



Seed Grant Research Program – 2010 Recipients

Cardiovascular/Pulmonary Diseases

- Name:** David Clifton
Institution: University of Texas Medical Branch at Galveston
Galveston, TX
Project Title: Simulating Sunrise and Sunset by Gradual Transitions Between Light and Dark and their Effects on Diurnal Plasma Cortisol and Melatonin
Grant Amount: \$2,500
- Name:** Jeffrey Costas, MS
Institution: Arizona College of Osteopathic Medicine at Midwestern University
Glendale, AZ
Project Title: The Effects of Voluntary Exercise and Prednisolone on Dilated Cardiomyopathy and Cardiac Fibrosis in Dystrophin-Deficient Mice
Grant Amount: \$2,227
- Name:** Kelsey Derricks
Institution: Boston University School of Medicine
Boston, MA
Project Title: The Influence of Stretch and Proteases on an Engineered 3D Extracellular Matrix
Grant Amount: \$2,500
- Name:** Darshan Doshi, MD
Institution: Columbia University College of Physicians and Surgeons
New York, NY
Project Title: Location of KCNE1 and KCNE2 relative to hERG in the I(Kr) potassium channel
Grant Amount: \$2,500
- Name:** Curtis Gabriel
Institution: Vanderbilt University School of Medicine
Nashville, TN
Project Title: The Role of Insulin Resistance in the Development of Atherosclerosis in a Mouse Model of Lupus
Grant Amount: \$2,499
- Name:** Ann-Johanna Giaccone, MD
Institution: Children's Hospital of Philadelphia
Philadelphia, PA
Project Title: Milrinone Pharmacokinetics and Pharmacodynamics in Newborns with Persistent Pulmonary Hypertension of the Newborn
Grant Amount: \$2,372
- Name:** Anna Kamp, MD, MPH
Institution: University of Michigan Medical School
Ann Arbor, MI
Project Title: Can Signal-Averaged Electrocardiogram Predict Appropriate Device Therapies in Pediatric Patients with Implantable Cardioverter-Defibrillators Placed for Hypertrophic Cardiomyopathy?
Grant Amount: \$2,497.32
- Name:** Rung-chi Li, PhD
Institution: Touro University College of Osteopathic Medicine
Vallejo, CA
Project Title: Study of neuronal ischemic sensitivity in a transgenic mouse model of Alzheimer's Disease
Grant Amount: \$2,500

Cardiovascular/Pulmonary Diseases (continued)

Name: Joshua Meisner, MS
Institution: University of Virginia School of Medicine
Charlottesville, VA
Project Title: Systemic Coordination of Distant Ischemia and Collateral Artery Growth for Therapeutic Arteriogenesis
Grant Amount: \$2,500

Name: Shreyas Roy, MD, CM
Institution: SUNY Upstate Medical University
Syracuse, NY
Project Title: Novel Mechanism-Driven Dual Modality Therapy for Prevention of the Acute Respiratory Distress Syndrome in Septic Shock
Grant Amount: \$2,500

Name: Hersh Sagreiya
Institution: Stanford University School of Medicine
Stanford, CA
Project Title: Complete re-sequencing of genes critical to response to warfarin therapy
Grant Amount: \$2,428

Name: Shiraj Sen
Institution: University of Texas Medical School at Houston
Houston, TX
Project Title: Metabolic Signals as Regulators of Cardiac Growth Pathways
Grant Amount: \$2,500

Name: Keri Seymour, DO
Institution: SUNY Upstate Medical University
Syracuse, NY
Project Title: The Effect of Statins and NO-statins on Thrombospondin-1 Induced Vascular Smooth Muscle Cell Proliferation in Hyperglycemic and Hypercholesterolemic Cells
Grant Amount: \$2,500

Name: Natalie Shaw, MD
Institution: Massachusetts General Hospital
Boston, MA
Project Title: The effect of obstructive sleep apnea on nocturnal GnRH/LH secretion in children in early puberty
Grant Amount: \$2,500

Name: Jesse Sulzer
Institution: Louisiana State University Medical Center, New Orleans
New Orleans, LA
Project Title: Effects of hypertonic saline resuscitation during acute alcohol intoxication
Grant Amount: \$2,500

HIV/AIDS

Name: Deanna Cettomai, MHS
Institution: Johns Hopkins University School of Medicine
Baltimore, MD
Project Title: Evaluation of the Utility of Screening Tools and Quantitative Sensory Tests in the Diagnosis of HIV-Associated Peripheral Neuropathy in Western Kenya
Grant Amount: \$2,500

HIV/AIDS (continued)

Name: **Andrea Dean**
Institution: The Warren Alpert Medical School of Brown University
Providence, RI
Project Title: An SMS-based peer-support group: A pilot project using mobile technology to increase social support for HIV-positive pregnant women in South Africa
Grant Amount: \$2,010

Name: **Caitlin Elgarten**
Institution: Columbia University College of Physicians and Surgeons
New York, NY
Project Title: Evaluation of antiretroviral treatment outcomes among HIV infected children in a comprehensive care clinic in La Romana, Dominican Republic
Grant Amount: \$2,500

Name: **James Lester**
Institution: Edward Via Virginia College of Osteopathic Medicine, Blacksburg
Blacksburg, VA
Project Title: A Nanomedical Approach to Prevention of Neurodegeneration and Encephalopathy Induced by HIV-1 gp120 and Tat Proteins
Grant Amount: \$2,500

Name: **Greg Rice**
Institution: Kansas City University of Medicine and Biosciences, College of Osteopathic Medicine
Kansas City, MO
Project Title: HIV Tat Effects on the Drug Efflux Pump P-glycoprotein in an Enterocyte/Lymphocyte Coculture System
Grant Amount: \$2,500

Leukemia

Name: **Laura DiNardo**
Institution: University of Pennsylvania School of Medicine
Philadelphia, PA
Project Title: Effects of PI-103 and Rapamycin Synergism on mTOR Inhibition in Pre-B Cell ALL
Grant Amount: \$2,500

Name: **Lynn Rudner**
Institution: University of Utah School of Medicine
Salt Lake City, UT
Project Title: oTg, a New Model of Heritable T Cell Malignancy and Apoptosis Resistance
Grant Amount: \$2,495

Neoplastic Diseases

Name: **Christopher Alvarez-Breckenridge**
Institution: Ohio State University College of Medicine
Columbus, OH
Project Title: Deciphering and circumventing the natural killer response to OV therapy
Grant Amount: \$2,500

Name: **Hans Arora**
Institution: Northwestern University, Feinberg School of Medicine
Chicago, IL
Project Title: Determining the Utility of Fe₃O₄@TiO₂-Doxorubicin Nanoconjugates for Enhanced Delivery and Cytotoxicity in a Drug-Resistant Ovarian Carcinoma Model
Grant Amount: \$2,500

Neoplastic Diseases (continued)

Name: Akash Chandawarkar
Institution: Harvard Medical School
Boston, MA
Project Title: Tumor vessel normalization effects of MMP-14 inhibition
Grant Amount: \$2,500

Name: Kevin Chen
Institution: Duke University School of Medicine
Durham, NC
Project Title: Glioma and CMV peptide vaccines: impact of peptide length and temozolamide-included lymphodepletion on immune response
Grant Amount: \$2,500

Name: Dustin Deming, MD
Institution: University of Wisconsin School of Medicine and Public Health
Madison, WI
Project Title: Mutation Directed Therapy for the Treatment of Colorectal Cancer
Grant Amount: \$2,500

Name: Allen Ho
Institution: Harvard Medical School
Boston, MA
Project Title: Identification of Genetic Targets for Tumor Initiation in Glioblastoma via Direct P53 Loss
Grant Amount: \$2,500

Name: Nicolas Kummer
Institution: New York Medical College
New York, NY
Project Title: Tumor invasion and matrix metalloproteinase-1 and -3 expression in papillary thyroid carcinoma
Grant Amount: \$2,500

Name: Ta-Chiang Liu, MD, PhD
Institution: Washington University in St. Louis School of Medicine
St. Louis, MO
Project Title: The role of toll-like receptor 4 in hepatocellular carcinoma
Grant Amount: \$2,500

Name: Su Luo
Institution: Massachusetts General Hospital
Boston, MA
Project Title: Identification of c-Kit variants to construct a global mutation profile of the Spitz nevus
Grant Amount: \$2,500

Name: Benjamin Nacev
Institution: Johns Hopkins University School of Medicine
Baltimore, MD
Project Title: Exploring the anti-angiogenic activity and mechanism of the drug danazol
Grant Amount: \$2,500

Name: Michael Nguyen, MS
Institution: Washington University in St. Louis School of Medicine
St. Louis, MO
Project Title: Stromal Retraction as a Predictive Tool in the Prognosis and Clinical Management of Urothelial Carcinoma of the Urinary Bladder
Grant Amount: \$2,500

Neoplastic Diseases (continued)

Name: **Toral Patel, MD**
Institution: Yale University School of Medicine
New Haven, CT
Project Title: Evaluation of Convection Enhanced Delivery of siMGMT-loaded Nanoparticles in a Rat Model of Glioblastoma Multiforme
Grant Amount: \$2,500

Name: **Jarrold Predina**
Institution: University of Pennsylvania School of Medicine
Philadelphia, PA
Project Title: Evaluation of Ad.IFN α as an adjuvant to standard esophageal carcinoma treatment
Grant Amount: \$2,500

Name: **Sarah Russell**
Institution: Keck School of Medicine of the University of Southern California
Los Angeles, CA
Project Title: Notch1 Over-Expression in Head and Neck Cancer: Its Relationship to HPV and the Potential for Therapeutic Intervention
Grant Amount: \$2,500

Name: **Preeti Sukerkar**
Institution: Northwestern University, Feinberg School of Medicine
Chicago, IL
Project Title: Bioorthogonal Contrast Agents for Cancer Detection by Magnetic Resonance Imaging
Grant Amount: \$2,500

Name: **Rabi Upadhyay**
Institution: University of Massachusetts Medical School
Worcester, MA
Project Title: Development of a Quantitative Intra-operative Fluorescence Protease Imaging System for Surgical Tumor Margin Detection
Grant Amount: \$2,500

2010 AMA Foundation Seed Grant Recipient Project Abstracts

* Abstracts posted below are approved by the researcher to be made publicly available

Cardiovascular/Pulmonary Diseases Recipients

Jeffrey Costas, MS

Arizona College of Osteopathic Medicine at Midwestern University

“The Effects of Voluntary Exercise and Prednisolone on Dilated Cardiomyopathy and Cardiac Fibrosis”

Abstract: Dilated cardiomyopathy and cardiac fibrosis contribute to cardiac dysfunction in patients with muscular dystrophy. Although there is no cure for this disease, anti-inflammatories and low-intensity exercise are treatments aimed at improving cardiac function by maintaining muscle mass and reducing fibrosis. However, the effectiveness of these treatments have not been sufficiently studied. In this study, we test the hypothesis that the inflammatory-suppressing drug methylprednisolone and submaximal exercise slows the dystrophic histological changes in the left ventricle of the hearts of mice unable to express dystrophin. Our results show that methylprednisolone treatment reduced cardiac fibrosis relative to other treatment groups, as indicated by picrosirius red staining ($P < 0.05$). Exercised mice had significantly less cardiac fibrosis than control mice and mice treated with both methylprednisolone and exercise ($P < 0.05$). Mice treated with both methylprednisolone and exercise had significantly thinner left ventricular walls than methylprednisolone treated and exercise treated mice. No significant differences were found in heart mass to body mass ratio among treatment groups, nor were significant differences in the septolateral diameter of the left ventricle found among treatment groups. We conclude that methylprednisolone alone is the most effective treatment of those included in our analysis for reducing cardiac fibrosis. However, methylprednisolone treatment combined with moderate exercise had a deleterious effect on left ventricular wall thickness and cardiac fibrosis. These findings suggest caution is warranted when prescribing exercise with methylprednisolone for the treatment of cardiomyopathy in patients with muscular dystrophy.

Kelsey Derricks

Boston University School of Medicine

“The Influence of Stretch and Proteases on an Engineered 3D Extracellular Matrix”

Abstract: Emphysema is triggered by the inflammatory response to prolonged contact with cigarette smoke, exposure to indoor and outdoor pollutants, or a genetic defect. Inflammatory cells lead to increased levels of elastases and reduction of the natural protease inhibitor, alpha 1-antitrypsin. This leads to an overall increase in extracellular matrix (ECM) protein degradation and improper repair mechanisms of the main ECM proteins: elastin and collagen. Overall, a net increase in collagen and elastin synthesis is met with a decrease in mechanical sustainability. Alveolar airspaces increase in size, decreasing the overall surface area-to-volume ratio and making gas exchange both more difficult and less efficient. The proposed study aimed to explore various mechanical environments to understand how tissue strain modulated the stability of newly produced elastin and collagen fibers in an emphysematic state. Gelfoam matrices seeded with neonatal rat lung fibroblasts were subjected to stretch at various strains while being exposed to proteolytic elastase digestion. The resulting biochemical properties of the constructs were evaluated. Preliminary findings from our study showed that increased elastin matrix is produced on the Gelfoam scaffolds with ascorbate treatment. The natural matrix elastin is more susceptible to damage by elastase as quantified using an ELISA. In addition, the newly deposited matrix provided a more stable robust environment for cell matrix interactions resulting in greater cell retention after stretching and elastase treatments. Finally, a trend towards greater destruction in static conditions was noted. It can be postulated that a greater number of elastase binding sites become available on elastin or that binding frequency is altered. Future studies should evaluate the role of frequency modulation, longer stretching timepoints, and the role of other matrix proteins and proteoglycans. Eventually, therapeutic techniques could be developed that utilized what we have learned about matrix production and destruction to modulate the mechanics of the lung and help stop or reverse the emphysematic process.

Curtis Gabriel

Vanderbilt University School of Medicine

“The Role of Insulin Resistance in the Development of Atherosclerosis in a Mouse Model of Lupus”

Abstract: Introduction: Patients with the autoimmune disease systemic lupus erythematosus (SLE) are more susceptible to cardiovascular disease (CVD). The mechanism behind this predisposition is not known, but is presumably related to the increased prevalence of several CVD risk factors. One of these risk factors, insulin resistance (IR), is associated with SLE and may contribute to the high CVD burden in these patients; however, the mechanism behind this association has not been adequately explored. We have previously shown that the B6.SLE congenic mouse model of spontaneous SLE is prone to developing premature atherosclerosis. We hypothesize that this strain is also predisposed to developing insulin resistance, which could contribute to atherogenesis. We tested this hypothesis in a diet-induced obesity model of metabolic syndrome. Methodology: We examined the glucose homeostasis in B6.SLE mice using glucose tolerance tests (GTT) and by measuring fasting insulin levels. Mice were fed either low-fat diet (LFD, 10% calories from fat) or high-fat diet (HFD, 45% calories from fat). Age- and gender-matched B6 mice were used as controls in all experiments. GTTs were completed after 5 and 10 weeks of feeding and the glucose bolus was normalized to lean body mass. Fasting insulin and glucose levels were measured at baseline and after 5 and 10 weeks of feeding. Results: LFD-fed B6.SLE

mice had lower glucose tolerance than B6 controls after 10 weeks of feeding. No difference was seen between the HFDfed groups at this time point. No difference in glucose tolerance was seen after 5 weeks of feeding. No difference was seen in fasting insulin or glucose between B6.SLE mice and controls in either diet group at any time point. Discussion: LFD-fed B6.SLE mice had worsened glucose tolerance at the ten week time point. Interestingly, HFD did not seem to exacerbate glucose intolerance in B6.SLE mice relative to B6 controls. This indicates that HFD could mask differences in metabolic phenotype between strains and that a LFD-fed model will be more useful for subsequent experiments. Future experiments will investigate the immunological mechanisms which give rise to IR in this model and will determine whether IR contributes to cardiovascular disease.

Joshua Meisner, MS

University of Virginia School of Medicine

“Systemic Coordination of Distant Ischemia and Collateral Artery Growth for Therapeutic Arteriogenesis”

Abstract: Development of a robust collateral vasculature provides a critical survival advantage for patients with cardiovascular disease. However, insufficient understanding of how collateral vessels grow (i.e. arteriogenesis) and the coordination of this process between hemodynamics, inflammatory cells, and local and systemic signaling cascades have hindered the development of clinically success therapies for stimulating arteriogenesis. Though poorly understood, evidence suggests that the spatially separated primary stimuli for collateral vessel growth—ischemia and altered shear stress—can feedback on each other. Analysis of such "long-range" feedback may provide key targets that can be harnessed for therapeutic treatment of vascular disease that are effective through systemic administration. To demonstrate the potential of the systemic feedback between ischemia and altered shear stress in guiding arteriogenesis, this study sought to quantify changes in shear stress between non-ischemic (where arteriogenesis is absent) and ischemic (where arteriogenesis occurs) hindlimb arterial ligation. To quantify these changes, we developed a method of using laser speckle flowmetry to measure changes in blood flow in intact gracilis collateral arterioles. Second, this study sought to measure whether the addition of an ischemic stimulus is sufficient to induce arteriogenesis (i.e. works synergistically with altered shear stress) in a model of non-ischemic hindlimb arterial ligation. Mice were preconditioned with ischemia through abdominal aortic occlusion or concurrently underwent arterial ligation in the forelimb coupled with non-ischemic hindlimb arterial ligation to test whether temporally or spatially separated ischemia, respectively, could stimulate arteriogenesis in the non-ischemic hindlimb arterial ligation model. Changes in arterial growth were measured by whole mount immunohistochemistry of gracilis collateral vessels. While initial results proved inconclusive, the methods developed for quantifying collateral blood flow in vivo and directly testing how remote ischemia can impact arteriogenesis will allow our laboratory and other laboratories to answer these questions in the near future.

Natalie Shaw, MD

Massachusetts General Hospital

“The effect of obstructive sleep apnea on nocturnal GnRH/LH secretion in children in early puberty”

Abstract: The transition from the quiescence of the reproductive axis in childhood to its reactivation during puberty is characterized by sleep-specific augmentation of luteinizing hormone (LH) activity. We hypothesized that LH secretion would be specifically associated with deeper stages of sleep, as is the case for growth hormone. Further investigation of this relationship is important to our understanding of the control of GnRH secretion during puberty and will address the question of whether obstructive sleep apnea (OSA), which affects 1% of children, interferes with normal maturation of the reproductive axis. To answer these questions, we enrolled 4 children, 11.8-14.4 yr-old, in Tanner stages II-IV, with OSA treated with continuous positive airway pressure (CPAP). Subjects underwent 2 studies, spaced 1-2 mo apart, consisting of frequent blood sampling for LH and polysomnography on or off CPAP in random order. ANOVA was used to compare pulse characteristics in each sleep stage controlled for time in that stage. We found that LH pulses occurred most frequently during stage N3 (slow-wave sleep) compared to N2, N1, REM, or wake following sleep onset (1.5 ± 0.3 [mean \pm SE] vs 0.4 ± 0.1 , 0 , 0.1 ± 0.1 and 0 pulses/hr of sleep stage, respectively; $p < 0.001$) and there was no effect of treatment (CPAP) on this relationship. Unlike pulse frequency, LH pulse amplitude did not vary by sleep stage. These findings suggest that slow-wave sleep may stimulate LH secretion during puberty. In children with OSA, one night of sleep without CPAP does not appear to disturb this temporal relationship.

Jesse Sulzer

Louisiana State University Medical Center, New Orleans

“Effects of hypertonic saline resuscitation during acute alcohol intoxication”

Abstract: Acute alcohol intoxication (AAI) impairs the hemodynamic response to hemorrhagic shock (HS) and fluid resuscitation (FR) with lactate Ringer's (LR), attenuates the HS-induced rise in plasma arginine vasopressin (AVP), and increases organ injury following HS. Studies suggest that AAI enhances nitric oxide (NO) inhibitory tone in the paraventricular nucleus (PVN) contributing to a blunted AVP response to HS. In contrast, AAI does not impair the AVP response to hypertonic saline (HTS), which we predict is due to suppressed PVN NO levels in AAI non-HS animals. Thus we hypothesized that FR with HTS would decrease NO in the PVN and enhances AVP release leading to improved blood pressure (MABP) recovery and decreased organ injury in AAI-HS Male Sprague Dawley rats received a 15h alcohol infusion ($2.5\text{g/kg} + .3\text{g/kg/h}$) or isovolumic dextrose (DEX) followed by HS ($40\text{mmHG} \times 60 \text{ min}$) and FR with HTS (7.5% ; 4ml/kg) or LR (2.4x blood volume removed). HTS decreased NO in the PVN and enhanced ($\sim 66\%$) circulating AVP vs LR in AAI- and DEX-HS 2h post-FR. HTS improved initial MABP recovery vs LR in AAI (109 vs 80 mmHG) and DEX (114 vs 83 mmHG) and decreased circulating

levels of alanine aminotransferase, an indicator of hepatic injury, following HS during AAI. Peripheral antagonism of the V1a receptor, the receptor responsible for the pressor effects of AVP, prior to FR prevented the pressor effect of HTS without altering MABP recovery in LR-treated animals. These results suggest that resuscitation with HTS in AAI+HS removes central inhibition of NO restoring circulating AVP and that the pressor effect of HTS is dependent on stimulation of AVP release. Furthermore, resuscitation with HTS can limit early organ damage following AAI-HS.

HIV/AIDS Recipients

Deanna Cettomai, MHS

Johns Hopkins University School of Medicine

“Evaluation of the Utility of Screening Tools and Quantitative Sensory Tests in the Diagnosis of HIV-Associated Peripheral Neuropathy in Western Kenya”

Abstract: OBJECTIVE: To investigate the utility of quantitative sensory testing (QST) and neuropathy screening tools in identifying moderate to severe peripheral neuropathy (PN) in resource-limited settings. BACKGROUND: PN is the most common neurological complication of HIV but is widely under-diagnosed and under-treated in resource limited settings. DESIGN/METHODS: We enrolled 240 HIV-infected outpatients in western Kenya using two-stage cluster randomized sampling. Non-physician healthcare workers (HCW) administered the Neuropathy Severity Score (NSS), Single Question Neuropathy Screen (SQNS), Subjective Peripheral Neuropathy Screen (SPNS), and Brief Peripheral Neuropathy Screen (BPNS). NSS was created by combining the SPNS with a functional status assessment. BPNS included assessment of symptoms and ankle reflexes. QST consisting of graduated tuning fork (TF), monofilament, and two-point discrimination (2PD) examinations was performed by study staff. Each tool was validated against the gold standard exam and clinical assessment of PN by trained study staff. RESULTS: The sample was 65% female with mean age 36.4 years, median CD4 count 324 cells/ μ L, and 45% were WHO Stage 3 or 4. Per the gold standard, 71% (170/240) of participants had PN with 18% (42/240) having moderate/severe PN. NSS, SQNS, and SPNS were 76% sensitive in diagnosing moderate/severe PN with specificities ranging from 56% (SQNS) to 61% (SPNS) and accuracies from 59% to 63%. BPNS had the lowest sensitivity (17%). Agreement between ankle reflexes assessed by HCW during administration of the BPNS and study staff was poor (κ = 0.09). Monofilament examination had the highest diagnostic utility for moderate/severe neuropathy with 68% sensitivity, 93% specificity, 89% accuracy, a positive likelihood ratio of 10.18 and a negative likelihood ratio of 0.35. TF was 76% accurate, 56% sensitive and 81% specific. 2PD was 75% sensitive, 53% specific and 57% accurate. Patients with moderate/severe neuropathy were significantly more likely to be older, taller, male and have lower nadir CD4 counts. CONCLUSIONS: SQNS, monofilament and TF examinations demonstrated high diagnostic utility in this study and may provide objective methods for routine clinical and research use in resource-constrained settings.

Caitlin Elgarten

Columbia University College of Physicians and Surgeons

“Evaluation of antiretroviral treatment outcomes among HIV infected children in a comprehensive care clinic in La Romana, Dominican Republic”

Abstract: BACKGROUND: Few studies have examined indications for and response to second-line therapy (SLT) among children in resource-limited settings with inconsistently available virologic monitoring. METHODOLOGY: We reviewed medical records of HIV-infected children initiating ART from 10/2004-12/2008 at CFLR. Primary outcome was switch to SLT, defined as initiation of a PI-based regimen and concurrent NRTI change. RESULTS: 111 children met study criteria. Median age was 6.1 years [inter-quartile range (IQR)=2.7-9.4], 50% were female, 50% were orphaned, 18% resided in sugar plantation settlements of primarily Haitian migrants (bateys). 96% were infected by mother-to-child transmission (MTCT), 8% despite MTCT-prevention interventions. Median baseline CD4 was 425 cells/ μ L [IQR=193-846]. Initial regimen was NNRTI-based for 87% (PI-based for remainder). Six died (5.4%): median time to death, 17 days [IQR=10-30]. No variable examined predicted mortality. Median follow-up time of the cohort after ART initiation was 33 months [IQR=22-44]. 13/86 switched to SLT (0.5 switches/10 person-years). Documented criteria for switch were the following: poor virologic response, 1; poor immunologic response, 5; clinical progression, 1; multiple criteria, 4; undocumented criteria, 2. Median time to switch was 18.7 months [IQR=13.4-26.1]. Second-line was didanosine+abacavir+lopinavir/ritonavir for all. Switch to SLT, compared with continued first-line, was associated with lower median baseline CD4 (187 versus 436 cells/ μ L, $p=0.028$) and baseline CDC clinical category C (100% versus 56%, $p=0.001$). Anemia, malnutrition, batey residence, treatment for TB co-infection and orphanhood did not predict switch. Of 14 starting SLT, one died 6 months post-switch; 13 survived (median follow-up: 18.6 months [IQR=11.6-26.3]). Median CD4 increases after 6 and 12 months were 166 and 331 cells/ μ L, respectively. CONCLUSIONS: Regimen switch was associated with advanced clinical and immunological disease at ART initiation. These findings suggest that prompt diagnosis and timely treatment of HIV are associated with durability of first-line regimens, hence minimizing need for switch. Response to second-line ART was excellent.

Greg Rice

Kansas City University of Medicine and Biosciences, College of Osteopathic Medicine

“HIV Tat Effects on the Drug Efflux Pump P-glycoprotein in an Enterocyte/Lymphocyte Coculture System”

Abstract: Background: Despite the development of effective antiretroviral therapies for the treatment of HIV, eradication has not been possible primarily because of viral persistence within reservoirs. Although the mechanism of maintenance of reservoirs is not well understood, overexpression of the drug efflux protein P-glycoprotein within the Gut Associated Lymphoid Tissue (GALT) may limit drug penetration into this important reservoir tissue. The HIV transcription protein Tat has been shown to upregulate P-glycoprotein in endothelial cells of the brain, another important viral reservoir, yet a relationship within the GALT has not been demonstrated. Our hypothesis is that HIV Tat increases P-glycoprotein expression within endothelial cells associated with the GALT, thereby limiting GALT drug penetration. Methods: To examine the effects of HIV Tat on P-glycoprotein expression, a culture of Caco-2 cell monolayers (model for intestinal enterocytes) and a transwell co-culture of Caco-2 cells and Jurkat cells (lymphocytes) were both exposed to 50nM recombinant Tat for 24 hours. Western blotting for Caco-2 cell P-glycoprotein was performed and samples normalized to β -actin. Western blot data was quantified as relative density using Image J software (NIH). Results: When exposed to Tat, average P-glycoprotein protein intensity in Caco-2 co-culture samples was 0.74, compared with an average of 0.43 for untreated co-culture controls (n=7, p=.0039). Average P-glycoprotein protein intensity in the Caco-2 monolayer exposed to Tat was 0.47, compared with an average of 0.78 in untreated controls (n=5, p=.077). Conclusions: Preliminary results indicate a statistically significant increase in enterocyte-specific P-glycoprotein in Caco-2/lymphocyte co-culture cells exposed to Tat when compared with untreated controls. When lymphocytes are absent, there is a non-statistically significant increase in enterocyte P-glycoprotein expression upon Tat exposure, suggesting a possible role for cell signaling by lymphocytes. These findings support our hypothesis that HIV Tat upregulates P-glycoprotein expression in enterocytes, which may play a crucial role in limiting drug transport into the GALT and facilitate the maintenance of HIV reservoirs in the GALT.

Leukemia Recipients

Laura DiNardo

University of Pennsylvania School of Medicine

“Effects of PI-103 and Rapamycin Synergism on mTOR Inhibition in Pre-B Cell ALL”

Abstract: mTOR integrates multiple signaling cascades to act as a critical mediator of lymphocyte survival and proliferation. The mTOR inhibitor (MTI) rapamycin (rap) suppresses proliferation and induces apoptosis of pre-B ALL in vitro and in human ALL xenografts, although lymphoid growth factors such as IL7 can overcome these effects. We hypothesized that PI3K inhibition would potentiate the action of MTI by blocking growth factor signals upstream of mTOR. PI-103 is a dual MTI and class I PI3KI. In contrast to rap, which selectively inhibits mTOR Complex (TORC) 1, PI-103 inhibits both TORC1 and TORC2. Murine and human pre-B ALL cell lines were treated with combinations of PI-103, rap, and IL7. Growth inhibition and cell death were assessed via MTT proliferation assays and Annexin V flow-cytometry, respectively. Post-translational modifications of downstream targets of mTOR such as S6, AKT, and 4E-BP1 were assessed by immunoblots. PI-103 alone decreased cell proliferation and increased cell death in a dose-dependent manner, with IC50 values of approximately 250 nM and 100 nM for human and murine pre-B ALL cell lines, respectively. Combinations of PI-103 and rap demonstrated synergistic inhibition at IC50 dosing on both human and mouse ALL lines. Additionally, co-treatment with 1 μ M PI-103 and 10 ng/ml rap fully inhibited cell proliferation, compared to 40% inhibition with rap alone. This same combination resulted in 50% cell death, versus 15% with rap alone. While IL7 fully reversed rap-mediated inhibition, the addition of 1 μ M PI-103 effectively blocked this IL7-mediated reversal. PI-103 + rap also decreased phosphorylation of S6, AKT, and 4E-BP1 more than that achieved with either agent alone. IL7 was able to reverse this effect (increased phosphorylation) when given with rap, but not in the presence of PI-103. These results suggest the presence of nonoverlapping inhibitory effects of these agents on mTOR signaling. Our data support the notion that treatment with combination therapy targeting multiple nodes of the growth factor-PI3K-mTOR pathway could improve efficacy and reduce MTI resistance in pre-B ALL.

Neoplastic Diseases Recipients

Christopher Alvarez-Breckenridge

Ohio State University College of Medicine

“The role of NK cells in the context of herpes simplex virotherapy for glioblastoma”

Abstract: It is controversial as to whether the host immune response hinders or improves the efficacy of oncolytic Herpes Simplex viral (oHSV) therapy of glioblastoma (GBM). Natural killer cells (NK) limit viral infections, and previous work suggests they may similarly attenuate virotherapy. Using both xenograft and syngeneic intracranial GBM tumor models, we used flow cytometry to evaluate the temporal pattern and phenotypic characteristics of NK cells present in the periphery and recruited to the site of oHSV infection. Within hours after infection and continuing through 72 hours following oHSV inoculation, NK cells were rapidly recruited to tumor bearing hemispheres and possessed an activated phenotype. This robust NK response was confirmed to be detrimental to OV efficacy through the enhanced survival of NK depleted mice inoculated with oHSV compared to oHSV treated mice possessing NK cells. In vitro, human NK cells preferentially lysed oHSV-infected GBM in a cell contact, perforin, and DNAM-1 dependent manner. Fusion proteins were used to detect currently unknown ligands for the NK natural cytotoxicity receptors (NCR) and decipher the critical NK activating ligands that mediate this response. Following oHSV infection of a panel of GBM stem cells and cell lines, we detected robust up-regulation of ligands for NKp46 and NKp30

specifically within virally infected cells. Additionally, blocking antibodies against either NKp30 or NKp46 abrogated NK mediated clearance of oHSV infected GBM. We have previously shown that immunomodulation with cyclophosphamide (CPA) and valproic acid (VPA) enhances oHSV efficacy. CPA administered prior to virus inoculation abrogated the oHSV induced NK and macrophage recruitment into the tumor at all time points tested compared to oHSV alone. Similarly, VPA treatment resulted in a decline in NK and macrophage recruitment at 6 and 24 hours post-oHSV; however, a robust increase at 72 hours-post-oHSV was seen, resembling the response seen with oHSV alone. VPA was also found to have a profound immunosuppressive effect on human NK cells in vitro. NK cytotoxicity was abrogated following exposure to VPA through down-modulation of cytotoxic gene expression and granzyme B protein levels. In addition, IFN- γ was suppressed in a Stat5/T-bet dependent manner.

Kevin Chen

Duke University School of Medicine

“Glioma and CMV peptide vaccines: impact of peptide length and temozolamide-included lymphodepletion on immune response”

Abstract: Background: Even with surgery, radiation and chemotherapy, median overall survival remains less than 15 months for patients with glioblastoma multiforme (GBM). Many groups have demonstrated expression of cytomegalovirus (CMV) antigens in greater than 90% of GBMs while normal brain is negative; thus, CMV epitopes are ideal targets for immunotherapy. Furthermore, although chemotherapy and radiation might intuitively have detrimental effects on immunotherapy, vaccine responses may actually be augmented during recovery from lymphodepletion. Thus, this project studied optimized peptide vaccine regimens as well as immunity against CMV-targeted peptide vaccines for eventual translation to GBM therapy. Methods: C57Bl/6 mice received adoptive lymphocyte transfer of ovalbumin (OVA)-reactive T-cells followed by TMZ-lymphodepletion, class I OVA vaccine and/or class II OVA peptide. Peripheral CD8+/OVA-tetramer+ T-cells were counted by flow cytometry. A mouse model of human immunity (HLA-A2.1) received HLA-A2.1-restricted peptide vaccine or pp65 peptide mix. Functionality of T cells was assessed by cytometric bead-array. A separate cohort received CMV glycoprotein B (gB) epitope conjugated to carrier protein KLH. Humoral response was evaluated using bead-linked ELISA and CMV neutralization assay. Results: CD8+/OVA-tetramer+ T-cells elicited by OVA class I peptide vaccine (0.69%) were not significantly affected by TMZ lymphodepletion ($p=0.63$) nor by concomitant administration of class II OVA peptide ($p=0.11$). The combination, however, of class I and class II OVA peptide during recovery from TMZ-induced lymphodepletion appeared synergistic: a 4-fold increase in CD8+/OVA-tetramer+ T-cells was observed (2.96%, $p=0.004$). Vaccines against pp65 peptide mix demonstrated splenocyte responses in IFN γ ($p=0.089$), TNF ($p=0.049$) and IL-2 ($p=0.021$). Significant titers of antibodies specific to a neutralizing epitope of gB were also elicited ($p=0.0003$) and these antibodies were functionally capable of neutralizing live virus ($p<0.05$). Conclusions: Immune responses to peptide vaccines appear to be maximized when co-administered with class II peptides in the context of recovery from TMZ-induced lymphodepletion. Feasibility of engendering functional cytotoxic and humoral responses to CMV peptide vaccines is demonstrated. Subsequent studies will apply optimized vaccine conditions to CMV peptide vaccines, followed by CMV-expressing tumor models. Translation of these preclinical studies offers a new therapeutic option for GBM patients.

Allen Ho

Harvard Medical School

“Identification of Genetic Targets for Tumor Initiation in Glioblastoma via Direct P53”

Abstract: Glioblastoma (GBM) is the most common form of brain tumor, and one of the most lethal cancers with a median survival of 12 months despite implementation of the most intensive therapeutic modalities and supportive care. Recent work by The Cancer Genome Atlas Research Network utilizing large cohorts of human cancer tissue through integrated multi-dimensional analyses have identified TP53 and PTEN as the two most commonly perturbed tumor suppressor genes. Half of the p53 suppression in this study was due to ARF deletions. Deletion of the Ink4a/ARF locus on chromosome 9p21 is also among the most frequent cytogenetic events in human cancer including GBM. Arf binds to and inactivates the MDM2 protein which can act as an E3 ubiquitin ligase to target p53 for proteasomal degradation. The Depinho laboratory has recently studied and demonstrated that Pten and p53 deficiencies together might be the root cause of gliomagenesis by affecting NSC self-renewal and differentiation potential, leading to the transformation of these NSCs into “cancer stem cells”. However, we have found that while p53^{-/-}Pten^{-/-} mice reliably generate tumors, our murine Ink4a/Arf^{-/-}Pten^{-/-} model does not. Therefore, there must be some additive effect of direct p53 inactivation at the neural stem cell level that is leading to tumorigenesis. My project is a characteristic and genetic comparison between these two mouse transgenic models to delineate the differences between p53 down-regulation via Ink4a/Arf deletion and direct p53 suppression. These genetic differences will help delineate the mechanism of tumor initiation in human glioblastoma.

Nicolas Kummer

New York Medical College

“Tumor invasion and matrix metalloproteinase-1 and -3 expression in papillary thyroid carcinoma”

Abstract: Aggressive papillary thyroid carcinoma (PTC) has a poor prognosis, and little is known about its pathogenesis. The BRAFV600E mutation accounts for up to 60% of PTC, and is associated with aggressive disease. We have investigated the clinical and functional relationships between the BRAFV600E mutation and both metalloproteinase expression and tumor invasion. Sixteen PTC samples were stratified into four arms (Arm-1, invasive and BRAFV600E, N=4; Arm-2, noninvasive and BRAFV600E, N=6; Arm-3, invasive and wt BRAF, N=2; Arm-4, non-invasive and wt BRAF,

N=4), and analyzed MMP-1 and -3 mRNA by quantitative real-time RT-PCR. Statistically significant differences in GAPDH-normalized MMP-1 expression (reported as $2dCT \pm SEM$; p determined by Wilcoxon signed rank test) were observed between ARM-1 (0.044 ± 0.009) and Arm-4 (0.009 ± 0.001 ; $p=0.001$), and the combined BRAFV600E groups, Arm-1 plus Arm-2 (0.032 ± 0.005), compared to the BRAF wt groups, Arm-3 plus Arm-4 (0.010 ± 0.002 ; $p=0.002$). Functional relationships between BRAFV600E, MMP-1 expression and invasive phenotype were explored using the BRAFV600E cell lines, BCPAP (PTC), and 8505C (anaplastic thyroid carcinoma). The chemical MEK1/2 inhibitor, U0126, significantly decreased MMP-1 levels in cell lysates and conditioned media of both cell lines, as measured by western blots and gelatin zymography, respectively. Similar results were observed using BRAF-targeting siRNA in BCPAP cells. Inhibition of BRAF signaling was validated by western blot for ERK phosphorylation for both U0126 and siRNA treatment groups. The BRAF wild type PTC cell line, TPC-1, and normal thyroid cell line NTHY-ori did not express MMP-1. Functionality of MMP-1 expression was demonstrated using the invasion/migration assay employing extracellular matrix coated chambers. Control BCPAP cells demonstrated a $64\% \pm 5$ invasion/migration index, which was significantly reduced by U0126 ($5\% \pm 3$ $p=0.0002$), BRAF siRNA ($25\% \pm 19$ $p=0.0088$), and an inhibitory MMP-1 antibody ($4\% \pm 3$ $p=0.002$) (Results reported as $100\% \times \text{invading cells/migrating cells} \pm SD$, p determined by t-test). These data demonstrate a correlation between BRAFV600E positive PTC and increased MMP-1 expression in clinical samples, and expose a functional and targetable relationship with an invasive phenotype, that may be exploited for clinical benefit.

Su Luo

University of Miami Miller School of Medicine; research completed at Massachusetts General Hospital
"Agminated segmental nevi demonstrating intranevic concordance of BRAF status"

Abstract: Agminated Segmental Nevi (ASN) are characterized by multiple discrete lesions that cluster within a confined developmental segment. Like other segmental conditions, this unusual clinical presentation of pigmented lesions is likely due to mosaicism. Recently, mutations in several RAS pathway genes have been described in various sporadic melanocytic nevi. However, given the rarity of ASN, no molecular analyses of these lesions have been reported. In this study, we hypothesized that the mosaic event may in fact be an activating change at one of the loci mutated in common pigmented nevi. In order to test this hypothesis, we performed genetic analysis on 11 distinct nevi from within the segment and an isolated nevus from outside of the affected region. Results from direct sequencing were cross-validated using the Luminex platform, an allele-specific, multiplex bead-based assay. Uniformly, all 11 segmental nevi revealed the identical BRAFT1799A mutation while the single nevus outside the segment and the patient's germline DNA were both wildtype for BRAF. Strict concordance among lesions within the ASN lends strong support to the notion that the activating change in BRAF may be the primary somatic event explaining this patient's mosaic pattern. These findings do provide novel insight into the molecular underpinnings of melanocytic mosaicism.

Toral Patel, MD

Yale University School of Medicine

"Evaluation of Convection Enhanced Delivery of siMGMT-loaded Nanoparticles in a Rat Model of Glioblastoma Multiforme"

Abstract: Treatment of intracranial diseases is severely limited by the inability of systemically-delivered agents to cross the blood-brain barrier. Convection-enhanced delivery is a promising technique for local delivery of agents in the brain, but it could be improved by the use of delivery vehicles that provide controlled release. Here, we present a novel method for fabricating ultrasmall polymer nanoparticles that are optimized for convective delivery. A single emulsion, solvent-evaporation process was used to fabricate poly(lactide-co-glycolide) (PLGA) nanoparticles loaded with coumarin-6 (C6). High-speed partial centrifugation was used to collect ultrasmall (60 – 100 nm) nanoparticles. Conventional techniques were employed to produce standard-sized (~150 nm) PLGA nanoparticles. Particle size and morphology was characterized by scanning electron microscopy (SEM). Eight rats ($n = 4$ per group) underwent stereotactic infusion of C6 nanoparticles (20 μ L; 100 mg/ml; 7.5 – 15 psi; 0.67 μ L/min) into the right caudate. Animals were sacrificed 30 minutes post-infusion; brains were harvested and frozen. Serial coronal slices were obtained and imaged using a fluorescent stereoscope. Slices were then analyzed and volume of distribution (Vd) was calculated using a custom Matlab script. SEM of the C6-loaded PLGA nanoparticles demonstrated that we were able to produce ultrasmall polymer nanoparticles (mean diameter: 71.3 nm, SD: 13.2 nm) with good morphology (spherical). In comparison, the standard-sized polymer nanoparticles, produced using published formulations, had a mean diameter of 147.3 nm (SD: 27.2 nm). Mean Vd for the ultrasmall particles was 73.62 mm^3 (SD: 13.18 mm^3), while mean Vd for standard-sized particles was 11.33 mm^3 (SD: 5.89 mm^3); a statistically significant difference ($p < 0.05$). These findings confirm that ultrasmall (<75 nm diameter) polymer nanoparticles can be delivered to a large volume in the brain through CED at a single site. We are now in a position to adequately address the infiltrative nature of malignant gliomas via direct interstitial infusion of drug-loaded nanoparticles. Ongoing work in our lab has demonstrated the feasibility of encapsulating and delivering both siRNAs and chemotherapeutics via these nanoparticles. We anticipate that this strategy will have significant clinical impact.

Jarrod Predina

University of Pennsylvania School of Medicine

"Evaluation of Immunotherapy as an adjuvant to standard esophageal carcinoma treatment"

Abstract: Purpose: Esophageal cancer is associated with a dismal prognosis. For resectable disease standard-of-care is neoadjuvant cisplatin/5-fluorouracil followed by surgery. However, over 40% of these patients will have recurrences. A major hurdle in improving outcomes is the lack of animal models to evaluate novel therapies. Our group has developed

the first readily reproducible syngeneic model of esophageal cancer, AKR. Using our model, we tested the hypothesis that gene-mediated cytotoxic immunotherapy (GMCITM) synergizes with neoadjuvant chemotherapy. Experimental Design: AKR tumor cells were injected into flanks of syngeneic, immunocompetent mice (n=200) in several experiments. Mice bearing tumors were treated with mouse-equivalent dosages of cisplatin/5-fluorouracil, Ad.HSV-tk/GCV, or combination treatment. The mechanism of Ad.HSV-tk/GCV was elucidated using CD8-depletion studies. Human cancer cell infectivity for Ad.HSV-tk/GCV was confirmed using in vitro transduction techniques. Results: Growth of AKR flank nodules was observed in 98% of mice injected with tumor cells. Treatment of mice bearing established AKR tumors with cisplatin/5-fluorouracil plus Ad.HSV-tk/GCV inhibited tumor growth by 80% and was superior to cisplatin/5-fluorouracil or Ad.HSV-tk/GCV alone (p=0.02). Of those mice receiving the combination, 40% were alive at 60 days versus none in the groups receiving monotherapies (p=0.04). The efficacy of Ad.HSV-tk/GCV was negated using CD8 T-cell depletion. Infectivity of human esophageal cancers was superior to AKR for all tested viral concentrations (p<0.05 at all concentrations tested). Conclusion: These results suggest that Ad.HSV-tk/GCV therapy augments cisplatin/5-fluorouracil for esophageal cancer and functions in a CD8 T-cell dependent mechanism. The use of Ad.HSV-tk/GCV provides a promising option to improve the prognosis in patients managed with preoperative cisplatin/5-fluorouracil.