

UNIVERSITY OF COLORADO DENVER
ANSCHUTZ MEDICAL CAMPUS

25TH ANNUAL STUDENT RESEARCH FORUM

AND

STUDENT RESEARCH AWARDS CONVOCATION

GRADUATE SCHOOL
SCHOOL OF MEDICINE
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DECEMBER 14, 2010
ANSCHUTZ MEDICAL CAMPUS
EDUCATION 2 NORTH/SOUTH

25TH ANNUAL
UNIVERSITY OF COLORADO DENVER
ANSCHUTZ MEDICAL CAMPUS

STUDENT RESEARCH FORUM

Tuesday, December 14, 2010

Poster Sessions

1:00-2:15 pm

2:15-3:30 pm

Awards Convocation

4:30 pm

ED2 South Room1102

ANSCHUTZ MEDICAL CAMPUS
EDUCATION 2 NORTH/SOUTH

The Forum Organizing Committee wishes to acknowledge with gratitude the financial support for medical student research provided by:

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The organizing committee is especially grateful to the following schools, departments, divisions, and programs for their generous contribution of a \$250 research prize awarded to the top scoring posters at the forum.

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ABSTRACTS

ALVAREZ-CALDERON, F

INHIBITION OF MITOCHONDRIAL RESPIRATION SYNERGIZES WITH TYROSINE KINASE INHIBITORS TO ELIMINATE BCR-ABL⁺ LEUKEMIA F Alvarez-Calderon (M.D., Ph.D., GS) M Gregory, N Serkova, J DeGregori

BCR-ABL kinase inhibitors are the standard therapy for chronic myelogenous leukemia (CML). While effective in controlling disease in chronic phase, these inhibitors often fail to eliminate BCR-ABL⁺ CML cells and also fail to achieve durable remissions for advanced CML and BCR-ABL⁺ acute lymphoblastic leukemias (ALL). Our lab employed a large-scale synthetic lethal shRNA screen to identify genes involved in CML survival upon treatment with the Bcr-Abl kinase inhibitor imatinib (IM). This screen identified several genes involved in energy utilization including pyruvate dehydrogenase. CML proliferation depends on Bcr-Abl-driven glycolysis for energy production. Upon IM treatment, I hypothesize that these cells are forced to rely on other sources of energy including mitochondrial ATP production, and that targeting alternative energy production pathways will enhance IM-mediated elimination of CML cells. Thus, I predicted that inhibition of mitochondrial respiration by knockdown or pharmacologic inhibition would cooperate with tyrosine kinase inhibitors to eliminate Bcr-Abl⁺ leukemias. My experiments have shown that knocking down pyruvate dehydrogenase in human blast crisis Bcr-Abl⁺ CML cells (K562) greatly sensitizes cells to IM in vitro. Moreover, I find that the mitochondrial ATP-synthase inhibitor Oligomycin A also synergizes with IM to kill CML cells in vitro. In addition, inhibition of mitochondrial ATP production with Oligomycin A enhances elimination of Bcr-Abl⁺ cells by the Bcr-Abl inhibitor dasatinib in vivo and prolongs disease-free survival in a mouse model of Bcr-Abl⁺ B-ALL. Targeting glucose utilization as an adjuvant drug therapy may prevent the survival of residual BCR-ABL⁺ CML cells upon IM or dasatinib treatment.

ANTONIOLI, AH

STRUCTURAL INVESTIGATION OF BROME MOSAIC VIRUS AH Antonioli, JA Hammond, and JS Kieft Department of Biochemistry & Molecular Genetics University of Colorado, Aurora, CO 80045

Brome mosaic virus (BMV) belongs to a class of tRNA-like structures (TLS) and is a positive-stranded icosahedral RNA plant virus that was first isolated in 1942. The BMV genome is made up of three 5' RNAs: RNA1 codes for a methyltransferase and helicase, RNA2 codes for an RNA-dependent RNA polymerase, and RNA3 codes for a viral movement protein and coat protein. All three RNAs have a highly conserved 3'tRNA like –structural domains that act as a substrate for tyrosylation. The smallest BMV aminoacylatable element is 134nts. The BMV TLSs have diverse roles in processes including translation. Additionally they can act as a viral coat protein nucleation sites and are important in viral intracellular localization. The sequence similarities as well as replication manner of the viruses suggest that understanding their function can improve our understanding of the viral life cycle. While TLSs act functionally similar to tRNAs, their unique structural differences include that they lack a consensus anticodon sequence. In BMV RNA3, the acceptor stem (A stem) is known to be a tRNA mimicking element. Tyrosyl-tRNA synthetase (TyrRS) can successfully aminoacylate the BMV TLS, although the exact mechanism is not well understood. The BMV TLS does not have a canonical anticodon loop and lacks the tyrosine anticodon triplet GUA. Experiments have indicated that the B2 hairpin orientation may have a role in positioning the TLS for the tyrosylation reaction. While TLSs have been defined as tRNA mimics, newer data indicate that these structures adopt tighter backbone packing and have dynamic structural elements. The experimental aim of this

work was to use crystallography to address whether addition of the upstream pseudoknot F in the BMV TLS impacts the folding and interaction of BMV TLS with TyrRS.

AUSTIN, EW

GHRELIN ADMINISTRATION ATTENUATES THE MYOCARDIAL INFLAMMATORY RESPONSE TO HYPOTHERMIC ISCHEMIA/REPERFUSION AND AFFORDS CARDIOPROTECTION

Everett W. Austin (M.D. Candidate, B.S.), N.G. Yousif (M.D.), L. Ao (B.S.), D. Fullerton (M.D.), X. Meng (M.D., PhD); University of Colorado Department of Surgery

Purpose of Study: During most cardiac surgical operations, the heart must endure the injuries of hypothermic ischemia followed by blood reperfusion. Mechanisms of inflammation are recognized to contribute to injuries of ischemia/reperfusion (I/R). Ghrelin is an endogenous peptide, principally produced by the gastric mucosa, and has recently been shown to have anti-inflammatory actions. We hypothesized that exogenous administration of ghrelin would reduce the myocardial injury associated with hypothermic ischemia and blood reperfusion. In a heterotopic heart transplant model, the purpose of this study was to examine the anti-inflammatory actions of ghrelin in the myocardium. **Methods Used:** Syngeneic mice (C3H/HeN strain) underwent heterotopic heart transplantation. Donor hearts were stored in cold, cardioplegic solution for 4 hours. Recipient mice received synthetic ghrelin (100 µg/kg, iv) prior to cervical implantation of the heart. Donor hearts were reperfused by the recipient's blood for 4 hours, and then harvested for analysis. Myocardial injury was evaluated by serum cardiac troponin-I (cTn-I) levels. Levels of monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1) and monocyte infiltration were assessed by ELISA, immunoblotting and immunofluorescence, respectively. Controls underwent the same procedure without ghrelin. Statistical analysis was performed using Student's t-test ($p < 0.05$ significant). **Summary of Results:** Ghrelin was cardioprotective, and this protection was associated with significant anti-inflammatory actions. Ghrelin treated animals exhibited less myocardial MCP-1 and ICAM-1 by 68.3% and 61.3% (both $p < 0.05$), respectively. These findings correlated with a 58.3% reduction of monocytic infiltrates in the myocardium ($p < 0.05$). These anti-inflammatory actions were associated with significantly reduced circulating levels of cTn-I ($p < 0.05$). **Conclusions:** Exogenous administration of the anti-inflammatory protein, ghrelin, was cardioprotective. It suppressed myocardial expression of MCP-1 and ICAM-1, and reduced mononuclear cell infiltration and myocardial damage. Based on these novel findings, we conclude that ghrelin is a potent suppressor of the myocardial inflammatory response and protects the myocardium against I/R injury.

BAHR, TM

CHRONIC OBSTRUCTIVE PULMONARY DISEASE IS ASSOCIATED WITH DIFFERENTIAL GENE EXPRESSION OF LYMPHOCYTE SUBSETS IN PERIPHERAL BLOOD MONONUCLEAR CELL.

T.M. Bahr, (M.S. SPH), C.D. Coldren, PhD, C. Schnell, BA CCRC, A.L. Friedlander, MD, K. Butterfield, K.J. Kechris, PhD, R.P. Bowler, MD PhD. Department of Biostatistics and Informatics, Colorado School of Public Health, University of Colorado Denver.

Although the majority of chronic obstructive pulmonary disease (COPD) patients are smokers, only a minority of smokers develop COPD. We hypothesize that smokers who develop COPD have differential gene expression in peripheral blood mononuclear cells (PBMCs) compared with smokers who do not develop COPD.

RNA from PBMCs were obtained from 83 smokers (23 current and 60 former) with and without COPD from a COPD Gene ancillary pilot study. Genome wide gene expression was assessed using Human Genome U133 plus 2.0 microarrays (Affymetrix). Following quality control adjustments, logistic and linear regression models adjusted for age, sex, race, smoking status,

and BMI were fit to determine the association between airflow obstruction and gene expression. Transcripts were mapped to their associated genes. A gene ontology (GO) analysis was applied to the top 1,000 most differentially expressed genes to identify over-represented functional classes of genes.

Of the final set of 12,277 filtered transcripts modeled, 352 were found to show statistically significant evidence of differential expression across case and control subjects (controlled at a false discovery of 0.10 for all 352 significant transcripts; unadjusted p-values range from 5.86×10^{-5} to 0.00343). GO analysis of differential expression genes revealed overrepresentation of GO terms associated with the immune system (all p-values $< 10^{-7}$) such as immune system process, immune response, and lymphocyte activation.

GO analysis of differentially expressed genes in PBMCs suggests that COPD is associated with alterations in the immune response in lymphocytes. These data suggest that PBMC gene targets could be used as novel diagnostic or therapeutic targets.

BAKER, KA

STUDY OF THE NEUROPHYSIOLOGY OF COGNITIVE DYSFUNCTION IN PARKINSON'S DISEASE. KA Baker, (MD, SOM), BM Kluger, Department of Neurology, University of Colorado, Denver, CO, and D Arciniegas, Department of Psychiatry, University of Colorado, Denver, CO.

Cognitive dysfunction affects 20- 40% of persons with Parkinson's disease (PD) at the time of diagnosis and is a significant risk factor for psychosis, dementia, nursing home placement and death. Among individuals with PD surviving 20 years or longer, cognitive dysfunction is the leading cause of nursing home placement and 75% eventually develop dementia. These statistics have not significantly changed since the 1920's despite major advances in the treatment of motor symptoms. This study builds on recent advances in cognitive neuroscience and an innovative neurophysiologic research technique to address this glaring gap in our knowledge and treatment of PD.

We will use magnetoencephalography (MEG) to investigate cognitive dysfunction in subjects with PD performing a demanding cognitive task (modified cued Stroop paradigm). For each trial, subjects will receive an instructional cue, either "Color" or "Word", indicating whether they are to name the color or read the word of the upcoming stimulus. The stimuli are color words written in colored font. The stimuli may be congruent (e.g. "red" written in red letters) or incongruent (e.g. "red" written in blue letters). Subjects will perform this task continuously for 30 minutes while in their on-medication state. We plan to collect data on 10-20 PD subjects and 10-20 age-matched controls.

Preliminary evidence shows that the Stroop paradigm is an effective method of determining cognitive dysfunction mediated by executive control, indicating that this study is capable of demonstrating cognitive differences between PD and non-PD populations.

We hypothesize that top-down networks, particularly those related to medial frontal structures affected by PD, are critical in mediating cognitive dysfunction. We predict that cognitive dysfunction will correlate with medial frontal brain activity.

BELAY, BW

INDUCTION OF HIV IN LATENTLY INFECTED, RESTING CD4+ T CELLS OF INFECTED INDIVIDUALS RECEIVING ANTIRETROVIRAL THERAPY: IMPLICATIONS FOR EDUCATION OF VIRUS Bietel W. Belay^{1,2,3}, Danielle Murray², Shawn Justement² and Tae-Wook Chun² 1. Howard Hughes Medical Institute 2. Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases 3. University of Colorado School of Medicine

The persistence of latently infected, resting CD4-T cells is one of the major obstacles in eradicating HIV infection by highly active antiretroviral therapy (HAART). Induction of viral replication in such infected cells has been suggested as one of the main strategies for

eliminating HIV from infected individuals receiving HAART. Valproic acid, OKT3, and IL-2 have previously been tested as virus purging agents without success. Recently, increasing interest has been shown in the novel antitumor-promoting phorbol ester, prostratin.

Prostratin has potent in vitro activity to induce HIV expression in latently infected cells, but its limited availability has hindered its therapeutic development. With the successful and practical synthesis of prostratin and its analogs by Wender et al., consideration for prostratin as an adjuvant for HAART has emerged again. We have tested four different prostratin analogs in vitro and analyzed their activation potency. All four analogs reactivated viral replication in resting CD4-T cells isolated from HIV-infected individuals receiving HAART with undetectable plasma viremia without cellular proliferation.

In addition, these analogs profoundly downregulated the expression of HIV receptors CD4, CXCR4, and CCR5. We are currently analyzing the purging capacity of the prostratin analogs to determine whether they can be used as effective adjunctive therapy for HAART to eradicate HIV.

BOGUNIEWICZ, J

THE IMMUNOMODULATORY ROLE OF HIGH DOSE INTRAVENOUS IMMUNOGLOBULIN IN A MURINE MODEL OF BILIARY ATRESIA. J Boguniewicz, RM Tucker, S Brindley, and CL Mack, Divisions of Allergy/Clinical Immunology and Gastroenterology/Hepatology, University of Colorado, Denver, CO

Background: Biliary atresia (BA) is an inflammatory, sclerosing disease affecting the bile ducts of neonates causing cholestasis. While the incidence of BA is low, it is the most common indication for pediatric liver transplantation. Current understanding of the pathophysiology of BA suggests that a viral insult initiates bile duct injury followed by progressive autoimmune-mediated inflammation and fibro-obliteration of the bile ducts. Intravenous immunoglobulin (IVIg) is composed of polyclonal, polyspecific immunoglobulin and has demonstrated clinical benefit in several other autoimmune and inflammatory diseases. One mechanism of IVIg action is to increase Treg production, thereby decreasing autoimmune-mediated injury.

Purpose of Study: To determine if markers of bile duct injury are diminished and liver Treg production is increased in IVIg-treated BA mice.

Methods Used: Neonatal BALB/c mice were injected with rhesus rotavirus (RRV) or Hanke's Balanced Salt Solution (HBSS) 12-18 hours after birth. On day 7, 9, 11 BA mice were given 1g/kg high dose IVIg or albumin by intraperitoneal injection. Survival in each group was monitored and, at 14 days post-injection, mice were sacrificed and blood, livers, and extrahepatic bile ducts were collected. Direct bilirubin assay was performed on serum from pools of 3-4 mice. Liver immune cells were isolated by Percoll density gradient and quantification of liver Tregs was performed by flow cytometry.

Summary of Results: IVIg did not improve survival in BA mice compared to albumin control. A significant decrease in direct bilirubin levels was observed in mice treated with IVIg (4.1 ± 0.9 mg/dL) compared to albumin (10.2 ± 1.5) (unpaired t-test, $p = 0.01$) and untreated BA controls (10.5 ± 1.9 ; $p = 0.001$). Increased Treg production was observed in IVIg-treated BA mice ($10.6 \pm 1.2\%$ FoxP3) compared to albumin (7.5 ± 0.1 ; $p = 0.06$) and untreated controls (5.9 ± 1.4 ; $p = 0.07$).

Conclusions: Immune therapy with high dose IVIg was associated with decreased bilirubin suggesting diminished bile duct injury. An increase in Tregs in IVIg-treated mice implies better control of autoimmunity and may be a possible mechanism of action for IVIg in BA.

BROCKER, C

ALDEHYDE DEHYDROGENASE 7A1 (ALDH7A1) IS A NOVEL ENZYME INVOLVED IN CELLULAR DEFENSE AGAINST HYPEROSMOTIC AND OXIDATIVE STRESS. C Brocker (PhD, GS)¹, N Lassen¹, A Pappa¹, K Kavanagh², U Oppermann² and V Vasiliou¹, ¹Department of Pharmaceutical Sciences, University of Colorado Denver, Denver, CO, USA and the ²Structural Genomics Consortium, University of Oxford, Oxford, UK.

Aldehyde dehydrogenase 7A1 (ALDH7A1) is homologous to plant ALDH7B1 which protects against various forms of stress such as salinity and dehydration. Mammalian ALDH7A1 is thought to play a major physiological role in lysine catabolism through the NAD⁺ dependent conversion of alpha-amino adipic semialdehyde (AASA) to alpha-amino adipic acid (AAA). In humans, deleterious mutations in ALDH7A1 are responsible for pyridoxine-dependent and folinic acid-responsive seizures. The continuing aim of this study is to characterize the biochemical properties and functions of mammalian ALDH7A1. Herein, we show that ALDH7A1 is a novel protein that protects against both hyperosmotic and oxidative stress by generating osmolytes and metabolizing reactive aldehydes. Human ALDH7A1 expression in Chinese hamster ovary (CHO) cells attenuated osmotic stress-induced cell death caused by either increased sucrose or sodium chloride. Moreover, protein expression significantly protected cells from treatment with both 4-hydroxy-2-nonenal (4-HNE) and hydrogen peroxide (H₂O₂). Knockdown of ALDH7A1 in human renal proximal tubular epithelial (HK-2) cells via siRNA increased susceptibility to sodium chloride-induced hyperosmotic stress. Purified recombinant ALDH7A1 efficiently metabolizes a number of aldehyde substrates including the osmolyte and methyl-group donor precursor, betaine aldehyde, lipid peroxidation (LPO)-derived aldehydes and the intermediate lysine degradation product, AASA. Human ALDH7A1 was crystallized and the structure supports the enzyme's diverse substrate specificities. ALDH7A1 tissue distribution studies in mice revealed highest expression in liver, kidney and brain, followed by pancreas and testes. ALDH7A1 protein is found in the cytosol, nucleus and mitochondria, thus making it unique among ALDH enzymes. In conclusion, ALDH7A1 is a novel ALDH expressed in multiple subcellular compartments that protects against both hyperosmotic and oxidative stress through the generation of osmolytes and removal of toxic aldehydes.

CALCATERRA, S

PSYCHOSTIMULANT-RELATED DEATHS: AMERICA'S DIRTY SECRET.

S Calcaterra MD (MPH, GS), I Binswanger MD, MPH Department of Medicine, University of Colorado, Denver, CO

Background: Methamphetamine and psychostimulants are highly addictive and widely abused substances. Little is known about medical conditions upon psychostimulant-related death, contributing substances found in drug screens upon death, and sociodemographic characteristics related to death. We reviewed a national database to examine sociodemographic characteristics of those who died with psychostimulants in their system, geographic trends in psychostimulant-related deaths, and other lethal conditions found upon death.

Methods: We conducted a death registry study of all deaths in the United States from 1999-2006 using the CDC Wonder Database which records data from death certificates of all U.S. residents. We searched the database using the ICD code T43.6, poisoning by psychostimulants with abuse potential, to determine the number of deaths, age-adjusted death rates, residence upon death, age group, race, gender, and contributing causes.

Results: During 1999-2006, there were 10,424 psychostimulant-related deaths. From 1999-2004, the death rate was 0.4 per 100,000 person-years; during 2005-2006 the rate climbed to 0.7 per 100,000 p-y. Psychostimulant-related deaths clustered in the Western United States. For instance, Nevada's death rate was 2.6 per 100,000 p-y from 1999-2006, and increased to

3.6 per 100,000 p-y during 2005-2006. Those aged 35-44 years had the highest rate of death. From 1999-2004 their death rate was 0.9 per 100,000 p-y; during 2005-2006 it increased to 1.4 per 100,000 p-y. American Indians/Alaska Natives had the highest increase in death from 0.3 per 100,000 p-y in 1999-2004 to 1.3 per 100,000 p-y in 2005-2006. The most common comorbid condition found upon psychostimulants-related death was polysubstance abuse, including alcohol and cocaine.

Conclusion: From 1999 to 2006, psychostimulant-related deaths rates increased, especially in the Western U.S. Polysubstance use was frequent upon those that died with psychostimulants. Alaska Natives and American Indians use of psychostimulants is rapidly increasing. Interventions are necessary to reduce the risk of death.

CHRISTOPHER, KL

CHARACTERIZATION OF ALPHA-CRYSTALLIN UPTAKE INTO EYE LENS CELLS AS A NOVEL TREATMENT FOR CATARACT AND RETINAL DEGENERATION. KL

Christopher(MD, SOM), NH Mueller, and JM Petrash, Department of Ophthalmology, Rocky Mountain Lions Eye Institute, University of Colorado School of Medicine, Aurora, Colorado.

Protein aggregation disorders are among the most common diseases afflicting humans and tend to have the fewest treatments available. In the eye, cataracts can be formed by aggregation of γ -crystallin protein subunits in the lens. Similarly, a mutation in the rhodopsin gene leads to aggregation and blindness in patients with retinitis pigmentosa. A family of small heat shock proteins called the α -crystallins show chaperone-like activity (CLA) in the eye as well as elsewhere in the body. During cataract formation, these proteins tend to accumulate at the membrane of lens cells and lose their CLA function. Replacing the pool of α -crystallin proteins is a novel therapeutic strategy to restore CLA in the eye and prevent protein aggregation. Using human lens epithelial (HLEB3) cells, we characterized the cellular uptake of one of the α -crystallin proteins, α B-crystallin, and monitored its activity once inside the cell. Recombinant α B-crystallin was purified and labeled with a fluorescent probe to enable us to monitor its real-time uptake and subcellular distribution. Uptake of wild-type α B-crystallin after packaging into a cationic polymer transfection reagent proved most effective, however the reagent caused reduced cell viability. Additional mechanisms to enhance protein delivery are being studied such as our modified α B-crystallin fused to a protein transduction domain. Stability of α B-crystallin once inside the HLEB3 cells was also investigated. Using gel electrophoresis methods, we observed that the protein was not being rapidly degraded by the cell. Additionally, a series of point mutants of α A-crystallin have been developed, expressed, and purified. These mutants have the potential for increased CLA and may be a more effective therapeutic option. Furthermore, a cell line with inducible aggregation-prone rhodopsin expression was developed and is being used to test the efficacy of treatment with our therapeutic α -crystallins to prevent or decrease the amount of protein aggregation in the cell. The α -crystallin family of small heat shock proteins remains a promising approach to treatment of protein aggregation disorders in the eye and elsewhere in the body.

MARRS CONNER, T

SINGLE-BOLUS DOSE CONIVAPTAN INDUCES HYPERNATREMIA AND DECREASED INTRACRANIAL PRESSURE IN A PORCINE NEUROSURGICAL MODEL. Tracy Marrs

Conner (MSIII), Alex Harbison, Marlin Dustin Richardson, Robert Neumann, and Allen Waziri, Department of Neurosurgery, University of Colorado, Denver, CO.

Observational human studies have suggested that conivaptan, a nonselective vasopressin receptor antagonist, may be useful for elevating serum sodium and decreasing intracranial pressure (ICP). However, no controlled studies have established details of safety or efficacy in this regard. Therefore, we performed a controlled trial evaluating sub-acute effects of single-bolus dose conivaptan in a porcine neurosurgical model. Female adult farm pigs were

anesthetized and underwent insertion of arterial and central venous lines prior to craniotomy and external ventricular drain (EVD) placement. Study animals were given a single bolus of conivaptan at 0.32 mg/kg over 30 minutes; fluid intake was standardized for study and control groups. All pigs were monitored at regular intervals for 8-10 hours, including arterial/central venous pressure, arterial blood gases, serum chemistry, urine output/specific gravity, and ICP. As predicted, administration of conivaptan induced a rapid, nearly 4-fold increase in hourly urine output in study animals when compared to controls. This change was associated with a modest decrease in CVP without concurrent cardiovascular instability. While serum sodium remained stable in controls, study animals demonstrated a linear increase in serum sodium averaging 16.6 (+/- 4.045) mEq/L above baseline ($p < 0.001$). Other serum chemistries remained stable. Notably, we observed a net decrease in ICP of 0.4 mmHg (+/- 3.949) in study animals, while ICP in controls increased an average of 8.33 mmHg (+/- 6.533) over the same interval ($p < 0.03$). Single bolus-dose conivaptan demonstrates clear efficacy for inducing hypernatremia and decreasing ICP without associated cardiovascular instability in an adult porcine model. These data warrant further study of conivaptan for ICP management in patients with brain injury.

CORDOVA, KN

INSIGHT INTO THE PATHOGENESIS OF RHEUMATOID ARTHRITIS FROM A FIBRINOGEN-INDUCED MOUSE MODEL OF AUTOIMMUNITY KN Cordova, (Ph.D., GS), RL Baker, G Barbour, K Haskins, and VM Holers, University of Colorado Denver, Aurora, CO

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes joint inflammation, leading to severe pain and loss of movement. Both genetic factors (i.e. the human HLA shared epitope DR4 allele) as well as environmental factors have been associated with susceptibility to RA. These environmental factors may trigger inflammation, prompting the conversion of peptidyl-arginine to peptidyl-citrulline in specific proteins. The presence of citrullinated proteins (especially fibrinogen) in the joints of RA patients correlates with disease severity. Synovial macrophages, fibroblasts, dendritic cells and B and T cells are all involved in the pathogenesis of RA, but T cells are specifically believed to be involved in initiating, controlling and driving antigen specific immune responses.

We and others have found that mice immunized with human fibrinogen (HF) or human citrullinated fibrinogen (HCF) develop mild inflammatory arthritis after day 25 characterized by increased levels of complement C3 and Ig staining in the cartilage and synovium, and the development of serum antibodies to both HF and mouse fibrinogen (MF). Additionally, there is a proliferative response to both HCF and HF, but not MF, in lymph nodes and spleens.

The purpose of this study is to determine if fibrinogen reactive T cells are important in the pathogenesis of arthritis. In these experiments, DBA1/J mice were immunized with HCF in complete Freund's adjuvant (CFA). Lymph nodes were taken at various time points throughout the disease course and either evaluated directly *ex vivo* or cultured *in vitro*. *Ex vivo* T cells were evaluated for the presence of activation markers and cytokine production after antigen stimulation by FLOW cytometry. T cell lines were cultured long term *in vitro* with antigen and IL-2, and evaluated in the same manner.

We found that following two immunizations with HCF, mice produced higher levels of CD4 cells with an activated effector phenotype (CD44^{hi}+CD62L⁻) than CFA injected mice and also and IL-17 were produced specifically by CD4 cells in response to challenge with HCF. Cultured T cell lines produced high levels of IL-17 and IL-6 when stimulated with HCF, and a line derived late in the disease induction protocol also produced IFN

F
. Our conclus
cells are activated over the disease course and exhibit an inflammatory phenotype that may be pathogenic in experimental autoimmune arthritis. Currently, T cell lines are being evaluated *in vivo* to determine if they are able to precipitate or accelerate disease in this model.

PULMONARY DENDRITIC CELL SPECIFICITY OF EFFEROCYTOSIS

*A. Nicole Desch*³, *Gwendalyn J. Randolph*¹, *Robert Mason*⁴, *Peter M. Henson*^{2,3*}, and *Claudia Jakubzick*^{2,3*} ¹Department of Gene and Cell Medicine, Mount Sinai School of Medicine, NY, NY ²Department of Pediatrics, ³Integrated Department of Immunology, ⁴Department of Medicine, National Jewish Health and UC Denver Anschutz Campus, Denver, CO *co-mentor

Every day billions of cells undergo apoptosis and are cleared by neighboring phagocytes through a process known as efferocytosis. The efficient removal of dying cells from tissue prevents the induction of inflammation. This is orchestrated through phagocyte recognition of exposed phosphatidylserine (PS) on the extracellular surface of apoptotic cells. Previously, we found that PS-exposing apoptotic cells in the lungs promoted an immunosuppressive response during acute inflammation. This data suggested the possibility of dendritic cells playing a role in this immunosuppressive response. In the lung there are two migratory dendritic cell (DC) subsets: CD11b^{Lo}CD103⁺ DCs (CD103⁺ DCs) and CD11b^{Hi}CD103⁻ DCs (CD11b^{Hi} DCs). Our affymetric genechip revealed that CD103⁺ DCs expressed candidate PS receptors and bridging molecules to a greater extent compared to CD11b^{Hi} DCs. Therefore, we investigated whether pulmonary CD103⁺ DCs preferentially acquire apoptotic cells and migrate to draining lymph nodes (LN) compared to CD11b^{Hi} DCs. To our surprise, CD103⁺ DCs alone took up apoptotic cells, while CD11b^{Hi} DCs did not. In contrast, non-cellular substances delivered in the lungs were acquired and trafficked to draining LNs by both DC subsets. This observation led us to name the CD103⁺ DCs as efferocytic DCs and CD11b^{Hi} DCs as nonefferocytic DCs. This finding demonstrates, for the first time, opposing roles for the two DC subsets in the lung based on their capacity to take up dying cells. Moreover, it has been shown *in vitro* that pulmonary CD103⁺ DCs are capable of cross-presentation, thus having potential implications for vaccine design in lung cancer and viral immunity.

A BRAIN SLICE CULTURE MODEL OF VIRAL ENCEPHALITIS REVEALS AN INNATE CNS CYTOKINE RESPONSE PROFILE AND THERAPEUTIC POTENTIAL OF CASPASE

INHIBITION *K.R. Dionne* (B.A.)^{1,2}, *J.S. Leser*³, *K.A. Lorenzen*⁴, *J.D. Beckham*^{3,4,5}, *K.L. Tyler*^{1,2,3,4,5,6,*} University of Colorado Denver - Anschutz Medical Campus, Aurora, CO 80045 Medical Scientist Training Program¹, Neuroscience Program² Departments of Neurology³, Medicine⁴, and Microbiology⁵ Denver Veterans Affairs Medical Center⁶, Denver, CO 80220

Viral encephalitis is a significant cause of human morbidity and mortality in large part due to suboptimal diagnosis and treatment. Murine reovirus infection serves as a classic experimental model of viral encephalitis. Infection of neonatal mice with T3 reoviruses results in lethal encephalitis associated with neuronal infection, apoptosis, and CNS tissue injury. We have developed an *ex vivo* brain slice culture (BSC) system that recapitulates the basic pathological features and kinetics of viral replication seen *in vivo*. We utilize the BSC model to identify an innate, brain-tissue specific inflammatory cytokine response to reoviral infection, which is characterized by the release of IL6, CXCL10, RANTES, and murine IL8 analog (KC). Additionally, we demonstrate the potential utility of this system as a pharmaceutical screening platform by inhibiting reovirus-induced apoptosis and CNS tissue injury with the pan-caspase inhibitor, Q-VD-OPh. Cultured brain slices not only serve to model events occurring during viral encephalitis, but can also be utilized to investigate aspects of pathogenesis and therapy that are not experimentally accessible *in vivo*.

D'SOUZA, RD

NICOTINIC RECEPTORS ENHANCE AND TEMPORALLY SCULPT OUTPUT RESPONSES TO SENSORY INPUT AT OLFACTORY GLOMERULI RD D'Souza (Ph.D., GS) and S

Vijayaraghavan, Department of Physiology and Biophysics, School of Medicine, University of Colorado Denver, Aurora, CO 80045.

Olfactory bulb glomeruli, the initial sites of synaptic processing of sensory odor information, exhibit a high concentration of nicotinic acetylcholine receptors (nAChRs). We examined the role of nAChRs in modulating electrical signaling in the glomerulus using whole-cell electrophysiology. Activation of glomerular nAChRs depolarized external tufted (ET) and mitral cells, and resulted in an increase in glutamate release at dendrodendritic synapses onto periglomerular (PG) cells. nAChR activation also led to an increase in GABA release onto PG, ET, and mitral cells. This release of GABA was, in part, dependent on the activation of glutamate receptors. The nAChR-mediated depolarization of mitral cells increased their excitability, but suppressed olfactory nerve-evoked responses in them. Our results suggest that activation of nAChRs in the glomerulus amplifies incoming sensory input by depolarizing the excitatory neurons in the bulb, while also providing excitation-dependent inhibition onto them that might filter out weaker signals. We thus provide a model demonstrating a role of nAChRs in modulating olfactory bulb circuit dynamics during enhanced odor detection and discrimination. Funding: NIH RO1 DC 008855

DURAZO, SA

MITOCHONDRIAL TARGETED DELIVERY: MECHANISMS OF UPTAKE. S.A. Durazo (Ph.D., SOP), R. Kadam, D. Drechsel, M. Patel, and U.B. Kompella, Department of Pharmaceutical Science, University of Colorado, Denver, CO.

Mitochondrial defects are hypothesized to underlie the pathologies of various eye disorders including retinal degenerations, optic neuropathies, and glaucoma. A critical rate limiting step in treating these disorders is targeted drug delivery to the mitochondria. As a first step in understanding the mechanisms of drug delivery to the mitochondria, the purpose of this study was to investigate the effect of membrane potential on the delivery of 20 drug molecules with varying physiochemical properties. The delivery of 8 cationic drugs (β -blockers), 6 neutral drugs (corticosteroids), and 6 anionic drugs (NSAIDs) was assessed using mitochondria isolated from the brain of male Sprague Dawley rats. Drugs of each class were exposed as a separate cassette to freshly isolated mitochondrial preparations in the absence and presence of a membrane potential disrupter for 1 hr. Drug uptake was quantified using high throughput assays for each cassette based on liquid chromatography tandem mass spectrometry (LC-MS/MS). When the most lipophilic compound in each series was compared, it was observed that the positively charged compound had the highest percent uptake (propranolol; 50%), followed by the neutral compound (budesonide; 30%) and the negatively charged compound (mefanamic acid; 20%). However, in the presence of the membrane potential disrupter only the positively charged compounds had reduced uptake. Within each series, mitochondrial uptake increased with an increase in drug lipophilicity, with the correlations being more significant for the positively charged and neutral compounds ($R^2 > 0.8$). For positively charged compounds, uptake is largely driven by the potential difference. Neutral and negatively charged compounds are possibly entering the mitochondria by a passive or transporter-mediated mechanism.

DYAMENAHALLI, K

SHAPE-MEMORY POLYMER COILS FOR TRANSCATHETER EMBOLIZATION OF ANEURYSMS. K. Dyamenahalli, (M.D.,Ph.D., GS), and R. Shandas, Department of Bioengineering, University of Colorado, Denver, CO.

Aneurysms are pathologically-weakened and dilated sections of blood vessels that are at increased risk of rupture. In the cerebral vasculature, rupture of an aneurysm can lead to catastrophic hemorrhagic stroke. Early intervention may involve craniotomy and placement of a clip at the base of the malformation. However, this surgical approach has lost ground to minimally-invasive embolization techniques, which involve delivery of small coils into the aneurysm through a catheter, producing an effective seal. Embolic coils have primarily been fabricated using metals such as stainless steel, platinum and nitinol. Although such coils are well-accepted clinically, metals are limited by their poor capacity for shape-memory, poor resistance to kinking, and the inability to fine tune stiffness, elute drugs or biodegrade. Furthermore, metal coils produce artifacts on magnetic resonance (MR) images, which necessitates fluoroscopy or CT imaging for follow-up evaluation, increasing ionizing radiation exposure to the patient. Synthetic polymers avoid the drawbacks of metals, but they are inherently radiolucent, making it difficult to track their placement using conventional methods. Recent efforts have yielded a radio-opaque shape-memory polymer (SMP) appropriate for embolic coil design. Here, we present thermo-mechanical, radiographic, and MRI data characterizing this novel polymer.

Embolic coils were fabricated using an acrylate-based SMP functionalized with iodine residues for radiographic visibility [EndoShape Inc]. Control SMP coils were prepared using a standard formulation of tert-Butyl acrylate and poly(ethylene glycol) dimethacrylate. Dynamic mechanical analysis was used to measure the glass transition temperature (T_g) for each polymer. Coils were evaluated for radiographic and MRI visibility in a water-filled, acrylic phantom using fluoroscopic and 3T MR imagers, with nitinol and commercial platinum [Cook Medical] coils as reference.

T_g s were measured at 26.5°C and 45.2°C for the iodinated and control SMPs, respectively. Fluoroscopic imaging yielded normalized linear x-ray attenuation coefficients of 1.00 (platinum), 0.702 (nitinol), 0.322 (iodinated SMP), and 0.009 (standard control SMP). T1-weighted, 3 mm MRI images revealed markedly distorted signal from both platinum and nitinol coils. The iodinated and standard SMP coils produced no MRI artifact, and individual coil wires were clearly visible on the MRI images.

Thus, iodinated SMP coils can be engineered with transition temperatures at or below body temperature, allowing for targeted release under physiological conditions, and with markedly enhanced radio-opacity. Moreover, these SMPs possess an inherent MRI signal, with no imaging artifacts.

EDWARDS, LE

A NOVEL MECHANISM FOR PROGRAMMING PROTECTIVE CD8+ T CELL MEMORY in CD4+ DEFICIENT HOSTS. LE Edwards, (M.D./Ph.D., SOM/GS) and RM Kedl, Integrated Department of Immunology, University of Colorado Denver and National Jewish Health, Denver, CO.

While CD4+ T cell deficient hosts produce a normal primary response to vaccination/infection their CD8+ T cell memory response is dramatically decreased compared to wild type (wt) controls. CD4+ deficient hosts progressively lose CD8+ memory cells during memory maintenance and the remaining cells are insufficient to protect against secondary challenge. Our laboratory uses a combined poly(I:C)/CD40-agonist vaccination that generates significant secondary responses, up to 80% antigen specific CD8+ T cells post-boost. Due to our ability to generate a dramatic secondary expansion in wt mice, we hypothesized that

combined poly(I:C) /CD40-agonist vaccination would overcome the need for CD4+ cells in the generation of CD8+ T cell memory. Here we demonstrate that, in contrast to vaccination with *Listeria monocytogenes* (LM), a CD4-dependent memory response, combined poly(I:C)/CD40-agonist immunization elicits protective CD8+ T cell memory even in class II deficient (CD4+ deficient) hosts. Though the CD8+ memory cells in the class II deficient hosts appear to have reduced survival, their capacity for secondary expansion is equal to, if not greater than, memory CD8+ T cells in wt hosts. The CD8+ memory cells generated by poly(I:C)/CD40-agonist immunization also protect both wt and class II deficient mice against vaccinia virus challenge. Interestingly, poly(I:C)/CD40 immunization is able to program protective central memory cells despite elevated levels of the transcriptional repressor Blimp-1 (PRDM1) which is detrimental to memory cell formation in infectious models. This suggests combined poly(I:C)/CD40 immunization utilizes a novel mechanism to program protective CD4-independent CD8 T cell memory. A gene expression study comparing poly(I:C)/CD40 immunization to LM was recently completed and preliminary results indicate that a unique combination of increased expression of both cell cycle inhibitors and survival factors following poly(I:C)/CD40 immunization may be responsible for the generation of protective central memory cells even in the absence of CD4+ T cells. Our results demonstrate that poly(I:C)/CD40 immunization utilizes a novel program to generate protective CD8+ T cell memory in a CD4+ deficient environment. These results have significant impact on both the basic immunology of CD8+ T cell memory as well as for the rational design of novel therapeutic vaccines.

ELKON, BD

MASS TRANSFER OF PEDIATRIC TERTIARY CARE HOSPITAL INPATIENTS TO A NEW LOCATION IN UNDER 12 HOURS: LESSONS LEARNED AND IMPLICATIONS FOR DISASTER PREPAREDNESS. BD Elkon (MD, MS4), JK Fuzak MD, LC Hampers MD, MBA, KJ Polage RN, MBA, JD Milton Rph, LK Powers RN, MBA, K Percell-de'Shong, RN, JE Wathen, MD Department of Pediatrics, Section of Emergency Medicine, The Children's Hospital, University of Colorado at Denver, University of Colorado School of Medicine, Aurora, CO.

Objective: To report experience with large-scale rapid transport of hospitalized children, highlighting elements applicable to a disaster event.

Study Design: This is a retrospective study of the relocation of an entire pediatric inpatient population. Mitigation steps included postponement of elective procedures, planned discharges, and transfer of selected patients to satellite hospitals. Drills and simulations were used to estimate travel times and develop contingency plans. A transfer queue was modified as necessary to account for changing acuity. The Hospital Incident Command System was utilized. Results: 13 critical care teams, 5 general crews, 2 vans, and 4 other vehicles transferred 111 patients 8.5 miles in 11.6 hours. Patients were transferred along parallel (versus series) circuits, allowing patients from different areas to be moved simultaneously. 64 patients (32 infants) were considered critical. 24 required ventilator support, 3 required inhaled nitric oxide, 30 required continuous infusions, and 4 had external ventricular drains. There were no adverse outcomes.

Conclusions: Mass inpatient pediatric transfers can be managed rapidly and safely with parallel transfers. Preexisting agreements with regional pediatric teams were imperative. Disaster preparedness concepts including pre-planning, evacuation priorities, recovery analysis and prevention/mitigation can be applied to this event.

BIOMECHANICAL CHARACTERIZATION OF THE CARTILAGINOUS ENDPLATE IN THE INFANT SPINE. Ryan P Farmer (M.D., SOM)¹, Rachel C. Paietta¹, Virginia Ferguson¹, Evalina Burger¹

The purpose of this study is to improve our understanding of the interface between mineralized and non-mineralized biological tissues. This is of great importance to ensure successful prosthetic implant fixation and to improve our understanding of the etiology of spinal disc degeneration and developmental disorders. In the spine, bone and cartilage meet at the osteochondral interface – a region that experiences high shear forces in normal use. We seek to characterize the gradient of properties from bone to cartilage within this interface using scanning electron microscopy, in both backscattered (BSE) and secondary electron (SEM) modes, to visualize the 2-D and 3-D structures of the cartilaginous endplate region in the human infant spine.

Within this interface, anchoring of the intervertebral disc (IVD) to the adjacent vertebral bodies by collagen fibrils will be assessed via immunohistochemistry staining for collagen types I, II, IV, and X. BSE imaging methods were developed using mature, ovine lumbar spine samples that were histologically embedded in poly(methylmethacrylate) (PMMA) prior to sectioning and imaging. Additional BSE imaging has also been performed on a human fetal (37 weeks gestational age) lumbar spine L3-L5. A second ovine lumbar vertebra is currently being in order to image the mineralized surface of the cartilaginous endplate.

The infant cartilage endplate (CE) showed a highly porous mineralized cartilage region that was connected to, but distinct from, the underlying subchondral bone (SCB) and is an area of rounded pores that likely contained chondrocytes. While the adult ovine tissue possessed similar characteristics, the mineralized CE was significantly more compact and of greater thickness and was thicker in the AF area than in the NP area. This thinning is likely an adaptation to greater hydrostatic forces within the NP region. In addition, the CE overlying the annulus contained tidemarks – a histological feature that is typically associated with thickening of the calcified region in articular cartilage and may indicate degenerative changes.

This work forms the basis for two separate studies of both human and ovine spinal tissues to understand how the mineralized CE region is altered with age in healthy subjects.

CHARACTERIZATION OF PHAGOCYtic MAMMARY EPITHELIAL CELLS DURING POST-PARTUM INVOLUTION. J Fornetti (Ph.D.,GS), P Henson, and P Schedin. Division of Medical Oncology, University of Colorado, Denver, CO.

Women are at an increased risk of breast cancer in the post-partum period, and women diagnosed during this time have a poorer prognosis due to increased metastasis. Our lab has identified post-partum mammary gland involution as a period of time that may contribute to increased metastasis. During early involution, mammary epithelial cells (MECs) obtain phagocytic properties and function in clearing the gland of the apoptotic milk-producing cells. Peak apoptosis and clearance precedes an influx of macrophages, which our lab has characterized as M2 macrophages. Importantly, subsets of M2 macrophages are tumor-promotional. If macrophages infiltrating the gland during late involution belong to this subset, this might be one way involution contributes to the poor prognosis of post-partum breast cancer. My project is directed characterizing the phagocytic MECs, and testing the hypothesis that phagocytic MECs contribute to the M2 macrophage phenotype by secreting factors necessary for M2 macrophage maturation. To test this hypothesis, I have developed an in vitro model to study phagocytic MECs. In this model, MECs are cultured to form a monolayer with high transepithelial electrical resistance (TER), and then treated with transforming growth factor beta 3, which results in decreased monolayer TER, disruption of tight and adherens junctions, and increased phagocytosis. Importantly, this model mimics the state of adherens junctions at the

onset of involution, in which we have found cleavage and re-distribution of the adherens junction protein e-cadherin. To test the hypothesis that phagocytic MECs promote M2 macrophages, I have harvested RNA, protein, and conditioned media from the phagocytic MEC assays in order to begin characterizing cytokine production, and the effect of phagocytic MECs on macrophage phenotype. By identifying factors involved in the promotion of M2 macrophages during involution, we may be able to identify novel targets for the treatment and prevention of breast cancers diagnosed in the post-partum period.

GALLIGAN, JJ

PROFILING IMPAIRED HEPATIC ENDOPLASMIC RETICULUM GLYCOSYLATION AS A CONSEQUENCE OF ETHANOL INGESTION Galligan, J.J. (PhD., G.S.); Fritz, K.S.; Smathers, R.L.; Shearn, C.T.; Tipney, H.; Hunter, L.J.; and Petersen, D.R. Departments of Pharmacology and Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO.

Chronic ethanol consumption remains a predominant cause of liver injury in the United States. The precise mechanisms underlying the progression of alcoholic liver disease (ALD) are poorly understood; however, alterations in post-translational modifications (PTMs) have been observed. Glycosylation is the most abundant and diverse cellular PTM, affecting protein folding and activity. Using a mouse model of ALD, 2-D gel electrophoresis and hydrazide chemistry were employed to assess the hepatic endoplasmic reticulum (ER) glycoproteome. Gradual decreases in glycosylation were observed during a time-course of ethanol ingestion, with marked alterations occurring at week 6. To identify proteins associated with the observed changes in glycosylation, LC-MS/MS was employed. This approach yielded 30 proteins, with 50.0% of those proteins identified being novel identifications for this PTM. Of the proteins identified, triacylglycerol hydrolase (TGH) consistently displayed decreased glycosylation. Impaired glycosylation of TGH has been shown to result in increased cellular storage of lipids, consistent with the pathologies associated with ALD. To further elucidate processes and mechanisms behind the progression of ALD, all 30 proteins were subject to bioinformatic analysis. Of interest, terms associated with ER protein folding, unfolded protein response (UPR) signaling and esterase activity were found significantly enriched in our samples. Impaired protein folding and UPR signaling has been implicated in rodent models of ALD, however, precise mechanisms behind its induction are currently unknown. Interestingly, impaired ER glycosylation is reported to induce potent UPR activation when chemically induced with tunicamycin (TM). Consistent with the actions of TM, our data suggest that dysregulation of hepatic protein glycosylation may be an important initiating factor for impaired ER protein folding and altered lipid homeostasis associated with ALD. (R37 NIH/AA009300 & F31 AA018606).

GALTON, C

OPEN-LABEL RANDOMIZED TRIAL FO THE SAFETY AND EFFICACY OF A SINGLE DOSE CONIVAPTAN TO RAISE SERUM SODIUM IN PATIENTS WITH TRAUMATIC BRAIN INJURY. Galton C¹, (MD, UCD School of Medicine) Deem S², Yanez D³, Souter M², Chesnut R⁴, Menon N², Treggiari M². ¹School of Medicine, University of Colorado, Aurora CO; ²Department of Anesthesiology and Pain Medicine, ³Biostatistics and ⁴Neurological Surgery, University of Washington, Seattle WA.

Hypertonic saline is used to reduce intracranial pressure in patients with traumatic brain injury (TBI). Aquaresis instead of the administration of high quantities of Na⁺ might be a safe alternative. We hypothesized that conivaptan would be safe, will maintain stable serum Na⁺ and decrease the overall Na⁺ load. We designed an open-label, randomized, controlled trial enrolling 10 subjects with TBI. Patients were assigned to receive a single dose of conivaptan (Vaprisol®) (n=5) or usual care (n=5). The primary endpoint was drug safety as indicated by serum Na⁺ increases greater than 1 mEq/hr, serum Na⁺ levels above the target range, and adverse events. Ten patients were included in the intention-to-treat analysis. The mean age

was 50±13 years in the conivaptan group and 48±20 years in the control group (p=0.83). Three patients experienced brief (less than four hours) Na⁺ increases >1 mEq/L/h. The average serum Na⁺ on day 2 was 143±6 and 142±7 (p=0.82), with no patients achieving Na⁺ levels > 160 mEq/L. The amount of Na⁺ administered in the first 48 hours was 818±724 mEq and 1076±1203 mEq (p=0.69). 48-hour urine output and fluid balance were not different (p=0.20 and 0.06, respectively). There were no related adverse events. These data suggest that a single dose conivaptan is safe in non-hyponatremic patients with severe TBI, who require increases in serum Na⁺ for the purpose of ICP control. Further studies to establish the effect of higher doses and longer administration are needed. This research was supported by Astellas Pharma.

GLISINSKI, K

WILDERNESS SEARCH AND RESCUE EPIDEMIOLOGY IN AND AROUND BOULDER COUNTY, COLORADO 2002-2008 K Glisinski, (MD SOM)¹, B Blok, (MD)¹, M Valley, MS¹, D Christenson², A Sheets, (MD)², TM. Larabee, (MD)¹ ¹University of Colorado Denver School of Medicine, Aurora, CO ²Rocky Mountain Rescue Group, Boulder, CO

Background: There is a growing body of literature related to the use of wilderness search and rescue teams, the types of encounters they face, and the type of training that they require. Boulder, Colorado is a mecca for outdoor activities, but search and rescue data for this region has not been documented in the medical literature. The Rocky Mountain Rescue Group (RMRG) is one of the main search and rescue groups in this area, and has been formally collecting data related to their activities since 2002. **Objective:** The purpose of this study is to examine the epidemiology of search and rescue activities in and around Boulder, Colorado using data available from RMRG. **Methods:** A retrospective analysis was performed on data available from RMRG for 2002-2008. Data points collected include number of calls, types of calls, location of calls, victim activity, rescue techniques used and volunteer hours required. Data was analyzed using descriptive statistics and linear regression models. **Results:** The number of calls, types of calls, location of calls, and types of required rescue did not change significantly over the study period. Injuries related to hiking and climbing activities were the most common activities requiring a search and rescue response. The types of activities associated with a response did not change significantly over time. Limited medical data was available for collection. **Conclusions:** The prevalence and nature of calls to RMRG did not change over time. This data is valuable for assessing volunteer training program requirements, and for assessing necessary evacuation and medical equipment needs for the near future. Improved medical data collection may be beneficial to better plan for future medical emergencies.

HALGRIMSON, WR
PATTI, BN

DEVELOPMENT OF AN ARTHROSCOPY SIMULATOR TRAINING PROGRAM FOR UNDERGRADUATE MEDICAL STUDENTS USING HAPTIC FEEDBACK SIMULATION. WR Halgrimson (M.D., SOM), BN Patti (M.D., SOM), VM Spitzer Ph.D., Center for Human Simulation, University of Colorado, Denver, CO.

The endoscope has a four decade history as a component of less invasive surgical techniques in numerous surgical subspecialties, including orthopaedics, gynecology, general surgery, and neurosurgery. In recent years simulation has emerged as an attractive means to both instruct and evaluate resident physicians in arthroscopic techniques while eliminating the morbidity and mortality risks associated with live patients. The University of Colorado Center for Human Simulation and Touch of Life Technologies have expanded upon the success of the Virtual Human Project by partnering with the American Academy of Orthopaedics (AAOS) to produce a diagnostic knee arthroscopy course. We aim to study the application of a standardized arthroscopy training program to undergraduate medical education.

The K.A.S.T. (knee arthroscopy simulator training) program at the University of Colorado School of Medicine is a newly developed simulator training course for undergraduate medical students interested in pursuing a career in surgery. Students are randomized to one of three instruction tracts and ultimately perform a live diagnostic knee arthroscopy on cadavers for evaluation. Attending physicians from the Department of Orthopaedics evaluate blinded video feeds of students' performance based upon three main criteria: errors and tissue damage, dexterity and arthroscopy skill, and degree of completion of a diagnostic arthroscopy of the knee. A subset of students then undergo refresher training and repeat evaluation after an additional six week "off-period" to study training durability.

We expect to demonstrate the utility of acquiring technical surgical skills through simulation and the applicability of this education to those students pursuing graduate medical education in surgical subspecialties. Ultimately we aim to demonstrate the value of surgical simulation training as an elective course to benefit University of Colorado undergraduate medical students.

HELLYER, JA

MATERNAL RISKS, COMMUNITY VIOLENCE EXPOSURE AND SELF-REPORTED ASTHMA AMONG CHILDREN IN FOSTER CARE. JA Hellyer, (MD, SOM), SE Culhane, EF Garrido, CLM Petrenko, and HN Taussig, Department of Pediatrics, University of Colorado, Denver, CO

Objective - - The occurrence of pediatric asthma has been associated with exposure to chronic stress. The goal of this study was to examine whether stress and asthma were associated in a high risk sample. The study examined the relationship between maternal and community risk factors and asthma in a sample of maltreated children placed in foster care.

Method – Interviews were conducted with 355 maltreated children (9-11 years old) who had been court-ordered into foster care within the past year. Measures included youth self-report of asthma and community violence exposure. County administrative records were abstracted for data on maternal risk factors (e.g. criminal history and substance use) and a cumulative risk index was created.

Results –The Maternal Risk Index significantly predicted the presence of asthma ($OR = 1.647$, $95\% CI = 1.2-2.3$, $p=0.002$). Child reported community violence exposure was not significantly associated with asthma.

Conclusion – Maternal risk factors were significantly associated with the presence of asthma in a maltreated, foster care population. This connection has important implications for clinicians who oversee the care of maltreated children as it may be an indication of which children in this already high-risk group have a greater propensity for physical health issues.

HERRING, MJ
WINSTON, BA

STRESSED BRAIN TUMOR CELLS SHIFT TO LIPID METABOLISM: IMPLICATION FOR THE SERETOME. MJ Herring, (M.D., SOM), BA Winston, (M.D., SOM), MW GRANER PH.D., Department of Neurosurgery, University of Colorado, Denver, CO.

Brain tumor-derived exosomes are believed to affect the microenvironment and neighboring cells' metabolic activity in order to manipulate the surroundings. Additionally, the unfolded protein response (UPR) in glioblastomas has been implicated in the tumor's highly invasive nature by influencing the secretome. The metabolomic profile of stressed tumor cells and their potential ability to transmit the metabolic response via exosomes could be invaluable to understanding tumorigenesis and possibly uncover targets for new therapies and biomarkers. To investigate this hypothesis, glioblastoma cell line U87 was grown in C13 glucose and treated with dithiothreitol (DTT) to induce the UPR. The metabolic profile of these cells was then analyzed using nuclear magnetic resonance. Cells treated with DTT were also probed for fatty acid synthase using western blotting techniques. The resultant data demonstrated a marked

shift of resources from glycolytic processes to lipid synthesis pathways. The western blot data parallels this observation as the DTT stressed cells showed an increased expression of fatty acid synthase. These findings suggest during periods of cell stress, tumor cells divert energy from glycolytic processes and increase fatty acid synthase expression in a cumulative effort to increase overall lipid metabolism. We hypothesize that the function of this cellular response is to synthesize exosomes that will ultimately influence the tumor microenvironment.

HIGGINS, DM

IMPACT OF VITAMIN D DEFICIENCY ON OUTCOME IN CRITICALLY ILL PATIENTS

DM Higgins (MD, SOM), KM Queensland, PE Wischmeyer, AJ Sufit, DK Heyland, Department of Anesthesiology, University of Colorado School of Medicine

Although as many as 1 billion people worldwide have a deficiency in vitamin D which has been associated with a myriad of disease conditions, the impact of this deficiency on outcome in critically ill patients is not clear. We hypothesize that vitamin D deficiency in ICU patients is a common condition and is associated with negative outcomes. We conducted a prospective multi-center study of baseline serum vitamin D levels in 195 patients admitted to the ICU to evaluate the incidence of vitamin D deficiency and associations with mortality, infection status, length of stay, and organ function. Infection status and clinical outcomes were documented for 28 days after admission. A Cox proportional hazard model, logistic regression and ANOVA models with adjustment for age, BMI and APACHE II score were used. Vitamin D deficiency or insufficiency (<60 nmol/L) was observed in 159 patients (82%). Vitamin D status was not associated with 28-day all cause mortality (hazard ratio (HR) 1.004; 95% CI 0.993-1.015). However, increased levels of vitamin D were associated with a shorter length of stay (HR 3.56; 95% CI 1.5-8.6 at 28 days). Vitamin D deficient and insufficient patients had a higher incidence of probable infections compared to patients with normal levels of vitamin D (OR 3.2; 95% CI 0.78-13.1, p=0.1). Patients with culture confirmed pneumonia had significantly lower levels of baseline vitamin D (38.6 nmol/L +/- 3.2) compared to those who did not have pneumonia (48.23 nmol/L +/- 1.9) (p<.01). Lower levels of baseline vitamin D also correlated with increased baseline SOFA scores (p<.05), and elevated serum Cr levels (p<.01). This is largest prospective cohort trial to date showing vitamin D insufficiency is a common condition in the ICU and the first data showing a significant correlation of Vitamin D deficiency with longer length of ICU stay, increased risk of pneumonia, and elevated organ failure scores. This may be due the vital role of vitamin D in regulating immune function.

HOLST, JA

GENOMICS OF ISOLATED CONGENITAL HEART DISEASE: USING PECONPI TO IDENTIFY PUTATIVE DEVELOPMENTAL REGULATORS OF CARDIAC GENES.

JA Holst (M.D., SOM), L Francey, MA Berman, H Fetting, JT Glessner, L Conlin, P Gruber and ID Krantz, Division of Human Genetics, The Children's Hospital of Philadelphia.

Congenital heart defects (CHD) are the most common human birth defects, affecting up to 1 out of every 100 births. Defects are seen as a component of multiple syndromes as well as in isolation. The molecular etiologies of many syndromic forms have been elucidated. Logically, if a gene harbors a mutation, it will be present in all cells of the body, thus affecting all tissues in which that gene is expressed resulting in a syndromic phenotype. The genetic etiology of the more common isolated forms of CHDs has remained much more elusive. We hypothesized that mutations in regulators (that "switch-on/off" genes in a temporal-spatial fashion) of syndromic cardiac genes will be responsible for isolated CHDs when disrupted. DNA from probands with CHDs was genotyped with SNP arrays, raw data analyzed with BeadStudio, copy number variations (CNV) identified with PennCNV software, PECONPI software was used to rank these deletions according to pathogenicity parameters including: increased non-coding sequence conservation across species, within 1 Mb of a known/putative syndromic heart gene and

decreased overlap with literature or control CNVs. Out of 408 probands, 3878 total CNV deletions were identified. Of these, 156 novel CNV deletions encompassed a conserved non-coding element (CNE) and 30 encompassed a CNE and were also within 1 MB of a CHD gene (novel CNVs contained no control or literature overlap). A small CNV upstream of Transforming Growth Factor Alpha (TGFA) was identified in 7 probands with ventricular outflow tract anomalies (4 with transposition of the great arteries (TGA)). The CNV encompasses a CNE, with no control or literature overlap. Further studies performed in the mouse demonstrate this element's ability to drive expression of a trans gene in the heart. The data to date suggests that deletion of this regulatory CNE is a potential cause of the CHDs in these probands. Further work is needed to identify the gene(s) regulated by this CNE and to fully elucidate the role that it plays in heart development. This suite of analytic software, using CNV deletions to localize CNEs that regulate cardiac genes, could be used to find genotype-phenotype correlations between other enhancers and correlated CHDs, as well as applied to other diagnoses.

JOHNSON, AW

TWO-PHOTON IMAGING OF AQUEOUS OUTFLOW STRUCTURES IN THE INTACT MOUSE EYE. AW Johnson, (M.D., SOM), DA Ammar, and MY Kahook. Department of Ophthalmology, University of Colorado Denver, Aurora, CO.

PURPOSE. To perform novel imaging of the conventional outflow pathway within the intact enucleated mouse eye using a non-invasive microscopy technique that visualizes the inherent fluorescence of the tissue.

METHODS. Two-photon microscopic (2PM) imaging techniques, including two-photon autofluorescence (2PAF) and second harmonic generation (SHG), were used to image through the sclera of the mouse eye into the trabecular meshwork region to image collagen and melanin content, respectively. Cardiac perfusion of fluorescein-conjugated dextran was used to label blood vessels within the eye to serve as an anatomical reference. Eyes were subsequently fixed and paraffin embedded; histologically stained sections were photographed for comparison to the 2PM images.

RESULTS. Three-dimensional analyses of multiple 2PM images reveal a well-defined SHG-vacant region adjacent to the iris and cornea that is consistent with the location of Schlemm's canal (SC). Each of these open regions is continuous with smaller tube structures that appear to be collector channels. These structures do not label in mice perfused with the vascular probe dextran, supporting the hypothesis that the enclosed spaces are filled with aqueous humor rather than circulating blood. The small trabecular meshwork region in the mouse eye is visible from the SHG signal of the collagen fibers in some, but not all images.

CONCLUSION. These results support the hypothesis that 2PM is useful for visually monitoring the conventional outflow pathway in animal models of glaucoma.

KAUVAR, EF

HOLOPROSENCEPHALY AND 18p: THE ROLE OF THE *TWISTED GASTRULATION HOMOLOG 1 (TWSG1)* GENE IN HUMAN BRAIN AND CRANIOFACIAL DEVELOPMENT.

EF Kauvar (MD, SOM), P Hu, DE Pineda-Alvarez, BD Solomon, A Dutra, E Pak, E Roessler, and M Muenke. Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

BACKGROUND: Holoprosencephaly (HPE) is the most common disorder of human forebrain and facial development. Presently understood etiologies include both genetic and environmental factors, acting either alone, or more likely, in combination. The majority of patients without overt chromosomal abnormalities or recognizable associated syndromes have unidentified etiologies. A potential candidate gene, *Twisted Gastrulation Homolog 1 (TWSG1)*, was previously suggested as a contributor to the complex genetics of human HPE based on (1)

cytogenetic studies of patients with 18p deletions, (2) animal studies of *TWSG1* deficient mice, and (3) the relationship of *TWSG1* to bone morphogenetic protein (BMP) signaling, which modulates the primary pathway implicated in HPE, Sonic Hedgehog (SHH) signaling.

OBJECTIVES: We sought to analyze a large cohort of patients with HPE for coding sequence variations in *TWSG1* and to perform fine mapping of 18p for a subset of patients with partial 18p deletions. **METHODS:** High Resolution Melting (HRM) followed by direct DNA sequencing were performed to test for sequence variations in *TWSG1*. Fine mapping of the HPE minimal critical region on 18p was performed using targeted fluorescence *in situ* hybridization (FISH) probes and array Comparative Genomic Hybridization (aCGH). **RESULTS:** After testing nearly 350 patients with HPE, we identified 2 patients with previously undocumented synonymous coding sequence variants and 1 patient with a previously undocumented missense variant. Fine-mapping of 10 patients with HPE and partial 18p deletions revealed: both *TWSG1* and *TGIF* deleted in 3 severely affected and 3 mildly affected patients; *TGIF* alone deleted in 2 severely affected patients and 1 mildly affected patient; *TGIF* deleted and *TWSG1* duplicated in 1 severely affected patient. **CONCLUSIONS:** Surprisingly, minimal evidence for alterations of *TWSG1* was found, suggesting that sequence alterations of *TWSG1* are neither a common direct cause nor a frequent modifying factor for human HPE pathologies.

ZIMMERMAN, M

SIROLIMUS DETERS THE RATE OF HEPATITIS C PROGRESSION FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION: A SINGLE CENTER EXPERIENCE. MA Kelly, (M.D., SOM), M Kaplan, MA Zimmerman, M Wachs, T Bak, and I Kam, Division of Transplant Surgery, University of Colorado Health Sciences Center, Aurora, CO.

Recurrence of hepatitis c virus (HCV) infection is a foreseeable problem following orthotopic liver transplantation (OLT), which over time leads to liver fibrosis and graft loss. While several animal studies have shown that sirolimus (SRL) acts to inhibit the rate of liver fibrosis, few studies in the human population support or refute these findings. Our center has had ample experience with using SRL as an effective immunosuppressive agent. In this study we sought to determine the difference in the rate of recurrence of HCV infection as well as the progression of infection in patients who received SRL as primary immunosuppression in comparison to patients who received calcinurin inhibitors (CNIs) post-OLT.

Patients transplanted for end-stage liver disease (ESLD) due to HCV were identified from our transplant database. These patients were categorized into two groups: CNI immunosuppression (control group) and SRL immunosuppression group. We controlled for 3 clinicopathologic variables (MELD, warm ischemic time, and cold ischemic time). Cox proportional hazards regression was used to examine the effect of Sirolimus on overall mortality as well as severe HCV recurrence (defined as a liver biopsy of stage 2 fibrosis or greater). Logistic regression analysis was used to test the association between Sirolimus and severe HCV recurrence within in the first year following OLT.

From January 2000 to July 2009, 313 patients underwent OLT for HCV. After applying exclusion criteria our study population was 232 patients, of which 60 patients received SRL as primary immunosuppression therapy. Overall, no effect was detected of SRL on mortality. For severe HCV recurrence within the study period, SRL had a statistically significant reduction in risk of the outcome by 58% ($p=0.011$) and a trend toward significant reduction in the odds for severe HCV recurrence within the first year following OLT ($p=0.072$).

While a debate exists about the effect of SRL therapy on recurrence of HCV infection following OLT, we demonstrated a significant reduction in risk in this single-center experience.

Furthermore, SRL therapy showed a trend toward significance in reducing the odds of severe HCV recurrence within the first year following OLT.

LIN, Y
ROSS, H

PREOPERATIVE VITAMIN D DEFICIENCY DOES NOT AFFECT POST OPERATIVE

HYPOCALCEMIA Yihan Lin, MD candidate and Hayley Ross, MD candidate. Faculty: Dr. Christopher Raeburn. Department of Surgery, University of Colorado, Denver, CO.

Background: Postoperative hypocalcemia is a common complication of thyroidectomies and parathyroidectomies. However, evidence in the literature is lacking as to whether variations in preoperative vitamin D impact postoperative hypocalcemia. This study seeks to determine the impact of preoperative Vitamin D levels on post operative levels of serum calcium and whether it is predictive of symptomatic hypocalcemia.

Methods: This is a retrospective cohort study of 103 patients who underwent thyroidectomies and parathyroidectomies from 2008 to 2010. Preoperative Vitamin D levels were obtained and patients were grouped by Vitamin D levels <30 (group 1, n=53) and patients with vitamin D levels ≥30 (group 2, n=50). The primary outcome measure was postoperative serum calcium level. Patient's lowest inpatient calcium was recorded as well as the most current calcium levels. All patients were evaluated for signs and symptoms of hypocalcemia, which included numbness, tingling, muscle spasms, positive Chvostek's sign, and positive Trousseau's sign. Results: The average preoperative Vitamin D level for patients in group 1 was 20.0 as compared to 39.4 in group 2. Average of the most recent serum calcium levels were 8.7 for group 1 vs 9.0 for group 2 (p=0.002). Lowest mean inpatient calcium levels recorded were equal at 7.9 (p=0.802). Symptomatic hypocalcemia was documented in 30% of patients in group 1 vs. 10% in group 2 (p=0.051). Average length of stay was 1.7 days for group 1 vs 1.3 for group 2 (p = 0.229).

Conclusion: Preoperative vitamin D deficiency is correlated with lower postoperative serum calcium levels as well as a 20% decrease in symptomatic hypocalcemia. However, it does not impact long term calcium levels as well as length of stay in hospital. These data suggest that measuring vitamin D levels preoperatively might affect short term effects of hypocalcemia, but do not affect long term hypocalcemia.

LUEBBERT, T

THE ROLE OF NETRIN-1 RECEPTORS IN MEDIATING ATTENUATION OF ACUTE KIDNEY

INJURY. Luebbert, T. (M.D., SOM), Dalton, J.H., Grenz, A., Eltzschig, H.K., University of Colorado, Denver, School of Medicine

Purpose: Ischemia is the most common cause of acute kidney injury (AKI). For example, patients undergoing surgical procedures requiring cross-clamping of the aorta or renal vessels experience AKI in up to 30% of cases. Similarly, AKI after cardiac surgery occurs in approximately 10% of patients and is associated with dramatic increases in morbidity and mortality. Our laboratory has previously shown that netrin-1 attenuates kidney injury and inflammation following renal ischemia. The current study will investigate which of the known netrin-1 receptors (UNC5A-D, neogenin, DCC, A2BAR) mediates renal tissue protection. These findings could provide new therapeutic options for patients with AKI.

Methods: Mice were anesthetized using intraperitoneal pentobarbital. Mice underwent renal ischemia (30 or 60 minutes) using a previously-described hanging-weight system to selectively occlude the left renal artery. The kidney was allowed to reperfuse (2 or 6 hours), then left nephrectomy was performed and the kidney was flash-frozen. Each experiment was completed on 3 wild-type and 3 mice with partial depletion of netrin-1 expression (Ntn1+/-). Homozygote mice gene-targeted for netrin-1 are not viable and die shortly after birth. Fold-changes in netrin receptor expression were determined via RT-PCR. Confirmatory studies will be completed using Western Blot for receptors that show significant fold-changes in mRNA expression.

Results: Preliminary data show an up-regulation of UNCB5 and A2BAR in wild type mice and heterozygote mice following renal ischemia and reperfusion compared to controls without

ischemia. We could not show any regulation of the other receptors so far. However, the number of experiments must be increased to confirm this initial data set.

Conclusion: Based on mRNA analysis, the kidneys seem to up-regulate the UNC5B and A2B receptors after renal ischemia. Further studies must be performed to confirm these preliminary findings.

MALLER, O

THE FUNCTION OF MICROENVIRONMENT IN BREAST CANCER DORMANCY

O Maller (PhD Candidate, GS)^{1,2}, T Lyons¹, K Hansen³, and P Schedin^{1,2}

¹ Div. of Medical Oncology; ² Cancer Biology Program; ³ Depart. of Biochemistry & Genetics, University of CO Denver,

Identifying the role of the microenvironment in tumor cell transition from a quiescent/non-motile state to a proliferative/invasive state may shed light on why some tumors remain dormant, while others do not. This study focuses on understanding the functional and compositional differences between 'tumor supportive' and 'tumor suppressive' extracellular matrix (ECM) as the underlying 'soil' that influences tumor dormancy. While a 'tumor suppressive' microenvironment may benefit a patient in the short term, a long-term consequence may be existence of residual tumor lesions that could cause progression and/or relapse if these suppressive attributes are lost.

To study a physiologically relevant 'tumor suppressive' microenvironment, we focused on the parous mammary gland since parity has been shown to reduce risk of mammary cancer in rodents and humans. To support this observation, we injected breast tumor cells into parous and nulliparous mouse mammary fat pads, and observed a decrease in tumor growth in the parous glands. We have previously shown that mammary ECM isolated from parous female rodents (parous matrix) inhibits mammary epithelial cell ability to form branching structures *in vitro* compared to nulliparous matrix. Thus, we hypothesize that parous matrix will impede mammary tumor growth and invasiveness. An *in vitro* tumor dormancy assay developed by Barkan et al. (Cancer Research, 2008) was utilized to test whether nulliparous matrix was conducive to tumor progression, while parous matrix had suppressive attributes. Mammary tumor cells previously shown to exhibit a dormant phenotype in this *in vitro* assay (D2.0R cells) stayed dormant on Matrigel and were suppressed on parous matrices. Yet, when these cells were exposed to nulliparous matrices they were "awakened" and formed larger organoids. Moreover, D2.0R cells on Matrigel and parous matrices had greater retention of epithelial features including increased junctional β -catenin and decreased cytoplasmic E-Cadherin. Phospho-ERK1/2 levels were also decreased in D2.0R cells on Matrigel and parous matrices. Additionally, a 40% decrease in organoid size was observed when D2.0R cells on nulliparous matrix were treated with a MEK1/2 inhibitor, suggesting that ERK signaling may be involved in activating these cells. Finally when breast tumor cells were co-injected into mouse mammary fat pads with either parous or nulliparous matrices, tumor growth was significantly reduced in the glands injected with tumor cells plus parous matrices. To address what may account for these functional differences, the composition of nulliparous and parous matrices is being evaluated via mass spectrometry.

Our goal is to identify differences in the ECM components of 'tumor suppressive' and 'tumor supportive' microenvironments to gain mechanistic insight into the clinical course of dormant breast tumor cells and how to target them.

MITCHELL, KV

MEDICAL EDUCATION REGARDING HEALTH CARE ISSUES FOR PEOPLE WITH DOWN SYNDROME. KV Mitchell (MD, MS), S Sillau, E Elias. Department of Medicine, University of Colorado, Denver, CO.

People with Down Syndrome (DS) have unique health care issues which cause secondary disability if left undiagnosed. Medical students, residents, fellows, medical school deans and residency directors have been surveyed regarding medical education of health care issues for people with DS and have responded that it is lacking. Little research has been done to survey practicing physicians to determine if they feel their education on this topic was adequate for the patients they see with DS. We surveyed 583 physicians across Colorado including family practitioners and pediatricians to ask them to rate their medical education regarding DS. We also asked them to describe their patient population, their awareness of certain health care issues for people with DS, their awareness and use of the published guidelines, and whether they would benefit from further education regarding DS. In response, 433 (74.3%) physicians completed the survey. Of those, 308 (71.1%) physicians described their medical school training as poor or fair while 248 (57.2%) physicians described their residency training as poor or fair. Pediatricians were more likely to rate their medical school training as good or excellent (OR, 2.7; 95% CI, 1.9-4.0, P<0.0001) as well as their residency training (OR, 7.5; 95% CI, 5.0-11.2; P<0.0001). They were also more likely to be aware of the published health care guidelines (OR, 10.2; 95% CI, 6.9-15.1; P<0.0001), to implement the guidelines (OR, 4.2; 95% CI, 2.2-7.8; P<0.0001), and to be aware of the specific health care issues for people with DS. Of all the physicians, 294 (67.9%) said they would benefit from special training regarding health issues in people with DS. These results show that improvement is needed in medical education regarding DS for physicians to be able to treat their patients adequately.

MORRIS, BL

DIAGNOSTIC ERRORS RESULTING IN MALPRACTICE CLAIMS: A DESCRIPTIVE STUDY

BL Morris, AB¹(MD, SOM); D Levin, MD¹; G Misky, MD¹; M Victoroff, MD¹ ¹University of Colorado School of Medicine, Aurora, Colorado

Background: Diagnostic errors represent a significant proportion of medical errors and are a leading cause of medical malpractice litigation.

Purpose for study: To describe the factors that lead to diagnostic error resulting in malpractice litigation.

Methods: COPIC Insurance Co. captures data on malpractice claims for covered providers. We identified claims in which "Wrong or Delayed Diagnosis" was a factor. Two experienced clinicians reviewed brief, nurse-generated narratives for each claim. They sorted wrong diagnosis claims into six broadly defined categories: 1) clinician error, 2) patient care delivery system error, 3) error due to patient non-adherence, 4) unavoidable procedural complication, 5) no apparent error occurred, and 6) further chart reviewed needed.

Results: Out of 33,189 reports (claims and occurrences) collected from 2002-2010, 4,141 events (12%) were coded as involving diagnostic error; of which 1,295 (31%, 4% of the total) were claims. After removing duplicate records in cases with multiple claims, 785 claims were reviewed. Clinician error was judged to have more frequently occurred among reviewed malpractice claims (286, 36% of total). Breakdowns in the delivery of patient care (failure to follow-up on abnormal labs or imaging, as well as procedural delays) accounted for 143 claims (18%).

Conclusion: In malpractice claims involving a wrong or delayed diagnosis, clinician error appears to be the most frequent contributing factor. Errors in patient care delivery systems were the second most frequent contributing cause leading to diagnostic error.

MURPHY, BV

INCREASED SUSCEPTIBILITY OF CD1D -/- MICE TO ACETAMINOPHEN-INDUCED LIVER INJURY. Murphy BV, (Ph.D, GS), Ju C. Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO 80045, USA.

The idiosyncratic nature, severity and poor diagnosis of drug-induced liver injury (DILI) make these reactions a major safety concern during drug development, as well as the most common cause for the withdrawal of drugs from the pharmaceutical market. Evidence suggests that aside from drug-induced direct damage to hepatocytes, an inflammatory innate immune response is triggered that may contribute to the overall pathogenesis of liver injury. The specific role that natural killer T (NKT) cells play in acetaminophen (APAP)-induced liver injury remains the topic of much controversy. CD1d is a major histocompatibility complex (MHC) class I-related molecule that functions in glycolipid antigen presentation to NKT cells allowing for their activation and development.

We found that upon APAP-challenge of mice, CD1d deficient mice (lack of NKT cells) are more susceptible to hepatic injury compared to WT mice. Additionally, CD1d -/- mice demonstrate higher protein adducts. This protective effect afforded by CD1d molecule appears to be dependent on the regulation of CYP2E1 that is significantly upregulated in CD1d -/- mice following starvation. Furthermore, we have observed a significant increase in ER stress (CHOP protein expression) in our CD1d -/- mice prior to APAP-challenge (after starvation), and 2, 8 and 48 hr after APAP-challenge. Lastly, when treating our CD1d -/- mice with an ER stress inhibitor tauroursodeoxycholic acid, TUDCA, we are able to decrease the observed increase in CYP2E1 protein levels.

Collectively, our data indicate a protective role of the CD1d molecule through its ability to regulate ER stress and thereby CYP2E1 expression during the pathogenesis of APAP-induced liver injury.

PARKER, JJ

DEVELOPMENT OF AN ORGANOTYPIC SLICE CULTURE MODEL OF HUMAN MALIGNANT GLIOMAS AND RESPONSE TO PHYSIOLOGIC MANIPULATORS. JJ Parker, (M.D.-Ph.D., GS) K Dionne, J White, R Massarwa, A Waziri. Department of Neurosurgery, University of Colorado, Denver, CO.

Malignant gliomas, including anaplastic astrocytomas and glioblastoma multiforme (GBM), are aggressive and deadly central nervous system tumors, which have evaded significant advances in treatment for many years. GBM has a dismal survival time from diagnosis of 12-15 months despite surgery, radiation, and chemotherapy. New treatments are desperately needed for patients. Therefore, strategies allowing for rapid translation from bench to bedside are critical to malignant glioma research. Traditional cell culture methods fail to recreate the unique brain microenvironment and extracellular matrix. To provide for a novel and highly physiological system that more accurately recapitulates human brain tumors in their native environment, we have begun the development of an *in vitro* system by which we can culture fresh *ex vivo* sections of human malignant glioma from tumor resections.

Glioma cell movement and normal neural progenitor cell tropism towards the tumor mass are two aspects of glioma biology which are thought to rely on gradients of chemotactic factors. The slice culture system is an ideal platform with which to study the effects of these gradients on tumor behavior. Hypoxia, a key pathologic characteristic of the GBM microenvironment, is known to drive to expression of various pro-angiogenic and pro-migratory factors including VEGF and PDGF via HIF-1 α stabilization.

Our preliminary data indicate that we can culture 400um slices of malignant gliomas for up to 15 days, and these slices retain GFAP+ cells via confocal imaging. Further, these cultures demonstrate cellular viability via the MTT assay at 13 days of culture. Lastly, when grown in

hypoxic conditions (1% O₂) tumor slices increase vascular endothelial growth factor (VEGF) secretion into the media, compared to secretion levels in normoxic (21% O₂) conditions.

PATTI, BN
DAWSON, AL

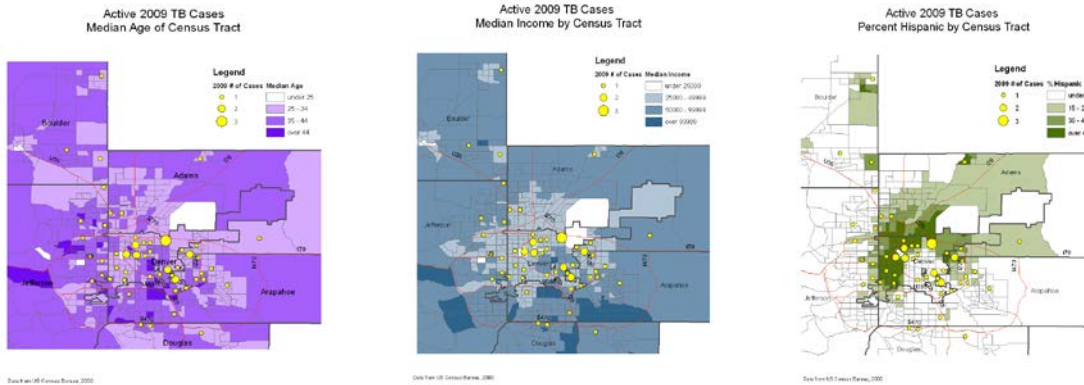
SPANISH ACQUISITION BEGETS ENHANCED SERVICE (S.A.B.E.S.): A NEW APPROACH TO MEDICAL SPANISH LANGUAGE EDUCATION FOR NOVICE SPANISH STUDENTS. BN Patti (M.D., SOM), AL Dawson (M.D., SOM), S Lowenstein. Department of Emergency Medicine, University of Colorado, Denver, CO.

S.A.B.E.S. is a for-credit, student-run medical Spanish elective course offered to first and second year medical students at the University of Colorado. The class teaches a functional medical Spanish curriculum which can be immediately applied in the clinical setting. The course combines training in medical vocabulary with mock patient interviews and weekly sessions with Spanish-speaking mentors. In previous years, beginning students reported that the course did not provide them with adequate language preparation and that they were unable to adequately apply their Spanish skills in a clinical setting. In response to this feedback, we wrote a textbook and designed and implemented a new beginners' curriculum in the 2009-10 academic year. This curriculum was designed to provide novice Spanish students with a foundation in basic language skills while maintaining a clinically-based teaching methodology. The eighteen-chapter textbook complements weekly lesson plans and provides a clinically-focused introduction to Spanish grammar and mechanics. The new textbook and curriculum were well-received by beginning level students and improved student performance and language command as evidenced by end-of-year performance in the C.A.P.E. (Center for Advancing Clinical Excellence), informal evaluation of student performance and progress, and student feedback. The textbook is being updated to include practice assignments and additional chapters. We also plan to integrate audio and video lessons into the curriculum with accompanying self-assessment components. The curriculum is currently being utilized in the 2010-11 school year.

PAWLAK, M

GEOGRAPHIC INFORMATION SYSTEM MAPPING OF 2009 ACTIVE TUBERCULOSIS IN DENVER METRO AND SOCIOECONOMIC PATTERNS. M Pawlak, (MPH) and R Reves. Denver Public Health, Denver, CO.

Geographic information system mapping of the Denver metro active tuberculosis cases in 2009 was performed to determine if socioeconomic patterns exist. The 2009 active tuberculosis data were obtained from Denver Public Health. The 2000 census tract data of median age, median income, and percent Hispanic in the Denver metro area was obtained from the United States Census Bureau. These data were overlaid in maps using ArcGIS 9.3 and analyzed to determine the association between the active tuberculosis cases and the socioeconomic data. In preliminary analysis, no association was found between 2009 active tuberculosis cases in Denver metro area and 2000 census tract socioeconomic data. Further analysis and alternative data may be more informative. Future mapping using 2010 census data and block group data may delineate an association in the socioeconomic patterns of active tuberculosis in Denver metro.



PELLINEN, J

SLEEP DISRUPTION IN MICE CAUSED BY THE FATTY ACID SYTHASE INHIBITOR C75

Jacob Pellinen¹ (MD, UC Denver SOM), Matthew Esposito¹ and Éva Szentirmai^{1, 2, 3}
¹WWAMI Medical Education Program and ²Department of Veterinary and Comparative Anatomy, Physiology and Pharmacology, Washington State University, Spokane ³Sleep and Performance Research Center, Washington State University, Spokane

Introduction

Rodents have two metabolically distinct phases corresponding to the 12-12 light dark cycles. The majority of energy intake, as well as the majority of lipogenesis, occurs during the dark phase. This metabolic cycle corresponds with distinct phases of sleep-wake activity. Fatty Acid Synthase (FAS) catalyzes lipogenesis, and central or peripheral administration of FAS inhibitor strongly suppress food intake and body weight. The aim of this experiment was to examine the disruptions in sleep, body temperature, and locomotor activity caused by the administration of the FAS inhibitor, C75, in mice.

Methods: Male C57B1/6 mice of the same age (approximately 5 months) and weight (29-32 grams) received EEG and EMG electrode implantations, and a intraperitoneal telemetry transmitter to record temperature and locomotor activity. For baseline measurement, groups of mice were injected intraperitoneally with RPMI 1640 (10 ml/kg), and for the experimental measurement, were injected in the same location with C75 (7.5 mg/kg, 15 mg/kg, or 30 mg/kg) on the following day. For the consecutive baseline and experimental days, sleep, locomotor activity, and body temperature were recorded following the injections, which were given approximately 10 minutes before dark onset.

Results: The FAS inhibitor, C75, significantly decreased locomotor activity and body temperature post-injection in a dosage-dependent manner. Following all doses of C75, wakefulness increased and REMS was suppressed. Sleep intensity (measured by slow wave activity during NREMS) was significantly decreased in a dosage-dependent manner for between 6 and 12 hours.

Conclusion: These data support the hypothesis that the disruption of lipid metabolism may disrupt normal sleep.

PLANK, TD

CAP-INDEPENDENT TRANSLATION FROM THE HIV-1 5' LEADER IN A PATHOGENICALLY RELEVANT CELL TYPE REQUIRES SPECIFIC RNA STRUCTURAL ELEMENTS. TD Plank, (Ph.D., GS), L Opincariu, and JS Kieft. Dept. of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO.

The HIV-1 viral life cycle is orchestrated in part through interactions between host cell proteins and the 5' RNA leader of the genomic RNA. One function of the 5' leader is the

recruitment of the host cell translation machinery for synthesis of viral packaging proteins. The majority of eukaryotic cellular mRNAs recruit the translation machinery through a mechanism dependent on the m7G cap. However, some cellular and viral mRNAs recruit the translation machinery independently of the cap through RNA elements known as internal ribosome entry sites (IRESs). Previous studies using HeLa cells have demonstrated that the HIV-1 5' leader can initiate translation as an IRES, however, HeLa cells are not the natural host cell for HIV-1. This discrepancy is significant, as IRES activity can vary dramatically between cell types. Here we report results of our investigation of the cell type dependence of the HIV-1 IRES. We have found that HIV-1 IRES activity is cell type specific, with robust activity in CD4+ T-cells, but not in all T-cell lines. This result suggests that the HIV-1 IRES may require a particular host cell factor(s) for optimal function. Furthermore, using a series of internal and deletion IRES mutants, we have mapped the secondary structural determinants of function for the HIV-1 IRES in CD4+ T-cells and have identified a possible RNA structure-based regulatory domain for HIV-1 IRES activity. We are now exploring the possibility that translation from the HIV-1 IRES is modulated during the cell cycle and RNA structure may play a role in this regulation. Together, our initial studies suggest a possible model for HIV-1 IRES function in a pathogenically relevant cell type.

PODLOGAR, MC

PATIENT/FAMILY CHARACTERISTICS IN AMERICAN INDIAN CHILD AND ADOLESCENT QUALITY OF CARE RESEARCH. MC Podlogar, (M.D., SOM), DK Novins, Department of Psychiatry, University of Colorado, Denver, CO.

Across the country, both rural and urban American Indian (AI) children and adolescents are at a higher risk than other U.S. ethnic groups for developing mental health problems such as depression, substance abuse, domestic violence, and suicide, and have a disparately high need for mental health services. New tribal healthcare service systems, as well as state and federal agencies (such as Medicaid and the IHS), could highly benefit from a systematic assessment of the quality of the mental health care they provide to AIs. Perhaps the best known framework is that proposed by Donabedian, which organizes the assessment of care into an assessment of its structure, process, and outcome. Additionally, health policy makers, providers, and consumers have recognized that culturally competent care addresses and eliminates racial/ethnic disparities in health care. We propose to introduce a fourth domain, "patient/family characteristics," to Donabedian's framework, and to investigate how this domain relates to the other domains of assessment, as well as to cultural appropriateness of care.

Data extracted from medical records of three research sites (with a subsample verified by an independent rater as a quality control mechanism) and interviews with administrators, clinicians, parents, and youth will be analyzed with the aid of qualitative analysis programs such as NVivo, looking for clinician, patient, and family perspectives of treatment in regards to the four examined domains of quality of care.

Collaboration with the partner sites and preliminary analysis of data extracted from medical records has defined patient/family characteristics to include sociodemographics, family structure, specific behavioral health difficulties, attitudes towards treatment, and barriers or support to care. Further research will analyze the relationship of these characteristics to structure, process, and outcome.

We hypothesize that the relationship between patient/family characteristics and structure, process, and outcome of mental health services will provide a useful model for subsequent research in more culturally sensitive AI quality of care studies.

POTOCKO, JR

NEUROENDOCRINE REGULATION OF CEREBROSPINAL FLUID AND INTRACRANIAL PRESSURE: EXPLORING THE POSSIBLE LINK TO CHRONIC CARBON DIOXIDE

EXPOSURE. JR Potocko, (M.D.,SOM), DJ Alexander, Space Medicine Division, Johnson Space Center, Houston, TX.

Astronauts endure a variety of unique environmental exposures during long-duration missions on board the International Space Station (ISS), including microgravity and elevated partial pressure of carbon dioxide (CO₂). Historically, various crewmembers on ISS and the Space Shuttle have reported the onset of headache and vision changes closely associated with acutely elevated CO₂ levels. Often a reduction in CO₂ provides an abrupt improvement in symptoms. Over the past decade, there has been growing concern about the combined effects of microgravity and chronic CO₂ exposure contributing to persistent intracranial hypertension (IH). Thus far, the most serious consequences of IH have included significant visual acuity changes as well as visual field deficits occurring in flight that persist for several months upon return to earth. These vision changes have correlated to elevated opening pressures on lumbar puncture, as well as papilledema on fundoscopy, optic nerve sheath expansion on ocular sonography, and globe flattening on MRI. Consequently, long-term space exploration will require an improved understanding of appropriate cabin CO₂ levels, as well as any countermeasures that may serve to protect crewmembers from debilitating symptoms. CO₂ must be chemically scrubbed from the cabin environment, using equipment that requires precious mass, volume, power and which produces heat and waste to be expended. Experiments are currently being developed by NASA to determine whether chronic low-level CO₂ exposure may be contributing to prolonged IH. These will include non-invasive studies such as imaging and cognitive testing, as well as lab tests of serum and cerebrospinal fluid (CSF). Based on reviews of medical and scientific literature, preliminary NASA flight data, and proposed mechanisms of CO₂ toxicity, this paper proposes that measurement of hormonal signaling molecules in CSF may help elucidate potential mechanisms of chronic CO₂ toxicity in the microgravity environment. Specifically, atrial natriuretic peptide (ANP) and arginine vasopressin (AVP) appear to play important roles in downregulating the production of CSF in the choroid plexus of humans with intracranial hypertension.

POWERS, MA

MORBIDITY OF TUBE THORACOSTOMY FOR TRAUMA IN SOUTH AFRICAN

TUBERCULOSIS AND/OR HIV PATIENTS. MA Powers (M.D., SOM), DM Arnett, KA Johnson, NR Lamborn, AP Thyssen, SS Patterson, KJ Abbott, RS Lieurance, M Stokke, and DB Richards, Department of Surgery, Division of Emergency Medicine, University of Colorado, Denver, CO, and Josef Korbel School of International Studies, University of Denver, Denver, CO.

Pneumothorax, the presence of air between the parietal and visceral pleura, results in lung collapse and varying impairment of the cardiovascular and respiratory systems. Thoracostomy tube placement is considered the standard of care for a pneumothorax caused by penetrating traumatic injury; however, current literature reports between 9-30% complication rate for all tube placement procedures. We hypothesize that adhesions of the lung to the parietal pleura caused by TB lead to an increased incidence of mechanical placement complications from tube thoracostomy after trauma. Similarly, we hypothesize that the compromise of the immune system associated with HIV also leads to an increased incidence of infectious complications from tube thoracostomy after trauma. This may be important because the risks to these patients may outweigh the benefits of tube thoracostomy in some clinical settings.

The goal of this study is to statistically determine whether patient groups with TB, HIV, or both have a higher incidence of complications from placement of a thoracostomy tube caused by traumatic penetrating pneumothorax as compared to negative controls. The outcome was determined by the acquisition of complications or lack thereof. A retrospective chart review (n=978) was completed that included all patients over the age of 18 who presented to the Emergency Department (ED) with penetrating traumatic pneumothorax and were subsequently treated with thoracostomy tubes at GF Jooste Hospital, Cape Town, South Africa, from July 1, 2008 to June 30, 2009. TB and HIV status of each patient was obtained both through the GF Jooste Medical Records and the South African National Electronic Tuberculosis Register (ETR), in order to cross-reference ED patients with their TB/HIV status. Complication data included trauma caused by tube placement and infection, as well as outcome. Preliminary data suggests that the rate of complications is near 25%, with the most prevalent complication being tube misplacement. Upon cross matching of TB/HIV status with individual complication data, analysis will include chi-square, regression, multiple regression, and discriminate analysis using SAS. In addition, we will be following up this study with a prospective study to test outcomes in TB/HIV patients with tube thoracostomy. Our aim is to create a clinical decision rule for patients with penetrating trauma and TB/HIV in order to decrease complications, which may include conservative treatment instead of thoracostomy tube placement.

REDDI, A

**TRANSLATING GLOBAL HEALTH RESEARCH INTO INTERNATIONAL POLICY:
EXPERIMENTS IN PUBLIC HEALTH, POLITICS, AND ADVOCACY**

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Introduction: In May 2009 the Obama administration unveiled a new Global Health Initiative (GHI). In addition to the reauthorization of the President's Emergency Plan for AIDS Relief (PEPFAR) to fund HIV/AIDS, tuberculosis, and malaria, the plan also supports maternal and child health (MCH) initiatives. The architects of the Obama administration's GHI recommend funding MCH at the expense of future funding increases for PEPFAR.

Unfortunately, the policies of the Obama administration resulted in a retrenchment in PEPFAR funding for new patients in Uganda resulting in "antiretroviral rationing."

Methods: We sought to demonstrate that policies that de-emphasize PEPFAR threaten to undermine rather than support MCH in countries with high HIV/AIDS prevalence. We published academic publications in *AIDS* and *Science* as well as in *The Huffington Post*, *The Washington Post*, and *The New York Times* to provide an evidence-based critique of the GHI.

Results: Our advocacy and the contributions of others convinced the Obama administration to recommit the necessary funds (\$400 million) to continue antiretroviral scale-up in Uganda.

Conclusions: These results demonstrate that medical students can contribute to international policy change by effectively communicating scientific results in the academic literature and popular press.

REIDY, RE

THEORY OF MIND IN CHILDREN AT RISK FOR DEVELOPING PSYCHOSIS. RE Reidy, (M.D., SOM), SK Hunter, and RG Ross, Department of Psychiatry, University of Colorado, Aurora, CO.

Purpose: Theory of Mind (ToM), the ability to appropriately attribute mental states to the self and others, is a cognitive ability that normally develops between three and five years of age (Wellman & Liu, 2004). Theoretically, psychosis may be related to deficiencies in both monitoring and interpreting intentions of the self and others (Frith, 1992). A large number of studies show that schizophrenic patients have poor performance on ToM tasks (see Brune, 2005) and deficits in ToM performance have been found in first-degree relatives of

schizophrenic patients (Anselmetti et al., 2009), suggesting that poor performance on ToM tasks may be an endophenotype for schizophrenia. Surprisingly, early impairments in ToM have not been studied in the children of schizophrenics.

Methods: Children with at least one parent meeting *DSM IV* criteria for diagnosis of schizophrenia or other psychotic disorder and children with no family history of psychosis are given tasks at 40 and 48 months of age to assess ToM development. The first is a locations false belief task which requires the child to predict where a protagonist will search for an object based on a false belief about the object location. The second task is a contents false belief task and requires the child to attribute knowledge about the contents of a mislabeled box to the self and others. The Dimensional Change Card Sort is being administered concurrently in order to analyze interactions with executive function, another endophenotype for schizophrenia.

Results: Thirty children have completed the study to date and preliminary data show children's scores significantly increase with age, as predicted. Of the thirty children who have completed the study, three have a parent with a diagnosis of schizophrenia or other psychotic disorder, thus the study currently lacks sufficient power to analyze differences in performance between conditions of parental mental health status.

Conclusions: We hypothesize that poor performance on ToM tasks is an endophenotype for schizophrenia and other psychotic illness and may be detectable early in life. Longitudinal analysis is hypothesized to show a significantly larger increase in task performance for children with no family history of psychosis.

RITCHIE, PJ

EFFECTS OF COLESEVELAM ON LDL-C, A1C, AND GLP-1 LEVELS IN PATIENTS WITH TYPE 1 DIABETES: A PILOT RANDOMIZED DOUBLE-BLIND TRIAL. PJ Ritchie (M.D., SOM), SK Garg, EG Moser, JK Snell-Bergeon, BJ Freson, RM Hazenfield, Department of Internal Medicine and Pediatrics, University of Colorado, Denver, CO.

Colesevelam is indicated to lower low density lipoprotein cholesterol (LDL-C) in hyperlipidaemia and improves glycaemic control in type 2 diabetes. This short-term pilot study was the first to evaluate its effects in type 1 diabetes.

This double-blind, randomized, investigator-initiated, single-center, 12-week pilot study evaluated 40 adults (age = 36.4 ± 9.4 years) with type 1 diabetes (duration = 20.4 ± 8.5 years) and hyperlipidaemia. It was powered to show a treatment difference of >10% LDL-C reduction. Subjects received 3.75 g/day colesevelam (n = 20) or placebo (n = 20) for 12 weeks. LDL-C and haemoglobin A1c (A1c) levels were assessed at screening (week -2), baseline (week 0) and every 4 weeks throughout the treatment duration. Glucagon-like-peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) levels were measured during 4-h meal (Boost Plus®) challenge tests (MCT) at baseline and 12 weeks.

Colesevelam treatment resulted in a significant reduction in LDL-C values at 4 weeks [-12.1% (95% CI: -20.1 to -4.1), $p = 0.004$] which was sustained for the study duration ($p = 0.005$ at 12 weeks). The treatment group also showed a significant change in A1c from baseline at week 4; however, this was not significant for the study duration. There was a significant median increase in GLP-1 levels during the first 2 h of the baseline MCT in the treated group but no difference at 12 weeks.

During this short-term pilot study, colesevelam treatment effectively lowered LDL-C in patients with type 1 diabetes. Improvements in A1c seen at week 4 were not sustained. Effects on glycaemic control in subjects with type 1 diabetes may be related to a postprandial rise in GLP-1 levels and require further clinical study.

ROBERTS, S

WHO'S AFRAID OF THE BIG BAD H1N1? S Roberts, (MPH, CSPH), L Hines, Department of Biology, University of Colorado, Colorado Springs, CO.

In 2009, the World Health Organization declared H1N1 a global pandemic, and US federal officials invested \$2 billion on vaccination efforts. Despite the huge campaign to persuade Americans to receive H1N1 immunization, recent polls indicate that only 21 percent did. The goal of this project was to assess the characteristics of individuals who opted to receive H1N1 vaccination utilizing data collected from free H1N1 vaccination clinics offered to students, faculty and staff at the University of Colorado at Colorado Springs (UCCS). Despite the ease and convenience, only 10% of the total UCCS population took advantage of the on-campus clinics. Furthermore, UCCS faculty and staff were more likely to be vaccinated (47% faculty/staff versus 7% students), although students were more likely to be in a high priority group (e.g. 24 years or younger). When compared to the UCCS student population as a whole, a significantly higher proportion of female students attended the H1N1 on-campus clinics (61% of vaccinated vs. 54% of total population, $P=0.0014$). In addition, there was a slightly higher representation of minority students (23% of vaccinated vs. 19% of total population, $P=0.018$). The notably low participation rate is likely to be partially attributed to the timing of vaccine availability, which was close to peak H1N1 activity in Colorado Springs. In spite of the high potential for H1N1 exposure and the convenience of vaccination, the experience from this college campus demonstrates that different implementation strategies are warranted, and these strategies need to be tailored to designated target populations.

ROBIN, T

THE ROLE OF EYA3 AS A TARGET OF EWS/FLI1 IN EWING'S SARCOMA. T Robin (MD, SOM; PhD, GS), L Reaves, E McKinsey, P Jedlicka, and H Ford. Program in Molecular Biology and Department of OB/GYN, University of Colorado, Denver, CO.

Ewing's sarcoma is an aggressive pediatric bone cancer most commonly affecting patients between 10 and 20 years of age. The vast majority of Ewing's sarcomas harbor a characteristic translocation between chromosomes 11 and 22, encoding the EWS/Flt1 fusion protein. Flt1 is an Ets transcription factor with a DNA-binding domain, whereas the EWS portion of the fusion contributes a transcriptional activation domain. EWS/Flt1 acts as a potent oncogenic transcription factor that is critical in the development of Ewing's sarcoma. EWS/Flt1 knockdown and rescue microarray data suggest that Eya3 may be a downstream target of EWS/Flt1, which we have validated using EWS/Flt1 overexpression and knockdown systems. Eya3 is important in DNA repair, and can interact with the Six family of homeoproteins as a bipartite transcription factor. The Six-Eya complex promotes proliferation and survival to facilitate the proper development of many tissues, but these features can also lead to aggressive cancers when re-expressed in adult tissues. We have shown that Eya3 knockdown in the human tumor-derived Ewing's sarcoma cells line, A673, decreases the rate of proliferation of these cells. Additionally Eya3 knockdown sensitizes Ewing's sarcoma cells to chemotherapeutics currently used to treat Ewing's sarcoma: doxorubicin, etoposide, and vincristine. Furthermore, clonal isolates of A673 Eya3 knockdown cells, expressing very low levels of Eya3, morphologically resemble their presumed cell of origin, the mesenchymal stem cell. These data suggest that Eya3 is an important target of EWS/Flt1 that may serve as a clinically significant novel therapeutic target for the treatment of Ewing's sarcoma patients.

ROBINSON, ZD

ROLE OF ANTIMICROBIAL PEPTIDES IN ESOPHAGITIS. ZD Robinson*, (MD,SOM), J Masterson^{ψ†*}, L Hosford*, S Fillon^{ψ†*}, and GT Furuta^{ψ†*},^ψDigestive Health Institute, †The Children's Hospital, *University of Colorado Denver School of Medicine.

Esophagitis is caused by exposure of the epithelia to allergens (eosinophilic esophagitis-EoE) or acid (gastroesophageal reflux disease-GERD) but the exact pathogenetic mechanisms of these diseases are unknown. Recent data supports a potential role for microbial flora in the initiation or perpetuation of esophagitis. We hypothesize that dysregulated innate defense molecules (defensins) of esophageal epithelia contribute to esophagitis. This study aimed to measure defensin expression in patients with esophagitis and in esophageal epithelial cells of an *in vitro* model of eosinophilic inflammation.

Defensin (hβD1 and 3) expression from esophageal biopsies (EoE, GERD and normal) was measured by qRT-PCR. Epithelia hβD1 and 3 expression of an *in vitro* model of esophagitis [esophageal epithelia-HET-1A cell line and human peripheral eosinophils-IL-5 (100pg/ml) and GMCSF (100pg/ml)] were measured via qRT-PCR.

Esophageal biopsies from EoE patients have decreased expression of hβD1 compared to normal (0.19 ± 0.22 vs 1.0 ± 0.61 , respectively; $p=0.019$). EoE patients treated with topical steroids have similar hβD1 expression levels compared to normal (1.0 ± 0.80 vs 1.0 ± 0.61 respectively; $p=0.56$). HβD3 expression in biopsies from EoE patients is significantly decreased compared to normal (0.15 ± 0.21 vs 1.0 ± 0.65 , respectively; $p=0.03$). HβD3 expression in EoE patients treated with topical steroids trends but is not significantly reduced compared to normal (0.21 ± 0.14 vs 1.0 ± 0.65 respectively; $p=0.06$). HβD3 expression in biopsies from GERD patients and GERD patients treated with proton pump inhibitors is significantly decreased compared to normal (0.10 ± 0.07 vs 1.0 ± 0.65 and 0.17 ± 0.22 vs 1.0 ± 0.65 ; $p=0.03$ and $p=0.03$, respectively). HET cell hβD1 and 3 expression are significantly reduced by IL-5 and GMCSF compared to HET cells cultured in media alone (0.71 ± 0.12 vs 1.05 ± 0.11 and 0.39 ± 0.14 vs 0.95 ± 0.24 ; $p=0.03$ and $p=0.01$, respectively). HET cells cultured with IL-5, GMCSF, and eosinophils have a trend towards decreased hβD1 expression compared to controls without eosinophils (0.77 ± 0.08 vs 1.24 ± 0.22 ; $p=0.07$, respectively).

Defensin expression is decreased in esophagitis. We speculate that dysregulated defensin expression contributes to esophagitis.

SIMON, ST

REPRODUCIBILITY OF GAMA-BAND RESPONSE RECORDED BY MAGNETOENCEPHALOGRAPHY AND ELECTROENCEPHALOGRAPHY ST Simon (MD SOM), DC. Rojas (PhD) Department of Psychiatry, University of Colorado, Denver, CO.

Patients with schizophrenia often exhibit unusual sensory experiences, ranging from visual or auditory distortions to dynamic hallucinations. Oscillatory neuronal electrical activity in the range of 30-50 Hz (the gamma-band oscillation) has been proposed to involve feature binding or inter-regional communication within the brain and is critically dependent on inhibitory neurotransmission within the cerebral cortex. Gamma-band oscillatory power has been reported to be impaired in persons with schizophrenia and is also found in the first-degree relatives of persons with schizophrenia, suggesting a potential heritable component.

Magnetoencephalography (MEG) and electroencephalography (EEG) technologies have repeatedly shown subjects with schizophrenia to have a low gamma-band evoked power in response to auditory stimuli. This makes the measure an exciting potential tool that could be useful to other schizophrenia researchers, but no one has yet performed test-retest measurements in the same group of subjects. While there have already been numerous reports of gamma-band abnormalities in schizophrenia with these technologies which are replicable across sites, no study looking at the measurement reliability of the gamma-band metrics have

been reported. This study is a necessity before such data can be used in large-scale projects such as genetic linkage analyses or as biomarkers in clinical trials.

Twenty healthy and normal hearing adults screened for personal and family history of mental illness and neurological disorders will be recruited. The subjects will be recorded in an auditory steady state response (ASSR) paradigm while having a 64-channel EEG recording followed by a 248-channel MEG recording on the same day. One week later, they will return and repeat the same procedures. We will be comparing two stimuli in the MEG and EEG runs – amplitude modulated white noise and click trains, binaurally presented at 75 dB SPL. Both produce strong 40 Hz responses. We will be looking at phase-locked response amplitude and phase-locking factors, in both sensor and source space. The reliability of the source localization will also be a secondary interest variable. Thorough analysis of ASSR-derived measures of gamma-band power and inter-trial phase-locking for retest reliability will ensue.

With five of twenty subjects completed, no results have yet been analyzed. We expect to see a high level of test-retest reliability with the MEG and expect it to be significantly greater than that seen in the EEG recording.

SIPPEL, T

GRANULOCYTES AS A SOURCE OF IMMUNOSUPPRESSION IN GLIOBLASTOMA

PATIENTS. T Sippel (PhD, GS), K Nag, J White, A Waziri, MD. Department of Neurosurgery, University of Colorado, Denver, CO.

Glioblastoma Multiforme (GBM) is an aggressive form of primary brain tumor that responds poorly to current therapeutics, including recently developed immunotherapies. These patients have a widespread immunosuppression rendering their immune system unable to respond to the tumor, even with therapeutic stimulation. Immunosuppression has been well described in patients with GBM, however the source of the immunosuppression remains unknown. Our lab has identified a population of cells within the PBMC fraction of blood which is expanded in GBM patients and correlates with the phenotype and function of myeloid derived suppressor cells (MDSCs) recently described in other cancer types. When this population is used in a mixed lymphoid reaction, normal donor T cell function is suppressed. MDSCs in GBM patients were identified by the markers CD11b+CD33loCD14-HLA-DR- using flow cytometry. These markers were found to be identical to those expressed on granulocytes. To confirm the granulocytic phenotype, MDSCs were also stained for the expression of the granulocytic markers CD15 and CD66 and found to be positive for both. It is well known that granulocytes contain the enzyme Arginase I (ArgI) within their granules and it is released upon degranulation. ArgI acts to break down L-arginine, which is an amino acid required for T cell function through expression of the T cell coreceptor CD3 ζ . We found ArgI to be highly expressed in GBM patient plasma and during *in vitro* mixed lymphoid reactions using GBM patients cells compared to normal donors. In addition, GBM patient T cells showed lower expression of CD3 ζ compared to normal donors and patients with other types of brain tumors, suggesting a role for ArgI in GBM immunosuppression. To overcome T cell functional suppression by ArgI, extracellular L-arginine was restored *in vitro* through L-arginine supplementation or ArgI inhibition with the specific inhibitor nor-NOHA. Both were able to rescue the function of GBM patient T cells *in vitro* to a level of normal donors. Our data suggests that restoring extracellular L-arginine levels in GBM patients would be a promising new therapeutic option to overcome T cell dysfunction in GBM patients and act as an adjuvant to make immunotherapies more effective in these patients.

SMITH, AL

THE ROLE OF THE MIR106b-25 CLUSTER IN SIX1-MEDIATED BREAST CANCER

METASTASIS. A.L. Smith, D.S. Micalizzi, R. Iwanaga, R.L. Vartuli, D.J. Drasin, H.L. Ford.

Program in Molecular Biology, Department of Obstetrics and Gynecology, University of Colorado, Denver, Co.

The Six1 homeoprotein is an important regulator of embryonic development, where it controls many cellular processes. While highly expressed in embryogenesis, its expression is significantly diminished in most adult differentiated tissues. Six1 is re-expressed in many cancers, including breast cancer, where it is overexpressed in 50% of primary tumors, and a striking 90% of metastatic lesions. We have recently identified Six1 as an important mediator of breast cancer metastasis where it upregulates the TGF β pathway, induces an EMT-like transformation, expands the tumor initiating stem cell population, and increases metastatic spread in mouse models. We have found that this induction of metastasis is dependent on a Six1-mediated upregulation of TGF β signaling.

In examining how Six1 may regulate TGF β signaling, we have directed our work in part towards miRNAs. miRNAs are non-coding small RNAs that control gene expression. After an initial screen for miRNAs that were differentially expressed with Six1 expression, we found a cluster of miRNAs that are upregulated with Six1 overexpression, the miR106b-25 cluster. This cluster contains three miRNAs, miR-106b, miR-93, and miR-25. We have found that all three miRNAs correlate with endogenous Six1 expression, and further, that they are controlled by Six1 expression. Interestingly, this cluster has previously been found to impair the growth suppressive functions of TGF β . TGF β is a multifunctional cytokine, which can act as a tumor suppressor in early tumorigenesis via growth inhibition, but can act as a tumor promoter in later stages of tumorigenesis via induction of an EMT. We have found both in vitro and in vivo, that Six1 overexpressing cells and tumors will lose the growth suppressive functions of TGF β . We propose that the Six1 mediated upregulation of the miR106b-25 cluster may be a mechanism by which TGF β switches from a tumor suppressor to a tumor promoter in this system.

Interestingly, we also find that overexpression of the miR106b-25 cluster mirrors some of the phenotypes of Six1 overexpression. Namely, we have found that miR106b-25 miRNAs are sufficient to increase the mammary stem cell population, induce β -catenin transcriptional activity, and a reduction of E-Cadherin and B-catenin from the membrane. Additionally, we find that the miR106b-25 cluster also upregulates the Type I TGF β receptor (T β RI) and leads to increases in p-Smad3 protein, suggesting that it has a role in activating TGF β signaling, which we know to be the mechanism of many Six1-mediated phenotypes. Therefore, these miRNA may not only play a role in mediating the Six1 induced switch of TGF β to a tumor promoter, but also may activate TGF β signaling.

SMITH, AR

PSYCHOSOCIAL MEDIATORS TO PHYSICAL ACTIVITY DURING THE PERINATAL PERIOD: A SYTEMATIC REVIEW OF THE LITERATURE

Andrea R. Smith, (M.D.), Jenn Leiferman, PhD, Department of Public Health, University of Colorado at Denver, Health Sciences Center

Purpose: Less than half of US women reported regular exercise during pregnancy and 46 percent of normal weight women, 46 percent of obese women, and 59 percent of overweight women gain in excess of the recommended gestational weight advised by the Institute of Medicine. This evidence suggests that a sedentary lifestyle contributes to excessive gestational weight gain, which identifies a need for effective intervention strategies that target mediators to physical activity (PA) in order to increase activity levels in perinatal women. The purpose of this study is to critically review prospective and intervention studies identifying potential mediators to PA during the perinatal period.

Methods: PubMed, MEDLINE, CINAHL, and PsychINFO were searched for prospective studies that examined potential mediators to PA as well as any intervention studies that aimed to increase PA levels during pregnancy up to two years postpartum. Fourteen prospective studies and 7 intervention studies were selected that met the inclusion criteria of this review. Articles were reviewed and discussed according to the population, study design, mediators examined, measured outcomes, results, conclusions and limitations of the studies.

Results: The prospective studies identified several significant correlates of PA during the perinatal period including exercise and barrier self-efficacy, social support, prepregnancy exercise behavior, safety concerns, and perceived stress. Three intervention studies significantly increased PA levels, three did not achieve significant increases in activity, and one study performed an intervention to alter the potential mediators to PA, but did not measure activity levels.

Conclusions: The most commonly reported significant predictors of PA during the perinatal period in this review were self-efficacy and prepregnancy exercise behavior. Several intervention studies followed theoretical frameworks and targeted various mediators to PA. However, very few mediators were directly measured to determine the efficacy of the interventions in altering the mediators and PA levels. Further research is needed to establish significant mediators in the perinatal period, as well as effectively alter these mediators in intervention programs.

STOERMER, KA

A ROLE OF ARGINASE 1 IN ALPHAVIRUS-INDUCED RHEUMATIC DISEASE.

KA Stoermer¹ (Ph.D., GS) and TE Morrison², Departments of ¹Immunology and ²Microbiology, University of Colorado Anschutz Medical Campus, Aurora, CO.

Mosquito-transmitted *Alphaviruses*, such as Ross River virus (RRV) and chikungunya virus (CHIKV), are enveloped, positive-sense single strand RNA viruses that cause debilitating inflammatory diseases of joints, tendons, and skeletal muscle tissue. To investigate the pathogenesis of the rheumatic disease caused by these viruses, we have developed murine models that recapitulate many of the disease signs of RRV- and CHIKV-infected humans. Previous studies of the RRV model showed that both macrophages and complement component C3 (C3) play a role in RRV virulence. The reduced severity of disease observed in C3-deficient mice was independent of effects on recruitment of inflammatory cells to musculoskeletal tissues, suggesting that complement may regulate macrophage effector functions. Arginase 1 (Arg1) expression by myeloid cells is a major regulator of immune, inflammatory, and tissue repair processes in malignant, fibrotic, and infectious diseases. We detected high levels of Arg1 expression in inflamed musculoskeletal tissues and inflammatory macrophages of both RRV- and CHIKV-infected wild-type mice at the peak of disease signs. In contrast, Arg1 expression was dramatically lower in RRV-infected C3-deficient mice. To further dissect the role of Arg1 in alphavirus-induced musculoskeletal disease, we developed an in vitro model using myoblast and macrophage cell lines. Co-culture of macrophages with RRV-infected differentiated muscle cells, but not uninfected cells, results in induction of Arg1 expression in the macrophages. In addition, direct inoculation of macrophages with RRV or CHIKV induced Arg1 expression. Interestingly, ultraviolet light-treated virus also induced Arg1 expression in macrophages, suggesting that viral replication is not required for induction. Taken together, these findings suggest that induction of Arg1 expression in macrophages at the sites of inflammation may promote tissue damage and more severe disease. Thus, Arg1 may be a novel target for development of therapeutics to control alphavirus-induced rheumatic disease.

DEASON-TOWNE, F

GOT Mg²⁺? THE EMERGING ROLE OF Mg²⁺ AVAILABILITY ON B CELL RECEPTOR SIGNALING. F

Deason-Towne (Ph.D., GS)*, SE Farias**, CC Wu**, C Schmitz*, AL Perraud*. Department of Immunology*, Department of Pharmacology**, University of Colorado, Denver, CO.

Hypomagnesemia in patients, as well as in animal models, results in a weaker humoral immune response. The molecular mechanisms underlying this magnesium (Mg²⁺) dependent modulation of the humoral response remains undefined. The recently discovered regulator of Mg²⁺ homeostasis, TRPM7, is a cation channel with a fused serine/threonine kinase domain. It has been demonstrated that TRPM7-kinase interacts with several phospholipase isoforms including PLC

our hypothesis that in response to the environmental availability of Mg²⁺, TRPM7 acts as a homeostatic sensor to attenuate BCR signaling by serine/threonine phosphorylation(s) of PLC

response is reduced under low Mg²⁺ conditions. We also identify distinct serine and threonine residues on PLC

using a complementation approach in PLC deficient DT40 B cells, we show that PLC the mutated phospho-serine site results in a reduced BCR Ca²⁺ response, and an even further reduced response under hypomagnesian conditions. Using targeted proteomics, we have confirmed the phosphorylation of these Ser/Thr sites in full-length PLC

same methodology of multiple reaction monitoring mass spectrometry also allowed us to identify further previously uncharacterized PLC

TRPM7 actively responds to the availability of environmental Mg²⁺, in turn modulating downstream BCR signaling responses through serine/threonine phosphorylation of PLC interaction could be a key piece of the puzzle in explaining why animal models of nutritional hypomagnesemia have weaker humoral immune responses.

TRIOLO, TM

MULTIPLE AUTOIMMUNE DISORDERS IN NEW ONSET TYPE 1 DIABETES. TM Triolo (MD, SOM), L Yu, DM Miao, LA Scrimgeour, GJ Klingensmith, GS Eisenbarth, and JM Barker, The Barbara Davis Center for Childhood Diabetes, University of Colorado, Aurora, CO.

Children with type 1A diabetes (T1D) are at risk to develop multiple autoimmune disorders including autoimmune thyroid disease (AIT), celiac disease (CD), and Addison's disease (AD). Assays are available for thyroid peroxidase autoantibodies (TPOAb) of AIT, tissue transglutaminase antibodies (TTG) of CD, and 21-hydroxylase antibodies (21OHAb) of AD. We analyzed these non-islet, organ-specific antibodies at type 1 diabetes diagnosis and determined the number of individuals diagnosed with multiple autoimmune disorders within six months of type 1 diabetes onset. Patients diagnosed with T1D were followed over an average of 2.11 years for additional autoimmune disorders. Eligibility included diagnosis of type 1A diabetes (defined by the presence of at least one diabetes related antibody), age 0 to 30 years, and diabetes antibodies obtained within 3 months of diabetes diagnosis. Subjects were followed for AIT, CD or AD. 491 subjects met eligibility criteria and consented for follow-up. 53.4% were male and the average age at diagnosis was 9.6 years. 487 (99.2%) were tested for four diabetes related antibodies near diagnosis and 398 (79.9%) had two or more positive diabetes related antibodies. Individuals with antibodies associated with AIT, CD, and AD at T1D diagnosis were followed for progression to clinical disease. Of the 487 with positive diabetes antibodies, 160 (32.9%) had AIT, CD or AD antibodies at T1D diagnosis. 25% (122/487) were positive for TPO antibodies, 11.7% (57/487) were positive for TTG antibodies and 1.0% (1/487) were positive for 21OH antibodies. Throughout follow-up, 42 (8.6%) had evidence for progression to clinical disease (AIT, CD, or AD) and of these, 2 individuals had multiple autoimmune diseases in addition to T1D. DNA was available for 93.4% of subjects. The highest risk diabetes related genotype HLA DR3/4-DQ8 was present in 27.2% (31/114) of TPO

antibody positive (NS), 39.6% (21/53) of TTG antibody positive (NS), and 60% (3/5) of 21OH antibody positive subjects (NS), compared with 26.3% (80/304) of those with no additional autoantibodies. In conclusion, one-third of patients newly diagnosed with T1D are positive for at least one organ specific autoantibody. Within 6 months of T1D diagnosis 15.2% (28/184) of these were diagnosed with an additional autoimmune disease. Presence of these other organ specific autoantibodies is not related to the highest-risk T1D HLA genotype.

WEIGANG, TA

THE IMPORTANCE OF A DIAGNOSIS OF A HIGH RISK CONDITION WHEN PROVIDERS ARE CONSIDERING A REFERRAL TO EARLY INTERVENTION. TA Weigang, (MD, MS), A Talmi, R Asherin

Departments of Psychiatry and Pediatrics, University of Colorado-Denver, Aurora, CO

Purpose: Early Intervention services have been shown to be effective resources for young children (under 3 years old) who, either are developmentally delayed or have a condition that puts them at a high risk of becoming developmentally delayed. These services are most effective when utilized as early as possible. A little over 2% of the pediatric population under 3 years old is enrolled in Early Intervention services, but research indicates that as many as 13% of the children under 3 years old may be eligible for these services (Rosenberg et. al 2008). The current study aimed to look at patterns of pediatric provider referrals in the presence of an abnormal score on a parent report child developmental screen (Ages and Stages Questionnaire (ASQ)). Specifically, the study was designed to confirm findings reported by Silverstein, et al (2006), who reported that the majority of pediatricians they surveyed thought that an established diagnosis of a high risk condition was important when deciding whether to refer a child to Early Intervention. Our study aimed to determine if there is a population of children without established high risk conditions, but who show signs of developmental delay and are not being referred to Early Intervention services?

Methods: A retrospective study was done by extracting data from electronic medical records of all well child checks (WCCs), of children under 3, done at an outpatient Child Health Clinic in a Children's Hospital between October of 2008 and April of 2010. Data was obtained by a computer query of all WCCs documented in the electronic medical records. A computer query and manual review of these records were used to identify all WCCs that had an ASQ completed during the visit. The records with abnormal ASQ scores were analyzed. Established high risk conditions used were those recognized by Early Intervention Colorado.

Results: Data analyses are still pending but will include: the percent of ASQs with abnormal scores, Early Intervention referrals generated, and the number of children with established high risk conditions.

Conclusions: Pending

WILLIS, VC

CL-AMIDINE, A PROTEIN ARGININE DEIMINASE INHIBITOR, REDUCES THE SEVERITY OF MURINE COLLAGEN-INDUCED ARTHRITIS. VC Willis, (Phd, GS) A Gizinski, NK Banda, CP Causey, B Knuckley, KN Cordova, Y Luo, B Levitt, M Glogowska, P Chandra, L Kulik, WH Robinson, WP Arend, PR Thompson, V. Michael, Holers, Rheumatology, University of Colorado Denver, Aurora, CO

Rheumatoid arthritis (RA) is associated with the development of autoantibodies to citrullinated self-proteins. Citrullinated synovial proteins, which are generated via the actions of the protein arginine deiminases (PADs), are known to develop in the murine collagen-induced arthritis (CIA) model of inflammatory arthritis. Given these findings, we evaluated whether N-alpha-benzoyl-N5-(2-chloro-1-iminoethyl)-L-ornithine amide (Cl-amidine), a recently described pan PAD inhibitor, could affect the development of arthritis and autoimmunity by treating mice in the CIA model with Cl-amidine on days 0-35. Cl-amidine treatment reduced total synovial

citrullination, decreased clinical disease activity by ~50%, and significantly lowered IgG2a anti-mouse type II collagen (CII) antibodies. Additionally, histopathology scores and total complement C3 deposition were significantly lower in Cl-amidine treated mice compared to vehicle controls. Synovial microarray analyses demonstrated decreased IgG reactivity to several native and citrullinated epitopes when compared to vehicle controls. Cl-amidine treatment had no ameliorative effect on collagen antibody-induced arthritis (CAIA), suggesting its primary protective mechanism was not mediated through effector pathways. The fact that the levels of citrullinated synovial proteins are reduced in mice treated with Cl-amidine is consistent with the notion that Cl-amidine derives its efficacy from its ability to inhibit the deiminating activity of the PADs. In total, these results suggest that the PADs are necessary participants in the autoimmune and subsequent inflammatory processes in CIA. Cl-amidine may represent a novel class of disease-modifying agents that modulate aberrant citrullination, and perhaps other immune processes, necessary for the development of inflammatory arthritis.

Three points argue that RA may begin in the lung: 1) The strong association of smoking with RA; 2) the high prevalence of lung disease in even early RA; 3) The existence of patients with lung disease who are ACPA+ with no clinical signs of RA. It is therefore possible that immune dysregulation to citrullinated autoantigens begins in the lung. To investigate if the lung is a site of initiation for RA we have utilized CT scans, pulmonary function tests (PFTs) and citrulline content levels of bronchoalveolar lavage (BAL) to determine if arthritis-free, ACPA+ first-degree relatives (FDRs) of RA patients show signs of lung disease. Our results indicate that arthritis-free ACPA+ FDRs show increased signs of lung disease and citrulline levels compared to arthritis-free ACPA- FDR controls.

YAN, C

NOVEL INDOLEQUINONE INHIBITORS OF THIOREDOXIN REDUCTASE ARE ACTIVE AGAINST HUMAN PANCREATIC CANCER. C Yan¹, (Ph.D., GS), Biehuoy Shieh¹, David Siegel¹, M Colucci², A Chilloux², JJ Newsome², J Gomez², CJ Moody², D Ross¹. ¹Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Denver; ²School of Chemistry, University of Nottingham, UK.

A series of novel indolequinones was developed as potent antitumor agents against human pancreatic cancer. Three general classes of indolequinones with varying substitutions at the indole 2- position exhibited marked growth inhibitory activity against human pancreatic cancer both in-vitro (MTT and clonogenic assays) and in-vivo (mouse xenograft models). The pancreatic cancer cell lines PANC-1, MIA PaCa-2, and BxPC-3 were used as in-vitro model systems and the IC₅₀ values of the indolequinones in all three cell lines were in the low nanomolar range. Indolequinones were also found to be efficient inducers of apoptosis in these cell lines at concentrations which induced growth inhibition. Selected indolequinones were screened against the NCI-60 cell line panel and their spectrum of activity was similar to established inhibitors of thioredoxin reductase. Indolequinones were therefore tested as inhibitors of thioredoxin reductase and were found to inhibit the enzyme in pancreatic cancer cells at concentrations equivalent to those inducing growth inhibitory effects. The mechanism of inhibition of thioredoxin reductase by the indolequinones was then studied in detail in cell-free systems using purified enzyme and the C-terminal selenocysteine of thioredoxin reductase was found to be the primary adduction site of the indolequinones using LC-MS analysis. Inhibition of thioredoxin reductase by indolequinones in pancreatic cancer cells resulted in a change of thioredoxin redox state and activation of the p38/JNK signaling pathway. Oxidized thioredoxin is known to activate apoptosis signal-regulating kinase 1 (ASK1), the upstream activator of p38/JNK in the MAPK signaling cascade, providing a potential mechanism for indolequinone-induced apoptosis. Our results demonstrate that thioredoxin reductase, p38 and JNK, are potential targets of antitumor indolequinones in pancreatic cancer cells.

SLUG MRNA IS HIGHLY EXPRESSED IN SQUAMOUS LUNG CARCINOMA AND ASSOCIATED WITH EXPRESSIONS OF EGFR AND ZEB1 IN PATIENTS WITH NON-SMALL CELL LUNG CANCER (NSCLC). JM Zhao (MD, SOM), K Yoshida, C Mascaux, MW Wynes, R Dziadziuszko, S Witta, FR Hirsch. Division of Medical Oncology, University of Colorado, Aurora, CO.

PURPOSE: Understanding biological markers for disease progression and metastasis in lung cancer is a key step for developing new treatment. Epithelial-to-Mesenchymal Transition (EMT), a mechanism involved in the processes of invasion and metastases and sensitivity to EGFR Tyrosine Kinase Inhibitors, is characterized by the upregulation of the transcription repressors slug (a member of the snail family) and ZEB1 (Zinc finger E-box Binding homeobox 1) and the resulting downregulation of E-cadherin. **METHOD:** In the present study, we performed quantitative RT-PCR analysis on resected tumors from 121 patients with NSCLC to measure the mRNA expressions of slug, ZEB1 and EGFR using beta-actin as the normalization signal. **RESULTS:** Slug mRNA was found to be significantly higher expressed in squamous lung carcinoma as compared with adenocarcinoma with a mean level of expression of 1.41 vs. 0.61 ($p = 0.0009$), respectively. In addition, slug was positively correlated with EGFR (Spearman $r = 0.33$, $p = 0.0011$) as well as with ZEB1 (Spearman $r = 0.24$, $p = 0.028$). **CONCLUSION:** Our results demonstrate that slug mRNA is more elevated in squamous lung carcinoma, indicating that slug could be a potential target for the treatment of this type of lung cancer. The positive correlation between slug and EGFR based on patient tumors are consistent with the previous cell line data that showed decreased expressions of slug and ZEB1 when EGFR pathways were inhibited. Our results offer new insights into the pathways of EMT activation and suggest a synergistic relation between EGFR activation, its downstream Myc product in the MEK/ERK pathway, and slug induction.