



Pediatric Research and Career Development Symposium

Tuesday, August 6, 2019

Health Sciences Research Building Auditorium and Café
Noon - 6:00 pm

Abstract Book

**Oral Presentations
Poster Presentations
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Oral Presentation Abstracts

In Order of Presentation

Presentation Time: 1:30 - 2:30 pm

A Patient-Specific 3D Printed Model for *In Vitro* Analysis and Treatment Planning of Pulmonary Vascular Disease

Presenting Author: Dr. Holly Bauser-Heaton; Emory University

Tomov, Martin; Do, Katherine; Serpooshan, Vahid; and Bauser-Heaton, Holly

RATIONALE: Pulmonary atresia (PA) with major aortopulmonary collateral arteries (MAPCAs) is a heterogeneous form of pulmonary artery stenosis, where the outcomes of surgical rehabilitation vary widely, leading to lifelong complications secondary to distal stenosis. As such, PA with MAPCAs is a pathology with high impact on patient quality of life and merits improved treatment options. Lack of an *in vitro* animal models of PA, however, has substantially limited research and training endeavors to treat these rare and complex congenital cardiac lesions. Here, we present a functional patient-specific *in vitro* platform, capable of sustained flow, that can be used to train proceduralists and surgical teams in current interventions, as well as in developing novel therapeutic approaches to treat a variety of vascular anomalies.

OBJECTIVE: To develop an *in vitro* model of pulmonary stenosis, based on patient data, that can be used as a patient specific training tool, surgical aid, and to model cardiovascular disease and interventions.

METHODS AND RESULTS: Starting with the PA patient cardiac scans obtained from computer tomography (CT) or three-dimensional (3D) rotational angiography, we generated digital 3D models of the arteries. Subsequently, *in vitro* models (i.e., phantoms) of PA with MAPCAs were first 3D printed using biocompatible resins, and next bioprinted using gelatin methacrylate hydrogel, to simulate neonatal vasculature or second order branches of an older PA patient. Printed models were further used to study creation of extraluminal connection between atretic pulmonary artery and a MAPCA using a catheter-based interventional method used in the clinics. Following the recanalization, engineered PA constructs were perfused using a bioreactor and flow was visualized using contrast agents and X-ray angiography.

CONCLUSIONS: A novel 3D printed model for vascular atresia was successfully created. We demonstrated the unique capabilities of this model to develop a novel technique for establishing blood flow in atretic vessels using patient CT and 3D rotational data, together with 3D bioprinting-based tissue engineering techniques. Advanced medical imaging and additive manufacturing technologies can enable fabrication of live, functional vascular phantoms for practice and development of various interventional procedures that can be translated into clinical applications.

Modeling Complement-Mediated Acute Lung Injury in Sickle Cell Mice

Presenting Author: Dr. Satheesh Chonat; Emory University

Chonat, Satheesh; Patel, Seema; Jeffers, Lauren; Cisneros, Eduardo; Koval, Michael; Archer, David; Joiner, Clinton; and Stowell, Sean

Sickle cell disease (SCD) is a common and life-threatening autosomal recessive hematological disorder that affects almost 100,000 people in the US and millions worldwide. Amongst acute complications in SCD, acute chest syndrome (ACS) is a leading cause of hospitalization and the most common cause of

death due to SCD. Since the pathophysiology underlying ACS is still unclear, current supportive interventions include oxygen therapy, antibiotics and RBC transfusions. Retrospective analysis of stored plasma samples from our SCD patients with ACS revealed acute hemolysis, and significantly increased levels of anaphylatoxins (C3a and C5a) and markers of the alternative complement pathway (Ba, Bb) during episodes of ACS compared to their own baseline values. To examine the underlying mechanism of the role of complement in ACS, we developed a pre-clinical model of acute lung injury (ALI) in humanized sickle cell (SS) mice. Injection of cobra venom factor (CVF) to SS mice, a commonly used approach to induce complement activation, resulted in rapid deoxygenation, hypopnea and bradycardia (all hallmarks of ALI in mice), followed by death. In contrast, CVF treated littermate control (AA) mice did not develop detectable hemolysis, pulmonary compromise or increase in mortality. The SS mice had markedly increased levels of plasma anaphylatoxin C5a and increased C3 deposition in kidneys and lungs by immunofluorescence when compared to their controls and those treated with vehicle. While erythrocytes in these SS mice had elevated levels of C3b/iC3b/C3c deposition when compared to AA-mice, no difference was noted in the total plasma C3, suggesting sickle erythrocytes are prone to complement mediated hemolysis. Plasma C5a elevation in SS mice treated with CVF was accompanied by inflammatory responses, including increased expression of P-selectin. Our data thus far suggest that complement activation in SS mice results in a rapid drop in hemoglobin, release of free heme and production of C5a, which is a potent pro-inflammatory mediator that can activate leukocytes, platelets and endothelial cells, all of which possibly play a role in ALI. These results demonstrate that inhibition of C3a or C5a production may represent pharmacological targets to treat ACS in patients with SCD.

Harnessing the Osteo-Inductive Property of JAGGED1 as a Maxillary Bone Regenerative Intervention

Presenting Author: Dr. Archana Kamalakar; Emory University

Kamalakar, Archana; Amanso, Angelica; Ballestas-Naissir, Samir; Bhattaram, Pallavi; Davis, Michael; Willett, Nick; Drissi, Hicham; Abramowicz, Shelly; and Goudy, Steven

Maxillary bone deficiency (MBD), a challenging clinical problem, results from aberrant maxillary bone development, trauma or surgery. While bone morphogenetic protein (BMP2) is used to ameliorate MBD in adults, in pediatric cases, it leads to significant pain, erythema and inflammation. Therapies to stimulate bone formation without triggering painful inflammation, in children with MBD, are still lacking. JAG1, a membrane-bound NOTCH ligand, is required for normal craniofacial development, and Jagged1 mutations in humans are known to cause MBD and Alagille Syndrome, which is associated with cardiac, biliary, and bone fractures. We previously recapitulated this phenotype in Wnt1-cre; Jagged1^{fl/f} (Jag1CKO) mice and demonstrated deficient maxillary osteogenesis by conditional deletion of Jagged1 in maxillary mesenchymal stem cells. We also discovered that a non-canonical JAG1-NOTCH1 signaling target, JAK2, induces differentiation of neural crest cells, osteoblast precursors during intramembranous ossification for maxillary development. Thus, we hypothesized that JAG1 delivery to craniofacial bones will induce bone formation. In this study, we investigate the targets downstream of JAG1-JAK2 in O9-1 cells, a cranial neural crest (CNC) cell line. Lysates of O9-1 cells, stimulated with recombinant JAG1-Fc bound to beads (5 μ M) for 30 minutes, were subjected to RNA sequencing. RNA-seq evaluation of the JAG1-stimulated CNCs revealed enhancement of multiple genes within the NOTCH pathway (Hes1), bone chemokines (CXCL1), craniofacial transcription factors (Snail1) and inhibitors of osteoclasts (Id1). Further, to test the feasibility of JAG1 delivery for induction of bone formation, we incorporated JAG1-Fc-Beads complex (5 μ M and 10 μ M) into 4% PEG-MAL hydrogels and delivered these treatments by maxillary onlay injection in mice as 3 doses (initial dose, week 4, week 8). After 12 weeks, we harvested the mouse skulls and quantified ectopic bone formation differences between experimental groups by MicroCT analysis. All data are presented as mean $\pm$ SD (n>3) and subjected to ANOVA and Tukey's post-test (p<0.05). We observed increased bone deposition, demonstrating the efficacy of PEG-MAL hydrogel delivery of JAG1. Collectively, these data suggest that JAG1 stimulates bone formation *in vivo*, independently of BMP2, and the identification of JAG1-induced NOTCH canonical and non-canonical targets could change the paradigm of treatment for maxillary hypoplasia in diseases like Alagille Syndrome.

Evaluation of W1282X CFTR in the FRT Model as a Means to Test Small Molecule Interventions

Presenting Author: Dr. Candela Manfredi; Emory University

Manfredi, Candela; Rab, Andras; Hong, Jeong; Joshi, Disha; Mahiou, Jerome; Mense, Martin; and Sorscher, Eric

CFTR W1282X encodes a premature stop codon that foreshortens and destabilizes CFTR peptide, blunts half-life of the transcript, and omits the carboxy-terminal PDZ anchoring domain, all of which diminish abundance of functional CFTR at the cell surface. Truncated W1282X CFTR exhibits residual activity with a significant CFTR gating defect potentiated by curcumin. G418, an established PTC readthrough agent, has negligible effect on W1282X under conventional *in vitro* conditions; although, prolonged administration at high drug concentrations leads to biochemical evidence of full length CFTR. We also show that G418 unexpectedly stabilizes both wild type and W1282X mRNA, and increases steady state levels of truncated protein, all of which improve surface localization of wild type and W1282X CFTR, even in absence of stop codon readthrough. To further investigate W1282X molecular phenotype, we developed protocols using FRT cell lines encoding wild type or W1282X CFTR with or without mini-introns surrounding exon 23 or legacy exon 20 (the location of W1282X), and show that W1282X CFTR EMG (expression minigene encoding intronic DNA) produces low levels of protein and mRNA compared to the corresponding cDNA without introns. Augmentation of W1282X CFTR surface protein following treatment with either Vx-809 or G418 was found to be much stronger (in proportion) in cells expressing W1282X with mini-introns. Moreover, short circuit current measurements indicate W1282X monolayers chronically exposed to Vx-809 or G418 are preferentially enhanced in the presence of intronic DNA. W1282X EMG expression is further increased by the nonsense-mediated decay inhibitor 14 (NMDi14), with stronger activation in the EMG context following co-administration of G418. These findings demonstrate importance of intronic DNA for model systems intended to study W1282X or other CFTR premature truncation variants. Our experiments also emphasize the unanticipated effect of G418 on CFTR mRNA and truncated CFTR protein levels, independent of stop codon suppression, as well as increased full length (wild type) CFTR following G418 in the FRT model system.

Presentation Time: 3:45 - 4:45 pm

Gamma Delta T-Cell Immunotherapy for T-Cell Acute Lymphoblastic Leukemia (T-ALL)

Presenting Author: Dr. Sunil Raikar; Emory University

Raikar, Sunil; Story, Jamie; Fleischer, Lauren; Knight, Kristopher; Doering, Christopher; and Spencer, Trent

BACKGROUND: Survival for patients with relapsed T-ALL remains extremely poor. Here, we capitalize on the promise of gamma delta ($\gamma\delta$) T cells, which unlike the more predominant alpha beta ($\alpha\beta$) T cells, do not require antigen presentation and identify targets in a MHC independent manner. Inherent cytotoxic mechanisms used by $\gamma\delta$ T cells include recognition of cellular stress molecules such as NKG2D and DNAM-1 ligands. These markers are overexpressed in T-ALL, making them susceptible to $\gamma\delta$ T-cell mediated killing. Moreover, stress ligand expression can be further upregulated through methods such as proteasome inhibition, enhancing the $\gamma\delta$ T-cell cytotoxic effect. We also predict that malignant T-ALL cells can be directly targeted by $\gamma\delta$ T cells using CD5-directed chimeric antigen receptors (CARs). The limited persistence of $\gamma\delta$ T cells negates the possibility of life-threatening immune suppression from T-cell aplasia, which may be seen with a CAR-based approach using $\alpha\beta$ T cells. Thus, we will test both an unmodified and gene-modified $\gamma\delta$ T-cell approach to target T-ALL.

STUDY DESIGN: $\gamma\delta$ T cells were expanded from PBMCs using our established laboratory protocol. NKG2D and DNAM-1 ligand expression changes were measured in three T-ALL cell lines, Jurkat, CCRF-CEM and MOLT4, after treatment with the proteasome inhibitor bortezomib. $\gamma\delta$ T-cell cytotoxicity was then measured against the cell lines after treatment with bortezomib using a flow cytometry based assay.

We also developed an anti-CD5 CAR with a CD28 co-stimulatory domain and are testing two different approaches to genetically modify $\gamma\delta$ T cells – a traditional lentiviral vector approach and a non-integrating adeno-associated viral (AAV) vector approach.

RESULTS: *Ex vivo* expanded $\gamma\delta$ T cells have inherent baseline cytotoxicity against T-ALL cell lines. NKG2D ligand expression is upregulated in T-ALL after treatment with bortezomib, leading to increased *in vitro* killing by $\gamma\delta$ T cells. As proof-of concept CD5-CAR functionality against CD5-positive T-ALL was confirmed using the natural killer cell line NK-92. Our initial transduction data shows that transgene expression is higher in $\gamma\delta$ T cells using the AAV vector.

CONCLUSIONS: The intrinsic cytotoxic activity of $\gamma\delta$ T cells can potentially be enhanced and directed towards cancerous T-ALL cells.

Postnatal Zika Virus Infection Causes Persistent Abnormalities in Brain Structure, Function, and Behavior in Infant Macaques

Presenting Author: Dr. Jessica Raper; Emory University

Raper, Jessica; Mavigner, Maud; Kovacs-Balint, Zsolia; Gumber, Sanjeev; Sanchez, Mar M.; Alvarado, Maria C.; and Chahroudi, Ann

To date, most studies have focused on the impact of zika virus (ZIKV) infection *in utero*, documenting its association with microcephaly, fetal brain lesions, and other serious birth defects. Considering the impact that ZIKV infection can have on the developing nervous system and given that the postnatal period is also a time of rapid brain growth, it is important to understand whether ZIKV infection during infancy could have similar neurodevelopmental consequences. To address this question, we used a highly clinically relevant rhesus macaque (RM) model. Infant RMs were infected with ZIKV at 5 weeks of age and we longitudinally monitored the animals until 12 months of age with neuroimaging, as well as behavioral and neurohistopathology assessments. Postnatal ZIKV infection resulted in long-term behavioral changes, including increased emotional reactivity, decreased social contact, increased slips and falls, as well as visual recognition memory deficits at one year of age. Structural and functional magnetic resonance imaging demonstrated that ZIKV-infected infant RMs had persistent enlargement of lateral ventricles, smaller amygdalae, hippocampi, and putamen, as well as altered functional connectivity between brain areas important for socioemotional behavior and cognitive function. These structural and functional brain changes may explain the observed alterations in socioemotional behavior and learning and memory function. Neurohistopathology at 12 months of age did not show any signs of continued viral infection, yet brain lesions were observed. One infant RM showed persistent mild neuronal and perivascular calcification in the putamen and another RM presented distended lateral ventricle of the occipital lobe. These neurohistopathology findings confirm and validate the alterations in structural and functional neuroimaging, including weak functional connectivity between the putamen and inferior temporal cortex. Overall, this study demonstrated that postnatal ZIKV infection of infants in this model has long lasting neurodevelopmental consequences.

Dysregulated Arginine Metabolism Correlates with Structural and Functional Myocardial Changes in Chronic Kidney Disease

Presenting Author: Dr. Loretta Reyes; Emory University

Reyes, Loretta; Winterberg, Pamela D.; George, Roshan P.; Kelleman, Michael; Harris, Frank; Brown, Lou Ann; and Morris, Claudia

IMPORTANCE: Cardiovascular disease is the leading cause of death in children and young adults with chronic kidney disease (CKD) yet the mechanisms underlying this increased cardiovascular risk are incompletely understood. Global arginine bioavailability ratio (GABR) has been found to predict increased risk for cardiovascular complications in different patient populations but has not yet been studied in CKD.

OBJECTIVE: To determine associations between GABR and myocardial dysfunction in CKD.

DESIGN: Plasma from 129X1/SVJ mice with and without CKD (5/6 nephrectomy model) at 8- and 16-week time points, and banked plasma from children with and without CKD (previously cared for at Emory/Children's Pediatric Nephrology) were analyzed for arginine, citrulline, ornithine, and ADMA by LC-MS/MS and arginase concentration/activity by ELISA/colorimetric assay. GABR was calculated via arginine/(ornithine+citrulline). Echocardiographic measures of myocardial dysfunction, including left ventricular hypertrophy (LVH), diastolic dysfunction and impaired ventricular strain were compared with plasma analytes.

RESULTS: In mice with CKD, low GABR correlated with worsening diastolic function (decreasing E/A ratio) [$r = 0.58$, $p = 0.01$] and increasing relative wall thickness (RWT), a measure of LVH [$r = -0.49$, $p = 0.03$]. Plasma arginase activity was significantly increased in CKD mice at 16-weeks [10.5 (8.4-11.7)] compared to controls [5.5 (1.5-10.0); $p \leq 0.05$] and to CKD mice at 8-weeks [7.0 (3.7-7.6); $p = 0.002$]. Increased arginase activity correlated with impaired global longitudinal strain, GLS, [$r = -0.34$; $p = 0.04$]. Children with CKD demonstrated a non-significant (ns) reduction in GABR compared to healthy controls [0.27 (0.17-0.46) vs 0.54 (0.28-0.63); $p = 0.065$]. Arginase concentration was lower in children with CKD compared to healthy controls (ns) but interestingly, arginase activity was significantly higher, despite the lower concentration [2.92 (2.04-5.34) vs 1.52 (1.23-2.15); $p = 0.04$]. A significant association was seen between increasing ADMA and lower E/A ratio in mice with CKD [$r = -0.34$; $p \leq 0.05$] and increasing relative wall thickness in children with CKD [$r = 0.54$; $p = 0.003$].

CONCLUSIONS: GABR, arginase activity and ADMA are all abnormal in CKD and correlate with myocardial dysfunction. Additional studies are needed to further elucidate the mechanisms underlying dysregulated arginine metabolism in CKD.

The Role of Type I Interferons in Factor VIII Inhibitor Formation

Presenting Author: Dr. Patricia Zerra; Emory University

Zerra, Patricia; Cox, Courtney; Baldwin, Hunter; Patel, Seema; Arthur, Connie; Lollar, Pete; Meeks, Shannon; and Stowell, Sean

FVIII replacement in hemophilia A can be complicated by neutralizing FVIII IgG alloantibodies (inhibitors) that can actively block FVIII activity and prevent optimal replacement efficacy. Currently, no prophylactic therapy prevents inhibitor development, likely due to poor understanding of key immune regulators governing inhibitor formation.

We previously identified that MZ B cells, a unique innate lymphoid population within the splenic marginal sinus, engage FVIII shortly following injection, and are also required for anti-FVIII antibody formation. These results suggest that MZ B cells play a key role in initiating and subsequently orchestrating an immune response to FVIII.

We next sought to define the innate immune signaling events influencing inhibitor formation. Recent studies suggest that type I interferons (IFNs) can regulate MZ B cell activation following exposure to blood-borne antigens. To determine whether a similar pathway is involved in inhibitor development, wild type (WT) or IFNR KO mice (deficient in the common receptor for type I IFN) were injected with FVIII followed by examination of anti-FVIII antibody formation. Compared to WT, IFNR KO recipients had significantly decreased anti-FVIII antibody titers, suggesting that type I IFN signaling regulates antibody formation against FVIII. Moreover, previous studies suggest that patients exposed to type I IFNs and/or viral infection, which can increase type I IFN formation, may experience a higher rate of inhibitor development. Thus, we next examined the effect of PIC, a viral-like nucleic acid used as a surrogate for viral infection, on FVIII inhibitor formation. Exposure to PIC prior to FVIII significantly increased anti-FVIII antibody titers. Importantly, this increase in anti-FVIII antibodies was dependent on MZ B cells, suggesting that PIC, which enhances type I IFNs, also increases inhibitor formation through a MZ B cell-dependent process.

Taken together, our results suggest that MZ B cells and type I IFNs regulate inhibitor development, and that environmental factors that augment type I IFN signaling can similarly enhance anti-FVIII antibody formation. These studies not only provide new insight into key aspects of inhibitor formation, but may also provide an important framework to develop rational approaches to prophylactically prevent inhibitor development following FVIII infusion in patients with hemophilia A.

Poster Presentation Abstracts

Presentation Topics

Noon - 1:15 pm

Cardiology, Cystic Fibrosis and Neurology

4:45 - 6:00 pm

Gastrointestinal, Infectious Disease and Leukemia

1. Longitudinal Changes of Cerebral Metabolite and Diffusivity Property in the Developing Brain

Presentation Time: Noon - 1:15 pm

Li, Chun-Xia; Meng, Yuguang; Mao, Hui; Chan, Anthony; and Zhang, Xiaodong

INTRODUCTION: Microstructural and neurochemical changes are generally seen in developing brains. However, it remains not fully understood how these biomarkers are temporally correlated during the brain maturation. In the present study, *in vivo* magnetic resonance spectroscopy (MRS) and diffusion tensor imaging (DTI) are employed to investigate the cerebral metabolite and microstructural changes during the developing brains of rhesus monkeys.

METHODS: Four infant rhesus monkeys were used in this study. *In vivo* MRS experiments were conducted at the age of the 6, 12, and 18 months on a Siemens 3.0 T whole body scanner using a single-loop surface coil. Animals were anesthetized using 1-1.5% isoflurane mixed with air and physiological parameters were monitored continuously. Single voxel MRS was acquired using a PRESS sequence with a $5 \times 5 \times 5$ mm³ voxel placed in anterior cingulate cortex (ACC). Spectra with and without water suppression were acquired at each voxel, respectively. Concentrations of metabolites, including N-acetylaspartate (NAA), creatine and phosphocreatine (tCr), total choline (tCho), myo-inositol (mI), Glutamate(Glu)/Glutmin (Glx), were derived from the spectra using the LC Model software. DTI with 30 gradient directions and $b=1000$ s/mm² was performed using a phase-arrayed volume coil in the same scan session. Repeated ANOVA and spearman correlation analysis between the MD and metabolite concentrations were performed ($P<0.05$).

RESULTS: NAA, mI, Glu and tCr in ACC showed significantly increase from 6 months to 18 months. Glx significantly increased from 6 months to 12 months. MD in ACC showed dramatic and progressive decrease over time. Progressive elevation of metabolite concentration was seen in mI, NAA, tCr and Glu which also were negatively correlated with MD change.

DISCUSSION and CONCLUSION: The present study on ACC of monkey infants reveals that the progressive elevation of NAA, tCr, mI and Glu was significantly correlated with the quick MD changes, suggesting that neuron /axon and glia cell experience quick maturation period during such early brain development. The results also demonstrate the evolution of each metabolite is correlated differently with the microstructural maturation during early brain development. Combined MRS/DTI examination could offer complementary information to characterize early brain maturation and related disorders.

2. Multiparameter MRI Examination of the Developing Monkey Fetal Brain in Uterus

Presentation Time: Noon - 1:15 pm

Li, Chun-Xia; Kempf, Doty; Milla, Sarah; Chan, Anthony; and Zhang, Xiaodong

BACKGROUND: Birth defects affect about 3% of all babies born in the United States each year, and are the leading cause of infant deaths. Nonhuman primates (NHP) share high similarities with humans including brain anatomy, physiology, immunology, reproduction, cognitive capacity and social behaviors, and are widely used in biomedical research including congenital malformations induced spontaneously or experimentally by drugs, toxic chemicals, ZIKA viral infection, et al. In this study, we exploited the advanced MRI techniques to examine rhesus monkey fetus in uterus on a clinical 3T MRI scanner.

METHODS: Monkey fetal MR images were collected with a Siemens 3T TIM Trio clinical scanner. A 4-channel Siemens FLEX coil was placed around the abdomen of the pregnant dam. The T2-weighted structural images, diffusion MRI images with $b=1000$ s/mm² and 30 directions, resting state functional MRI data were acquired using parallel imaging technique. The animal was breathing spontaneously and anesthetized with ~1.5% isoflurane during scanning and monitored physiologically. The structural, diffusion tensor imaging (DTI), rsfMRI data were processed and evaluated using the Siemens Syngo software, FSL and AFNI software respectively. The multi-parameter MR Images of fetal brain of a pregnant rhesus monkey (5 years old, 2nd trimester) were demonstrated.

RESULTS: The cerebral gyrus and sulcus of the fetal brain are well-delineated using T2-weighted images. The grey matter and white matter (corpus callosum) of whole fetal brain are clearly demonstrated on fractional anisotropy (FA) and diffusivity (mean, axial, radial) maps. Also, functional connectivity of medial prefrontal cortex to posterior cingulate cortex is observed and illustrated. In particular, fetus motion was substantially reduced due to anesthesia, allowing for improvement of DTI image quality with high resolution.

CONCLUSION: Our preliminary MRI data of monkey fetus *in utero* demonstrate that MRI is a robust approach to longitudinally examine the fetal brain structural anatomy, grey matter and white matter abnormality, and structural and functional connectivity in the developmental brain non-invasively. NHP highly mimics the reproduction of human in comparison to rodents. Application of the contemporary MRI techniques in monkey fetus *in utero* can facilitate translational research of birth defect substantially.

3. Selective Death Induction of HIV-1 Infected Myeloid Reservoirs

Presentation Time: 4:45 - 6:00 pm

Gavegnano, Christina; Shepard, Caitlin; Holler, Jessica; Coggins, Si'Ana; and Kim, Baek

BACKGROUND: Existing antiretroviral therapy (ART) cannot efficiently eliminate HIV within the CNS. HIV persistence in myeloid sanctuaries represents a major barrier to eradication, and drives HIV associated neurocognitive dysfunction (HAND), which occurs in up to half of HIV-infected individuals even with well-controlled viremia. Safe, specific agents that selectively eliminate key cells harboring the myeloid reservoir are urgently needed. Our group has identified two safe, FDA approved agents rufinamide and bergenin (non-HIV indication) that demonstrate selectivity for killing of only HIV-infected macrophages.

METHODS: Cell isolation: Primary human macrophages were isolated from healthy donors and differentiated with GM-CSF. Memory T cells or activated CD4 T cells were isolated with magnetic beads (Miltenyi).

HIV INFECTIONS: Macrophages were infected with HIV-1ba1 (MOI 0.5) for 72 hr in the presence of 1 or 10 μ M rufinamide or bergenin, HIV alone, or HIV+VPX (positive control). Effect on HIV infection was quantified (intracellular/extracellular p24, 2-LTR circles). Effect on cell killing of HIV-infected cultures+drug versus HIV infection alone, and HIV uninfected cells were quantified (FACS live/dead stain and MTT assay). Effect on dNTP pools and SAMHD1/pSAMHD1 were quantified.

RESULTS: Rufinamide and bergenin do not kill uninfected macrophages. Both agents demonstrate selectivity for killing HIV-infected macrophages, and increase cell killing in HIV-infected cultures 3-4 fold versus HIV-infected cultures without drug. Both agents significantly accelerate HIV replication in

macrophages (intracellular and extracellular p24, and 2-LTR circles) versus HIV infection alone. Both agents increase dATP levels in macrophages, but do not modulate SAMHD1/pSAMHD1 levels.

CONCLUSIONS: Rufinamide and bergenin demonstrate selectivity for killing only HIV infected macrophages, and increase cell killing in HIV-infected cultures at least 4-fold versus HIV infected cultures without drug. Agents accelerate HIV replication in macrophages, implying acceleration results in selective cell death of infected macrophages. Acceleration of replication is coupled with increase in dATP, but not SAMHD1/pSAMHD1; regulation of acceleration and cell death is conferred by increased dNTPs, but not directly by SAMHD1. Bergenin and rufinamide demonstrate selectivity towards killing only HIV-infected macrophages, warranting further mechanistic studies to evaluate the use of these agents towards elimination of myeloid derived viral sanctuaries systemically and within the CNS.

4. Mitochondrial Protein Acylation as a Post-Translational Signature of Cardiac Energy Dysfunction

Presentation Time: Noon - 1:15 pm

Peoples, Jessica; Ghazal, Nasab and Kwong, Jennifer

Mitochondria are tightly regulated organelles critical for energy production and signaling. This essential role of mitochondria in fueling cellular functions underscored in issues with high energy demands, such as the heart, as mitochondrial energy dysfunction is a hallmark of a wide range of cardiac diseases ranging from primary mitochondrial cardiomyopathies to heart failure. To date, however, we lack therapeutics that directly target mitochondrial dysfunction. Thus, understanding how mitochondria respond to energy dysfunction and direct the cellular response to energetic crisis holds great promise for the design of metabolism-targeted strategies to support the diseased heart. However, fundamental knowledge of the molecular effectors of mitochondrial signaling operant *in vivo* and in the heart remains incomplete. Here we generated tamoxifen inducible and cardiac mitochondrial phosphate carrier (SLC25A3) deletion mice (SLC25A3 KO) as a novel model for cardiac mitochondrial energy dysfunction and to identify molecular pathways that are specific to mitochondrial energetic stress *in vivo*. SLC25A3 is a mitochondrial transporter that imports inorganic phosphate (Pi) into the matrix. Because Pi is required for ATP synthesis, SLC25A3 is critical for mitochondrial energy production. We have previously found that SLC25A3 KO mice have impaired mitochondrial ATP synthesis and develop a mitochondrial cardiomyopathy that recapitulates features observed in patients with SLC25A3 mutations. Here, we find that cardiac mitochondrial proteins from SLC25A3 KO mice are highly modified by acetylation and malonylation, two posttranslational lysine modifications known to impair mitochondrial function. Mechanistically, proteomics analyses revealed that SLC25A3 deletion induces a highly interconnected network of metabolic changes that converge on alterations in acetyl-CoA and malonyl-CoA flux. Importantly, acetyl-CoA and malonyl-CoA are the requisite substrates for protein lysine acetylation and malonylation. Our results suggest that mitochondrial intermediary metabolites play an important role in the mitochondrial response to energy dysfunction, mitochondrial protein acylation reflects cellular energetic state, and critically, that alterations in acylation patterns are a metabolic signatures of energy dysfunction.

5. CD8 Depletion Plus Signaling of the Non-Canonical NF- κ B Pathway Reverses Latency in SIV-Infected, ART-Suppressed Rhesus Macaques

Presentation Time: 4:45 - 6:00 pm

Mavigner, Maud; Brooks, Alyssa; Mattingly, Cameron; Schoof, Nils; Vanderford, Thomas; Bosinger, Steve; Sampey, Gavin; Galardi, Cristin; Dunham, Richard; Margolis, David; Silvestri, Guido; and Chahroudi, Ann

Worldwide, nearly two million children are infected with human immunodeficiency virus (HIV). While antiretroviral therapy (ART) greatly reduces the mortality and morbidity of HIV infection in both adults and infants, it is not curative due to the persistence of the virus in reservoirs. The leading approach to reduce

viral reservoirs, referred to as “kick and kill”, aims at inducing HIV expression from latently-infected memory CD4+ T-cells to promote their clearance. Latency reversal agents (LRAs) tested to date have been only modestly effective. Targeting the non-canonical NF- κ B pathway (ncNF- κ B) with small molecule mimetics of the second mitochondrial activator of caspases (SMACm) is a promising LRA approach *in vitro*. Furthermore, as CD8+ T cells are required for maintaining viral suppression on ART, experimental depletion of CD8+ T cells may act synergistically with LRAs in reactivating virus production.

We compared the *in vivo* LRA activity of the SMACm AZD5582 in ART-suppressed SIV-infected rhesus macaques (RMs) in the presence or absence of CD8+ T cells. Eighteen SIVmac239-infected RMs on ART for 593-595 days were administered weekly i.v. doses of AZD5582 at 100 ug/kg. Six RMs also received 50 mg/kg of the CD8 α -depleting antibody MT-807R1 24h prior to AZD5582 treatment. On-ART plasma viral loads were monitored to assess for latency reversal.

Experimental CD8+ T-cell depletion was successful with >95% of peripheral CD8+ T cells depleted by day 5 after antibody administration. No major adverse events were seen with AZD5582 treatment in the presence or absence of CD8+ T cells. On-ART viremia was observed in 6/6 (100%) RMs treated with AZD5582 after CD8+ T-cell depletion versus 5/12 (42%) RMs treated with AZD5582 only. Following AZD5582 treatment, episodes of viremia above 60 copies/ml were seen in 24.2% versus 20.8% of viral load measurements for the CD8-depleted+AZD5582 group vs. the AZD5582 only group, respectively.

These studies show that activation of ncNF- κ B signaling pathway via AZD5582 results in SIV-RNA expression in the blood and tissues of SIV-infected, ART-suppressed RMs and that the LRA activity of AZD5582 can be potentiated by CD8+ T-cell depletion.

6. Effect of Metabolic Regulation on Maturation of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Presentation Time: Noon - 1:15 pm

Li, Dong; Gentillon, Cinsley; Jha, Rajneesh; and Xu, Chunhui

Heart diseases continue to be a major cause of death in developed countries despite the recent advances in medical treatments. Human induced pluripotent stem cell (hiPSC) has emerged as an attractive cell model for studying human heart diseases *in vitro* and further rendered cardiomyocytes derived from patient-specific hiPSC (hiPSC-CMs) great potentials for therapeutic applications. Several protocols have been established to derive hiPSC-CMs using growth factors, small molecules or matrix proteins; however, the maturity of differentiated hiPSC-CMs are not comparable with the cardiomyocytes in adult hearts, which constrains their therapeutic potentials for treating heart diseases. Numerous approaches have been employed attempting to promote the maturity of hiPSC-CMs, including long-term culture, substrate modification, cell alignment improvement, chemical and mechanical stimulation. Here we show that the use of a small molecule for metabolic regulation during three-dimensional cardiac differentiation could promote the maturity of hiPSC-CMs. We treated the hiPSC-CMs with the small molecule starting from day12 of the differentiation for 16 days. Flow cytometry analysis revealed that the small molecule-treated hiPSC-CMs displayed significantly increased mitochondrial membrane potential compared with vehicle-treated hiPSC-CMs. Quantitative real-time PCR revealed a significant increase in mitochondrial DNA (mtDNA): nuclear DNA (nDNA) ratio in the small molecule-treated hiPSC-CMs. In addition, the small molecule significantly upregulated the expression of genes controlling oxidation phosphorylation and mitochondrial fusion, indicating an increased mitochondrial function. Consistent with these observations, the small molecule treatment significantly increased the expression of a subset of genes encoding cardiomyocyte structural proteins, Ca²⁺ handling proteins and Na⁺/K⁺ channels. Furthermore, a subset of genes involved in the metabolic regulation were also upregulated, including adrenergic receptors, glucose transport, fatty acid transport and activation. Our results demonstrate the small molecule is capable of promoting the molecular and structural development of hiPSC-CMs that lead to more maturation. We will further evaluate the effect of the small molecule on functions of hiPSC-CMs and examine the underlying mechanisms. We anticipate our research to provide insights on how the small molecular candidates contribute to the improvement of hiPSC-CMs maturity.

7. Viral and Immune Dynamics in a Nonhuman Primate Model of Pediatric HIV Infection: Studies to Inform Cure Strategies

Presentation Time: 4:45 - 6:00 pm

Obregon-Perko, Veronica; Bricker, Katherine; Uddin, Ferzan; Berendam, Stella; Fouda, Genevieve; Bar, Katharine; Shaw, George; Silvestri, Guido; Permar, Sallie; and Chahroudi, Ann

Currently available antiretroviral therapy (ART)-based measures to prevent mother-to-child transmission of HIV-1 do not address the scenario of new maternal infections during the peri- or post-partum periods, creating a risk for transmission to infants during breastfeeding. With lifelong drug exposure in HIV-infected children associated with accelerated non-AIDS complications and the risk of triple drug class virologic failure, there is a critical need for strategies to achieve viral remission. Expanding on a nonhuman primate model of pediatric infection recently published in our laboratory, here we aimed to characterize immune responses, the viral reservoir, and rebound kinetics in orally SHIV-infected ART-treated infant rhesus macaques.

Sixteen 4-week-old Mamu B08-/B17- rhesus macaques born in Summer 2018 were orally administered SHIV.CH505.375H.dCT in two inoculations 24 hrs apart and placed on ART at 8 wpi. Median viral loads at peak and immediately prior to ART were ~500,000 and 100,000 copies/mL, respectively. Males and females had comparable replication kinetics, but there was a trend for lower pre-ART viral loads in Mamu A01+ animals. Cell-associated viral DNA was detectable in blood and lymph node CD4+ T cells pre-ART and declined in both compartments by up to 2 logs after ART initiation. SHIV DNA was also readily detected in rectal biopsy CD4+ T cells at 16 wpi, where higher levels were associated with an increased frequency of CCR5+CD4+ T cells ($p < 0.0001$). Consistent with observations from HIV-infected children, SHIV-infected infant macaques showed little to no decline in peripheral CD4+ T-cell frequencies during acute infection. Env-specific gp140, gp120, and gp41 binding antibodies were first detected at 3-4 wpi and displayed a gradual, significant decline after ART initiation.

In summary, we have begun to characterize viral and immune dynamics in a pre-clinical nonhuman primate model that displays key features of pediatric HIV infection and uses a chimeric SHIV expressing clade C HIV Env, a subtype highly relevant to the current epidemic and one that allows investigation of HIV envelope-targeting strategies. Ongoing work involves whole-body PET/CT imaging to visualize anatomical sites of infection before and after ART interruption.

8. Developing High-Throughput Screening Methods for Testing Drug-Induced Cardiotoxicity

Presentation Time: Noon - 1:15 pm

Rampoldi, Antonio; Maxwell, Joshua; Liu, Rui; Fu, Haian; Du, Yuhong; and Xu, Chunhui

Current drug development relies on animal models which have limitations to predict cardiotoxicity in humans as a side-effect response to drugs, mainly due to physiological differences between human and animal cardiomyocytes. Cardiotoxicity is a severe side effect of some drugs, including those for chemotherapeutics, and is a risk factor for long-term morbidity of patients. The drugs can induce an electrophysiological dysfunction and structural damage to cardiomyocytes, resulting in loss of contractility and arrhythmia. There is the need to develop a new physiologically relevant model that can be used to reliably investigate drug-induced cardiotoxicity in high-throughput systems. Cardiomyocytes derived from human-induced pluripotent stem cells (hiPSC-CMs) can serve as a novel human cell-based model for the characterization of cardiac defects. To study cell contractility, we performed live cell imaging to analyze changes in intracellular Ca²⁺-transients, since dysregulation of intracellular Ca²⁺-handling plays an important role in the pathogenesis of cardiac arrhythmias. Using a Ca²⁺-sensitive fluorescent dye and an automated high-throughput microscope, we detected two categories of Ca²⁺-release events, regular and irregular (arrhythmic) Ca²⁺-transients in hiPSC-CMs in a highly efficient and scalable manner. Ca²⁺-transients were categorized as regular if the Ca²⁺-transients had mostly consistent amplitudes and beat periods (rapid upstroke and decay kinetics), and instances of Ca²⁺-release in-between transients. Ca²⁺-transients were categorized as irregular if they exhibited oscillations of diastolic cytosolic Ca²⁺,

indication of an arrhythmic events. The arrhythmic events were further classified in 4 subtypes based on the severity of Ca²⁺oscillations: Type A (single notch), B (multiple notches), C (ectopic beat) and T (tachyarrhythmic). The number of cells exhibiting regular or irregular Ca²⁺transients was counted, and percentages of the cells in each category were calculated for each condition tested. Our combination of hiPSC-CM arrhythmia modeling and high-throughput intracellular Ca²⁺screening has the potential to predict drug-induced proarrhythmic effects in a highly efficient manner and improve the development of therapeutics for potentially life-threatening conditions.

9. Withdrawn

10. Quantifying Stem Cell-Derived Cardiomyocytes Traction Forces as a Function of Differentiation Using Molecular Tension Probes

Presentation Time: Noon - 1:15 pm

Rashid, Sk Aysha; Forghani, Parvin; Xu, Chunhui; and Salaita, Khalid

Heart disease often arises from abnormal contractile profiles of the unit muscle cells (cardiomyocytes), which comprise a major component of the human heart. There is a direct correlation between heart diseases and loss of functional cardiomyocytes. Accordingly, human induced pluripotent stem cell derived- cardiomyocytes (hiPSC-CMs) are being investigated as a promising cell source for replacement therapeutics due to their ease of availability and high proliferation rates compared to primary cardiac cells. hiPSCs can potentially serve as a source of patient-specific cardiomyocytes, which is desirable, unlike human embryonic stem cells (hESC), as their use is noncontroversial. Therefore, methods that can characterize the molecular properties of hiPSC-CMs are severely needed. While most methods rely on profiling the chemical properties (protein/gene expression), there is a need of developing methods for characterizing their mechanical properties, given the importance of mechanics to cardiomyocytes. Current technologies such as traction force microscopy, atomic force microscopy and optical edge detection are useful; however, these approaches are either serial in nature, or their spatial (~μm²), and force (~nN) resolutions are limited, and therefore, unable to map the pN forces applied by integrin adhesion receptors in CMCs.

In this project, we address this need by demonstrating the use of molecular tension fluorescence microscopy (MTFM) to directly map integrin tension of CMs and its relationship with differentiation. MTFM probes used in this study are comprised of an extendable DNA hairpin linker or DNA duplex, flanked by a fluorophore and quencher pair. Forces applied by CMs lead to mechanical melting of DNA and an increase in fluorescence. Skin-derived hiPSCs were differentiated toward cardiomyocytes in a feeder-free environment using activin A/BMP4. Post-differentiated cardiomyocytes in 2D and 3D format at early (>14days) and late stages (> 24 days) were dissociated. Next, we measured the fluorescence signal generated by MTFM probes upon plating these cells. Varying ages of hiPSC-derived CMs were tested allowing us to quantify traction forces as a function of differentiation. These results indicate that the mechanical properties of cardiomyocytes can be used for functional screening of the unit muscle cells.

11. A Role for the SIX1 Homeobox Gene in CALM-AF10 Leukemogenesis

Presentation Time: 4:45 - 6:00 pm

Aumann, Waitman; Lavau, Catherine; Harrington, Amanda; Conway, Amanda; and Wechsler, Daniel

BACKGROUND: The CALM-AF10 translocation is detected in ~10% of T-cell acute lymphoblastic leukemias (T-ALLs), and in some acute myeloid leukemias (AMLs). CALM-AF10 leukemias are characterized by high expression of proleukemic HOXA genes. Since HOXA genes are difficult to target, we hypothesized the identification of non-HOXA CALM-AF10 effector genes could potentially yield novel therapeutic targets. To this end, we took advantage of our prior observation that the nuclear export factor CRM1/XPO1 tethers CALM-AF10 to HOXA genes by interacting with a nuclear export signal (NES) in

CALM. We used RNA-sequencing and microarray to determine that SIX1, similar to HOXA genes, is increased in CALM-AF10 leukemias and decreased in response to CRM1 inhibition.

OBJECTIVE: To evaluate the role of SIX1 in CALM-AF10 leukemias.

DESIGN/METHODS: RT-qPCR and Chromatin Immunoprecipitation were performed using both bone marrow progenitors and murine embryonic fibroblasts (MEFs) transduced with CALM-AF10 or an empty vector, with and without LMB. The ability of SIX1 to enhance self-renewal of hematopoietic progenitors was examined by measuring the colony-forming ability of transduced fetal liver progenitors. CRISPR-Cas9 was used to silence SIX1 in Human Embryonic Kidney 293 (HEK293) cells.

RESULTS: RT-qPCR confirmed overexpression of SIX1 in both CALM-AF10 transduced MEFs and CALM-AF10 leukemias, with decreased SIX1 expression observed in the presence of LMB. ChIP analysis showed that CALM-AF10 binds the SIX1 gene locus. Overexpression of SIX1 in fetal liver cells was sufficient to increase the self-renewal potential of these colony-forming progenitors. SIX1 was successfully knocked out in HEK293 cells, resulting in slowed HEK293 proliferation.

CONCLUSIONS: SIX1 is a homeobox gene highly expressed during embryogenesis with expression normally silenced post embryogenesis. Increased SIX1 expression has been reported in numerous solid tumors; but SIX1 involvement in leukemogenesis is uncertain. We have determined that SIX1 is upregulated in the presence of CALM-AF10, and increases the self-renewal potential of hematopoietic progenitors. Despite decreased proliferation rates in HEK293 cells with SIX1 knocked out, SIX1 is not essential for cell survival and inhibition could be effective in impairing CALM-AF10 leukemia cell proliferation. Thus, SIX1 may play a pathogenic role in leukemogenesis and could be a novel therapeutic target in CALM-AF10 leukemias.

12. Using Human Induced Pluripotent Stem Cell Cardiomyocytes to Model Inflammation

Presentation Time: Noon - 1:15 pm

Saraf, Anita; Rampoldi, Antonio; Li, Dong; Jha, Rajneesh; Liu, Rui; Maxwell, Joshua; and Xu, Chunhui

Pro-inflammatory markers, such as TNF- α , are upregulated in multiple congenital cardiac diseases, such as those modified for single ventricular Fontan physiology. Animal models for congenital heart disease are limited; hence, understanding the pathophysiology of inflammatory factors on cardiomyocytes is challenging. Furthermore, evaluating long term influence of inflammation *in vitro* is limited by the short *in vitro* life span of primary cardiomyocytes. We investigated the influence of long-term exposure of TNF- α at various doses on cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs). TNF- α at 1, 10, 20 and 100 ng/mL and 4 days exposure, did not affect differentiation of hiPSC-induced cardiomyocytes. Viability of hiPSC-induced cardiomyocytes decreased at higher concentrations of TNF- α (20, 100 ng/mL). Production of mitochondrial reactive oxygen species increased at 10, 20 and 100 ng/mL of TNF- α . hiPSC cardiomyocyte contraction and relaxation velocity and beat rate decreased significantly at all tested concentrations of TNF- α . In conclusion, hiPSC cardiomyocytes can be effectively used to model the influence of inflammatory factors, such as TNF- α *in vitro* and can be used as a translational system replicating cardiomyocyte behavior.

13. Exploring the Role of NUP214 in Mediating the Interaction Between CRM1 and the HOXA Gene Locus

Presentation Time: 4:45 - 6:00 pm

Harrington, Amanda; Aumann, Waitman; Tope, Donald; Lavau, Catherine; and Wechsler, Daniel

BACKGROUND: Chromosomal translocations resulting from fusion of CALM and AF10 genes are recurrent abnormalities in 5-10% of T-cell acute lymphoblastic leukemias. These leukemias display elevated HOXA gene expression, although the mechanism by which CALM-AF10 transactivates the HOXA locus is unclear. CALM contains a Nuclear Export Signal (NES) that is required for CALM-AF10-

mediated leukemogenesis. The NES mediates the interaction with the nuclear export receptor protein CRM1, which typically functions to translocate proteins from the nucleus to the cytoplasm through the nuclear pore (NUP). We have shown CRM1 can substitute for CALM – a CRM1-AF10 fusion protein is leukemogenic *in vitro* and *in vivo*. In traversing the nuclear pore, CRM1 interacts with NUP components, including NUP214. Additionally, NUP214 is involved in leukemogenic chromosomal translocations that also cause increased HOXA gene expression. Together, these observations strongly suggest that NUP214 may mediate the ability of CRM1-AF10 to activate HOXA gene expression.

OBJECTIVE: To investigate NUP214 as a candidate protein that mediates the interaction between CRM1 and HOXA genes.

METHODS: Using a CRM1-AF10 fusion construct in which the CRM1 NUP214 binding sites have been mutated to impair binding (CRM1NUP-AF10), we performed HOXA7-luciferase reporter and methylcellulose colony assays, and carried out *in vivo* mouse transplantation.

RESULTS: Both CRM1-AF10 and CRM1NUP-AF10 activate the HOXA7-Luciferase reporter assay. However, CRM1NUP-AF10 was unable to transform hematopoietic stem cell precursors in *in vitro* colony-forming assays, and mice transplanted with CRM1NUP-AF10 did not develop leukemia.

DISCUSSION: Investigating how CRM1 interacts with the HOXA locus will further elucidate a role for CRM1 as a transcriptional activator of leukemogenic HOXA genes. We have demonstrated the importance of interaction between CRM1 and NUP214 by demonstrating abrogated leukemia development in mice transplanted with a mutated CRM1-AF10 fusion wherein NUP214 can no longer bind to CRM1. We plan to synthesize a NUP214-AF10 fusion protein to evaluate the potential for NUP214-AF10 induced leukemogenesis, to further investigate this mechanism. Our finding that mutation of NUP214 binding sites on CRM1 interferes with leukemia development warrants further exploration of NUPs as candidate proteins for HOXA gene activation, and may establish a CRM1/NUP interaction as a novel therapeutic target.

14. T Cells Play a Causal Role in Diastolic Dysfunction During Uremic Cardiomyopathy

Presentation Time: Noon - 1:15 pm

Winterberg, Pamela D.; Robertson, Jennifer M.; Kelleman, Michael; George, Roshan P.; and Ford, Mandy L.

BACKGROUND: Uremic cardiomyopathy is characterized by left ventricular hypertrophy, diastolic dysfunction, and impaired ventricular strain, and contributes to increased mortality in patients with chronic kidney disease (CKD). Emerging evidence suggests a pathogenic role for T cells during heart failure. We aimed to determine whether T cells contribute to the development of uremic cardiomyopathy.

METHODS: To model uremic cardiomyopathy, CKD was induced via 5/6th nephrectomy in male 129X1/SvJ mice. Echocardiography was performed at 8 weeks to assess heart function. Sequencing was performed on RNA isolated from mice with uremic cardiomyopathy for pathway analysis (Ingenuity, Qiagen). Hearts were digested and interrogated via flow cytometry to assess infiltration of leukocyte populations. T-cell memory (CD44 vs CD62L) and activation markers (OX40, PD-1, KLRG1) were assessed in spleen and lymph nodes via flow cytometry. T-cell cytokine (TNF α , IL-2, IFN- γ) secretion was determined via intracellular staining and flow cytometry after *ex vivo* stimulation with PMA/Ionomycin. T cells were depleted in mice with CKD using anti-CD3 antibody injections and heart function assessed by echocardiogram. Finally, Spearman correlation coefficients were used to evaluate for associations between T-cell populations in blood and echocardiography measurements in children with CKD.

RESULTS: Mice with CKD had increased memory T cells (CD44^{hi}) with markers of sustained activation (PD-1, KLRG1, OX40) and increased cytokine secretion potential (TNF, IL-2, IFN- γ). Heart transcripts from CKD mice showed differential expression in pathways needed for T-cell responses:

leukocyte extravasation, antigen presentation, and T-cell co-stimulation and differentiation. T cells infiltrated the hearts of CKD mice by 2 weeks. T-cell depletion improved diastolic function (E/A ratio: 1.2 ± 0.23 vs 0.9 ± 0.14 ; $p < 0.01$) and increased myocardial strain (-25.6 ± 1.4 vs -20.4 ± 1.5 ; $p = 0.02$) in CKD mice without altering hypertension or degree of renal dysfunction. In children with CKD, increasing frequency of T cells expressing PD-1 and/or CD57 in blood was associated with worsening diastolic function on echocardiogram (E/e').

CONCLUSIONS: CKD results in accumulation of pro-inflammatory T cells that contribute to myocardial dysfunction. Future research may inform novel therapies targeting T-cell function to mitigate uremic cardiomyopathy.

15. Targeting Galectin-9 Mitigates Obesity-Induced Chemoresistance in B-Cell Acute Lymphoblastic Leukemia

Presentation Time: 4:45 - 6:00 pm

Lee, Miyoung; Hamilton, Jamie; Talekar, Ganesh; Michael, Langston; Ross, Anthony; Rupji, Manali; Dwivedi, Bhakti; Scharer, Christopher; Boss, Jeremy; Graham, Douglas; DeRyckere, Deborah; Porter, Christopher; and Henry, Curtis

The incidence of obesity is rising with greater than 40% of the world's population expected to be overweight or obese by 2030. This is alarming given that obesity significantly increases mortality rates in patients with various cancer subtypes, including leukemia. The survival difference between lean and obese patients is largely attributed to altered drug pharmacokinetics in patients receiving chemotherapy; whereas, the direct impact of the obese microenvironment on cancer cells is rarely considered. Here, we show that adipocyte-secreted proteins upregulate the surface expression of Galectin-9 (Gal-9) on human B-acute lymphoblastic leukemia cells (B-ALL) which promotes chemoresistance. Antibody-mediated targeting of Gal-9 on B-ALL inhibits the cell cycle checkpoint which promotes cell death *in vitro* and significantly extends the survival of obese mice challenged with aggressive B-ALL. Our studies reveal that adipocytes directly induce the upregulation of Gal-9 on B-ALL cells which can be targeted with antibody-based immunotherapies to overcome obesity-induced chemoresistance.

16. Early CF Lung Damage is Linked to Increased Methionine Sulfoxide via Deregulated Myeloperoxidase Activity

Presentation Time: Noon - 1:15 pm

Margaroli, Camilla; Horati, Hamed; Giacalone, Vincent; Kilgore, Matthew; Bradley Heath; Dobosh Brian; Silva, George; Veltman, Mieke; Sarkar, Surupa; Xiong, Niya; Peng, Limin; Tiddens, Harm; Scholte, Bob; Guglani, Lokesh; Janssens, Hettie; and Tirouvanziam, Rabin

RATIONALE: Cystic fibrosis (CF) lung disease progressively worsens from infancy to adulthood. Early CF is associated with increased neutrophils, myeloperoxidase (MPO), and methionine sulfoxide (MetO). Early CF neutrophils exhibit pathological changes, including hypersecretion of MPO, implicating a possible role of hypochlorous acid (HOCl), an oxidant produced by MPO that generates MetO, in early CF pathogenesis and disease progression.

OBJECTIVE: To identify the significance and mechanisms of HOCl evolution in early CF, and to test exhaled breath condensate (EBC) as an alternative to bronchoalveolar lavage fluid (BALF) for early CF surveillance.

METHODS: Infants at Erasmus MC or Emory were diagnosed with CF by newborn screening and enrolled in prospective studies (I-BALL or IMPEDE-CF, respectively) to collect BALF and CT scans biannually between the ages of one to six years. EBC was also collected at Emory. In total, we analyzed 48 CF patients (mean age, 3.1 ± 1.6 years) and 19 disease controls (mean, 2.3 ± 2.1 years) enrolled at Emory. CT scans were scored via PRAGMA-CF to quantify lung damage. A neutrophil transmigration

model was used to test whether CF airway adaptation produces outcomes noted in early CF specimens. Metabolomics was performed by LC-MS, and MPO specific activity was quantified by an immunocapture method. Neutrophil production of HOCl was measured by taurine chloramine assay.

RESULTS: We replicated the finding that BALF MetO and MPO are correlated with lung damage in early CF, and noted significantly increased methionine oxidation at follow-up visits. Early CF BALF contained active MPO, and had greater MetO abundance compared to disease controls. MetO and other metabolites were detectable in early CF EBC. CF sputum supernatant protected free MPO from inhibition and caused transmigrating neutrophils to accumulate MPO, but did not increase neutrophils' unstimulated HOCl production.

CONCLUSIONS: Lung damage in early CF is closely connected to MPO, MetO, and other metabolites related to neutrophil ingress. MetO and other metabolites may provide opportunities to monitor this pathway via EBC analysis. Further studies are needed to establish whether HOCl is a causative agent of disease progression in early CF, and to establish therapies to limit harm.

17. Identifying Novel CRM1-Interacting Proteins in CALM-AF10 Leukemogenesis by Proximity Based Labeling

Presentation Time: 4:45 - 6:00 pm

Kazi, Rafi; Aumann, Waitman; and Wechsler, Daniel

BACKGROUND: While progress has been made in the treatment of pediatric leukemias, certain leukemias still have a poor prognosis. CALM-AF10 leukemias, which account for ~10% of childhood T-cell Acute Lymphoblastic Leukemia (T-ALL) and a subset of Acute Myeloid Leukemia (AML), are particularly difficult to treat. Our laboratory discovered that the CALM protein contains a nuclear export signal (NES) that is critical for CALM-AF10-mediated leukemogenesis. The NES interacts with the CRM1-nuclear export receptor, and we have determined that CRM1 is essential for transcriptional activation of HOXA genes by CALM-AF10. How CRM1 interacts with HOXA genes is poorly understood, since CRM1 does not contain a recognized DNA binding domain. To identify proteins that mediate the interaction between CRM1 and DNA, we take advantage of a proximity-based labeling approach using BioID2, a biotin ligase, fused to CALM-AF10. This permits biotinylation of nearby proteins which may be identified using mass spectrometry.

OBJECTIVE: Identify novel proteins that interact with CRM1.

DESIGN/METHODS: We created plasmids in which BioID2 is fused to HA-tagged CALM-AF10 and a CALM-AF10 mutant unable to bind CRM1, (CALM(NES*)-AF10). To ensure that BioID2 does not sterically interfere with CALM-AF10 and inhibit its activity, we used luciferase reporter and colony forming assays, and confocal microscopy. Human Embryonic Kidney 293 (HEK293) cells were transiently transfected with expression plasmids, followed by incubation with biotin. Purified biotinylated proteins will be analyzed using mass spectrometry.

RESULTS: CALM-AF10 and CALM(NES*)-AF10 fusion plasmids containing BioID2 were synthesized and confirmed via Sanger sequencing. Western blot demonstrated expression of fusion proteins containing BioID2. Similar to CALM-AF10, BioID2-CALM-AF10 activates the HOXA7 luciferase reporter 6-7-fold versus empty vector, transforms hematopoietic progenitor cells (HPCs) upon serial replating, and localizes primarily to the cytoplasm. Expression of biotinylated proteins in transfected HEK293 cells following biotin exposure was confirmed by Western Blot. Purified biotinylated protein samples have been submitted for analysis.

CONCLUSIONS: The presence of the BioID2 moiety does not interfere with CALM-AF10 activity. Furthermore, the BioID2 ligase is active when fused to CALM-AF10. Based on these findings, biotinylated protein samples have been submitted to the Mass Spectrometry Core and further results will be presented at the conference.

18. The Effects of Maternal Diet During Pregnancy on the Offspring's Response to Gastrointestinal Injury in a Mouse Model

Presentation Time: 4:45 - 6:00 pm

Barbian, Maria; Owens, Joshua; Denning, Patricia; Patel, Ravi; and Jones, Rheinnallt

INTRODUCTION: The intestinal microbiome plays a critical role in the development of the gastrointestinal tract. Intestinal microbial colonization is essential to nutrition, energy metabolism and conditioning of the host immune system. Microbial dysbiosis plays an important role in the pathogenesis of pediatric gastrointestinal diseases, such as necrotizing enterocolitis and inflammatory bowel disease. Recent studies have demonstrated that maternal diet during pregnancy impacts the offspring's microbiome. Our project aims to study the effects of different antenatal diets on the offspring's gut development by evaluating the offspring's response to gastrointestinal injury. The gut microbiome produces butyrate with supplementation of certain probiotics or with ingestion of a high fiber diet. Butyrate is a powerful histone deacetylase inhibitor. *In vitro* studies demonstrate that butyrate plays a role in modulating immune and inflammatory responses and improving intestinal barrier. We hypothesize that antenatal ingestion of butyrate will provide long lasting protective effects against gastrointestinal injury by improving the offspring's intestinal barrier and gastrointestinal immune response.

METHODS: Mating pairs of wild type mice C57BL/6 were randomly assigned to experimental groups. Once paired, the experimental group had 1% butyrate added to drinking water. When pups were born, the water was exchanged for water with no additives. The control group had water with no additives at all times.

At 3 weeks of age, pups from weaned and placed in cages according to their experimental group.

At 6 weeks of age, colitis was induced using a dextran sulfate sodium (DSS) model, followed by sacrifice. Colitis was measured by disease activity index (DAI), colon length and histological assessment.

RESULTS: The severity of colitis in the experimental group offspring was significantly lower compared to the controls (statistically significant), supported with lower DAI score and longer colon lengths.

CONCLUSION: This data suggests that maternal ingestion of butyrate during pregnancy may elicit protective effects against gastrointestinal injury in offspring at 6 weeks of age. These findings may be secondary to a butyrate-induced microbiome alteration or epigenetic effects of butyrate. Further experiments to explain these results are underway, including evaluation of expression of tight junction proteins and microbiome evaluation between the groups.

19. Obesity Attenuates T-Cell Function Which May Impact the Efficacy of T-Cell Based Immunotherapies in Pediatric Leukemia

Presentation Time: 4:45 - 6:00 pm

Ross, Anthony; Lee, Miyoung; Hamilton, Jamie; Talekar, Ganesh; and Henry, Curtis

BACKGROUND: Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood. Despite vast improvements in the treatment of pediatric ALL, recent epidemiologic studies have shown that children who are obese at diagnosis have poorer overall survival and higher relapse rates. These studies suggest that obesity-induced microenvironmental changes impact leukemia progression and therapeutic responses. Compromised immune responses have been observed in obese patients, which is of particular interest given the increasing use of immunotherapy in hematologic malignancies. An immunotherapy that has shown success in refractory/relapsed B-ALL is chimeric antigen receptor T-cell therapy (CAR-T) which requires functional T cells to target and effectively eliminate leukemia cells. The impact of obesity on the efficacy of CAR T-cell based immunotherapies is currently unknown.

OBJECTIVE: To determine the effect of obesity on non-engineered T-cell and CAR T-cell function.

DESIGN/METHODS: We performed *in vitro* T-cell activation assays on human Jurkat T cells, as well as, primary mouse T cells. T cells were stimulated for 72 hours in the presence of either control media or conditioned media (stromal conditioned media (SCM) or adipocyte conditioned media) followed by flow cytometry to determine expression of surface markers (CD44, TIM-3, and PD-1), intracellular production of cytokines (IFN- γ and TNF- α), and cytolytic machinery (Perforin and Granzyme B). Similar experiments were conducted on *ex vivo* T cells isolated from lean and obese mice, as well as, patient samples from Emory Biorepositories. We also used qPCR to examine T-cell receptor (TCR) machinery in *ex vivo* mouse samples. All experiments were performed in triplicate and statistical analysis was conducted using ANOVA with p-value <0.05.

RESULTS: Compared to T cells activated in control media or SCM, T cells activated in the presence of adipocyte secreted factors exhibited an exhausted phenotype highlighted by increased surface expression of the immunoinhibitory marker PD-1, and their failure to produce the effector mediators IFN- γ , TNF- α , Perforin, and Granzyme B. These compromised responses maybe attributed to downregulated TCR signaling machinery as seen in our *ex vivo* qPCR experiments.

CONCLUSIONS: T cells activated in the presence of adipocyte secreted factors have reduced functional potential which may compromise the efficacy of CAR T-cell based therapies.

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