2020 Annual Child Health Research Centers *'Virtual'* Meeting

October 12 – 13

ABSTRACT BOOK

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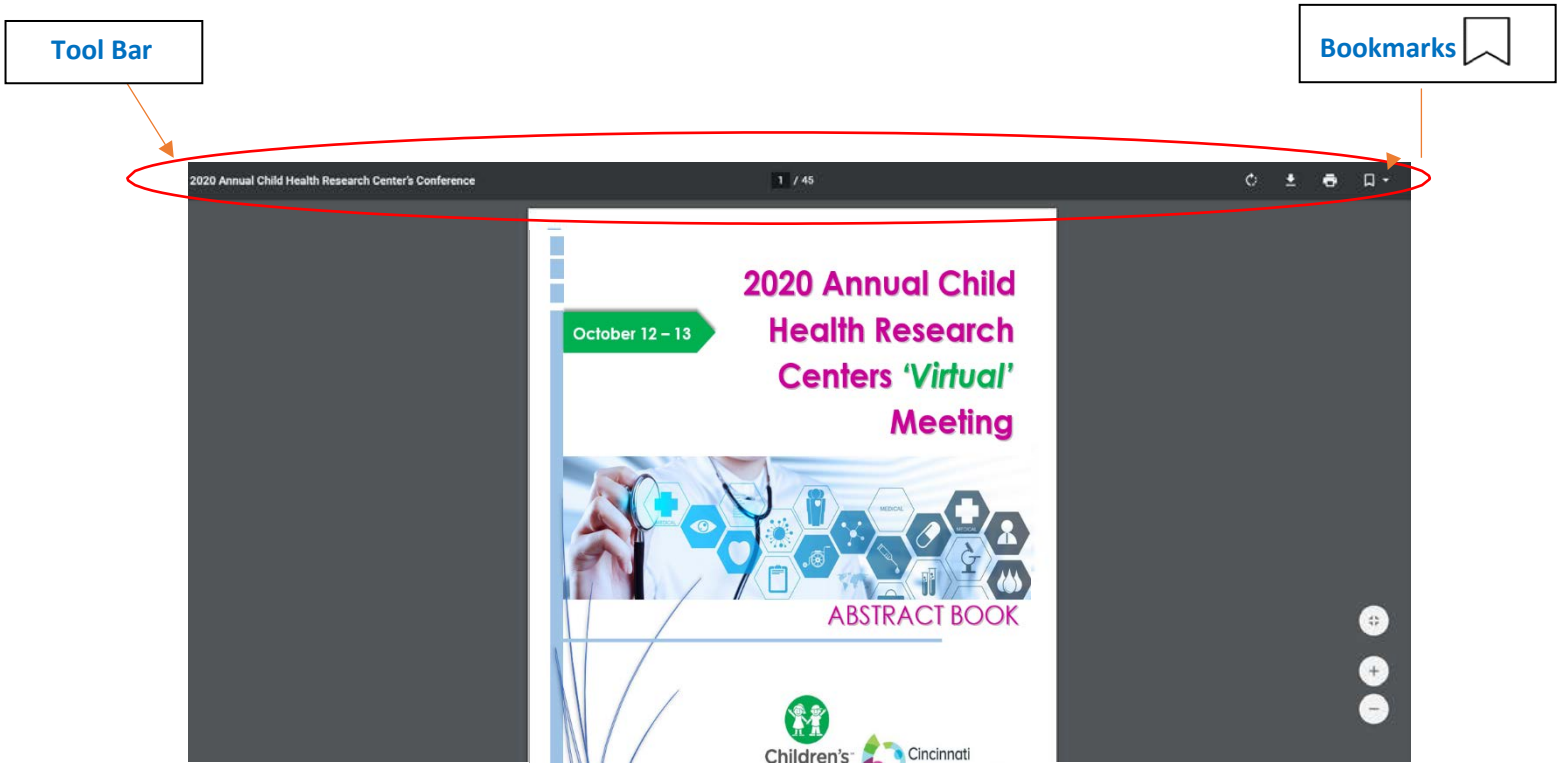


TABLE OF CONTENTS

Abstracts listed in order of presentation.

Day 1 | Session 1 | Oral Presentations

Day 1 | Session 2 | Oral Presentations

Day 1 | Sessions 1 and 2 | Poster Presentations

TABLE OF CONTENTS

Abstracts listed in order of presentation.

Day 1 | **Sessions 1 and 2** | **Poster Presentations** ***(continued)***

Day 1 | **Session 3** | **Oral Presentations**

Day 2 | **Session 4** | **Oral Presentations**

TABLE OF CONTENTS

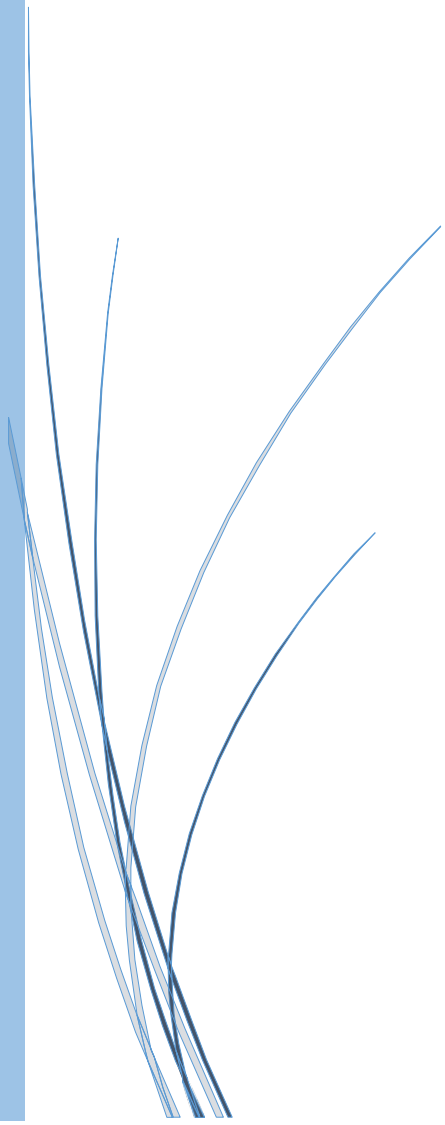
Abstracts listed in order of presentation.

Day 2 | **Session 4** | **Oral Presentations** ***(continued)***

Day 2 | **Sessions 3 and 4** | **Poster Presentations**

DAY 1

ORAL PRESENTATIONS



DAY 1 | SESSION 1 | **ORAL PRESENTATION 1**

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Ecological Networks Driving Composition and Function in the Vaginal Microbiome

The female genital tract (FGT) microbiome is emerging as a new target in sexual and reproductive health, impacting risk for prematurity, infertility and acquisition of sexually transmitted infections, including HIV. In order to target the microbiome for health benefit, it is necessary to gain a greater understanding of the functional traits of vaginal microbes, and the rules that govern their community assembly and stability. Toward this end, we have applied batch culture methods of individual isolates to determine strain-specific nutritional requirements of FGT species. Additionally, we have adapted a miniature bioreactor system to enable longitudinal cultivation of individual taxa or complex communities. We have determined the carbohydrate utilization profiles of more than 200 individual isolates of FGT bacteria, finding significant variation in carbohydrate utilization traits both within and across species. Using the bioreactor system, we have successfully cultivated simplified, synthetic communities, as well as complex co-resident communities representative of bacterial vaginosis *in vivo.* We have found that the availability of complex carbohydrates, like glycogen, maintains greater diversity in communities, while simple sugars, like glucose, promote outgrowth of single, rapidly-dividing species. Importantly, while certain species tend to grow to greater abundance in these communities, low-abundance members nevertheless continue to replicate and are not lost over time. Finally, cultivation in community bioreactors has enabled robust growth of fastidious bacteria, such as *Megasphae ra spp*. that grow poorly in isolation. These data demonstrate the power of community bioreactor culture as a system to study the biology and ecology of vaginal microbes. This system provides an invaluable model to test factors that promote the outgrowth of lower abundance or fastidious members, such as occurs during community state transitions *in vivo*. Moreover, using these longitudinal cultures, we can empirically test methods and formulations to disrupt bacterial vaginosis communities with health-promoting *Lactobacillus spp.* Ongoing work is using longitudinally collected human vaginal samples to identify changes in micro and macro-nutrient availability that associate with community compositional and transcriptional changes. We will then test the impact of these factors on *ex vivo* bioreactor cultures.

DAY 1 | SESSION 1 | **ORAL PRESENTATION 2**

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Long-Term Anatomical and Functional Deficits from Late-Gestation Transient Prenatal Hypoxia

OBJECTIVE: Late-gestation prenatal hypoxic-ischemic injury, most commonly known as neonatal hypoxic-ischemic encephalopathy, is a common and sometimes devastating global brain injury from loss of oxygen and nutrients immediately prior to birth that results in long-term neuroanatomic abnormalities and neurodevelopmental deficits. Antenatal risk factors leading to placental insufficiency, like maternal obesity and hypertension, are frequently associated with worse outcomes from hypoxic injury. Existing murine models for prenatal hypoxic injury focus on postnatal brain injury with unilateral injury, limiting the capacity to understand the effects of global hypoxia on developing networks. In this study, I used a mouse model to characterize the long-term effects of prenatal hypoxia on adult neuroanatomy and neurologic function.

STUDY DESIGN: Pregnant C57BL/6N mice were exposed to normoxia or to 2-8 hours of 5% inspired oxygen during late gestation (embryonic day 17.5). Immunoblots of hypoxia inducible factor 1α (HIF1α) and quantitative PCR of the HIF1α target vascular endothelial growth factor A (Vegfa) were used to determine the acute effects of hypoxia on the fetal brain. For 8-hour hypoxia exposures, survival and weight of offspring were monitored and compared with outcomes in animals exposed to normoxia. Adult mice from 8-hour exposures were subjected to high resolution *ex vivo* magnetic resonance imaging (MRI) studies to determine the long-term neuroanatomic effects of prenatal hypoxia. In addition, animals underwent functional studies focused on anxiety, motor function, and seizure threshold.

RESULTS: Transient prenatal hypoxia increased both HIF1α and Vegfa levels, consistent with acute hypoxic insult. The induction of these hypoxia markers were most consistent at 8 hours of hypoxic exposure, thus further postnatal experiments were completed at this time point. There was no difference in gestational age at birth, litter size at birth, or offspring survival. There was an early and persistent decrease in weight in hypoxic females compared to normoxic controls. Similar to findings seen in children with prenatal hypoxic injury, *ex vivo* MRI demonstrated that prenatal hypoxic mice had *ex vacuo* ventriculomegaly and decreased cortical thickness, although these effects were primarily observed in only females. A battery of behavior studies in adult mice revealed that prenatal hypoxia was associated with abnormal reactions to anxiety-producing stimuli and repetitive behaviors. Hypoxic females were more likely to have decreased grip strength, but neither sex had a difference in motor learning or coordination. Hypoxic males, by contrast, had a tendency to have a lower seizure threshold.

CONCLUSIONS: These data suggest that late gestation transient prenatal hypoxia is a clinically relevant, viable model for studying long-term effects of hypoxia on the developing brain, and that hypoxia results in sex-dichotomous structural and functional deficits. Current studies are focused on understanding the cell-type specific and sex-specific mechanisms driving these long-lasting effects of insult to the developing brain and on determining the effects of antenatal risk factors on outcomes.

DAY 1 | SESSION 1 | **ORAL PRESENTATION 3**

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The Rhesus Macaque Serves a Model for Human Lateral Branching Nephrogenesis

BACKGROUND: Premature infants are at risk for chronic kidney disease later in life due to low nephron endowment. Lateral branching nephrogenesis (LBN), not occurring in the mouse, is a poorly understood but critical period of human nephrogenesis contributing to the majority of human nephrons. Here, we analyze third trimester LBN in the rhesus macaque at the molecular and morphological level.

METHODS: The morphology of third trimester rhesus kidneys was assessed by immunostaining after tissue clearing. 3D renderings were created using Bitplane Imaris. Single-cell RNA sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq) were performed on four kidneys from four rhesus using cold protease digestion and 10xGenomics platform. Unsupervised analyses using ICGS2 was used to identify distinct cell populations and GO-Elite was used to compare rhesus with human and mouse datasets. RNAScope validation studies were performed on human archival tissue.

RESULTS: The gestational period of the rhesus lasts 165 days. We determined that cessation of nephrogenesis in the rhesus occurs between 136- and 147+-days gestational age (GA). LBN was observed along the ureteric stalks at 126-138 days GA analyzed in this study. We noted rosette-like patterns of the ureteric tips and nephron progenitor cells (NPC) in both rhesus and human third trimester archival samples. scRNA-seq was performed on four cortically enriched rhesus samples 129-131 days GA revealing 37 transcriptionally distinct cell clusters. C25 was predicted to contain the naïve NPCs and included CITED1, MEOX1, and EYA1. snRNA-seq yielded 5,972 nuclei, corresponding to 29 ICGS2 clusters. We found a single cluster (c26), with a near identical GO-Elite enrichment profile to that the naïve NPC scRNA-Seq cluster (c25). snRNA-seq C26 contained many unique markers not found in the matching scRNA-seq c25. GO-ELITE showed that late-gestation rhesus NPC markers more closely aligned to late-gestation murine NPC, whereas the 2nd trimester human NPC more closely to mid-gestation murine NPCs.

CONCLUSION: The rhesus is the first animal model to demonstrate LBN, suggesting that LBN is conserved in old world primates. We identified novel genes upregulated during LBN development. snRNA-seq of naïve NPCs nascent transcripts may provide mechanistic insights that would otherwise be missed.

DAY 1 | SESSION 2 | **ORAL PRESENTATION 4**

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Impaired Glucose Tolerance is Prevalent in Adolescents and Women with Turner Syndrome with Normal Hemoglobin A1c

BACKGROUND: Diabetes mellitus contributes to morbidity and mortality in women with Turner syndrome (TS). However, there is little data on glycemic status in those with TS who do not meet American Diabetes Association criteria for diabetes mellitus.

METHODS: This study compared glycemic excursions of adolescents and adults with TS to age and sex-matched controls using oral glucose tolerance tests (OGTT) and continuous glucose monitoring (CGM), excluding those with a hemoglobin A1c greater than 6% (42 mmol/mol).

RESULTS: Many non-diabetic subjects with TS demonstrated abnormal glycemic excursions during OGTT. An increased prevalence of impaired glucose tolerance at the two-hour time point and elevated one-hour and two-hour glucose values were noted in the TS group when compared to controls. No individuals in either group met OGTT criteria for diabetes. There was no difference in average glucose or glycemic variability metrics between groups on multiday CGM monitoring. One individual with TS was noted to have intermittent diabetic-range hyperglycemia.

CONCLUSIONS: Asymptomatic dysglycemia is commonly present in individuals with TS as detected by OGTT. Surprisingly, CGM was less sensitive in detecting these abnormalities. Longitudinal follow-up with OGTT and CGM may be helpful to further describe the progression from impaired glucose tolerance to diabetes in this population.

DAY 1 | SESSION 2 | **ORAL PRESENTATION 5**

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The Role of MSC-Derived Exosomes in the Septic Brain

BACKGROUND: Multi-organ dysfunction is a key cause of mortality in sepsis due to:

- Hyperimmune state
- Impaired cellular energy production

The Central Nervous System (CNS) is one of the most affected organs in sepsis and hyperinflammation leads to encephalitis and altered mental status. In murine models of sepsis inflammation is reduced when energy production is restored (i.e., improved aerobic cellular respiration). Mesenchymal stem cells (MSCs) increase cellular energy production via small extracellular vesicles (sEVs). sEVs can cross the blood-brain barrier and alter CNS function.

HYPOTHESIS: MSC-derived sEVs alleviate the hyperimmune state in the septic brain by restoring aerobic cell respiration.

METHODS: Fecal material in a cecal slurry, was administered by intraperitoneal injection to induce polymicrobial sepsis in mice. Mice were evaluated every two hours for sepsis severity and euthanized when a score of ≥15 was reached (maximum score = 28) with the goal for mice to reach a score of ≥15 at 16 -20 hours post-injection. A single dose of MSC-derived sEVs (6.5 x 10E+10 particles/mL) was administered 6h post-cecal slurry injection. Oxidative phosphorylation and glycolysis were measured via oxygen consumption and extracellular acidification rates respectively. Histopathology was utilized for cytokine quantification (IL-1, IL-6, IL-10, IFN-gamma, TNF-alpha).

RESULTS: There is a switch in energy production of brain from septic mice treated with MSC-derived exosomes favoring oxidative phosphorylation. Septic mice with no treatment continued to produce energy mostly by glycolysis. There was also an increase in survival of treated mice compared to controls.

CONCLUSIONS: IV administered MSC-derived sEVs can alter cell respiration in CNS and partially restore oxidative phosphorylation.

sEVs might be alleviating hyperinflammation in the septic brain by decreasing cytokine production. MSC derived sEVs could be a potential adjunct treatment in septic encephalitis for improved outcomes.

DAY 1 | SESSION 2 | **ORAL PRESENTATION 6**

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Modeling Acute Lung Injury in Sickle Cell Mice

Sickle cell disease (SCD) is a common and life-threatening autosomal recessive hematological disorder that affects 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. Unfortunately, supportive care remains the primary approach to alleviate these complications. This in part reflects an incomplete understanding of the pathophysiology and accompanying pharmacological targets that could specifically mitigate acute disease complications. 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 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.

Complement activation via injection of cobra venom factor (CVF) results in acute intravascular hemolysis and death in humanized sickle (SS) but not wild type AA mice. To specifically address the role of complement in the development of ALI following CVF injection, we generated SS mice deficient in complement component C3 (SS x C3 KO). Injection, of CVF into SS x C3 KO mice failed to induce detectable hemolysis, pulmonary compromise or mortality, demonstrating a critical role of C3 in this process. As hemin has previously been shown to likewise induce ACS, the role of C3 in hemin-induced ALI was likewise examined. While injection of hemin into SS mice with C3 resulted in rapid death, consistent with prior reports of hemin-induced ALI and death in this mouse model, similar hemin exposure in SS x C3 KO mice failed to induce a similar change in mortality, providing a critical link between hemin and complement in the development of ALI and death in SS mice. Our data thus far suggest that complement activation in SS mice results in hemolysis, release of free heme and production of C5a, which is a potent pro-inflammatory mediator, 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.

DAY 1 | SESSION 3 | **ORAL PRESENTATION 7**

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A Mouse Model of X-Linked Clustering Epilepsy

OBJECTIVE: It is hypothesized that the unbalanced expression of the X-linked gene, PCDH19, results in aberrant neural development, functionally expressed as the varying phenotypes observed in X-linked clustering epilepsy (XCE). To explore this hypothesis, we are utilizing a transgenic mouse model to examine the histologic and physiologic implications of mosaic expression of PCDH19.

METHODS: Female PCDH19-/- mice were bred with X-linked GFP male mice to obtain female offspring expressing GFP on the wild-type allele and no fluorescent marker on the PCDH19 null allele.

Brains from female offspring were harvested at varying timepoints from E10-P60 and processed for histological analysis. Seizure latency was assessed with a hyperthermic insult at P16. Prolonged EEG recordings were performed on P40-P60 female mice to asses for spontaneous seizures.

RESULTS: Brains of PCDH19+/- female mice revealed a unique cell segregation pattern in which there were distinct populations of cells expressing GFP (PCDH19+) separated from those lacking GFP (PCDH19), most notable in the cortex and CA1 region of the hippocampus. This cell segregation pattern was observed at E10 and persisted through adulthood. Preliminary studies suggest a lowered seizure threshold in PCDH19+/- mice during hyperthermia. Prolonged EEG recordings did not show increased interictal epileptiform discharges or spontaneous seizures in adult PCDH19+/- mice vs. controls.

CONCLUSIONS: Our mouse model supports the hypothesis that the mosaic expression of PCDH19 leads to aberrant neuronal segregation between the wild type and knockout cell populations. PCDH19+/- mice may have a lower seizure threshold to a hyperthermic insult in the early postnatal period, though spontaneous seizures were not observed in adulthood. Further work is necessary to characterize the molecular mechanisms underlying this early cell segregation and the physiologic implications at varying time points during development.

DAY 1 | SESSION 3 | **ORAL PRESENTATION 8**

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A Novel Anti-LILRB4 CAR-T Cell for the Treatment of Monocytic AML

BACKGROUND: Nearly half of children diagnosed with acute myeloid leukemia (AML) will die from relapsed or refractory disease, or due to treatment related toxicities. Novel treatment strategies are needed to improve poor outcomes in pediatric AML. We have previously demonstrated Leukocyte immunoglobulin like receptor-beta 4 (LILRB4) is highly expressed on monocytic AML, with a restricted pattern of expression on normal cells of monocytic lineage. Targeting LILRB4 by chimeric antigen receptor (CAR)-T cells may offer specific treatment against monocytic AML, while minimizing on-target/off-tumor toxicity to myeloid progenitors and other healthy cells.

STUDY DESIGN: We have generated a 2nd generation *humanized* anti-LILRB4 CAR, which we have optimized and validated for translation to clinical use. The single chain variable fragment is derived from a novel humanized rabbit monoclonal antibody with high affinity and specificity for LILRB4, spliced to the 41BB costimulatory domain, followed by the CD3- zeta activation domain. This construct was expressed in primary human- T cells by lentiviral transduction and expanded in optimized cell culture conditions. CAR-T cell effector function and potential toxicity was evaluated in various *in vitro* and *in vivo* assays. Additionally, we have completed further expression profiling of LILRB4 on specific tissues of interest by both IHC and flow cytometry.

RESULTS: Utilizing our lead clinical vector encoding anti-LILRB4 CAR, we validate anti-LILRB4 CAR-T cells display strong *in vitro* effector function of cytotoxicity, cytokine release and T-cell expansion, when cultured with LILRB4^{+} monocytic AML cell lines and primary patient samples compared to control- T cells. Using an aggressive monocytic AML-mouse xenograft model, anti-LILRB4 CAR-T cell treated mice display significantly decreased leukemia burden and significantly prolonged survival compared to mice in control conditions. Lastly, expression profiling utilizing novel rabbit-anti human anti-LILRB4 antibodies demonstrates LILRB4 is not expressed on tissue resident macrophages in the CNS, lung or liver.

CONCLUSIONS: We demonstrate the construction and validation of a novel anti-LILRB4 CAR-T cell which displays efficient leukemia cell killing in both *in vitro* and *in vivo* disease models, while importantly sparing normal hematopoiesis. LILRB4 displays a favorable pattern of expression, restricted only to aberrant leukemia cells and *hematopoietic* monocyte derived cells. The construct utilized in this study may be directly translated to be used in clinical trials, and may offer a new treatment strategy to improve outcomes in pediatric AML.

DAY 1 | SESSION 3 | **ORAL PRESENTATION 9**

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Evaluation of CAR T Cells in Pediatric Ependymoma

BACKGROUND: Ependymoma is the third most common pediatric brain tumor and current treatment still results in a 10-year relapse rate of over 70% in the highest risk groups. The treatment refractory nature of ependymoma to standard therapies strongly supports the development of novel interventions. Ependymoma tumor cells express HER2 and there are active clinical trials treating children with ependymoma using local delivery of second-generation HER2 CAR T cells.

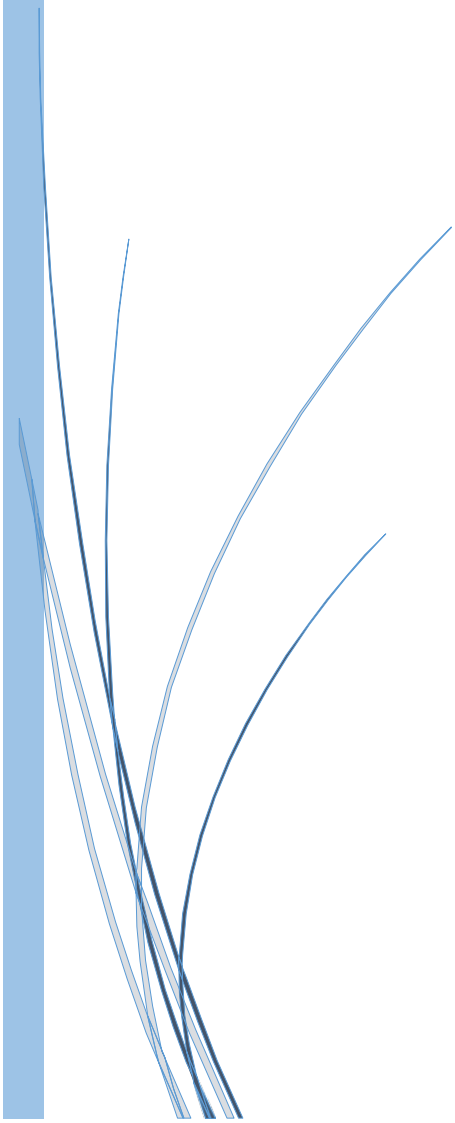
METHODS: Two high-risk patient-derived ependymoma cell lines, MAF811 and MAF928, that display HER2 surface expression are used for testing. We tested second-generation HER2-BBz CAR T cells *in vitro* and *in vivo*.

RESULTS: HER2 CAR T cells effectively kill ependymoma tumor cells in culture, but this strategy cannot eradicate the same tumor cells in mice when implanted in the fourth ventricle of the brain. HER2 CAR T cells proliferate and traffic into the tumor, but this causes a dramatic influx of immune cells, tumor swelling and lethal toxicity in a subset of mice. Mice that survive this initial tumor swelling, display significant tumor shrinkage but all tumors eventually start growing again. Ependymoma tumor cells release high amounts of inflammatory chemokines that strongly attract neutrophils and monocytes to the tumor, compared to other brain tumors, and can downregulate HER2 expression to escape recognition by CAR T cells.

CONCLUSION: The immunosuppressive microenvironment as well as tumor heterogeneity make HER2 CAR T cells ineffective in ependymoma. Studying these two hurdles in CAR T cell therapy is critical to effectively treat brain tumors with CAR T cells.

DAY 1

POSTER PRESENTATIONS



DAY 1 | SESSION 1 | **POSTER PRESENTATION 1**

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Resident Memory T Cells in Human RA Synovium Display Restricted TCR Clones

BACKGROUND: Resident memory T cells (TRM) are site-specific memory T cells that take up long-term residence in peripheral tissues and aid in pathogen defense. Dysregulated TRM have also been implicated in autoimmune diseases by driving localized recurrent inflammation. As rheumatoid arthritis (RA) is characterized by recurrent joint inflammation, we asked whether TRM can be identified in human RA synovium.

METHODS: Multicolored tyramide-based immunostaining was performed on formalin-fixed paraffin embedded synovium tissue obtained from RA patients. CD3, CD8, CD4, CD45RO, CD69 and CD103 surface antigens were assessed by immunofluorescence microscopy using the Mantra quantitative pathology imaging and analysis platform. TRM were identified by their protein expression signature. We utilized 10X Genomics droplet-based single cell RNA sequencing to evaluate gene expression of individual T cells. Leukocytes were harvested from disaggregated RA synovial tissue and memory T cells (CD3+CD45RO+) were enriched by FACS sorting prior to 10X sequencing. Individual cells were clustered based on gene expression profile and compared to known TRM gene expression signatures. TRM gene expression signatures were also assessed in sorted T cells from RA synovium across a spectrum of synovial inflammation and chronicity. We utilized 10X-integrated targeted amplification of full-length VDJ sequences of both alpha and beta chains of T cell receptor (TCR) constant regions to profile the immune cell repertoire.

RESULTS: Using Mantra multispectral immunofluorescence imaging, we visualized T cells bearing a signature consistent with TRM (CD3+CD45RO+CD69+CD103+) in RA synovium. Single-cell RNA sequencing revealed a subset of RA synovial T cells with a gene expression signature consistent with TRM. These cells were more prominent in late-stage non-inflamed RA synovial tissues, primarily expressed CD8, and demonstrated a transcriptome consistent with immune cell activation and

chemokine signaling. They displayed a restricted TCR profile within the top 0.5% of predominant T cell clones.

CONCLUSION: We identified T cells with a cell surface expression profile consistent with TRM in human RA synovium. Single-cell RNA sequencing showed a subpopulation of memory T cells from RA synovium with a transcriptomic signature consistent with TRM identified in other tissues. Prominent in late-stage non-inflamed RA synovial tissue, these cells are restricted in their TCR sequences and represent some of the most abundant T cell clones in the RA synovium. They also express genes involved in immune cell activation and recruitment, suggesting an active role in inflammation.

DISCLOSURES: none

DAY 1 | SESSION 1 | **POSTER PRESENTATION 2**

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Identifying Targets to Enhance Beta Cell Number and Response in Diabetes Using Single-Cell Functional Genomics

BACKGROUND/OBJECTIVE: Type 2 diabetes (T2D) is a multifactorial disease characterized by insufficient number and function of the insulin-secreting pancreatic beta cells. Insulin secretion is a tightly-controlled process, and the beta cell secretory defects associated with T2D remain unclear. There is strong evidence that beta cells vary greatly in their insulin secretory capacity, even within one person. My goal is to identify new targets responsible for heterogeneous and decreased beta cell function in T2D, with potential to therapeutically enhance insulin secretion. This study utilizes an existing dataset to identify preliminary pathways and targets that can be interrogated in our future single-cell functional genomic analyses.

STUDY DESIGN: An existing single-cell RNA-sequencing dataset was re-analyzed to further investigate beta cell subtypes. This dataset included pancreatic islet cells from 12 non-diabetic (ND) and 6 T2D human donors. K-means clustering was performed to identify islet cell types, and beta cells were then subjected to further sub-clustering, differential expression, and pathway analyses.

RESULTS: We identified four distinct beta cell subtypes in ND islets, and three beta cell subtypes in T2D islets, based on their transcriptomic profiles. The four ND beta cell subtypes were distinguished by insulin expression, futile glucose cycling, insulin receptor signaling, and a delta cell-like phenotype, among other things. The three T2D beta cell subtypes overlapped somewhat with three of the ND beta cell subtypes, but there was no T2D beta cell subtype with elevated insulin expression.

CONCLUSIONS: These findings suggest that beta cells normally exist in at least four heterogeneous states. Although all beta cells are identified based on insulin expression, we found that some beta cells expressed higher amounts of insulin, while other beta cells seemed more poised to respond to insulin in a paracrine fashion. Furthermore, some beta cells exhibited increased expression of genes involved in futile glucose cycling, which is associated with decreased glucose-stimulated insulin secretion. Lastly, the beta cell subtype with a delta cell-like phenotype expressed the hormone somatostatin, which is a general suppressor of endocrine hormone secretion and may contribute to decreased insulin secretion.

Our next study will investigate insulin secretion and transcriptomics in the same individual human beta cells, in order to more directly correlate differences in gene expression with functional changes.

DAY 1 | SESSION 1 | **POSTER PRESENTATION 3**

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Transcriptional Characterization of Pathogenic B Cells in Pediatric Liver Transplant Rejection

Late acute cellular rejection (ACR) occurs in ~20% of pediatric liver transplant recipients at >6 months post- transplantation, and unlike early ACR, has been associated with increased risk of progression to chronic rejection and allograft loss. Currently there are no treatments for chronic rejection other than retransplantation. Donor specific antibodies (DSA) have been associated with late ACR, chronic rejection, and allograft fibrosis in pediatric liver transplant recipients, but the cellular and molecular mechanisms by which DSA form and contribute to late ACR is unknown. Although B cells infiltrate the allograft during ACR, it is unknown what costimulatory or activation markers they express, whether they are playing a pathogenic or regulatory role during late ACR, whether they are allospecific or bystanders, or whether they differentiate into allograft-specific plasma cells which produce DSA. Single cell RNA sequencing (scRNA-seq) provides transcriptional profiling of immune cell subtypes in an unbiased fashion at the resolution necessary to infer cell-cell interactions, molecular pathways, costimulatory molecules, and antigen receptor specificity which may have gone unrecognized in prior studies which have exclusively examined bulk tissue RNA or peripheral blood gene expression profiles.

To test the hypothesis that B cells/plasma cells contribute substantially to allograft injury in late ACR subjects with DSA, but not in patients without DSA and begin to understand the role of B cells/plasma cells in late ACR, we will determine the whole cell transcriptome and CDR3 sequences of the B cell antigen receptor (BCR) of liver-infiltrating B cells and plasma cells during ACR in DSA+ vs. DSA- subjects. To date, we have performed such sequencing on two patients – one with ACR and one without – and identified clonally expanded B cell populations and plasma cells within the rejecting allograft. Ongoing experiments will sequence liver and peripheral blood B cells to determine whether the liver infiltrating B cell populations are alloreactive.

DAY 1 | SESSION 2 | **POSTER PRESENTATION 4**

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Super Elongation Complex as a Targetable Dependency in H3K27M+ Diffuse Intrinsic Pontine Glioma

Mutations in the histone 3 gene (H3K27M) are the eponymous driver in diffuse intrinsic pontine gliomas (DIPGs), aggressive pediatric brain tumors for which no curative therapy currently exists. To identify specific epigenetic dependencies which arise as a consequence of the H3K27M mutation, we performed an shRNA screen targeting 408 genes classified as epigenetic/chromatin-associated molecules in patient-derived DIPG cell lines. This identified AFF4, a component of the super elongation complex (SEC), as both highly expressed and necessary for DIPG cells to maintain growth and self-renewal. We hypothesized that SEC overexpression occurs as a consequence of the H3K27M mutation and that this relative abundance overcomes repressive transcriptional regulation in order to suppresses differentiation and promote self-renewal of DIPG tumor stem cells. We interrogated the role of AFF4 in DIPG using an shRNA lentiviral approach. We demonstrate a significant decrease in in vitro clonogenicity and stem cell maintenance following AFF4 depletion. We employed RNA-seq-based gene set enrichment analysis to delineate differentiation programs under AFF4 regulatory control. Finally, we sought to determine whether CDK9, the catalytic subunit of the SEC, represents a therapeutic vulnerability in DIPG. Using Pol II ChIP-seq following CDK9 pharmacologic inhibition, we demonstrate that the loss of regulatory transcriptional input in DIPG may be reversed via CDK9 inhibition and that this acts as a potent inhibitor of DIPG cell growth both in vitro and *in vivo* PDX models.

DAY 1 | SESSION 2 | **POSTER PRESENTATION 5**

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Exploring the Circadian-and Sleep-Dependence of NG2-Glia Functions

NG2-glia comprise the largest population of regenerative cells in the postnatal and adult CNS (approximately 5% of the total population). These cells play major roles in brain development and response to injury and disease, as they can actively proliferate, migrate towards injury, and differentiate into multiple cell types. However, it is unknown how circadian rhythmicity and/or sleep-wake state, both of which influence nearly every physiologic function in the body, may affect NG2-glia functions. Thus, it is our goal to evaluate the cellular and molecular mechanisms that control and are affected by the clock- and sleep-dependent programming of *in vivo* NG2-glia, both in the healthy, developing brain and in the injured brain. To accomplish this, we propose a multi-disciplinary approach with several cutting edge techniques, including non-invasive piezoelectric sleep recording, Translating Ribosomal Affinity Purification (TRAP), RNAScope, whole-brain clearing and light sheet microscopy, and a "hit and run" model of traumatic brain injury. Thus far, our preliminary data demonstrate that NG2-glia express 24-hour cycling of key molecular clock RNA and proteins. Furthermore, these NG2-glia may also exhibit cycling in cell density in cortex, suggestive of fluctuation in proliferation. These data serve as the foundation upon which we will perform unbiased translatomics from NG2-glia throughout the circadian day, both in development as well as in response to a mouse model of traumatic brain injury to determine the clock- and sleep-dependent cellular programming in NG2-glia, with a focus on key cellular functions such as proliferation, migration, and maturation.

DAY 1 | SESSION 2 | **POSTER PRESENTATION 6**

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Arginine Dysregulation Correlates with Cardiac Remodeling in Mice with Chronic Kidney Disease

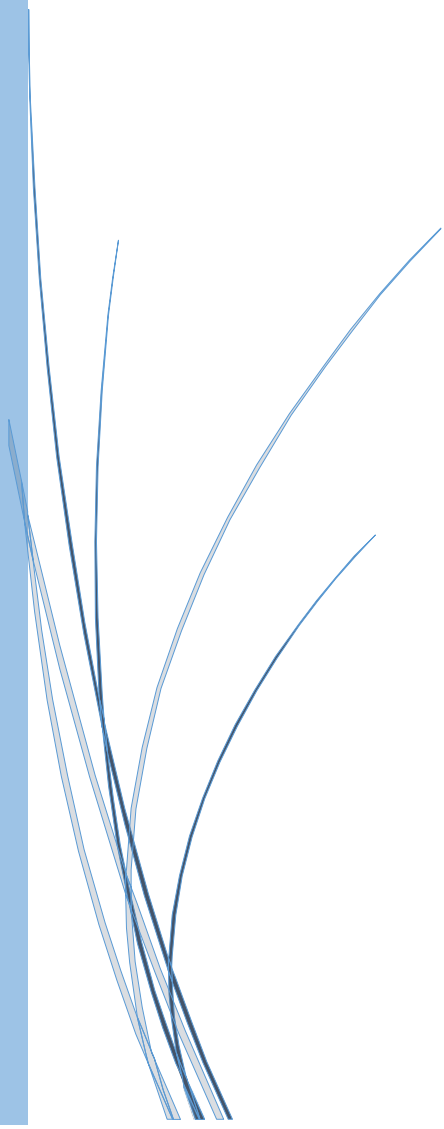
BACKGROUND: Nitric Oxide (NO) is critical for cardiovascular homeostasis. Arginine, the sole nitrogen donor for NO synthesis, is the common substrate for NO synthase and arginase enzymes. Low global arginine bioavailability ratio (GABR), a potential endothelial dysfunction biomarker, has been associated with increased mortality in adults with heart failure. Arginine metabolism is dysregulated in chronic kidney disease (CKD) and is associated with changes in myocardial function. This study aimed to determine the relationship between metabolites/enzymes in arginine metabolic pathways with cardiovascular measures in CKD mice and investigate the effect of arginine supplementation.

METHODS: CKD established in male 129X1/SvJ mice via 5/6th nephrectomy. Plasma collected at 8- and 16-weeks post-surgery was analyzed for arginine, citrulline, ornithine, asymmetric dimethylarginine (ADMA) via LC-MS/MS; GABR=arginine/(ornithine+citrulline). Arginase activity was analyzed via colorimetric assay. Echocardiographic measures of left ventricular hypertrophy (LVH), diastolic dysfunction and ventricular strain were obtained at 8- and 16-weeks. In a separate experiment, CKD and control mice received chow supplemented with arginine or alanine (nitrogen control) for 12 weeks. Plasma and echocardiograms obtained at 8- and 12-weeks were examined as above. Blood pressure was measured noninvasively.

RESULTS: In CKD mice, low GABR correlated with decreasing E/A ratio (measure of diastolic dysfunction) [r=0.58; p=0.01] and increasing relative wall thickness (RWT) (measure of LVH) [r=-0.49, p=0.03]. Plasma arginase activity was significantly increased in CKD mice at 16-weeks [median (IQR) 10.5 (8.4-11.7)] compared to controls [5.5 (1.5-10.0); p</= 0.05] and to CKD mice at 8-weeks [7.0 (3.7-7.6); p=0.002]. Increased arginase activity correlated with impaired ventricular strain [r=-0.34; p=0.04]. ADMA was significantly increased in CKD mice at 16-weeks [7.2 (7.1-7.3)] compared to controls [7.1 (7.1-7.1); p=0.036] and correlated with lower E/A ratio [r=-0.34; p</=0.05]. After 12-weeks of supplementation, arginase activity was significantly lower in arginine supplemented CKD-mice compared to arginine supplemented normal mice [7.8 (3.1-8.5) vs 14.3 (10.0-15.6); p=0.004] and compared to alanine supplemented CKD-mice [18.8 (12.9-19.7); p=0.006]. In arginine supplemented CKD-mice, blood pressure was significantly lower at 12 weeks compared to 8 weeks; p=0.03.

CONCLUSIONS: In a mouse model of CKD, dysregulation in arginine metabolism correlates with myocardial dysfunction; hypertension is ameliorated with arginine supplementation.

DAY 2
ORAL PRESENTATIONS



DAY 2 | SESSION 4 | **ORAL PRESENTATION 10**

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Structural Requirements for GRK2-Mediated Inhibition of the MALT1 Proto-Oncoprotein

BACKGROUND: Stimulation of the B-cell or T-cell receptor triggers assembly of a signaling complex that contains the proteins CARMA1, BCL10 and MALT1 ("CBM"). This CBM complex induces activation of the NF-κB transcription factor and lymphocyte proliferation in response to antigen. While germline mutations that cause loss of CBM activity result in severe combined immunodeficiency, somatic gain-of-function mutations that cause constitutive CBM activity underly multiple lymphoid malignancies including Activated-B-cell Diffuse Large B-cell Lymphoma (ABC-DLBCL). This spectrum of disorders associated with aberrant CBM activity suggests that appropriate regulation of MALT1, the downstream effector protein, is critically important to normal lymphocyte function. BCL10, the upstream regulator of MALT1, associates with MALT1 in unstimulated lymphocytes. Antigen receptor stimulation induces recruitment of BCL10/MALT1 to the CBM complex where BCL10 serves as the linker between CARMA1 and MALT1. Multiple studies established that in ABC-DLBCL, the CBM complex is constitutively assembled, leading to deregulated MALT1 signaling. Inhibiting MALT1 reduces growth and viability in these tumors. We recently identified G-protein-coupled receptor kinase 2 (GRK2) as a novel MALT1-binding protein and demonstrated that GRK2 inhibits MALT1-dependent NF-κB activation. Further, we found that knockdown of GRK2 in ABC-DLBCL enhances tumor growth, suggesting that GRK2 may act as a tumor suppressor by inhibiting MALT1. We now seek to characterize the molecular mechanisms by which BCL10 and GRK2 dually regulate MALT1 to either activate (BCL10) or inhibit (GRK2) downstream oncogenic signaling.

METHODS: We sought to identify the specific site(s) within BCL10, MALT1 and GRK2 that mediate the BCL10-MALT1 and MALT1-GRK2 interactions. We created a series of expression constructs encoding specific protein fragments and analyzed the ability of discreet structural domains to

interact using co-immunoprecipitation. Additionally, we tested the ability of specific GRK2 fragments to inhibit CBM-dependent NF-κB activation using an NF-κB reporter assay.

RESULTS/CONCLUSIONS: We found that BCL10 interacts with the Ig1-2 (Immunoglobulin-like) domains of MALT1, while GRK2 interacts with the N-terminal MALT1 death domain. Additionally, we demonstrated that a GRK2 fragment containing only the αN and RH domains (AA 1-173) as well as a fragment lacking the αN (AA 30-C) each block CBM-dependent NF-κB activation. These results indicate that BCL10 and GRK2 interact with distinct domains within MALT1 and that GRK2RH, the only domain retained in both GRK2 fragments, is sufficient to inhibit MALT1. These results advance our understanding of how BCL10 and GRK2 promote activation and inhibition, respectively, of the MALT1 oncoprotein. We are utilizing this information to advance the development of a MALT1 inhibitor for the treatment of MALT1-dependent leukemia and lymphoma.

DAY 2 | SESSION 4 | **ORAL PRESENTATION 11**

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Increased IFNγ and IL-17 Secretion in Polyarticular Juvenile Idiopathic Arthritis T Helper Cell Cultures

BACKGROUND/PURPOSE: Juvenile idiopathic arthritis (JIA) is the most common autoimmune arthritis in children and its pathogenesis is unknown. The polyarticular and extended oligoarticular JIA subtypes share genetic associations near important T cell genes. Effector T cells, including T helper (Th) cells, differentiate becoming a lineage that secretes characteristic cytokines upon activation.

Proinflammatory cytokines including interferon gamma (IFNγ) and interleukin 17 (IL-17) are increased in JIA, and cytokines are targeted by clinically important biologic therapies. This study assesses Th cell differentiation and cytokine secretion in young children with polyarticular JIA and pediatric healthy controls.

METHODS: Peripheral blood mononuclear cells (PBMCs) were collected from 20 JIA patients, 20 age-matched control children, and healthy adults. Children were 2 to 8 years old (average 62 months) at time of sample collection. JIA patients are polyarticular (n=18) or extended oligoarticular (n=2), female (n=16), and ANA positive (n=19). Some samples were collected at diagnosis (n=5). PBMCs were analyzed for naïve and memory T cells by measuring cell surface expression of CD3, CD4, CD8, CD197, and CD45RA with flow cytometry. Th cell differentiation and activity were measured in an ex vivo assay wherein PBMCs are treated with or without cytokines to make Th0, Th1, Th2, and Th17 cell cultures. Cultures are then stimulated, followed by assessment of secreted effector cytokines, cell RNA, and cell protein. Cell proliferation in the *ex vivo* cultures was measured using a fluorescence-based assay.

RESULTS: PBMC cell surface staining found no difference in the number of CD3+, CD4+, or CD8+ cells between groups. JIA and healthy pediatric control PBMCs have significantly more CD4+ and CD8+ naïve (CD197+, CD45RA+), less CD4+ central memory (CD197+, CD45RA-), and less CD8+ effector memory (CD197-, CD45RA-) cells than adults. No difference was identified in the JIA and control proliferation indices for CD3+, CD4+, or CD8+ Th0 and Th1 cell cultures. JIA Th0, Th1, and Th17 cell cultures had increased secretion of IFNγ, IL-17, or both IFNγ and IL-17.

CONCLUSIONS: JIA Th0, Th1, and Th17 cell cultures have an increase in proinflammatory cytokines, IFNγ and IL-17, that is not found in Th2 cultures. This suggests that factors present during the differentiation process can drive or repress the abnormal production of cytokines in JIA cultures. Our evidence does not

support differences in input PBMCs or CD3+ proliferation as the mechanism for these findings. Like other studies, we highlight the importance of using pediatric age matched controls. Analysis of the activated and repressed pathways in JIA Th cell cultures is being pursued to identify pathogenic molecular pathways, which can provide new therapeutic targets.

DAY 2 | SESSION 4 | **ORAL PRESENTATION 12**

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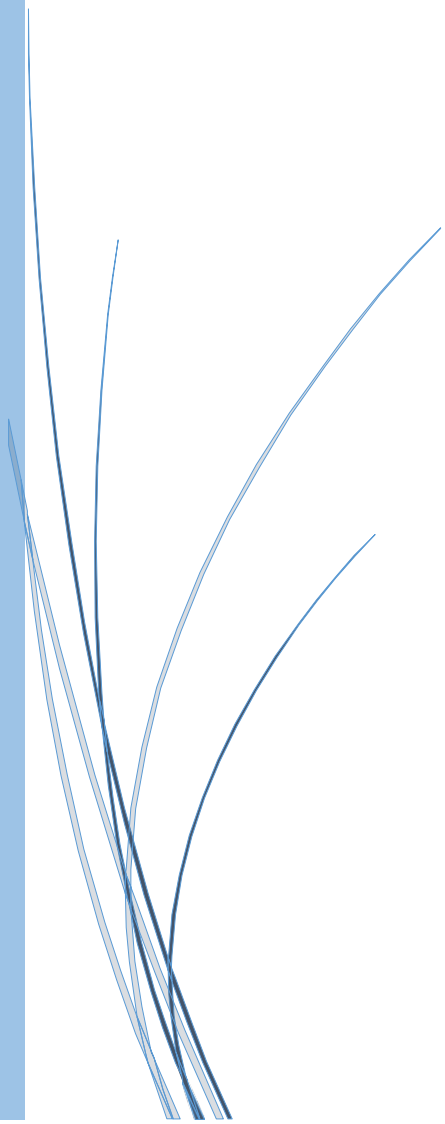
Monoacylglycerol Acyltransferase 1 Knockdown Exacerbates Hepatic Ischemia-Reperfusion Injury in Mice with Hepatic Steatosis

BACKGROUND AND AIMS: Nonalcoholic fatty liver disease (NAFLD) is becoming the most common indication for liver transplantation. The growing prevalence of NAFLD not only increases the demand for liver transplantation, it also limits the supply of available organs because steatosis predisposes grafts to ischemia-reperfusion (IR) injury. Thus, many steatotic grafts are deemed unsuitable for transplant and discarded. We have shown that monoacylglycerol acyltransferase 1 (MGAT1), an enzyme that converts monoacylglycerol to diacylglycerol, is highly induced in animal models and patients with NAFLD and is an important mediator in NAFLD-related insulin resistance. Herein, we sought to determine whether Mogat1 (gene encoding MGAT1) knockdown in mice with hepatic steatosis would reduce liver injury and improve liver regeneration following experimental IR injury.

APPROACH AND RESULTS: Antisense oligonucleotides (ASO) were used to knockdown expression of Mogat1 in a mouse model of NAFLD. Mice then underwent surgery to induce IR injury. We found that Mogat1 knockdown reduced hepatic triacylglycerol (TAG) accumulation, but unexpectedly exacerbated liver injury and mortality following experimental IR surgery. The increased liver injury was associated with robust effects on the hepatic transcriptome following IR injury including enhanced expression of proinflammatory cytokines and chemokines and suppression of enzymes involved in intermediary metabolism. These transcriptional changes were accompanied by increased signs of oxidative stress and an impaired regenerative response.

CONCLUSIONS: We have shown that Mogat1 knockdown in a mouse model of NAFLD exacerbates IR injury and inflammation and prolongs injury resolution, suggesting that Mogat1 may be necessary for liver regeneration following IR injury and targeting this metabolic enzyme will not be an effective treatment to reduce steatosis-associated graft dysfunction or failure.

DAY 2
POSTER PRESENTATIONS



DAY 2 | SESSION 3 | **POSTER PRESENTATION 7**

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Oncogenic N-Ras Mitigates Oxidative Stress-Induced Apoptosis of Hematopoietic Stem Cells

Leukemic relapse is believed to be driven by transformed hematopoietic stem cells that harbor oncogenic mutations or have lost tumor suppressor function. Recent comprehensive sequencing studies have shown that mutations predicted to activate Ras signaling are highly prevalent in hematologic malignancies and, notably, in refractory and relapsed cases. To better understand what drives this clinical phenomenon, oncogenic NrasG12D was expressed within the hematopoietic system in mice and its effects on hematopoietic stem cell (HSC) survival interrogated. We observe that N-RasG12D confers a survival benefit to HSCs and progenitors following metabolic and genotoxic stress. This effect is limited to HSCs and early progenitors and is independent of autophagy and of cell proliferation. Notably, N-RasG12D-mediated HSC survival was not affected by inhibition of the canonical Ras effectors, such as MEK and PI3K. Inhibition of the non-canonical Ras effector pathway protein kinase C (PKC) however, ameliorated the protective effects of N-RasG12D. Mechanistically, N-RasG12D lowers levels of reactive oxygen species (ROS), which correlates with reduced mitochondrial membrane potential and ATP. Inhibition of PKC, importantly, restored the levels of ROS to that of control HSCs and abrogated the protective effects granted by N-RasG12D. Thus, N-RasG12D activation within HSCs promotes cell survival through the mitigation of ROS and targeting this mechanism may represent a viable strategy to induce apoptosis during malignant transformation of HSCs.

DAY 2 | SESSION 3| **POSTER PRESENTATION 8**

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Novel Transcript Variant in MYOD1 in Rhabdomyosarcoma

INTRODUCTION: Rhabdomyosarcoma, a skeletal muscle cancer that mostly affects children, is thought to occur when normal muscle differentiation goes awry. Current therapies are frequently associated with moderate to severe long-term side effects in survivors and as such, there remains a need for the development of new therapies and increased insight into the oncogenic pathways that lead to rhabdomyosarcoma development in children. We hypothesized by combining computation analysis of RNA sequencing data with molecular biology tools, we could identify alternatively spliced genes that may alter the normal muscle cell differentiation pathway providing myoblasts with a selective advantage leading to rhabdomyosarcoma development.

METHODS: We developed a computation algorithm investigating RNA seq data of rhabdomyosarcoma specimens. After identifying genes of interest, we created retroviral vectors which were then transduced into mouse fibroblasts and human rhabdomyosarcoma cell lines. Phenotypes were then analyzed using a variety of molecular biology techniques.

RESULTS: RNA seq analysis of 4 myogenic basic helix-loop-helix proteins, MyoD, Myf5, Myogenin, and MRF4, evidenced exon skipping in MyoD and Myf5 in 34% and 38% of RMS specimens, respectively, while no exon skipping was identified in normal human muscle specimens. RT PCR confirmed the expression of an alternatively spliced form of MyoD where exon 2 is absent, MyoDδexon2, in JR-1 and Rh30 (rhabdomyosarcoma) cells. Retrovirus expression vectors with wild type MyoD and GFP or MyoDδexon2 and RFP were created and used to transduce mouse fibroblast and human rhabdomyosarcoma cells. Fibroblasts transduced with MyoDδexon2 demonstrated MyoDδexon2 expression by Western blot. Ectopic expression of wtMyoD induced morphologic changes and muscle gene expression by RT PCR in mouse fibroblast and Rh18 rhabdomyosarcoma cells. By using GFP and RFP tags, a co-culture assay for growth competition was able to demonstrate a selective growth advantage to cells ectopically expressing MyoDδexon2 over wtMyoD in 10T1/2 fibroblasts.

CONCLUSION: We can conclude through computational analysis of RNA Seq data and molecular biology evidence of alternative splicing/exon skipping in 2 of the 4 myogenic bHLH proteins in rhabdomyosarcoma. Functional analysis of the alternatively spliced form of MyoD, MyoDδexon2,

demonstrates expression of MyoDδexon2 in addition to a selective growth advantage to 10T1/2 fibroblasts ectopically expressing MyoDδexon2 in co-culture growth competitions. Ongoing investigations include functional assays to determine if MyoDδexon2 is a dominant negative mutation which would support its role as an oncogene in rhabdomyosarcoma development. Additionally, we are working to confirm our computation analysis results using direct human rhabdomyosarcoma samples from our institution.

DAY 2 | SESSION 3 | **POSTER PRESENTATION 9**

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Enhancing Prednisone Sensitivity of High-Risk B-Cell Acute Lymphoblastic Leukemia Through PI3Kδ Inhibition

BACKGROUND: Although treatment for children with B-cell acute lymphoblastic leukemia (B-ALL) is generally effective, patients with high-risk (HR) disease continue to have inferior outcomes. The prognosis is even worse for patients with positive minimum residual disease (MRD) after induction, the initial phase of chemotherapy. Immunotherapies have shown promise in refractory disease clearance but are followed by unacceptably high relapse rates (20-50%), leaving hundreds of patients with few effective treatment options. Glucocorticoids (GCs) are a key component of B-ALL chemotherapy regimens. Rapid response to GC monotherapy is good predictor of favorable outcomes, while slow response to GCs correlates with poor outcomes. We hypothesize that enhancing GC toxicity specifically in B-ALL cells during induction chemotherapy can reduce the number of patients who are end of induction MRD positive and therefore improve overall outcomes. In this work we target PI3Kδ, a component of the pro-survival B-cell receptor pathway that restrains GC signaling. PI3Kδ expression is restricted to lymphoid cells, making it an attractive target. We previously demonstrated synergy between dexamethasone (dex) and the FDA-approved isoform-selective PI3Kδ inhibitor idelalisib (idela) in B-ALL cell lines, patient specimens, and patient-derived xenograft models (Kruth et al Blood 2017). Since prednisone (pred) is a weaker GC than dex and is frequently used during induction therapy for patients with HR B-ALL, we now test the combination of pred and idela in HR B-ALL.

METHODS: To test the combination of idela and pred, peripheral blood or bone marrow of newly diagnosed B-ALL patients at the University of Iowa Stead Family Children's Hospital was obtained for Ficoll separation and isolation of B-ALL cells. Freshly isolated B-ALL cells were treated with serial dilutions of idela and pred for 72 hours before determining cell viability with PrestoBlue (Invitrogen) and identifying pred potentiation (SynergyFinder). To understand the mechanism, we measured changes in regulation of cell death genes using RNA-seq in NALM6 cells treated with GCs alone, idela alone, and the combination.

RESULTS: Idela enhances pred toxicity in almost all freshly isolated HR B-ALL patient specimens in cell viability assays, in some cases dramatically. This potentiation appears to occur through enhanced regulation of almost all cell death genes, including some not regulated by GCs alone.

CONCLUSION: Idela sensitizes B-ALL to pred in nearly all specimens tested through enhanced regulation of cell death genes. Based on this evidence, we propose adding idela to induction therapy for patients whose disease has inadequate clinical response in the first week of therapy but demonstrates this potentiation in *ex vivo* studies.

DAY 2 | SESSION 4 | **POSTER PRESENTATION 10**

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Epidemiology of Influenza Among Infants with Medically Attended Visits 2011-2019 in Southwestern Pennsylvania

BACKGROUND: Influenza affects more than 15 million infants worldwide causing nearly 400,000 severe cases and 10,000 deaths annually. In the United States, children less than 2 years of age have the highest pediatric hospitalization rate for influenza and account for 20-24% of pediatric influenza deaths. An immature immune response, lack of previous exposure to influenza or vaccine, and underlying conditions such as prematurity increase susceptibility to influenza infection. Understanding the epidemiology of influenza in this population is a first step to identify and optimize interventions that may reduce the burden of disease among these young children.

OBJECTIVE: We seek to describe the epidemiology of laboratory-confirmed influenza in infants 12 months of age or younger using a large retrospective cohort in southwestern Pennsylvania.

METHODS: This study utilized a retrospective cohort of infants 12 months of age or younger who were born from January 1, 2011 to December 31, 2019 within the University of Pittsburgh Medical Center (UPMC) health system who subsequently had two or more well-child encounters at UPMC between 0 to <15 months of age and could be linked to their mother's EHR. We then excluded infants who did not have at least one influenza rapid antigen, culture, or PCR test obtained from November 1 to May 31 of each included year in the context of a clinic, urgent care/emergency or hospitalization visit. Infants who tested positive for influenza on any influenza test were considered positive for influenza; otherwise they were considered negative. We then described population and influenza characteristics using proportions and medians. Infant exposure to influenza vaccine was validated using EHR data and the Pennsylvania Statewide Immunization Information System (PA-SIIS), which provides vaccine administration date, lot, and vaccine type.

RESULTS: We identified 2,160 infants who met inclusion criteria for this study. 296 (13.7%) infants had laboratory-confirmed influenza. 66.3% of infants with influenza were positive on rapid antigen testing, 26.6% on PCR, 4.1% on both and 3.4% on culture. The proportion of positive influenza tests ranged from 1.0% in the 2011-2012 influenza season to 28.1% in 2017-2018 influenza season. Influenza A predominated each season for a total of 209 (70.6%) cases over all seasons, except during the early 2019 season (data end December 2019) where influenza B accounted for 87.0% (40/46) of cases. The median age at influenza diagnosis was 7.2 months (range 2 weeks to 12 months). 102 (34.5%) infants were less

than 6 months of age at diagnosis. Among infants with influenza, 184 (62.2%) were not vaccinated prior to illness, 32 (10.8%) had one dose of vaccine, and 80 (27.0%) had two doses of vaccine.

CONCLUSION: Influenza accounts for a significant portion of disease among infants with medically attended visits who are tested for influenza. Although infants are predominately affected by Influenza A, the predominance of Influenza B in the 2019-2020 influenza season highlights the importance of continued influenza surveillance in this population. Infants less than 6 months of age who are not eligible for influenza vaccine remain vulnerable to infection. This supports the importance of future investigation of interventions that target this population including maternal influenza immunization and breastfeeding are warranted.

Flu Season (July 1-June 30)	# of infants tested for flu Nov 1-May 31	# positive	% Positive	# positive A/B
*-2011	10 (0.5)	1	10.0%	A=0 B=1
2011-2012	103 (4.8)	1	1.0%	A=1 B=0
2012-2013	223 (10.3)	28	12.6%	A=18 B=9 Unk A or B =1
2013-2014	130 (6.0)	10	7.7%	A=10 B=0
2014-2015	197 (9.1)	28	14.2%	A=23 B=5
2015-2016	103 (4.8)	6	5.8%	A=5 B=1
2016-2017	242 (11.2)	19	7.8%	A=12 B=7
2017-2018	424 (19.6)	68	28.1%	A=56 B=12
2018-2019	501 (23.2)	89	17.8%	A=77 B=12
2019*-	227 (10.5)	46	20.3%	A=6 B=39 A&B=1
Overall	2,160	296	13.7%	A=208 B=86 UNK A or B=1 A&B=1

	Not Immunized prior to illness	1 dose vaccine prior to illness	2 doses vaccine prior to illness	Total
Not Immunized prior to illness	184 (62%)	32 (10.8)	80 (27.0)	296
	0			

DAY 2 | SESSION 4 | **POSTER PRESENTATION 11**

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Raman Spectroscopy Reveals Distinct Tissue Biochemical Composition Associated with Eosinophilic Esophagitis

Objectives: Elucidating esophageal biochemical composition in eosinophilic esophagitis (EoE) can offer novel insights into its pathogenesis, which remains unclear. Using Raman spectroscopy, we profiled and compared the biochemical composition of esophageal samples obtained from children with active (aEoE), inactive EoE (iEoE), and non-EoE controls, and examined the relationship between spectral markers and validated EoE activity indices.

Methods: *In vitro* Raman spectra from aEoE (n=8; spectra=51), iEoE (n=6; spectra=48) and non-EoE controls (n=10; spectra=75) were acquired. Mann-Whitney test was used to assess the differences in their Raman intensities [median (IQR)] and identify spectral markers. Spearman's correlation was used to evaluate the relationship between spectral markers and endoscopic and histologic activity indices.

RESULTS: Raman peaks attributable to glycogen content (936/1449 cm^{-1}) was lower in aEoE [0.20 (0.18-0.21)] compared to non-EoE controls [0.24 (0.23-0.29)]. Raman intensity of proteins (1660/1209 cm^{-1}) was higher in aEoE compared to non-EoE controls [3.20 (3.07-3.50) vs. 2.91 (2.59-3.05)]; P=0.01), while that of lipids (1301/1260 cm^{-1}) was higher in iEoE [1.56 (1.49-1.63)] compared to aEoE [1.40 (1.30-1.48); P=0.02). Raman peaks attributable to glycogen and lipid inversely correlated with eosinophilic inflammation and basal zone hyperplasia. Raman mapping substantiated our findings.

CONCLUSIONS: This is the first study to identify spectral traits of the esophageal samples related to EoE activity and tissue pathology, and profile tissue-level biochemical composition associated with pediatric EoE. Future research to determine the role of these biochemical alterations in development and clinical course of EoE can advance our understanding of EoE pathobiology.

DAY 2 | SESSION 4| **POSTER PRESENTATION 12**

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RXRA DT448/9PP Generates a Dominant Active Variant Capable of Inducing Macrophage Maturation

BACKGROUND: Retinoid receptors (retinoic acid receptors (RARs) and retinoid X receptors (RXRs)) play important roles in myeloid maturation in both mice and humans. Retinoid treatments, both in vitro and in vivo, facilitate hematopoietic stem cell maturation and lineage commitment. RARA is well known as a fusion partner to PML in acute promyeloid leukemia. Transcriptome analysis shows that RAR and RXR are expressed across AML, most notably in myelomonocytic leukemia. Despite this, clinical application of retinoids in hematologic malignancies remains restricted to acute promyelocytic leukemia (APL, ATRA, a pan-RAR ligand) and cutaneous T-cell leukemia (CTCL, bexarotene, a pan-RXR ligand).

STUDY DESIGN: Using mouse RXR-KO MLL-AF9 leukemia cells (derived from Mx-Cre x Rxraflox/flox x Rxrbflox/flox bone marrow cells) and retroviral RXRA rescue, we found that RXRA acts as a tumor suppressor. We evaluated a series of RXRA variants in RXR-KO MLL-AF9 cells to determine which functional domains (DNA- and ligand-binding domains) are required for its tumor suppressor activity.

RESULTS: As expected, both the ligand- and DNA-binding domains are required for tumor suppression. We also identified a point mutation in RXRA, DT448/9PP, as a constitutively active variant capable of inducing macrophage maturation. When retrovirally expressed in RXR-KO MLL-AF9 leukemia cells, RXRA DT448/9PP induced adhesion to plastic, loss of colony-forming capacity, cell cycle exit, and macrophage cytomorphology. This maturation effect was also seen in human myelomonocytic leukemia cell lines. This phenotype has not been reported with other RXRA variants and is distinct from the response to retinoid treatments. In reporter assays, RXRA DT448/9PP was constitutively active with ligand-independent transcriptional activity, increased coactivator binding, and reduced co-repressor binding. We are now comparing the molecular response of MLL-AF9 leukemia cells to ligand-independent RXRA DT448/9PP and ligand-treated wildtype RXRA by RNAseq and using proximity-dependent biotin labeling to determine the protein-protein interactome of wildtype and DT448/9PP RXRA.

CONCLUSIONS: Additional clinical potential for retinoids as therapeutic agents likely exists. RXRA DT448/9PP leads to myeloid maturation that has not been seen with exogenous retinoid ligand treatments. We hope that by identifying the regulatory pathways triggered by the RXRA DT448/9PP variant, we may be able to further optimize therapeutic retinoids in non-APL AML.

Presenting Author Index