fluorocytosine) to the anticancer drug 5-FU (5-fluorouracil) in the infected cancer cells. Toca 511 was confirmed to spread through glioblastoma cells with high efficiency and expressed high levels of CD, exhibiting ~3-fold higher levels of prodrug conversion to 5-FU in vitro compared to wild-type, rapid prodrug conversion in vivo, and enhanced anti-tumor efficacy in multiple animal models. Additional improvements to this unique vector technology are currently being pursued under an NINDS-funded multi-institutional U01 program, including (1) development of improved cGMP manufacturing processes and characterization of diffusion-adjusted titers, replicative stability, transgene expression levels, prodrug conversion activity, in vitro cytotoxicity, in vivo tumor transduction efficiency, biodistribution, and therapeutic efficacy of concentrated clinical-grade vector lots, (2) convection-enhanced delivery methods to improve initial delivery of the virus to intracranial tumors via stereotactic injection, and (3) ^{19}F-NMR spectroscopic methods for non-invasive imaging to monitor conversion of 5-FC to 5-FU in vivo. Toca 511 is currently in Phase I investigational clinical trials for patients with recurrent high-grade glioma (rHGG) (www.clinicaltrials.gov: NCT01156584 and NCT01470794) in combination with Toca FC (an extended-release formulation of 5-FC).

704. The recombinant tight junction opener JO-1* decreases hypoxia in tumors

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Hypoxia or oxygen deprivation is a key factor in tumor progression and resistance to therapy due to its effect on various metabolic, molecular-genetic, pathophysiologic adaptive processes including neoangiogenesis and activation of immunosuppressive T cells. The most important regulatory factor of the hypoxia-signaling pathway activity in cells is hypoxia-inducible transcription factor 1 (HIF-1).

We have recently developed a small recombinant protein (JO-1*) that binds to desmoglein 2 (DSG2) and triggers the activation of pathways that are reminiscent of an epithelial-to-mesenchymal transition (EMT), including the phosphorylation of MAP kinases and the downregulation of epithelial junction proteins. In epithelial tumors, JO-1 mediated the transient opening of intercellular tight junctions thus increasing the intratumoral penetration and efficacy of anti-cancer drugs.

Here we report that intravenous injection of JO-1* into mice with tumors >600mm3 decreases intratumoral HIF-1-dependent transgene expression. We generated cancer cells that expressed luciferase under the control of a HIF-1-responsive promoter. This was achieved by transducing lung cancer (A549) cells and primary ovarian cancer (ovc316) cells with a hypoxia/luciferase reporter lentivirus vector. Clones of transduced cells were analyzed for luciferase expression with and without chemically (with CoCl2) induced hypoxia. Two clones with the highest induction factor were selected for in vivo studies. Mice with A549 tumors were subjected to non-invasive in vivo imaging for luciferase expression. With tumor growth lucerifase signals (normalized to tumor volume) increased, suggesting hypoxia. When tumors reached a volume of 600mm3, mice were intravenously injected with JO-1*. Images were taken before JO-1* injection (0 hours) and 1, 3, and 6 hours after JO-1* injection. JO-1* significantly decreased normalized luciferase signals. Furthermore, there was no change in luciferase signals upon JO-1* injection into mice with tumors that expressed luciferase under a promoter that was not controlled by HIF-1. Future studies will include measuring intratumoral oxygen concentrations using polarographic oxygen electrodes.

The finding that JO-1* decreases hypoxia is relevant for cancer radiotherapy and immunotherapy which is often inefficient in hypoxic environments. It will also give us a means to monitor the effect of JO-1* in cancer patients by non-invasive imaging of glucose metabolism. We are currently testing the hypothesis whether JO-1* can enhance radiotherapy.

705. Development of a Clinical AAV-Based Vector Encoding a Microdystrophin Transgene for the Treatment of Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is a recessively-inherited muscle wasting disorder caused by mutations within the dystrophin gene. Gene therapy using adeno-associated viral (AAV) vectors to deliver dystrophin expression cassettes remains a promising treatment option that would be applicable to all patients regardless of their underlying genetic mutation. We have previously shown that highly truncated dystrophins can significantly ameliorate the dystrophic phenotype in small and large animal models of DMD following delivery with recombinant AAV vectors. Studies in mdx and mdx:utrn-/- mice indicated that while several versions of microdystrophin, including a highly functional hinge-2 containing protein (H2μDys), could dramatically improve the dystrophic phenotype, they did not fully restore normal muscle physiology. Comparisons of functional alterations in microdystrophin structure have resulted in improved microproteins, one of which involved substitution of hinge 2 (H2) with hinge 3 (H3). Banks et al., observed that a H2μDys caused ringbinden in a variety of muscles from mdx mice, whereas a H3μDys prevented muscle degeneration while minimizing structural and mechanical abnormalities. To further characterize the therapeutic potential of a H3μDys, we have performed comprehensive studies in a broad range of muscle types including the diaphragm and heart. We systemically injected 2-week-old mdx mice with rAAV vectors expressing a hinge 3 microdystrophin. Eight months later injected mice exhibited a marked reduction in serum creatine kinase levels and improved histopathology, consistent with a whole-body reduction in muscle degeneration. In all muscles analyzed, the dystrophin-positive myofibers displayed a dramatic reduction in central-nucleation. When compared with untreated mdx mice, there was a significant increase in the specific force-producing capacity in the EDL muscles of treated mdx mice, reaching ~75% of wild-type levels, as well as a marked increase in resistance to eccentric contraction-induced injury. This human H3μDys expression cassette was further improved by incorporating an

optimized muscle-specific gene regulatory element and a codon optimized microdystrophin cDNA. The data suggest that the optimized microdystrophin cassette is promising for use in human clinical trials of gene therapy for DMD.

706. Novel therapeutic nanoparticles for in vivo delivery of low dose siRNA in liver cells and for the treatment of liver fibrosis associated nonalcoholic steatohepatitis

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siRNA is poised to be the next therapeutic drug. Potent siRNA can silence any gene, including non-druggable genes, at picomolar concentration. As a result, there is now a great deal of interest in using siRNA *in vivo* to better understand diseases but also to be use as a therapeutic molecule. The goal of this study was to develop new in vivo delivery nanoparticles to deliver siRNA in liver cells by screening a library of lipid based formulations. ***Method***s : An siRNA targeting FactorVII (FVII) was complexed with each formulation and injected intravenously at a 1mg/kg to 0.0125 mg/kg doses. Bid 3nd protein silencing was evaluated 48 hours after injection. The nanoparticles resulting in initial FVII knockdown were further optimized by design of experiment (mixture DOE) and evaluated for their ability to deliver other type of RNAi molecules. For NASH study, plasma and liver tissue were collected for determination of NASH features by histopathology, cell death assessment including TUNEL assay and immunoblotting using mitochondrial fractions. Hepatic stellate cell (HSC) activation was determined by real time PCR and liver fibrosis quantitated by image analysis of Sirius-red stained sections. ***Results:*** After a single intravenous injection of FVII siRNAs (0.05mg/kg) complexed with this new reagent (formulation 401), we observed more than 90% mRNA and protein level reduction in liver cells for more than 2 weeks and this silencing was dose dependent with an ED50 < 0.02mg/kg. We also observed a reduction of Cholesterol and LDL after silencing the APOB gene with this reagent. In addition, by mixing the siRNAs together, we were able to knockdown at least 4 genes at the same time after a single injection.

Finally we showed that we can use these formulations to deliver an siRNA against a key pro-apoptotic gene (Bid 3) for treatment of fibrosis in NASH mice model. C57BL/6 mice were placed on choline-deficient L-amino acid defined (CDAA) diet for NASH mice model. After 19 weeks of CDAA diet, mice with severe fibrotic-NASH, were injected with Bid 3 siRNA/Formulation 401, weekly for three weeks at1.5 mg/kg (week1) and 0.15 mg/kg (week 2 and 3). At the end of the treatment, Bid 3 mRNA was suppressed to 50% ($p<0.003$) and Bid 3 protein was reduced to 10% ($p<0.002$). In Mice treated with Bid 3 siRNA, liver fibrosis was improved as assessed by Sirius red quantitation and mRNA expression of fibrosis genes such as TIMP-1 ($p<0.03$) or CTGF ($p<0.05$). These changes were associated with marked reduction on TUNEL-positive cells and reduction on mitochondrial BAX.

Conclusion: We have identified novel therapeutic lipid nanoparticles for the delivery of siRNA with an ED50 of less than 0.02mg/kg. This study also demonstrates that these formulations with a pro-apoptotic gene (Bid 3) siRNA can be used to improve liver fibrosis associated with experimental NASH Gene. These findings are consistent with evidence that apoptosis triggers HSC activation and liver fibrosis and suggest that Bid 3 inhibition may be useful as an antifibrotic NASH therapy.

707. Directed evolution of artificial viruses

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It is long known that viruses can selectively deliver their genetic payload to cells. This has spawned a huge number of investigations into the use of viruses as vectors for gene therapy. Even though some marginal

successes have been achieved, a lot of hurdles still have to be overcome before gene therapy can reach its full potential. In the past researchers have tried to tackle these problems through the rational design of such carriers; an approach that as of now has not been very successful. Instead of rationally altering the viral coat protein to achieve changes in e.g. their tropism, we used high-throughput combinatorial techniques to create a very large library (>10^9 clones) of random mutants. By applying selective pressure on this library, only those clones which possess desired properties remain. This way, the process resembles natural evolution, with the exception that we can choose what to select for.

We give a proof of principle for this method using polyomavirus VP1 virus-like particles (VLPs) as model delivery system. VLPs are aggregates of viral structural proteins. They resemble the native viral capsid in structure, tropism and transduction efficiency, but do not contain any viral genetic material. One class of promising VLPs is those derived from polyomaviruses. The polyomavirus coat protein VP1 is one of the three structural proteins of the virus. After overexpression, VP1 proteins self-assemble to form VLPs. These VLPs are able to encapsidate double-stranded DNA in a sequence independent fashion and transfect mammalian cells, show good immune evasion, and have a high insert capacity for peptides. This makes them a good starting point for directed evolution.

To combine the beneficial properties of different polyomaviruses, we recombined the VP1 DNA sequences of different polyomavirus species through a technique called DNA shuffling to form novel polyomavirus VP1 hybrids. A vast library of hybrid VP1 genes was created, with an average of 5.7 crossovers per VP1 gene. These libraries are expressed in eukaryotic 293TT cells while supplying SV40 VP2/3 and large T antigen in trans. From these cells hybrid VLPs are purified. These VLPs have been subjected to selections based on packaging and transfection. As a proof of principle we demonstrate an enrichment of the wild-type VP1 gene from an excess of non-functional mutants, showing the potential of this system.

708. The NanoAssemblr™ Platform: Microfluidics-based Manufacture of Limit Size Lipid Nanoparticles for Nucleic Acid Delivery

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The NanoAssemblr™ Instrument is a novel, scalable microfluidics-based system for the development and manufacture of nanoparticles. Nucleic acid nanoparticles can be developed as therapeutics, or used as research tools for functional genomics and drug/biomarker target validation studies. Traditional methods for producing nanoparticles are inconsistent, difficult to scale, and labor-intensive. The NanoAssemblr™ uses custom engineered microfluidic chips to enable millisecond mixing of nanoliter reaction volumes containing nanoparticle components. This well-controlled process mediates bottom-up self-assembly of nanoparticles with process and formulation dependent characteristics, including limit-sized lipid nanoparticles (lsLNP), the smallest stable structure possible for a lipid nanoparticle (LNP). Rapid mixing of nanoparticle components with nucleic acids provides precise control over nanoparticle formation and size, resulting in nucleic acid solid-core nanoparticles ranging in diameter from 15-200 nm with low polydispersity (PDI < 0.1). Size is an important determinant of nanoparticle biodistribution and behavior in vivo. siRNA-LNP generated using the NanoAssemblr™ exhibit “solid core” morphology when visualized by cryo-TEM[1]. The size of these highly potent siRNA-LNP can be controlled by the NanoAssemblr™ using process and/or formulation parameters[2,3]. For example, the limit size of the siRNA-LNP is dependent on the percentage of stabilizing PEG-lipid in the formulation. 1% & 5% PEG-lipid produced 55nm and 28nm particles respectively (PDI < 0.05; encapsulation efficiency > 95%). Individual NanoAssemblr™ microfluidic mixers are capable of producing LNP at 0.5-20 mL/min. Seamless scale-up is achieved by parallelization of microfluidic mixers. Parallelization ensures that large scale reaction conditions remain identical to single

mixer devices. Pilot studies have demonstrated an n-fold increase in LNP output by arraying n mixers in parallel, with no differences in LNP characteristics (eg. Size) with scale. The NanoAssemblr™ Reagents are used with the NanoAssemblr™ instrument to prepare siRNA nanoparticles that reproducibly silence genes in vitro and in vivo. The NanoAssemblr™ Liver Reagents allow for gene silencing in hepatocytes in vivo resulting in > 95% reduction in protein levels in the blood at 24 hours (<0.3 mg/kg siRNA) and > 80% reduction after 14 days (<1 mg/kg siRNA). The NanoAssemblr™ Neuron Reagents mediate uptake into 100% of primary neurons and enable > 80% reduction in target protein levels at concentrations ≤ 0.8 μg siRNA/mL.

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709. Preclinical development of an AAV vector for the treatment of X-linked retinitis pigmentosa due to RPGRorf15 mutation

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Mutations in retinitis pigmentosa GTPase regulator (RPGR) account for >70% of X-linked retinitis pigmentosa (RP) and approximately 15% of all RP cases. These mutations cause a progressive blinding disease which typically begins with night blindness in the first decade and results in central vision loss by the forth decade. The disease afflicts approximately 10K people in the United States and 220K people worldwide. To develop a clinical candidate, we evaluated dose-efficacy profiles of AAV vectors expressing mouse and human RPGRorf15 in the RPGR knock-out mouse model. This model recapitulates the major features of the human disease over a 20 month frame including loss of rod and cone photoreceptors, mislocalized cone opsins, and reductions in electroretinogram (EGR) amplitudes. Homologous and heterologous RPGRorf15 transgenes were evaluated to determine the effect of species differences on bioresponse in the mouse model. All of the vectors employed a 295 bp human rhodopsin kinase promoter, a CMV/beta-globin intron and the human beta-globin polyadenylation site. The mouse RPGRorf15 vectors were packaged in AAV8 or AAV9 capsids and were administered by subretinal injection at 6 weeks or 12 months of age. The human RPGRorf15 vector used an AAV8 capsid and was administered at 6 weeks of age. The animals were evaluated at multiple time points by ERG and optical coherence tomography (OCT) and histopathology was performed at 20-24 months of age. The AAV8 mouse RPGRorf15 vector showed a bell-shaped dose-response curve with increased photoreceptor layer thickness and statistically significant scotopic and photopic ERG improvement at the 3e8vg/eye dose, but not at 1e8 or 1e9 vg/eye doses. Pronounced photoreceptor toxicity was observed at 1e10 vg/eye. The AAV9 mouse RPGRorf15 vector also showed therapeutic effect at 3e8 vg/eye but not at 1e9 vg/eye. At the 3e8 vg/eye dose of the AAV9 mouse RPGRorf15 vector, outer nuclear layer (ONL) thickness averaged 6 layers in vector treated eyes and 3 layers in vehicle-injected control eyes. OCT findings were consistent with this and indicated that the ONLs of treated eyes were 2 to 3-fold thicker than controls. Interestingly, the AAV8 human RPGRorf15 vector also produced similar degree of efficacy as the AAV9 mouse RPGRorf15 vector but at the 1e9 vg/eye dose. It was toxic at the 1e10 vg/eye dose. Areas of retinal thinning correlating with regions of very high RPGRorf15 expression were found in retinas receiving 1e9 vg/eye of vectors expressing the mouse gene but not the human gene. The toxicity of the mouse vectors may be due to the super-physiologic levels of RPGRorf15 protein produced by the 1e9 vg/eye dose. The different efficacy and toxicity profile of the human RPGRorf15 vector may be due to the heterologous transgene. This data demonstrates that an AAV8 human RPGRorf15 vector is able to inhibit rod and cone degeneration in a mouse model of RP. Vectors administered at 6 weeks and 12 months of age both showed efficacy,

indicating that this therapy may be relevant to juvenile and adult patients. The AAV8 human RPGRorf15 vector fits our criteria for further clinical development.

710. Cytotoxic T-cell Surface Engineering with Chemically Self-Assembled Antibody Nanorings (CSANS)

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Recent advances with bispecific antibodies have demonstrated that T-cells can be targeted to cancer cells, resulting in selective cell destruction. An alternative approach is adoptive T-cell therapy, which relies on gene transfer technologies for the expression of tumor targeting single chain antibodies (scFvs), referred to as chimeric antigen receptors (CARs). Although promising, the efficiency of gene transfer, cell-to-cell CAR gene expression variability and a dependence on time consuming plasmid re-engineering in order to alter the targeting receptor pose significant hurdles. To address these concerns, we have developed a new modular approach for the rapid engineering of cell surfaces. The approach relies on our discovery that when scFv-DHFR-DHFR fusion proteins are combined with bivalent methotrexate (bisMTX), chemically self-assembled antibody nanorings (CSANS) are formed with tunable valencies of 2, 4 and 8 scFv's. Recently, we have prepared bisMTX-phospholipid conjugates, which when treated with DHFR-DHFR-anti-EpCAM scFv fusion proteins, containing either a 13 or 1 amino acid linker between the DHFRs, form bivalent and octavalent anti-EpCAM lipid-CSANS (L-CSANS), respectively. The anti-EpCAM L-CSANS were shown to rapidly incorporate into the membranes of cells, including activated T-cells. The cell surface modified cells were shown to selectively bind to EpCAM+ cells, such as the breast cancer cell line, MCF-7 cells. Upon treatment with a non-toxic DHFR inhibitor, trimethoprim, which leads to CSAN disassembly, the cells were shown to quickly disengage from each other. In addition, activated T-cells that had been modified by anti-EpCAM L-CSANS demonstrated selective cytotoxicity to the target MCF-7 cells. Thus, we have shown that targeting L-CSANS have the potential to be a flexible and modular approach for the rapid engineering of T-cell surfaces for T-cell immunotherapy.

711. Specific siRNA delivery to primary activated T cells for anti-inflammatory therapy

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Activated T cells play a key role in immune response and immune system related diseases such as chronic inflammatory diseases, viral infections, autoimmune disease, transplant rejection, Crohn disease, diabetes, and many more.1 Therefore, a specific treatment targeting activated T cells (ATCs) bears a therapeutic benefit since the activated rather than naïve T cells play a central role in immune response cascades. However, T cells are hard-to-transfect cells and therefore not easily accessible for gene therapy. Using the overexpression of transferrin receptor (TfR) on ATCs, our group has examined siRNA delivery with a transferrin-low molecular weight polyethylenimine conjugate (Tf-PEI) in primary ATCs.1 We are the first to report ATC-targeted siRNA delivery for knockdown of the Th2 transcription factor GATA-3. We synthesized Tf-PEI conjugates via three different conjugation routes, an oxidation protocol,2 a crosslinking route using the homobifunctional linker dimethyl suberimidate (DMSI),3 and a crosslinking route using the heterobifunctional linker succinimidyl 3-(2-pyridyldithio) propionate (SPDP).4 The uptake of fluorescently labeled siRNA into activated T cells mediated by the DMSI and SPDP conjugates was more efficient than with Lipofectamine2000, a commercially available transfection reagent. When T cells were not yet fully activated, however, TfR expression was very low compared to fully activated T cells. Accordingly, no siRNA was taken up into those cells, confirming the specificity of the targeting ligand.1 siRNA delivered by the biodegradable, SPDP conjugate significantly knocked down target gene expression by 70% in primary ATCs in a sequence-specific manner. Measurements of the effect of GATA-3 knockdown on the levels of Th2 cytokines as anti-inflammatory therapy are currently under way.

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712. MicroRNA-155 confers encephalogenic potential to Th17 cells by promoting effector gene expression

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Th17 cells are central to the pathogenesis of autoimmune disease, and recently specific noncoding microRNAs (miRNAs) have been shown to regulate their development. However, it remains unclear if miRNAs are also involved in modulating Th17 cell effector functions. Consequently, we examined the role of miR-155 in differentiated Th17 cells during their induction of Experimental Autoimmune Encephalomyelitis (EAE). Using adoptive transfer experiments, we found that highly purified, MOG antigen-specific Th17 cells lacking miR-155 were defective in their capacity to cause EAE. Gene expression profiling of purified miR-155-/- IL-17F+ Th17 cells identified a subset of effector genes that are dependent upon miR-155 for their proper expression through a mechanism involving repression of the transcription factor Ets1. Among the genes reduced in the absence of miR-155 was IL-23R, resulting in miR-155-/- Th17 cells being hypo-responsive to IL-23. Taken together, our study demonstrates a critical role for miR-155 in Th17 cells as they unleash autoimmune inflammation, and finds that this occurs through a signaling network involving miR-155, Ets1 and the clinically relevant IL-23-IL-23R pathway.

713. DNA Mini Strings: The Gold Standard for Transgene Delivery

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Background: The DNA plasmid (pDNA) vector represents the current conventional technology driving therapeutic gene transfer, whether for use toward mal/non functional gene replacement, DNA vaccination, or production of recombinant proteins in mammalian cells. The conventional pDNA vector suffers from several safety and efficiency limitations: 1) it imparts adverse immune responses to bacterial sequences required for maintenance and amplification in prokaryotes; 2) its bioavailability can be compromised due to size; 3) it may be genotoxic due to its potential to integrate into the host chromosome and yield an oncogenic event. We have constructed an in vivo platform for the production of mini linear DNA vectors with covalently closed ends (lcc DNA) that we call DNA mini strings. Mini strings are devoid of unwanted bacterial sequences, encoding only the gene(s) of interest and necessary complementary eukaryotic expression/enhancement genetic elements. Results: Transfection of DNA mini strings encoding the enhanced Green Fluorescent Protein gene (eGFP) into rapidly dividing and slow dividing human cells resulted in significantly higher transfection efficiency, bioavailability, and cytoplasmic kinetics compared to the parental plasmid precursor and isogenic covalently closed "DNA minicircle"

counterparts. As we previously noted in prokaryotes, we show here that integration of lcc DNA into the mammalian host genome results in chromosomal disruption and subsequent apoptotic elimination of potentially oncogenic vector integrants from the cell population, thus improving the safety profile of mini strings. Conclusion: Mini strings are the gold standard of transgene delivery. Mini lcc DNA vectors significantly improve the bioavailability, immunocompatibility, safety, and efficiency obstacles encountered by conventional pDNA and other circular DNA vectors.

714. Restoring a minimal motor unit potential and peripheral motor conductivity in lower extremities in a 15 years old girl with complete loss of spinal cord continuity at Th2-Th3 after combine autologous bone marrow derived cells and mesenchymal stem cells transplantation

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The present standard of care for spinal cord injury (SCI) patients consists of stabilization of the injured region and subsequent long-term conservative and rehabilitation treatment. However, there is a lack of treatment enabling SC regeneration. Cell therapy is an evolving modality that might lead to spinal cord regeneration. A 15 years old girl with total spinal cord interruption at Th2-Th3 level was enrolled to experimental cell therapy procedure that involved transplantation with autologous bone marrow derived cells (BMC) and mesenchymal stem cells (MSC) combined with intense neurorehabilitation including physiotherapy. The patient had Th1 sensation level and paraplegia with sphincters palsy and without ability to fix her trunk, at the time of admission (3 months after SCI). The patient was scored ASIA A. Neurophysiology examination (EMG and ENG) showed bilateral axonal damage of both motor and sensory neural fibers with no motor unit potential and peripheral motor nerve conduction of lower extremities. The standard therapy did not bring any positive results.

Autologous BMC were injected intravenously (3.2x109) and intraspinally (0.5x109) 10 weeks after the SCI and with two rounds of MSC, a month (13x106) and five months later (36.5x106). Sterility of BMC and sterility and genetic correctness of in vitro cultured MSC transplanted cells was confirmed. There was no complications connected with cells transplantation procedure and no side effects were noted until the last follow-up evaluation 9 months after cell therapy beginning.

First improvement was noticed 4 weeks after the first round of MSC transplantation as seen as a decrease in sensation level to Th6-Th7 and increased bladder filling sensation. Ability to control the trunk was fully restored. No objective improvement was seen at that time in neurophysiological examination.

Further improvement was noticed 6 weeks after second round of MSC transplantation. Further decrease in sensation level to Th8-Th9 was noted together with restored bladder control however still without rectal control. Muscle strength at left lower extremity improved from plegia to deep paresis (1° in Lovett scale). Moreover ability to move her lower extremities against gravity supported by the movements in her quadriceps was restored. Neurophysiologic examination (EMG and ENG) at that time demonstrated slightly decreased bilateral surface sensation at the level of Th8/Th9 and L1/L2. The patient ASIA score changed to B. Minimal motor unit potential of lower extremities was restored with predominance on the left side. Peripheral motor nerve conduction of lower extremities became almost normal.

9 months after first cell transplantation the girl is able to control the trunk and sit independently and is able to stand independently in a standing frame. Additionally the patient did not developed spasticity or neuropathic pain as well as skin ulcers during the whole treatment period.

Patient is approaching the third round of MSC transplantation at the moment and is still undergoing intense neurorehabilitation.

715. Genomic correction of Duchenne Muscular Dystrophy patient-derived iPSCs using TALENs

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Targeted genome editing of human induced pluripotent stem cells (hiPSCs) is a useful technology for disease modelling and for future iPS cell therapy. Artificial nuclease based genomic editing technologies, such as Zinc-finger Nucleases (ZFNs) or TAL Effector Nucleases (TALENs), have been demonstrated to be effective and efficient in many organisms, including iPSCs. In this study, we aim to restore the mutated dystrophin protein in hiPSCs derived from a Duchenne Muscular Dystrophy (DMD) patient by TALENs. DMD is a severe muscle degeneration disease caused by the disfunction of dystrophin protein. Here, we derived iPS cell lines from a DMD patient who lacks the exon 44 in the Dystrophin gene. The truncated dystrophin protein without C-terminus is expressed in this patient because of one base pair shift from the reading frame. To restore the reading frame after the deleted exon 44, we devised a strategy to induce a (3n+2) base pair deletion or a (3n+1) base pair insertion by TALEN-mediated base excision. We have constructed 15 pairs of TALENs and tested the activities by using a Single Strand Annealing (SSA) assay. We found that 2 pairs of TALENs show high recombination activity. After introduction of TALENs into DMD-iPSCs, we sucessfully identified the deletions and/or insertions at the target site by restriction enzyme digestion and deep sequencing analysis. Our genomic surgery approach for Dystrophin gene by TALENs should facilitate the gene therapy using iPS cells for DMD patients.

716. A versatile method for titration of chimeric antigen receptor-expressing retroviral vectors based on translocation of CD3-epsilon

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Chimeric antigen receptors (CARs) are artificial membrane proteins with modular structure, designed to recognize extracellular antigens through a antibody moiety and to transduce a T cell activating signal thereafter. Thus, antigen-induced T cell activation is independent of antigen presentation by major histocompatibility complex (MHC) molecules, and is believed to be independent of the endogenous TCR-CD3 complex due to the presence of CD3-zeta chain signaling modules in the intracellular portion of CARs. However, it has been reported that CARs containing CD3-derived transmembrane domains induced an increased membrane expression of the endogenous CD3 complex. In the present study we explored the effects of multiple CAR designs on the membrane expression of endogenous CD3. To that end, we used a mutant variant of the Jurkat T cell line (J.RT3-T3.5) whose TCR beta chain gene is inactivated. As a consequence, membrane expression of TCR alpha chain and CD3 complex is inhibited at post-transcriptional level, but can be rescued upon introduction of an exogenous TCR beta chain.

We found that transduction of JRT3-T3.5 cells with CARs containing CD8 or CD28 transmembrane domains linked to the intracellular CD3-zeta domain mediated stabilization and surface expression of endogenous CD3-epsilon. Secondly, using a PSCA-specific CAR, we found that CD3 surface expression correlated with CAR expression and with the viral dose used for transduction. PSCA-CAR-expressing cells were functional as evidenced by upregulation of CD137 (4-1BB) and secretion of interferon-gamma upon specific recognition of target antigen. Finally, expression of a CSPG4-specific CAR, that failed to be stained by Protein-L or anti-mouse-immunoglobulin antibodies, was efficiently detected by CD3 translocation in J.RT3-T3.5 cells, indicating the utility of the line to titer the biological activity of different CAR constructs.

Our results show that presence of a CD3-derived transmembrane region is not necessary for upregulation of endogenous CD3-epsilon chain upon transduction of a CAR. In addition, we show that assessment of CD3-epsilon translocation in CAR-transduced J.RT1-T3.5 cells can be used as a standardized method for titration of CAR-expressing vector preparations, which is not affected by the binding properties of extracellular region of the immune receptor.

717. Dual Specific Suicide Gene Expression plasmid Delivery Using Bio-reducible Polymer for Hepatocellular Carcinoma Gene Therapy

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Hepatocellular carcinoma (HCC, also called hepatoma) is the most common type of a primary cancer of the liver. Currently, surgical resection and liver transplantation are considered as best treatment options. However, the narrow chance of treatments or recurrence of tumor after the surgical treatment brings less survival rate. For this reason, HCC remains one of the most difficult tumors to cure. To overcome the limitation of current therapy methods, gene therapy proposed as a potential future treatment strategy. For the successful and safe gene therapy, controllable therapeutic gene is one of the most important factors to minimize side-effects in normal tissues. In this study, we developed a novel hypoxia and hepatoma dual specific therapeutic gene expression plasmid. The constructed plasmids were transfected into various cell lines using bio-reducible polymer, poly (amidoamine) (PAMAM) conjugated arginine-grafted poly (cystaminebisacrylamide-diaminohexane) (ABP) (PAM-ABP). pAFPS-Luc and pAFPL-Luc plasmids were constructed with the alpha-fectoprotein (AFP) promoter and enhancer for hepatoma tissue specific gene expression. Then, pEpo-AFPL-Luc was constructed by insertion of the erythropoietin (Epo) enhancer for hypoxic cancer specific gene expression. In vitro transfection assay showed that pEpo-AFPL-Luc transfected hepatoma cell increased gene expression under hypoxic condition. To confirm the therapeutic effect of hypoxia and hepatoma dual specific gene expression plasmid, herpes simplex virus thymidine kinase gene (HSV-TK) was introduced for cancer cell killing. The pEpo-AFPL-TK was transfected into hepatoma cell lines in the presence of ganciclovir (GCV) pro-drug. Caspase-3/7activity, MTT and TUNEL assays demonstrated that pEpo-AFPL-TK transfected cells showed significant increasing of death rate in hypoxic hepatoma cells compared to control or pSV-TK. Therefore, the hypoxia/hepatoma dual specific gene expression plasmid delivery by the bio-reducible polymer might be useful for safe and efficient hepatoma gene therapy.

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718. Expression of the immunomodulatory cytokines interleukin-10 and interleukin-6 are increased in equine MSCs cultured in an inflammatory environment in vitro

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Joint injury and inflammation is accompanied by an increase in the production of catabolic cytokines including IL-1μ and TNF-α produced by synoviocytes and chondrocytes as well as an influx of inflammatory mediators produced by WBCs entering the joint. Catabolic cytokines and inflammatory mediators up-regulate destructive enzymes including MMPs and ADAMTSs which lead to disruption of cartilage homeostasis and progressive degradation of the extra-cellular matrix (ECM). MSCs are being investigated as a cell source for tissue regeneration however, full differentiation and engraftment of cells

at sites of damage have proved challenging. The immunomodulatory properties of MSCs have recently been investigated. IL-10 is a known anti-inflammatory cytokine and is constitutively expressed by MSCs. IL-10 has been shown to play a role in connective tissue ECM homeostasis and appears to have a protective role in inflamed joints. IL-10 may decrease the up-regulation of catabolic cytokines during inflammatory events. The role of IL-6 in joint pathology remains largely unknown with recent studies suggesting it has an anabolic effect on chondrocytes and may protect against ECM degradation. Our hypothesis was that equine MSCs cultured in an inflammatory environment would have alterations in immunomodulatory genes. Bone marrow was collected from the sternabrae of 2-5 year old horses for primary MSC isolation. Cells were cultured to passage 2 prior to re-plating in 24-well plates at a concentration of 5 x 104 cells/cm2. Cells were cultured in 24 hours prior to treatment. Media was replaced with serum-free media 4 hours prior to stimulation. MSCs were stimulated with LPS at 25ug/ml, 50ug/ml and 100ug/ml or rhTNF-α at 25ng/ml and 50ng/ml. Cells were lysed 24 hours after treatment and RNA was isolated. Gene expression was determined using qRT-PCR for IL-6, IL-10, IL-1μ, TNF-α with 18S as a housekeeping gene. Stimulation of MSCs with LPS (100 μ g/ml) significantly increased IL-10 expression over control ($p=0.049$). Stimulation of MSCs with TNF-α (25μ g/ml) increased IL-10 expression however, this increase was not significant. IL-6 expression was significantly increased when MSCs were stimulated with LPS at 25 μg/ml, 50 μg/ml and 100 μg/ml and TNF-α at 25 μg/ml ($p<0.05$). Although, MSCs constitutively expressed TNF-α, this was not significantly altered by any of the inflammatory treatments. IL-1 μ expression was significantly increased when MSCs were stimulated with LPS at 50 μg/ml and 100 μg/ml and TNF-α at 25 μg/ml ($p<0.05$). MSCs in an inflammatory environment in vitro appear to up-regulate expression of IL-10 and IL-6. These cytokines may play an important protective role in joint inflammation through their immunomodulatory effects. Intra-articular injection of MSCs in the inflamed joint may be beneficial due to increased expression of these protective cytokines as they may modulate the response of chondrocytes during inflammation. Both of these cytokines may be a promising for management of acute and chronic arthritis however, further investigation in the effects of IL-10 and IL-6 in equine cartilage is required. In the future, prolonged therapeutic levels of anabolic cytokines may be achieved by intra-articular injection of MSCs overexpressing IL-10 using an ex vivo gene therapy approach.

719. Gene Delivery Using Therapeutic Ultrasound To Mesenchymal Stem Cells: Targeted Antiangiogenic Tumor Therapy

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INTRODUCTION: Genetically engineered stem cells, such as mesenchymal stem cells (MSC), is an exciting therapeutic concept that offers the promise of therapy for an array of disorders and malignancies. MSC have been demonstrated to migrate to tumors and ischemic tissue thus can be used as a carrier of therapeutic DNAs. Ultrasound (US) is a physical method of delivery (a non-viral approach) used to deliver genes into cells and tissue. Non-viral vectors are emerging as substitutes to the viral ones since they are considered safer, easier to prepare and lack immunogenic response introduced. Ultrasound Contrast Agents (USCA) are gas-filled microbubbles that are used to increase transfection levels. Angiogenesis inhibition offers a powerful strategy in the treatment of cancer. One of many known angiogenesis inhibitors is the hemopexin-like domain fragment (PEX). In the present study, we aim to design and characterize a gene delivery system based on therapeutic US (TUS) for the transfection of MSC. The transfected cells will be then used as gene delivery vehicles for the therapy of cancer. METHODS: Biological In-Vitro activity of TUS-transfected secreted PEX: pDNA encoding for PEX was added to the MSC and were exposed to 1 MHz TUS at 20% Duty Cycle, 2 W/cm2 for 20 minutes with USCA. Conditioned media was taken from TUS-transfected MSC and was added to human prostate cancer cells (PC3), primary endothelial cells (HUVEC) and MSC. Viability was measured using AlamarBlue. Apoptosis was measured using PI and Annexin-V-FITC. In-Vivo studies: PC3 cells were inoculated subcutaneously (s.c) in mice, after the tumors have reached a volume of 100 mm3, TUS-PEX-transfected MSC were injected intravenously (i.v). All mice were sacrificed after 21 days and tumores weight and volume were measured. Immunohistochemistry (IHC) assays of apoptosis (Caspase-3), cell proliferation (Ki67) and micro vessel

density (CD31) were performed on the harvested tumors and compared to controls. RESULTS AND DISCUSSION: The PEX secreted from the transfected MSC significantly reduced the viability of the HUVEC and PC3 cells by (20%) and (40%) respectively, and did not affect the MSC (compared to control). The PEX increased the percentage of HUVEC apoptotic cells from 6%±3 (control) to 18%±2. In-Vivo studies: Tumor growth was significantly inhibited 21 days after treatment resulting in tumor volume of 157±39 mm3 compared to 410±100 and 423±56 mm3 in controls ($p<0.05$). MSC were found in tumors of treated mice compared to control, and compared to other organs in the treated mice.
IHC of tumor sections revealed that there was a significant change in the % vascularization, proliferation and apoptosis compared to controls. These results indicate that PEX transcribed in tumors and was biologically active and retained its efficacy. CONCLUSIONS: Our study demonstrates that TUS can efficiently transfect MSC while maintaining their viability. The TUS-transfected MSC secret the PEX into their media and reduce the viability of PC3 cells and increase the apoptosis in HUVEC cells while sparing non-cancer cells In-Vitro and In-Vivo. These results suggest that TUS-transfected MSC may be used as carriers of therapeutic DNAs in order to migrate to tumors and ischemic tissue.

720. The role of S100A4 in glioblastoma and cancer stem cell

Shih-Hwa Chiou, Jun-Ming Han, Hsin-I Ma

Cancer stem cell (CSC) hypothesis indicated that cancer cells are heterogeneous, and only rare tumor cells have stem cell properties, such as the ability to proliferate extensively and form new tumors, termed cancer stem cells (CSCs). Evidence of cancer stem cells has been published in human cancers including tumors of the breast, brain, head and neck, and colon, etc. Therefore, searching the critical regulation factors of CSCs as targets provides a new direction for cancer therapies. CSCs have been isolated by their phenotype to extrude Hoechst 33342 dye; hence, CSCs stain only weakly this dye and are referred to the CD133-positive marker and side population (SP). S100A4, a calcium-binding protein, promotes tumor metastasis and tumor vascularisation, and S100A4 protein expression is associated with patient outcome in many human cancers including ovary, pancreas, bladder, and breast, etc. To confirm that S100A4 is associated with the characteristics of CSCs, an alternative cancer stem cell isolation method by specific cell surface marker, CD133, were adopted. Glioblatoma CD133+ cells sorted by flow cytometry also displayed up-regulated expression of S100A4. These results suggested that S100A4 may be positively correlated with cancer stem cell properties. However, the biomolecular role of S100A4 in medulloblastoma is still unclear.

721. Biodistribution and toxicology studies of AAV2/rh8-CBA-β-hexosaminidase after intracranial delivery in C57/Bl6 mice

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Tay-Sachs disease (TSD) is an autosomal recessive neurodegenerative disease resulting from a lack of functional lysosomal enzyme β-N-acetylhexosaminidase A (Hex A). Hex A is a heterodimer of both α and β subunits, encoded by the HEXA and HEXB genes, respectively. TSD is caused by mutations in HEXA, which leads to a decrease in the hydrolysis of GM2 ganglioside and its accumulation in the CNS. Patients with infantile onset TSD, the most severe form of the disease, suffer from rapidly progressing cerebral and macular degeneration, and ultimately, death before age five. Biodistribution and toxicology studies were performed in C57/Bl6 mice using equimolar amounts of two separate AAV2/rh8 vectors containing the mouse Hex α-subunit (AAVrh8-mHexA) or β-subunit (AAVrh8-mHexB) driven by a CMV enhancer/Chicken β-actin promoter. One hundred and eighty animals were divided into three groups: low dose (1.17x10e9 vg total, 18M/18F, toxicology study only), high dose (1.17x10e10 vg total, 36M/36F), and a PBS control

group (36 M/36F). Control or test article was infused stereotaxically into the left brain lateral ventricle and bilaterally into the thalamus. Animals from all groups were sacrificed evenly at 7, 30 or 90 days. The primary safety endpoints of this study were the histopathological examination of brain and peripheral tissues and detection of vector genome in the blood, brain and peripheral organs. CBC and serum chemistry analysis were performed to measure acute toxicity. Animals were monitored daily and no clinical observations of adverse reactions were noted. Histopathological examination revealed trauma at the injection sites, as well as test article related findings that exhibited a dose and time relationship. These findings included vacuolization and gliosis at the injection site, eosinophilic inclusions, and lymphocytic and macrophage aggregates. These findings did not result in impaired motor function as determined by accelerating rotarod testing in control and vector treated day 90 animals. Injection sites in the thalamus had up to 1x10e7 vector genome copies/µg gDNA at day 7, which decreased less than a log by day 90. Uninjected sites within the brain maintained up to 1x10e4 copies at day 90. Vector DNA was present to some extent in all peripheral tissues assayed, most notably liver, where vector DNA increased from day 7 to day 90. No appreciable differences were detected in hematology and serum chemistry parameters between control, low and high dose animals. Ultimately, these studies demonstrate that robust diffusion of vector throughout the brain can be achieved after a few localized injections using the AAVrh8 vector. However, further studies are needed to elucidate the causes and implications of the histopathological findings. Studies including modifications to the promoter elements and dose are currently being explored to reduce transgene expression in the case of harmful overexpression.

722. Qualification for human umbilical cord derived mesenchymal stem cells (hUC-MSCs) for regenerative medicine

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Mesenchymal stem cells were regard as a useful tool for cell therapy. For clinical trials, quality control for isolation, characterization andmethods for guideddifferentiationof stem cells were requited. In this study, we were confirmed maintenance of potency of isolated and culturedhUC-MSCduring ex vivo expansion and cryopreservation. Mesenchymal stem cell specific marker expression was analyzed by FACS and itsdifferentiation potency was confirmed by guided differentiation of adipocyte, osteocyte, chondrocyte and hepatocyte after expanding over 15 doublings in vitro. Bacterial contamination, mycoplasma, endotoxin, adventitious virus testwere performed for detection of contamination, and karyotyping also carried out forchromosomal mutation during in vitro culture. After culture or thawing of hUC-MSCs, the stem cell marker expression of CD31, CD34 and CD45 were under 10%, however CD90 was over 90% by FACS analysis. Any contamination and mutation were detected and the proliferation and differentiation potency were changed during in vitro culture and cryopreservationof hUC-MSCs. These results could be used as standard methods of maintenanceof hUC-MSCs for cell therapy products and clinical application.

723. Attenuation of TGF-β induced liver fibrosis by bone morphogenetic protein 2.

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Liver fibrosis is the result of the accumulation of extracellular matrix components (ECM) with subsequent destruction of liver architecture and liver cell dysfunction. Activated hepatic stellate cells have been identified as major collagen-producing cells in liver fibrosis. These cells are activated by fibrogenic cytokines such as TGF- β1. Increasing evidence shows that TGF-β1 is a key mediator in the process of liver fibrosis. Bone morphogenetic protein (BMPs) are the largest family within the TGF-β superfamily and are involved in several important processes in development. In our previous study, we demonstrated that BMP-2 against TGF- β-induced renal fibrosis. However, little is known about the role of BMP-2 in TGF- β-induced liver fibrosis. In this study, we investigated the underlying mechanism of BMP-2 in the regulation of TGF-β-induced cellular fibrosis in hepatic stellate cells. The purpose of this study was to determine whether mutual regulatory mechanisms exist between BMP-2 and TGF-β1 in liver fibrosis

724. Subpopulation of T-lymphocytes in leukoconcentrates obtained from bone marrow donors in the setting of stable hematopoiesis

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Adoptive immunotherapy based on bone marrow donors' lymphocytes has become an integral part of post-transplant preventive measures as well as of the recurrent hemoblastoses therapy.

Aim: the paper is aimed at analyzing the cell counts of leukoconcentrates, including those of subpopulational T-lymphocytes obtained from HLA-matched related bone marrow donors in the setting of stable hematopoiesis.

Materials and methods: cell counts and T-lymphocyte subpopulation ratios in 34 leukoconcentrates are analyzed. Leukapheresis (AMICUS-2, COBE-Spectra-11, MCS+ -21) has been performed in 26 HLA-matched relative donors (13 male and 13 female) aged 15 to 61 (median age 33). 20 of the donors have undergone leukapheresis once whereas in remaining 6 donors the procedure was performed repeatedly: twice – in 4 donors, three times – in 2 donors. Intervals between repetitions of leukapheresis ranged from 4 to 28 weeks (median period 4 weeks). The time between bone marrow harvesting and leukapheresis ranged from 16 to 204 weeks (median period 20 weeks).

Bone marrow recipients with hematological malignancies have been treated with transfusions of leukoconcentrates in order to prevent or treat posttransplant recurrent episodes of the disease.

The qualitative composition of T-lympocyte subpopulations in leukoconcentrates, was evaluated using methods of immunophenotyping and flow cytometry (FACS Canto II (BD)). Monoclonal antibodies to the following antigens were used: CD3 (PerCP), CD4 (PE), CD8 (FITC), CD 25 (FITC), CD 56 (FITC) (BD). Immunophenotypes of the following T-lymphocyte subpopulations: T-lymphocytes (absolute CD3+ count), T-helper cells (CD3+/CD4+); T-cytotoxic cells (CD3+/CD8+), regulatory cells (CD4+/CD25high), T-NK cells (CD3+/CD56+) and NK-cells (CD3-/CD56+) were identified.

Results: Median absolute mononuclear count in leukoconcentrates equaled 11.2 x 109/l, that of lymphocytes 7.7 x 109 /l and of CD3+ cells – 5.8 x 109 /l, whereas the median of the relative CD3+ cell count – 50.3%.

Median relative and absolute subpopulation counts in the lymphocytic gate were found to be as follows: CD3+ - 73.5% (5.7 x 109/l), CD3+/CD4+ - 42% (3.2 x 109/l), CD3+/CD8+ - 24% (1.6 x 109 /l), CD4+/CD25high – 2.6% (0.23 x 109 /l), CD3-/CD56+ - 9% (0.7 x 109 /l), CD3+/CD56+ - 4% (0.3 x 109 /l).

When the total cell count (mean 12.2 ± 3.2 x 109 /l vs 13.3 ± 6.6 x 109 /l) and CD3+ cells counts (mean 5.7 ± 1.7 x 109/l vs 6.2 ± 2.4 x 109/l) in the leukoconcentrates obtained 4 weeks apart from each other are compared, no decrease of these data is observed.

Conclusion: The data on the cell counts and the ratios of T-lymphocyte subpopulations in leukoconcentrates obtained from HLA-matched bone marrow donors under the conditions of stable hematopoiesis can be used as reference values of the cell composition.

Saturday, May 18, 2013

Oral Abstract Session 410

10:15 am – 12:15 pm

Room: 150 DEFG

Late Breaking Abstracts

725. AAV/RNAi-mediated improvement of anti-malaria vaccination strategies

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Malaria, caused by protozoan Plasmodium parasites, remains one of the most prevalent infectious diseases and a major health burden due to the lack of an efficient, safe and globally applicable vaccine. Recent work has demonstrated the induction of sterile immunity in mice through successive triple vaccination with genetically attenuated parasites (GAP) that arrest during liver-stage development. This suggests that a better understanding of the host factors controlling the Plasmodium life cycle in the liver will aid in the design of potent vaccination strategies.

Towards this aim, we comprehensively dissected the mutual interactions of the hepatic miRNA machinery and Plasmodium in mice at multiple time points after inoculation with wildtype (wt) or GAP sporozoites. Strikingly, we measured an up to 8-fold down-regulation of five core RNAi factors (mRNA and protein levels) including Drosha and Exportin-5, which implies a fundamental role of liver miRNAs in Plasmodium development. Indeed, concurrent liver miRNA profiling via microarrays and qRT-PCR revealed a dysregulation of 31 miRNAs that we bioinformatically linked to cellular key processes with putative roles in parasite infection, such as cytoskeleton remodeling and lipid metabolism. The strongest effect, an up to 10-fold up-regulation, was noted for miR-155, a miRNA known to exert critical functions in mammalian immunity. Its parasite-dependent increase was further validated in mice co-infected with a miR-155-sensitive AAV8/luciferase reporter and Plasmodium sporozoites.

We then created AAV8 vectors for in vivo miR-155 inhibition or over-expression and studied their effect on GAP-mediated protective immunity and on parasite prepatency after wt challenge. Interestingly, mice treated with a miR-155 sponge vector exhibited a reduction of sterile immunity, as evidenced by higher numbers of positive mice and shorter prepatency times as compared to empty vector controls. Vice versa, we found a trend for an improvement in GAP vaccination capacity upon miR-155 over-expression as single AAV/GAP injections already sufficed to confer complete immunity to subsequent wt challenges.

To dissect the underlying mechanisms, we focused on two well-known miR-155 targets with negative regulatory functions in immunity, SOCS1 and CTLA4. Remarkably, AAV8/shRNA vector- or antibody-

mediated in vivo suppression of SOCS1 or CTLA4, respectively, closely recapitulated the results with miR-155 over-expression, hinting that the JAK-STAT pathway (SOCS1) and secondary T-cell responses (CTLA4) could play a role in GAP-induced immunity.

Our work provides the first in vivo evidence for a substantial involvement of the mammalian miRNA/RNAi machinery in Plasmodium development and in anti-parasitic immunity. The high compatibility of our new avenues to improve the protective capacity of attenuated parasites by mi/shRNA over-expression from well established hepatotropic AAV vectors brings us one important step closer to the ultimate goal of a globally applicable anti-malarial vaccination regime.

(* = equal contributions)

726. Post-entry Restriction of AAV Vectors by A PHD Finger Protein

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Gene transfer vectors based on adeno-associated virus show great promise for use in human gene therapy. Although AAV vectors can efficiently transfer genes to various cell types in vitro and in vivo, variable gene transduction efficiency can be seen among different AAV serotypes with the same transfer vector sequences. It is also shown that concurrent proteasome inhibitor treatment improves transduction by AAV vectors. Those observations may suggest the existence of cellular factor(s) which mediate post-entry restriction of AAV vectors. In this study, we screened a siRNA library, which covers 600 known and putative human genes in the ubiquitin-proteosome pathway, for AAV9 vector infectivity. Through screening of the library in HeLa cells, we identified 12 candidate genes. To verify our screening results, we tested the effects of disruption of those candidate genes by different shRNA or siRNA constructs. Knockdown of three genes, which encode a PHD finger protein (PHFP), a LIM domain protein (LDP) or a Rab family protein (RFP), repeatedly increased transduction efficiency of a luciferase-expressing AAV9 vector over 10-fold. Disruption of those genes also increased transduction of AAV2, AAV6 and AAV8 vectors, but did not affect the transduction by HIV- and adenovirus-based vectors. Of note, expression of a siRNA-resistant PHFP mutant in target cells rescued the block of AAV9 vector, thus ruling out the possible off-target effects of PHFP siRNA treatment. Disruption of PHFP expression increased the infectivity of AAV vectors with different internal promoters or different transgenes. PHFP knockdown also increased AAV transduction in other cell types, including primary human fibroblasts. To understand the mechanisms of increased vector transduction, we compared vector genome copy numbers in infected cells with or without PHFP knockdown. Real-time PCR analysis did not find significant increases in cytoplasmic or nuclear vector genome copy numbers, suggesting that disruption of PHFP does not enhance the vector entry. In summary, our data demonstrated PHFP as a cellular factor which affects the transduction efficiency of AAV vectors at the post-entry stage. We are in the process of analyzing the vector trafficking and AAV genome release in the cells with specific disruption of PHFP, LDP or RFP.

727. AAV-mediated Bevacizumab Maintenance Therapy Inhibits Ovarian Cancer In Vivo

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Ovarian cancer is the second most common gynecologic malignancy and the most common cause of gynecologic cancer death in the US. Despite advances in surgery and chemotherapy, tumor drug resistance leads to a recurrence of disease in most patients. Based on the knowledge that angiogenesis plays a role in the development and progression of ovarian cancer, and that vascular endothelial growth

factor (VEGF) mediates the growth of ovarian cancer, we hypothesized that persistent suppression of VEGF in the peritoneum would be efficacious in suppressing the growth of ovarian cancer. To test this hypothesis, AAVrh10.BevMab, a Rhesus serotype 10 adeno-associated viral vector coding for bevacizumab (Avastin), a humanized monoclonal antibody directed against human VEGF-A, was evaluated for the capacity of a single administration to persistently suppress the peritoneal growth of ovarian cancers. Based on initial studies demonstrating that AAVrh10.BevMab would mediate sustained levels of anti-VEGF in vivo following a single administration in mice, a model of ovarian cancer was established with 5x10⁶ A2780 human ovarian cancer cells deposited intraperitoneally in nude immunodeficient mice. The next day, 10¹¹ genomic copies/mouse of AAVrh10.BevMab or AAVrh10antiPA (a control vector expressing an irrelevant antibody) were administered intraperitoneally. Assessment of AAVrh.10 delivered bevacizumab showed high levels of expression in the peritoneum at wk 3 (250±45 ng bevacizumab/mg total protein). Tumor weight and A2780-derived human DNA were measured on day 14, 21 and 24. On day 14, control vector (AAVrh10.antiPA) treated mice showed detectable tumor growth whereas the AAVrh.10BevMab treated-mice had no visually detectable tumors (control 0.11±0.8 g vs treated not detectable, p<10⁻³). By day 21, the difference was 55-fold greater tumor weight in the control group (control 1.1±0.7 g vs treated 0.02±0.01 g, p<10⁻³) and on day 24, the difference was 15-fold (control 2.2±0.6g vs treated 0.15±0.2, p<10⁻⁷). Tumor burden was also quantified by quantitative PCR for the tumor-derived human DNA in tissue from the mouse peritoneum. In the control vector-treated group, human DNA accounted for 8, 35 and 42% of the total DNA (mouse and human) in mouse abdominal cavity on day 14, 21, and 24, respectively, whereas in the AAVrh10.BevMab-treated group, human DNA was only 1, 3 and 10% on day 14, 21 and 24, respectively. In addition, 87.5% of mice from the control group developed bloody ascites (74±32 µl) while the AAVrh10.BevMab treated group did not develop ascites. Together, these data provide evidence that AAVrh10.BevMab treatment substantially slows tumor growth. In conclusion, a single administration of AAVrh10.BevMab provides high and sustained expression of bevacizumab in vivo that significantly inhibits tumor growth in a human ovarian cancer mouse model, suggesting that AAVrh.10BevMab administration could be a new strategy to treat ovarian cancer through persistent and continuous bevacizumab expression.

728. Human iPS-derived antigen-targeted T cells are functional innate-like cytotoxic effectors and lyse CD19-positive tumor cells

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Pluripotent stem cells may be harnessed to generate long-lived tumor-targeted T lymphocytes for cancer immunotherapy. We previously demonstrated that human T cells targeted to the CD19 antigen, which is expressed on the vast majority of leukemias and lymphomas, can eradicate B cell malignancies in mice, using chimeric antigen receptors that redirect T cell specificity in HLA-independent fashion. We also showed that “second generation” CARs that provide combined activation and co-stimulatory signals enhance T cell expansion upon repeated antigen exposure to antigen and in vivo T cell persistence. Merging iPS and CAR technology, we characterize here the phenotype and anti-tumor function of human iPS-derived T cells that are genetically targeted to a predetermined tumor-associated antigen. For this purpose, we generated iPS cells from peripheral blood lymphocytes (T-iPS) and we consecutively genetically engineered them to express 1928z, a second-generation CAR specific to the B-cell lineage antigen CD19 currently used in clinical trials. Stably transduced 1928z-T-iPS lines were then directed to differentiate in vitro towards first the hematopoietic and then the T lymphoid lineage. By day 30 of differentiation around 80% of the cells were CD3+TCRαβ+ and expressed CD8a (13%) and CD56 (20-30%). The generated T cells expressed the 1928z CAR on the surface (1928z-TiPS-T). When cultured in the presence of cells expressing CD19, 1928z-TiPS-T cells rapidly responded by forming clusters, increased the expression of activation markers (CD69, CD25) and secreted type 1 cytokines (TNF-α, IFN-γ and IL-2). In

order to further characterize the T-iPS derived T cells we performed a whole genome expression array of day 30-35 CD3+ cells and compared their profile to that of known lymphoid cell subsets. 1928z-TiPS-T cells were clearly distinguished from B cells and clustered together with T cells and NK cells. More interestingly, they showed the highest correlation with the expression profile of cultured γΔ-T cells. We next sought to investigate if 1928z-TiPS-T cells are expandable and show clinically relevant cytotoxic function. After 3 weekly stimulations on CD19 positive cells we were able to expand 1928z-TiPS-T cells around 1000-fold. The expanded cells had an effector memory phenotype and up-regulated the expression of natural cytotoxicity receptors. More importantly, expanded 1928z-TiPS-T cells showed highly specific lytic activity against CD19 positive tumor cells lines in vitro. In conclusion, we demonstrate that TiPS-derived CAR+ T cells display key features of T lymphocyte function and can specifically eradicate target tumor cells. The genetic modification of human pluripotent stem cells with CARs is, thus an effective approach to rapidly and efficiently generate antigen-specific T cells with a clinically relevant function. The iPS cell technology not only offers new perspectives to generate naïve T cells of a predetermined specificity, but is also an excellent platform for safe genetic modifications to augment the potency of immune effectors.

729. Penton-dodecahedral particles trigger opening of intercellular junctions and facilitate viral spread during adenovirus serotype 3 infection of epithelial cells.

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We have recently reported that a group of human adenoviruses (Ads) uses desmoglein 2 (DSG2) as a receptor for infection. DSG2 is a desmosomal junction protein that is expressed in the epithelium of the airway, urinary, and gastro-intestinal (GI) tracts. Among DSG2-targeting viruses are the widely distributed serotypes Ad3 and Ad7, as well as newly emerged, highly pathogenic strains derived from serotype Ad14. We found that the DSG2 interacting domain(s) within Ad3 are formed by several fiber knobs. This specific mode of Ad3-fiber knob-DSG2 interaction provides a high avidity and is functionally relevant for opening of junctions between epithelial cells. Binding of Ad3 to DSG2 triggers autocatalytic cleavage of DSG2 and activation of pathways that are reminiscent of an epithelial-to-mesenchymal transition. The ability to open epithelial junctions appears to be important for Ad3 penetration into, and spread within epithelial tissue. Crucially involved in this process are penton-dodecahedra (PtDd) formed by the Ad3 penton base and fiber at a vast excess during viral replication. Earlier, we demonstrated that recombinant purified PtDd (produced in insect cells) open the junctions between neighboring epithelial cells. Here we show in immunofluorescence studies on polarized human epithelial cells, infected with a replication competent Ad3 vector, that PtDd are released from cells before viral cytolysis and trigger restructuring of epithelial junctions. To better study the role of PtDd in viral infection, we generated an Ad penton base virus mutant (D100R and R425E) that was greatly disabled to form PtDd. The wild-type and penton mutant Ad3 vectors were replication competent and had parts of the E3 region substituted with a GFP expression cassette. The Ad3 mutant was identical to wtAd3 in the efficiency of progeny virus production in 293 cells. To study viral spread, we infected polarized epithelial colon cancer (T84) cells that were grown in transwell chambers in multiple layers with viruses from the apical side. While the initial transduction efficiency (MOI 2pfu/cell) was comparable for both viruses, more than 5 fold less de novo produced penton mutant virus was released at the basal side into the outer chamber nine days after infection. Less efficient viral spread of the penton mutant virus was also visualized (based on GFP expression) in transduction studies on T84 tumor cell spheroids. Furthermore, we injected the viruses intravenously into mice carrying xenograft tumors derived from human lung cancer A549 cells. The histology of these tumors reflected that of most human solid tumors; with strong DSG2 signals and well developed epithelial junctions. Three weeks after virus injection, tumors were analyzed for GFP expression. The percentage of GFP-positive tumor cells was significantly less in mice injected with the penton mutant virus. Our findings demonstrate that PtDd support viral spread in solid tumors and have therefore implications for conditionally replicating, oncolytic adenoviruses based on Ad3.

730. An oncolytic adenovirus able to express a full-length functional anti-Her2 monoclonal antibody from tumor cells

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Background and hypothesis: Monoclonal antibodies are useful tools for treatment of cancer and many other diseases. However, overall clinical benefits – determined by on-target activity - have been limited by off-target systemic toxicity. Thus, local production from eg. a vector would be appealing, but has been difficult to achieve. We hypothesized that we could arm an oncolytic adenovirus with the cDNAs for the heavy and light chains of trastuzumab, and use the replication competent platform for production on an intact, fully functional antibody from tumor cells, resulting in high local and lower systemic concentrations. Design: We constructed a chimeric serotype 5 oncolytic adenovirus, Ad5/3-D24-tras, coding for human trastuzumab antibody heavy and light chain genes, connected by an IRES. We assessed trastuzumab production by the virus construct and evaluated the efficacy in Her2+ tumor models in vitro and in vivo. Results: Ad5/3-D24-tras retained oncolytic potency in vitro. Analysis of supernatants of tumor cell lines revealed correct assembly of full length antibody which was fully functional when compared to commercial trastuzumab. In vivo, Ad5/3-D24-tras treatment enhanced ($p<0.05$) antitumor efficacy compared to replicating control virus Ad5/3-D24 in a HER2+ mouse tumor model. Conclusions: Although traditionally antibody production has been thought to be restricted to B-cells, we were able to use the virus to induce production by tumor cells. This approach has translational appeal and could be utilized also in the context of many other antibodies.

731. Non-toxic and highly efficient targeting of a human chromosomal locus with a lentiviral vector-associated meganuclease

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Site specific endonucleases - Zinc Finger Nucleases, TALE Nucleases or naturally occurring Meganucleases (MN) - can be engineered for custom recognition of any genetic locus and used for gene targeting. Because the prolonged expression of these nucleases into cells may be toxic, due to the accumulation of DNA double strand breaks, efficient methods for their transient delivery are needed. Meganucleases (MN) can be produced as single chain proteins and are advantageous for developing approaches of protein transduction. We have previously described a system where I-SceI, a prototypical monomeric MN, is packaged as a protein into a non-integrating lentiviral vector and delivered along with a targeting construct. Here, we further explore the possibilities of associating I-CreI-derived single chain MNs to lentiviral vectors.

CLS4076 is a I-CreI-derived single chain MN with a unique cutting site on human chromosome 14. It displays a high level of activity when introduced by DNA transfection. This MN was associated with Non Integrative Lentiviral (NILV) particles alone or as a fusion protein with either HIV Vpr or Cyclophilin A. The meganuclease activity was initially measured in a quantitative extra-chromosomal assay based on the recombination-mediated rescue of a luciferase reporter gene. Targeted homologous recombination (HR) at the CLS4076 chromosomal locus was measured in HEK293H cells. Whilst a control NILV encoding CLS4076 was able to mediate up to 5% HR, up to 20-30% HR was observed when the MN protein was associated to the NILV particle. In addition, no cellular toxicity was observed for NILV associated with the MN protein. The results of gene targeting studies using this protein delivery method in human primary T or CD34+ hematopoietic cells will be presented.

732. Non-viral gene delivery to human embryonic stem cellderived retinal pigment epithelium

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Age-related macular degeneration (AMD) is an eye disease characterized by progressive loss of central vision attributable to degenerative and neovascular changes in the macula, a highly specialized region of the ocular retina responsible for fine visual acuity. Recent and promising developments towards an effective cure for choroidal neovascularisation secondary to AMD include the treatment with antibodies/antibody fragments, aptamers and fusion proteins directed against the vascular endothelial growth factor (VEGF) protein. However, in order to maintain therapeutic levels of these biomacromolecules in vivo, repeated intravitreal injections are required: this can be costly and inconvenient for patients. Moreover, anti-VEGF therapy benefits only a minority of patients in the early stages of wet AMD, when the retinal pigment epithelium (RPE) is mostly still intact. For these reasons, we propose a combination of cell therapy, to reconstruct a functional RPE monolayer (Subrizi A et al., Biomaterials 2012), and gene therapy, for long lasting secretion of neurotrophic and/or anti-angiogenic factors. In this study, we have assessed the feasibility of non-viral gene delivery to human embryonic stem cell-derived retinal pigment epithelium (hESC-RPE), a pigmented cell culture model of the outer blood retina barrier. The differentiation of RPE from pluripotent hESCs was performed as floating aggregates using an RPE-basic differentiation method previously described (Vaajasaari H et al., Mol. Vis. 2011). In order to select the optimal transfection time period, hESC-RPE cells were transfected at different times (up to two months) post-plating. A reverse transfection strategy (Reinisalo M et al., J. Control. Release 2006), where the nanoparticulates are freeze-dried on the bottom of well plates, was compared to the conventional transfection method. Cytotoxicity was evaluated with AlamarBlue assay. Sustained transgene expression (170-420 ng/ml) was achieved by using a plasmid with viral CMV promoter containing genetic elements from Epstein-Barr virus, OriP and EBNA-1, which are essential for its episomal maintenance. We have also used a reporter plasmid with human RPE-specific tyrosinase promoter, whose activity was, as expected, two orders of magnitude weaker compared to CMV. The carriers used for transfection were branched polyethylenimine (bPEI, average Mw 25 kDa, used as positive control), cationic liposomes made of N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium methylsulfate (DOTAP) and 1,2-dioleoyl-3-phosphatidylethanolamine (DOPE) (1:1 by mol), and cationic micelles composed of amphiphilic star or linear block copolymers, with a poly(n-butyl acrylate) (PBuA) core-forming block and a water-soluble cationic poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) shell-forming block. Transgene expression levels (170-420 ng/ml) obtained with the best combinations of carrier/plasmid, transfection time and low toxicity were therapeutically relevant, when compared to the minimal concentration of bevacizumab (1 µg/ml) and ranibizumab (60 ng/ml) required for in vitro VEGF neutralization (Klettner A and Roider J, IOVS 2008). In conclusion, we have successfully transfected human embryonic stem cell-derived retinal pigment epithelial cells using polyplexes, lipoplexes and core-shell micelles. The cells expressed the reporter gene at therapeutically relevant levels and the synthetic vectors exhibited modest toxicity.

Saturday, May 18, 2013

Poster Session III

3:45 pm – 5:45 pm

Room: Exhibit Hall C/D

Late Breaking Abstracts III

733. Correction of canine Factor VII (FVII) deficiency by AAV-mediated expression of canine FVII zymogen

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FVII deficiency is an orphan (vs rare) autosomal recessive coagulation disorder (1 in 500,000 people) with clinical manifestations including muscle hematomas, hemarthrosis and central nervous system bleeds. Treatment is by infusion of fresh-frozen plasma, plasma-derived FVII (pdFVII) concentrates and low-dose recombinant FVIIa. Based on clinical data, FVII deficiency is a good model of an orphan coagulation defect to be treated by gene transfer since hemostasis can be achieved with only a mild elevation of plasmatic FVII levels to reach above 10-15% normal (50-75 ng/ml). We decided to demonstrate the efficacy of a gene transfer approach using AAV-mediated gene delivery in a canine model of FVII deficiency that we have previously described (Callan MB et al., JTH 2006). This is the only existing large animal model of FVII deficiency: canine FVII activity is <4% and the specific mutation represents the majority of human FVII deficiency cases. Previously, we described amelioration of the bleeding phenotype of hemophilic dogs using AAV-mediated expression of a canine activated FVII transgene (Margaritis P et al., Blood 2009). Here, we used the zymogen version of our canine transgene (cFVII) for expression in FVII deficient dogs, that alleviates any species incompatibilities within the canine hemostatic system while minimizing immunogenicity. Recombinant AAV serotype 8 vector expressing cFVII from a liver-specific promoter (human alpha-1 antitrypsin) was generated. We administered 4.95E13 vector genomes/kg via the portal vein in a 3 yr old male FVII deficient dog under coverage of normal canine plasma. We monitored cFVII expression using a prothrombin time (PT) clotting assay (sensitive to FVII) as well as rotational thromboelastometry (ROTEM) that measures overall clot kinetics and strength. Within two weeks after vector administration, transgene expression reached a plateau indicated by a PT of 22.6±2.7 sec, significantly reduced from pre-treatment values (121.7±1.2 sec, $P<0.05$) and well-below that of hemostatically normal dogs (32.7±2.1 sec, $P<0.05$). ROTEM analysis using two indicative parameters (clot time [CT] and time to maximal clot velocity [MAXV-t]) showed similar results: at the plateau, cFVII expression resulted in a CT and MAXV-t of 288±79 and 307±82 sec (pre-treatment 482 and 503 sec, respectively), comparable to hemostatically normal values (289 sec). These values have been stable for 8 months (ongoing) indicative of sustained cFVII expression. No adverse events have been observed and serum chemistries have remained within normal limits at all times. In conclusion, this is the first example of successful treatment of FVII deficiency in a large animal model using AAV-mediated FVII zymogen gene transfer. The AAV dose administered resulted in >100% and sustained expression of cFVII. Since even 10-15% of normal FVII levels can ameliorate the FVII deficiency phenotype, our data immediately suggest that the therapeutic AAV vector dose can be much lower; experiments towards that goal are currently under way. Overall, our data hold promise for the AAV-mediated treatment of FVII deficiency and broaden the scope of novel approaches to rare inherited bleeding disorders.

734. Abstract Withdrawn from Presentation

735. Optimization of dual-function vectors for Alpha-1 Antitrypsin Deficiency

Qiushi Tang, Christian Mueller, Lina Song, Terence Flotte UMass Medical School

Alpha-1 antitrypsin (AAT) deficiency is a genetic disorder that results in both a loss of function and toxic gain of function. In patients, a single amino acid change (Glu342Lys) causes the protein to polymerize in the liver and results in decreased serum levels. This result in a lung disease due to the loss of antiprotease function of AAT, the liver polymerization causes disease due to hepatocellular toxicity as accumulating protein in the endoplasmic reticulum. Current FDA approved therapies target the lung disease by increasing serum levels of AAT via IV recombinant protein augmentation. However there is no single intervention to target both liver and lung disease. Our group has previously developed dual-function rAAV vectors for AATD, where a CBA promoter expresses both miRNAs against AAT and a miRNA-resistant normal AAT cDNA to achieve simultaneous knockdown of mutant AAT in the liver whilst augmenting

serum levels of the normal protein (Mueller et al Mol. Ther. 2012. 20). In previous experiments we achieved over 80% knockdown of mutant AAT while augmenting serum levels normal AAT. However we observed that expression of miRNA-resistant AAT was decreased as compared to a cassette without miRNAs. In fact as we increase the number of miRNAs from 3 to 6 the expression miRNA-resistant AAT decreased even more. Since both knockdown and augmentation are key components to developing a dual-function vector for AATD, we set out to optimize the design of the cassette to minimize the loss of AAT expression will ensuring maximal knockdown of mutant AAT. We therefore screened the previously published miRNAs individually and determined that the artificial anti-AAT miR-914 had the highest efficacy of knockdown. This miRNA was then cloned in the 3'UTR and into the intron of CBA promoter of GFP cassette, packaged into rAAV8 vector and delivered via the tail vein to PiZ transgenic mice at a dose of 1x1012 VPs. Sera analysis of the mice indicates that both constructs achieved a decrease of more than 95% of serum AAT levels. Importantly this was significantly higher than the 80% knockdown we previously reported and is likely due to use of rAAV8 instead of rAAV9. Also this data confirms that it is possible to achieve a high degree of knockdown with a single miRNA in the expression cassette. knockdown efficacy was same amount two testing vectors. To determine expression of the miRNA-resistant AAT we cloned the artificial anti-AAT miR-914 into the CBA intron, the 3'UTR or at both location of a cassette that expressed a c-Myc tagged miRNA-resistant AAT. Again mice were inj. at same dose rAAV8 vectors. Interestingly the serum levels of AAT as assayed by a c-Myc ELISA were significantly different for all three groups. rAAV8 vectors where the miRNA was in the intron achieved levels that were 2-fold higher than another two groups. At 5 weeks the serum levels had not plateaued yet so these differences could diverge even more as the study continues.

In conclusion we have screen for the optimal miRNA and determined that miRNA placement at this dose does not influence knockdown, it does influence expression of the AAT cDNA. Further studies at lower doses will seek to determine if miRNA placement has processed at different efficiencies. Importantly this optimization and the use of rAAV8 maximizes knockdown and augmentation which will be important as dual-function vector get translated into the clinic.

736. Targeting dendritic cells with lentiviral vectors induces tolerance of effector T cells and protects mice from experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is an autoimmune disorder characterized by self-reactive T cells that target myelin components of the central nervous system (CNS). The present treatment relies on delaying and ameliorating disease symptoms. The aim of this project was to induce permanent, antigen-specific tolerance of previously activated T cells involved in MS. The strategy includes the ex vivo modification of autologous hematopoietic stem cells (HSC) with lentiviral vectors that express myelin oligodendrocyte glycoprotein (MOG), an auto-antigen involved in experimental autoimmune encephalomyelitis (EAE), an animal model of MS which closely resembles the human disease. The MOG-transgene is under the control of a dendritic cell (DC)-specific promoter to transcriptionally target antigen expression to DCs. After re-infusion, the vector-transduced HSC will give rise to MOG-expressing DCs that, in a non-inflammatory condition, are expected to tolerize self-reactive T cells and, therefore, prevent/revert EAE. We demonstrated that all mice that had received HSC transduced with MOG-lentivirus vector were protected from EAE induction, while all mice that received HSC transduce with a control lentivirus vector developed EAE. In tolerized mice MOG-specific T cells were depleted and Foxp3+ regulatory T cells (Tregs) were generated, which appeared to be functional, as they were able to suppress the in vitro proliferation of MOG-specific T cells. Most importantly, we have demonstrated that tolerized mice did not develop any signs of autoimmune disease even when infused with pre-activated, MOG-specific effector T cells, which rapidly induced EAE in the control mice. The ability to revert the pathogenic MOG T cells highlights the potential of this strategist for a therapeutic model.

737. Novel adenoviral vectors induce robust T cell responses to HSV2 and significantly boost responses after repeat homologous administration

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Adenovirus vectors elicit potent CD8+ cellular responses greater than any other genetic vaccine approach in both animal models and clinical trials, including poxviruses, lentiviruses, alpha viruses and plasmid DNA, but can be limited by pre-existing antibodies against the vector backbone. Here we highlight results demonstrating protection in mice and guinea pigs against live HSV2 infection. This protection correlates with induction of strong T cell responses using two novel, low sero-prevalence non-human primate vectors expressing HSV2 UL19 and UL47 antigens. We show that a single intramuscular vaccination with two antigens, UL19 and UL47, expressed from these novel non-human primate adenoviral vectors provided protection against primary and recurrent disease in mice and guinea pigs, respectively, following intravaginal HSV2 challenge. We demonstrate the novel GC45 and GC46 vectors stimulated robust and multi-functional cellular responses equivalent or superior to the Ad5 vector. In addition, each of the vectors produced a long-lasting pharmacokinetic profile of antigen-specific CD8+ IFN-gamma+ T cell responses ~3-4% above background 26 weeks after a single vaccination. Heterologous prime boost using both vectors demonstrated substantially boosted T cell responses as previously shown with other adenoviral vectors. However, very interestingly and unanticipated, we found that unlike other replication-deficient adenoviral vectors, homologous prime boost regimens resulted in a very effective and further enhancement of T cell responses with GC45 and GC46. These are the first adenoviral vectors to our knowledge that show significant increases in antigen-specific T cell responses following repeat administration with the same vector. Based on the robustness of these studies, our data support the advancement of these newly identified non-human primate vectors and the two previously identified HSV2 UL19 and UL47 antigens for further vaccine development.

738. TALEN-based Individualized Gene Medicine for Epidermolysis Bullosa

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Recessive dystrophic epidermolysis bullosa is characterized by a functional deficit of the type VII collagen protein due mutations in the COL7A1 gene. Gene augmentation therapies are challenging for this disorder due to the large size of the gene as well as the risk of insertional mutagenesis. Cellular therapies are associated with suboptimal therapeutic response due to graft rejection/graft versus host disease. To abrogate these risks we explored the possibility of using the engineered transcription activator like effector nucleases (TALENs) in a precision genome-editing approach for correction of a patient specific mutation. We derived primary fibroblasts from this patient and report the ability of TALENs to induce site-specific double stranded DNA breaks leading to homology-directed repair from an exogenous donor template. This process resulted in permanent COL7A1 gene correction in primary human cells that could be expanded to therapeutically relevant numbers. Towards realizing the goal of ex vivo localized and systemic therapies we reprogrammed the fibroblasts into inducible pluripotent stem cells and showed multi-lineage tissue generation in mice including skin-like structures that expressed normal type VII collagen protein that was properly deposited at the dermal epidermal junction. To determine the safety

profile of this reagent we employed an unbiased, deep sequencing-based genome wide screen and show on target activity and three off target loci that, importantly, were at least 10 kb from a coding sequence. In totality, these data further establish TALENs as viable tools for broad human genome targeting in a patient specific manner for permanent in situ gene correction. Further, the ability to generate inducible pluripotent stem cells from gene corrected fibroblasts and the excellent safety profile of this reagent as determined by this first reported deep sequencing data set for TALENs provides proof-of-concept that will cooperatively facilitate human translational application.

739. Prevention of Anti-Capsid Immune Response Following Systemic AAV9 Dosing in Infant Rhesus Monkeys

Darin Falk

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AAV vectors confer long-term transgene expression when delivered to the neuromuscular system. However, there is high likelihood that re-dosing will be required, especially in inherited metabolic and neuromuscular diseases treated from an early age. Delivery of AAV vectors in nonhuman primates leads to anti-capsid antibody formation, which lessens the efficiency or may preclude the potential for re-administration of the same vector. Given the extensive experience with in utero administration of AAV vectors leading to non-responsiveness to vector transgene, we sought to evaluate the potential to create a similar state in postnatal animals by B-cell ablation prior to vector exposure. Four animals were selected for the study after screening dams for AAV9 antibodies. Two fetal monkeys were administered rAAV9 prenatally (one fetus in the early second trimester and one fetus twice in the second and third trimesters; 1x1013 vector genomes [vg] under ultrasound guidance). All animals were delivered at term by Cesarean section, umbilical cord blood was collected, then infants were raised in the nursery for postnatal studies. Blood samples were collected at birth then weekly for complete blood counts (CBCs), flow cytometry (B and T cells), clinical chemistries, and AAV9 antibody ELISA. Two newborns were included to test the utility of pre-exposure B-cell ablation on the ability to re-administer AAV9 vectors after birth. Rituximab (58 mg/kg, ~700 mg/M2) was administered intravenously at 10 days postnatal age immediately prior to dosing with rAAV9 (1x1013 vg), followed by repeat administration of Rituximab (~350 mg/M2) and rAAV9 at 2 months postnatal age. In addition to Rituximab, one animal also received Sirolimus orally once daily (2-4 mg/kg) until ~4 months postnatal age. No adverse events were observed in any of the animals assessed; body weights, CBCs, and clinical chemistry panels were all within normal limits. Results also indicated infant monkeys administered Rituximab had the expected ablation of all CD20+ B-cells until 10 weeks following the last dose. Anti-AAV9 antibody was detected in the one animals with repeat fetal dosing. The monkey that received both Rituximab and Sirolimus maintained non-responsiveness to AAV9 capsid proteins. The biodistribution of AAV9 vector will be evaluated at the terminal endpoint. These findings demonstrate that effective B-cell blockade in infant monkeys can lead to non-responsiveness to AAV capsid proteins which is critically important for the opportunity to re-administer AAV vectors in planned human clinical studies.

740. Inhibition of ocular neovascularization by subconjunctival gene delivery of calreticulin-derived peptide, vasostatin 112

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The majority of diseases that cause catastrophic vision loss, such as age-related macular degeneration (AMD), diabetic retinopathy (DR), retinal vein occlusion (RVO), retinopathy of prematurity (ROP) and

ocular tumours, do so as a result of pathologic ocular neovascularization. Thus, development of cost effective and less-invasive treatments for targeting retinal and choroidal angiogenesis are a priority in ophthalmology. Vasostatin, the N-terminal domain (amino acids 1-180) of calreticulin, is a potent inhibitor of angiogenesis isolated from culture supernatants of an EBV-immortalized B cell line. We have recently identified the functional domain of vasostatin into a peptide fragment of 112 residues, vasostatin-like peptide 112 (VS112). VS112 which could will be superior to current therapeutic approaches for it specifically targets endothelial cells and it also has anti-inflammatory properties (ongoing inflammation is a major contributor to neovascularisation in DR and AMD). In this study, we first showed that VS112 inhibits angiogenic activity in several assays of angiogenesis including human endothelial cell migration and tube formation and vessel sprouting from rat aortic ring explants. Importantly, VS112 almost completely abolished vascular growth in a rat model of basic fibroblast growth factor (bFGF)-induced corneal angiogenesis, highlighting the anti-angiogenic potency of VS112 in vivo. We then investigated the therapeutic potential of VS112 in laser-induced choroidal neovascularization (CNV) models in rats through subconjunctival gene delivery by an adenoviral vector which releases peptide slowly over 4 weeks. Serial fluorescent angiography (FAG) analysis indicated that subconjunctival VS112 gene delivery significantly reduced CNV lesions on all subsequent days. Histological analysis revealed attenuated CNV lesions and choroidal vascularity in the VS112-treated eyes. The present study provides evidence supporting that sustained delivery of a vasostatin-like anti-angiogenic peptide (VS112) using a minimally invasive procedure promises to revolutionise the management of ocular neovascularisation.

741. Transient Epigenetic Gene Therapy as a New Therapeutic/Prophylactic Avenue for Aging/ Degenerative Diseases and Neurodevelopmental Disorders

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Coined as a translational application (Bertolotti, 2005) of human promoter-specific siRNA-mediated transcriptional gene silencing (promoter methylation; Morris et al., 2004) and promoter demethylation mediated by non-coding antisense-RNA (Imamura et al., 2004), transient epigenetic gene therapy is aimed at long-term transcriptional gene silencing/activation through the transient action of promoter/enhancer-specific siRNAs/dsRNAs or short sense/antisense RNAs/oligonucleotides (oligos). Unlike canonical RNA interference (RNAi) mediated by siRNAs that target cytoplasmic mRNAs for post-transcriptional gene silencing through sequence-specific mRNA cleavage or translational repression, promoter-specific small RNAs/oligos-mediated transcriptional gene silencing/activation is a long-lasting epigenetic process that involves DNA methylation/demethylation and post-translational histone modifications. Therefore, transient epigenetic gene therapy provides a new dimension to basic gene therapeutics in which the transient action of promoter/enhancer-specific small RNAs/oligos can be used for long-term up-regulation/down-regulation of target genes. As an original arm of our proposed Universal Stem Cell Gene Therapy platform (Bertolotti, 2006), transient epigenetic gene therapy has exciting applications for inherited diseases (e.g. allele-specific silencing, activation of “fetal” genes). However, our current focus is to open new therapeutic/prophylactic avenues for 1) aging/degenerative diseases (e.g. aging-associated degeneration of hematopoietic stem cells, infarcted/ischemic myocardium, Alzheimer's & Parkinson’s diseases), 2) for neurodevelopmental disorders originating from deleterious early life epigenetic programming (e.g. depression, schizophrenia, autism) and 3) for the genesis of clinical-grade induced pluripotent stem (iPS) cells. One of our immediate paradigmatic target is SIRT3 the lentiviral transgenic over-expression of which has just been shown to rescue the regenerative capacity of hematopoietic stem cells (HSCs) in aged mice (Brown et al., 2013). Our approach is thus discussed in terms of sustained transgene-free targeted up- or down-regulation of critical endogenous genes into relevant aging cell/stem cell populations either through in vivo or ex vivo protocols culminating in the conversion of resident cells into therapeutic ones or in the ex vivo genesis of iPS cells. Of note, for the genesis of clinical-grade iPS cells, the transient epigenetic arm of our proposed Universal Stem Cell Gene Therapy platform stands as a potential alternate to the current most promising approaches that rely on transfection of microRNAs or synthetic modified mRNAs.

742. Successful Systemic Cancer Targeting by Adenovirus Vector with AB-loop Redesigned Fiber via Reducing Liver Sequestration

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Adenovirus (Ad) represents one of the most frequently used gene therapy vector for clinical applications. However, after intravenous administration, Ad vectors in circulation quickly diminish before reaching the target tumor predominantly due to liver sequestration.

Recent studies suggested the complex of Ad-hexon and a blood coagulation factor might interact with Kupffer cells for Ad sequestration in the liver. On the other hand, hepatocytes are reported to play a major role for liver trasnsduction. Although hexon-coagulation factor complex and Coxsackie-Adenovirus receptor (CAR) on the surface of hepatocytes are supposed to be important for liver transduction, their effect on the liver sequestration of the vector has not been fully elucidated.

The AB-loop region of the fiber knob has one of the major responsible domains for CAR-binding. We have shown AB-loop redesigned cancer targeting Ad, which was identified with our direct library screening system from a high diversity Ad-formatted library, enabled efficient targeting cancer cells. Therefore, our new vector is applicable for the development of novel systemic therapy with Ad vector, and provides a suitable tool to analyze the effect of CAR binding to liver sequestration of adenoviral vectors. We hypothesize that the cancer targeted Ad with AB-loop redesigned fiber would enable efficient systemic treatment of cancer.

Two independent Ads with redesigned AB-loop, AdML-VTIN (targeting mesothelin) and AdML-VRLL (targeting PC3 cells against an unknown target), were successfully isolated from the high-throughput screening of same Ad library by infectivity. The in vitro binding and viral burst data indicated that these isolated Ads with AB-loop redesigned fibers successfully achieved selective infectivity for targeting cells with CAR-binding ablation. In intra-tumoral injection of AdML-VTIN, significant selective replication and efficient antitumor-effect were observed in mesothelin-expressing pancreatic cancer (Panc-1) xenografts.

Upon systemic administration in the nude mice with Panc-1 subcutaneous xenografts, AdML-VTIN and AdML-VRLL showed significantly lower liver sequestration compared to the wild-type Ad5 at 1 hour after the vector injection from tail vain (1/10). In PC3 xenograft model, the trend was similar to Panc-1 model. In addition, the copy number of mesothelin-retargeted virus was significantly higher in the Panc-1 tumor at 7 days after the vector injection than that of Ad with wild-type fiber (1000-fold). These data indicated that two independent-isolated cancer targeted Ad vectors with redesigned AB-loop exhibited significant CAR-binding ablation, dramatically augmented delivery to the tumors, and significantly reduced liver sequestration upon systemic administration.

In this study, the CAR-binding ablation with AB-loop redesigning enables efficient transductional-retarget and systemic vector delivery to cancer with reduction of Ad sequestration in the liver. This new genetically AB-loop redesigned Ad vector may embody a next generation of systemic therapy for cancer.

743. Prevention of radiation-induced salivary hypofunction by delivering hSFRP2 into submandibular glands

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Dry mouth, xerostomia, is a common side effect of irradiation treatment in patients with head and neck cancers which leads to significant patient morbidity. Secreted frizzled-related protein 2 (SFRP2) is an interesting protein, which can inhibit apoptosis, reduce fibrosis, enhance regeneration/proliferation and promote morphogenesis. SFRP2 is a Wnt inhibitor, and also considered a tumor suppressor gene. Based

on these biological functions, we hypothesized that overexpression of SFRP2 may be able to prevent radiation induced salivary gland damage without negatively affecting cancer therapy. To test our hypothesis, we constructed a hybrid adenoretroviral vector encoding human (h)SFRP2 (AdLTR2EF1ƒÑ-hSFRP2) and transduced murine submandibular glands (SMGs) 24 hours before beginning fractionated IR (6 Gy x 5 days). Our results demonstrate that the hSFRP2 gene transfer (108-1010 particles/gland; both SMGs/mouse) could preserve saliva flow rate while saliva flow rates, from animals transduced with the Ad-control vector were >50% lower than those of a non-IR group after 1,2 and 8 months. Gland and body weights from the SFRP2 gene transfer group were the same as the non-IR group and significantly higher than that of the Adcontrol-treated group. Initial results also demonstrated that SFRP2 gene transfer did not affect growth of the mouse squamous cell carcinoma, SCC VII. Further studies are required to fully understand the mechanisms by which hSFRP2 gene transfer protects murine SMGs from IR damage.

744. Abstract Withdrawn from Presentation

745. Abstract Withdrawn from Presentation

746. Determining the physiologic effect of B-Type Natriuretic Peptide on cardio-renal homeostasis

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The natriuretic peptide family has 3 structurally related members: atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). B-type natriuretic peptides are produced in cardiomyocytes and secreted into circulation where they take part in regulating a variety of cardiovascular and cellular functions. BNP regulates blood pressure and maintain salt-water balance by promoting natriuresis, diuresis, and vasodilation. BNP has also been shown to have anti-fibrotic properties, and counteract the renin-angiotensin-aldosterone system/AVP release. In our preliminary studies, we have demonstrated that adeno-associated virus (AAV) serotype 9-based vectors facilitate long-term cardiac BNP overexpression in spontaneously hypertensive rats (over 12 months), leading to reduced blood pressure and fibrosis, improved cardiac function and prolonged survival. To further understand the physiological roles of BNP, we developed and analyzed BNP knockout (KO) rats.

Results: BNP KO males and control rats were monitored for three months. Echocardiographic analysis showed the thickening of septal walls, increased left ventricular mass, and decreased ejection fraction. Blood pressure (BP) measurements revealed increased BP. Real-time PCR analysis showed statistically significant increases in angiogenesis and fibrosis markers (Col1, TGF-beta, Timp1, FN1) in heart, lung and kidney. Histology of sectioned cardiac tissue supported qualitatively increased fibrosis in KO rats, when compared with age-matched wildtype controls. Histology also shows micro-cyst like structures, glomerular sclerosis, basement membrane thickening and interstitial fibrosis in BNP KO rat kidney sections when compared to age-matched wildtype controls.

In contrast, BNP KO females did not show significant elevation of BP or fibrosis markers in heart and lung, although they suffered higher morbidity/mortality rates, and showed elevated blood BUN and Cre, increased renal fibrosis markers by real-time PCR. Further nine month followup confirmed exaggerated increases in BP, accelerated cardiac remodeling, and increased renal fibrosis in BNP KO animals compared to age-matched control rats.

Conclusions: Genetic disruption of BNP in ss/Dahl background was characterized by progressive BP elevation, decreased cardiac function, and increased fibrosis in cardiac tissue and auxiliary organs (kidney and lung) in rats. Our study supports the cardio-renal protective properties of BNP, and suggests the potential of BNP therapy for kidney disease, specifically in retarding cyst formation. To determine the

preventative effects of long-term BNP production on renal function, cyst formation, and the development of interstitial fibrosis, we aim to use AAV vectors to facilitate long term expression in a rat model of developing polycystic kidney disease.

747. Bortezomib induced unfolded protein response synergizes with oHSV to induce necroptic cell death in solid tumors

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Background: Oncolytic virus (oHSV) treatment is a promising biological therapy currently being evaluated in human patients for safety and efficacy. Bortezomib is a potent and selective proteasome inhibitor, which is currently FDA approved for treatment of multiple myeloma. Bortezomib has shown positive clinical benefits either alone or as combination therapy to induce chemo-/radiosensitization or to overcome drug resistance. We investigated the impact of oHSV (34.5ENVE) in combination with bortezomib in the multiple solid tumor models including ovarian, glioma and head and neck cancers.

Methods: To determine the interaction between oHSV and bortezomib pretreatment of cells in cancer cell killing, the combination index (CI) was calculated using Chou-Talalay analysis. Impact of treating cancer cells with bortezomib prior to infection with 34.5ENVE was measured by quantifying viral replication and infection. Impact of 34.5ENVE on bortezomib mediated cell killing was evaluated by western blot analysis flow cytometry and caspase 3/7 activity. Production of reactive oxygen species (ROS) was measured by CellROX and MitoSOX. The NADPH oxidase inhibitor, dipheylene iodide (DPI) and mitochondrial electron transport inhibitor, Rotenone, JNK inhibitor were utilized to investigate the mechanism of synergistic cell killing. In vivo, subcutaneous and intracranial tumor xenografts were utilized to study the impact of bortezomib and 34.5ENVE on anti-tumor efficacy.

Results: Treatment of cells with bortezomib and 34.5ENVE displayed strong synergism in multiple cancer cell line killing (ovarian, head&neck, and glioma cells) (CI index < 0.8). Bortezomib pretreatment increased virus replication (p value <0.001). Bortezomib treatment of cells induced ER stress, evident by strong induction of Grp78, heat shock proteins, CHOP, PERK and IRE1α (western blot analysis). Heat shock proteins have been shown to be important for virus replication. While cells treated with 34.5ENVE and bortezomib did not show a further increase in ER stress, apoptosis or blockade of autophagy compared to bortezomib alone treated cells they displayed much higher ROS levels (suggesting necroptosis as a possible mechanism). Moreover, Treatment of cells with a ROS scavenger rescued the increased synergistic killing. Increased phosphorylation of JNK and c-Jun was observed in cells treated with bortezomib and 34.5ENVE. Pretreatment with necroptosis inhibitor (necrostatin-1) and JNK inhibitor (SP0600125) reversed bortezomib and 34.5ENVE induced synergistic killing (CI>0.8). In vivo, the combination of bortezomib and 34.5ENVE significantly enhance anti-tumor efficacy and prolonged survival of mice.

Conclusions: The combination treatment of bortezomib and 34.5ENVE synergistically increase cancer cell killing, and provides a significant rationale for combination therapy of bortezomib and 34.5ENVE to achieve synergistic efficacy.

748. Assessing Small Vector Pharmacology Using the Cre-LoxP System in Mice

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Reporter genes for optical imaging are frequently used in the place of therapeutic transgenes to provide insight into where and for how long expression will likely occur. Large vectors with high transgene capacity can generally accommodate many types of reporter genes and even multiple reporters simultaneously. However for self-complementary adeno-associated virus (scAAV) vectors or replication-competent adenoviruses, some reporter genes are too large to fit within their genome capacity. To circumvent these problems, we first used Cre recombinase as a “cat’s paw” to activate reporter genes embedded in transgenic mice. The small Cre gene was introduced into self-complementary adeno-associated virus (scAAV) vectors with limited packaging capacity. Injection of scAAV-Cre vectors into LoxP-inactivated luciferase and mRFP/mGFP mice enabled simultaneous three reporter tracking. In this system, mice bear exactly one copy of luciferase and one copy of RFP-GFP, so each reporter gene is either “on” or “off” in a cell providing a quantum method for measuring vector delivery. To better identify which cells are being transduced, we developed an "inverse" reporter system in which the low capacity vectors carries an inactivated form of a reporter gene rather than Cre recombinase. For this use, the inactivated reporter vector can be injected into mice that express Cre recombinase in a tissue-specific fashion. When the inactivated vector is delivered to non-Cre-expressing cells, it remains inactive. In contrast, when it transduces Cre-expressing tissues in the transgenic mouse, only these specific tissues activate the reporter providing for specific cell transduction in the absence of reporter background. These cat's paw reporter systems provide a stringent system to detect low level transduction at cell-specific levels in vivo. They also allow packaging very small genes like the 1 kbp Cre cDNA into vectors with limited size capacity.

749. Abstract Withdrawn from Presentation

750. Hydrodynamic Delivery of mIL10 Gene Protects Mice from High-Fat Diet-induced Obesity and Glucose Intolerance

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Along with the changes of life style and diet structure, the prevalence of obesity has been increasing globally. Obesity can be induced by several factors among which high fat diet (HFD) remains as the dominant one. HFD-induced obesity is associated with low-grade inflammation, insulin resistance and glucose intolerance. The objective of this study is to assess the effect of IL10, an anti-inflammatory cytokine, on blocking high fat-diet induced obesity and obesity-associated metabolic disorders by hydrodynamic delivery of IL10-containing plasmid. Animals fed with regular chow or high fat diet received two monthly injections of plasmids containing green fluorescence protein (GFP) or mouse IL10 (mIL10) gene. Blood concentration of mIL10 reached approximately 200 ng/ml on day 7 in animals receiving mIL10 plasmid DNA. The transfection efficiency of liver cells was the same in animals fed with regular chow and high fat diet. No difference was seen in animals on regular chow when injected with plasmids containing either GFP or mIL10 gene. Overexpression of mIL10 prevented weight gain of animals on high fat diet. Glucose tolerance and insulin tolerance tests showed mIL10 maintained insulin sensitivity and prevented glucose intolerance. The mechanistic studies reveal that mIL10 suppressed macrophage infiltration and reduced the development of crown-like structures, leading to repressed transcriptions of pro-inflammatory cytokines in adipose tissue. Collectively, these data suggest that maintaining a higher level of IL10 through gene transfer could be an effective strategy in preventing diet induced obesity and its associated complications.

751. Heparin affinity purification of extracellular vesicles

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Introduction: Isolation and purification of extracellular vesicles (EVs) from in vitro and in vivo biofluids is still a major challenge and the most widely used isolation method still remains ultracentrifugation (UC) which requires expensive equipment and only partially purifies EVs due to co-precipitation of proteins and lipids. Affinity purification of biomolecules is an efficient way to achieve high purity without requiring expensive equipment. Previously we have shown that heparin blocks EV uptake in mammalian cells in culture, implying a possible heparin/EV interaction (Maguire et al., 2012) Here we investigate whether heparin can be utilized to isolate and purify EVs from conditioned media.

Materials and Methods: We compared three methods of purification: heparin affinity, ultracentrifugation and ExoQuick-TCTM. For heparin purification, one ml of Affi-Gel® Heparin Gel (Bio-Rad, Hercules, CA) was incubated with 1 ml of concentrated conditioned media (4°C). On day two, the supernatant was collected, as well as three PBS washes. Lastly, one ml of 2M NaCl was added to the beads and incubated overnight at +4°C. On day three the supernatant (eluate) was collected for downstream analysis. Another ml of concentrated conditioned media was ultracentrifuged at 100,000 x g for 90 minutes and the pellet resuspended in PBS and used in parallel with heparin-purified microvesicle. The last ml of concentrated conditioned media was used to isolate EVs with the commercially available kit ExoQuick-TC™ (System Biosciences, Mountain View, CA).

Results: Here we show that we can purify EVs from conditioned media using heparin-coated agarose beads. We confirm binding of EVs from two cancer cell lines (U87 and 293T) and normal HUVE cells. We directly compare heparin-purified EVs to UC prepared EVs and ExoQuick-TCTM for the following characteristics: (1) purity by silver-staining of SDS PAGE gels, (2) morphology by transmission electron microscopy (TEM), (3) EV markers by immunoblot and RNA analysis, and (4) functionality by labeled EV uptake into mammalian cells. Heparin-purified EVs were of a higher purity in terms of total protein content than UC EVs. Furthermore, they retained the RNA content, morphology, and functionality of UC EVs. Currently we are evaluating heparin-based affinity for the isolation of EVs associated with adeno-associated virus (AAV) vectors.

Conclusions: Heparin-purified EVs contain EV-specific RNA, DNA, and proteins and are functional at uptake into recipient cells. We have discovered a simple and effective way to isolate a highly pure population of EVs using their apparent affinity for heparin.

752. Preclinical study of AAV-sTRAIL: pharmaceutical efficacy, tissue distribution, and animal safety

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Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor (TNF) family known to induce apoptosis in a number of cancer cell lines. The recombinant soluble TRAIL (sTRAIL) has been in clinical trial for various human malignancies. However, it has a relatively short half-life time (4-5 hours) in human body. This study has evaluated the potency of AAV-CAG-sTRAIL in a mouse xenograft model and the animal safety in mouse and nonhuman primates. Mice with established human colon HCT116 or lung A549 tumors were given one i.p. injection with 1.5E11 v.g., 1.5E10 v.g. and 1.5E9 v.g. of AAV-CAG-sTRAIL, respectively. The tumor inhibitory efficacy was observed for 30 days after the administration. Tumor inhibitory rates were reached to 40-70%, which is equivalent to the positive control of CTX (50 mg/kg). For the study of tissue distribution, 1.5E9 v.g. and 1.5E10 v.g. of AAV-CAG-sTRAIL were i.m. injected to BALB/c mice, and the copy number of AAV-CAG-sTRAIL in blood and various tissues was detected for 1 day to 12 weeks after administration. It was shown that AAV-CAG-sTRAIL was mainly distributed in blood, bone marrow, spleen, liver, and the injected side of muscle. No distribution

was found in all critical organs, such as heart, bone marrow, brain and reproductive system. Four to 12 weeks later, beside the minor amount of AAV-CAG-sTRAIL was remained in the injection side, no virus particles could be detected in all organs, suggesting that AAV-CAG-sTRAIL is rapidly cleared up with the time course, and could not be cumulated in tissues. Meanwhile, same amount of AAV-CAG-sTRAIL were i.t. injected to nude mice with established ACC-2 tumors, the copy number of TRAIL gene in the tumor and organs were detected. Similar results to the tissue distribution were observed. Safety evaluations in mice and cynomolgus monkeys revealed that no abnormalities associated with AAV-CAG-sTRAIL administration. In conclusion, these studies have characterized the pharmaceutical efficacy, tissue distribution, and animal safety of AAV-CAG-sTRAIL in rodents and monkey. The data from these studies are useful to predict the pharmacokinetics and safety of AAV-CAG-sTRAIL in the clinical trials.

753. Non-myeloablative preconditioning with Ack-2 for cell therapy for Hunter disease

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Mucopolysaccharidosis type II (MPSII; Hunter syndrome) is a lysosomal storage disorder caused by a deficiency in the enzyme iduronate 2-sulfatase (IDS). At present, the permanently therapeutic approaches for MPSII are bone marrow transplantation (BMT). And Hematopoietic stem cell (HSC)-targeted gene therapy (GT) might represent an alternative approach for overcoming the shortage of BMT. But these therapies have some limitations. In BMT, strong conditioning regimens, such as the use of a large dose of chemotherapeutic agent and irradiation, can sometimes cause severe side effects, such as infection and bleeding. And auto-HSC-based GT need preconditioning to make the space to settle down for transplanted-gene-modified-cell. Therefore, establishing milder conditioning regimens is very important. We studied the efficacy of administering a novel sequential treatment of parenteral ACK2, an antibody that blocks KIT, followed by low dose irradiation (LD-IR) for conditioning of BMT for wild-type and Hunter syndrome mice. This protocol did not make 100% but comparatively higher chimerism in white blood cells of peripheral blood of recipient mice. And this chimerism o remained the same level at 16 weeks after transplantation. GAG levels were significantly reduced in some organs of animals treated with this protocol. The extent of reduction in these mice was almost identical to that with 100% chimera, which indicates reconstitution with 100% donor cells is not required to obtain a therapeutic effect following bone marrow transplantation. Our findings suggest type ACK2-based preconditioning as a novel non-myeloabrative preconditioning for HSC transplantation for Hunter syndrome.

754. Human Placenta-Derived Mesenchymal Stem Cells Augments Spinal Cord Functional Recovery in SCID Mice Model

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The potential of human placenta-derived mesenchymal stem cell (PD-MSC) transplantation as a method of repair in the spinal cord injury (SCI) may serve a number of different purposes that span various

therapeutic targets. This animal study have demonstrated that transplanted human PD-MSCs modify the inflammatory environment in the acute setting and reduce the effects of the inhibitory scar tissue in the subacute/chronic setting to provide a permissive environment for axonal extension. Histological studies have suggested that in the control tissues reactive gliosis with spongiosis, rosenthal fibres and perivascular lymphocytic infiltrates were seen. Also an area of infarction was present in control sections. While histology of SCI with PD-MSC infusion showed mild increased cellularity with preserved neuronal architecture. Inflammatory granulation tissue was seen at the site of injury. Human placenta-derived MSC transplantation immediately following the SCI resulted in functional locomotor recovery and normal bladder functions. In addition, grafted cells may provide a source of growth factors to enhance axonal elongation across spinal cord lesions. Other studies have suggested that BM-MSCs may help to replace lost or damaged neuronal tissue. Preliminary clinical data indicates that autologous bone marrow cell transplantation and/or GM-CSF administration can be used to treat patients with SCI without any immediate serious complications. Future studies on placenta-derived MSCs on the SCI model will provide insight into how these therapeutic benefits was obtained.

755. Correlation of molecular parameters with lipoplex structure and transfection efficacy in pyridinium-based cationic lipids

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Although promising, cationic lipid-mediated gene delivery can still be improved. In particular, significant improvements are needed in lipid design, lipoplex formulation and intracellular delivery. One of the key factors that influences gene delivery is the final macromolecular shape of the lipid-DNA complex. Of particular importance is the role played by lamellar and inverted hexagonal lipid phases in controlling different stages of the process. Pyridinium-based cationic amphiphiles represent a class of nonviral lipid vectors that have shown much promise as gene delivery vectors. In this study, the synthesis of pyridinium-based cationic lipid vectors, namely Di16:0 and Di16:1, is described, together with the structural characterization of the lipid-DNA complexes from small-angle x-ray scattering (SAXS) and the preliminary in vitro lipid-DNA lipoplex delivery into CHO-K1 cells. All lipoplex formulations studied exhibited plasmid DNA binding, protection from DNase I degradation and concentration dependent cytotoxicity. The transfection efficiency of Di16:0 and Di16:1 formulations containing the co-lipid cholesterol outperformed those containing DOPE, and both Di16:0 and Di16:1 formulated with cholesterol outperformed a commercial control vector. Finally, these results provided a basis to explore correlations with specific structural parameters in a quantitative relationship derived from a defined lipofection index (LI*) given by: LI* = CR(R/R+1)(logP_mix) (logP_+/|ΔlogP|) (|1-S_mix |). Where, for a given formulation, CR is the charge ratio of cationic lipid cations to DNA phosphate anions, R is the molar ratio of neutral co-lipid to cationic lipid, logP_mix is the molar weighted average logP of the cationic and neutral lipid, logP_+ is the logP of the cationic lipid, and |ΔlogP| is absolute difference in logP between the cationic lipid and the neutral lipid, |1-S_mix| is a term relating the molar weighted average molecular packing parameter of the mixture to the lamellar-hexagonal phase transition.

756. Transplantation of human adipose tissue-derived stem cells provides behavioral improvement, clinical onset delay and life extension in mouse

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that selectively affects motor neurons in the cortex, brain stem, and spinal cord. The precise pathogenic mechanism remains unknown and there is no effective therapy. We evaluated the therapeutic effects of human adipose tissue-derived stem cell (ADSC) in an animal model of ALS. ADSCs were transplanted into a mutant human SOD1G93A transgenic ALS model mouse (ALS mouse) by intravenous or intraventriclar injection (IVC). Delayed onset of clinical symptoms (28 days) and prolonged survival of animals (24 days) were observed in the transplanted ALS mice, and good survival of engrafted ADSCs was illustrated in the spinal cord. Investigation of cytokines in ADSCs demonstrated that ADSCs secrete high levels of neurotrophic factors such as NGF, BDNF, IGF1 and VEGF, and that reduction of apoptotic cell death by these cytokines was confirmed in cultured neural cells and in the spinal cord of ALS mouse. These results indicate that transplantation of ADSCs in ALS mice provides neuroptective effects by production of neurotrophic factors, delays disease progression, and prolongs survival of ALS mice.

Late Abstracts - Author Index

Late Abstracts - Author Index

Late Abstracts - Author Index

Late Abstracts - Author Index

Late Abstracts - Author Index