

blocking complement-dependent injury thereby reducing risk of lung transplant rejection. **OBJECTIVES/GOALS:** Our goal was to determine if pre-existing autoimmune autoantibodies, such as those resulting from cigarette smoke (CS), contribute to graft rejection in lung transplantation (LTx) and if autoreactive-mediated graft injury is complement-dependent. **METHODS/STUDY POPULATION:** For in vivo experiments, we utilized our emphysema mouse model. Briefly, eight-week-old C57BL/6J mice are exposed to 3R4F reference cigarette smoke 5 hours per day, 5 days a week for 6 months. Upon completion, cigarette smoked (CS) mice and control (NS) mice received syngeneic orthotopic left-lung transplant from age-matched C57BL/6J donors. To determine if pre-existing autoreactivity mediated graft injury was complement-dependent we treated CS-LTx mice with a novel, bifunctional complement inhibitor. Autoantibody levels were measured by ELISA and lung injury was assessed by blinded histopathological analyses. Complement inhibition was verified by immunofluorescence. **RESULTS/ANTICIPATED RESULTS:** We found that CS-exposure leads to production of autoreactive antibodies towards extracellular matrix (ECM) components and contributes to graft injury. Interestingly, LTx into CS exposed mice further increased de-novo ECM autoantibody development. Lastly, treatment with our novel, bifunctional complement inhibitor blocked autoantibody spreading and significantly reduced graft rejection. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** These data demonstrate that smoking induces pre-LTx autoreactivity to ECM proteins that promotes graft injury following LTx. Furthermore, complement inhibition reduces autoantibody production and protects the graft from injury.

89723

CD105-targeted CAR T cells for the treatment of acute myeloid leukemia*

Konstantinos Lontos, Yiyang Wang, Andrew Frisch, Mason Colbert, Jason Lohmueller and Greg M. Delgoffe

¹University of Pittsburgh and ²Tsinghua University

ABSTRACT IMPACT: Our work might lead to a new treatment for patients with acute myeloid leukemia **OBJECTIVES/GOALS:** Acute myeloid leukemia (AML) is a devastating hematologic malignancy, with dismal 5-year survival. Chimeric antigen receptor (CAR) T cells have been approved for B cell malignancies but not for AML. The goal of this study is to explore the safety and efficacy of CAR T cells targeting CD105 (endoglin) to treat AML. **METHODS/STUDY POPULATION:** We have constructed human and murine CAR T cells targeting CD105. The CARs were created by sequencing the V(D)J regions of hybridomas and designing single chain variable fragments that target CD105 which were subsequently introduced in a CAR backbone via Gibson assembly. The CAR T cells were produced via transduction using retrovirus or lentivirus. Leukemia cell lines were assessed for CD105 expression with flow cytometry. Killing assays were performed via measurement of luminescence of target cells after co-culture with CAR T cells. Activation assays were performed with co-culture of CAR T cells and target cells and measurement of activation markers with flow cytometry. To assess in vivo efficacy and safety, murine CAR T cells were infused into C57BL/6J mice carrying B16 melanoma after lymphodepletion. **RESULTS/ANTICIPATED RESULTS:** All human leukemia cell lines assessed (Nalm6, MOLM-14, MV4-11, Kasumi-1, THP-1) expressed some degree of endoglin apart from the T cell leukemia Jurkat. Human CD105 CAR T cells were activated by co-culture with leukemia cell lines and effectively killed leukemia cells in vitro in a

CD105-specific manner. Murine CAR T cells killed efficiently both murine solid tumors (B16 melanoma) and murine leukemias (C1498) in vitro. Murine CAR T cells did not exhibit any toxicity when infused after low-dose lymphodepletion (cyclophosphamide 100mg/kg) but caused significant morbidity after higher doses (cyclophosphamide 200mg/kg). Murine CAR T cells delayed the growth of B16 melanoma in immunocompetent mice. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** We have constructed human and murine CD105 CAR T cells with excellent activity in vitro. The activity of human CD105 CAR T cells in xenografts and the biologic relevance of the toxicity of murine CD105 CAR T cells in humans needs to be further investigated. CD105 CAR T cells might prove an important therapeutic option for patients with AML.

91074

Identification of monoclonal antibodies with broad reactivity against the malaria parasite variant surface antigen responsible for severe malaria[†]

Raphael Reyes¹, Louise Turner², Isaac Ssewanyana³, John Rek³, Bryan Greenhouse⁴, Sebastiaan Bol¹, Thomas Lavstsen² and Evelien M. Bunnik¹

¹Department of Microbiology, Immunology and Molecular Genetics, The University of Texas Health Science Center, San Antonio, TX, USA, ²Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, ³Infectious Disease Research Collaboration, Kampala, Uganda and ⁴Department of Medicine, University of California San Francisco, San Francisco, CA, USA

ABSTRACT IMPACT: This study aims to provide insight into naturally acquired immunity against severe malaria, thereby laying the foundation for the design of novel vaccine candidates to prevent severe disease as well as monoclonal antibody therapies to treat severe malaria. **OBJECTIVES/GOALS:** Severe malaria is caused by parasite surface antigens that contain high sequence diversity. Nevertheless, *P. falciparum*-exposed individuals develop antibody responses against these antigens. Our goal is to isolate antibodies with broad reactivity to understand how disease protection is acquired. **METHODS/STUDY POPULATION:** Our study cohort consists of Ugandan adults living in a malaria-endemic region with high transmission intensity, who are protected against severe malaria. Using fluorescently labeled probes of parasite surface antigens, we have isolated antigen-specific B cells from these donors. We then expressed the corresponding monoclonal antibodies in vitro. These antibodies were screened against a library of variant surface antigens to determine antibody breadth and potential to inhibit interaction of the parasite surface antigen with host receptors, a critical step in pathogenesis. Additionally, using a panel of variant surface antigen mutants, we have predicted the epitopes targeted by the broadest monoclonal antibodies. **RESULTS/ANTICIPATED RESULTS:** We have identified three monoclonal antibodies with exceptionally broad reactivity and inhibitory activity against our panel of severe disease-inducing variant surface antigens. We have identified two major sites targeted by these broadly reactive antibodies. The first site was associated with the largest breadth, but limited inhibitory potential, while the second site showed high-affinity antibody binding and inhibition of receptor binding. Interestingly, two of these three antibodies were very similar in structure, even though they were isolated from different donors. Isolation of antigen-specific B cells from additional donors will enable us to identify how common such broadly reactive antibodies are and allow the identification of additional epitopes **DISCUSSION/SIGNIFICANCE OF**

FINDINGS: This study is the first to isolate broadly reactive antibodies that are likely to protect against severe malaria in naturally immune individuals. Further characterization of antibody-antigen interactions will inform the development of this surface antigen as a vaccine candidate for malaria.

99529

The Role of ATF6-Mediated Signaling in PARP Inhibitor Resistant Ovarian Cancer

Alexandra and Benjamin Bitler

University of Colorado Anschutz Medical Campus

ABSTRACT IMPACT: This work has the potential to identify targetable pathways conveying resistance to PARP inhibitors that may improve ovarian cancer patient outcomes. **OBJECTIVES/GOALS:** High grade serous ovarian cancer is the deadliest gynecologic malignancy. PARP inhibitors are an FDA approved targeted therapy that is being used more and more frequently in the clinic. It is vital to understand mechanisms driving resistance to this therapy in order to develop treatments to improve patient responses. **METHODS/STUDY POPULATION:** RNA-sequencing and transcription factor analysis was used to identify pathways of interest. An AP-1 transcriptional reporter assays was used to confirm results of the transcription factor analysis. An unbiased lentiviral shRNA screen was used to identify AP-1 subunits promoting PARP inhibitor resistance. Lentiviral transduction allowed for the knockdown ATF6. Comet assays and two-plasmid systems were used to determine levels of DNA damage and levels of DNA damage repair respectively. **RESULTS/ANTICIPATED RESULTS:** PARP inhibitor resistant cell lines have increased WNT signaling which promotes to increased DNA damage repair. PARP inhibitor resistant cell lines also have increased AP-1 transcriptional activity, ATF6 expression, and active p38. ATF6 knockdown and p38 inhibition is sufficient to resensitize cells to PARP inhibition. Upon treatment with PARP inhibitors, ATF6 knockdown as well as p38 inhibition lead to increased DNA damage in PARP inhibitor resistant cell lines. RNA-sequencing reveals a significant overlap in downregulated genes in cells treated with a β -catenin inhibitor and cells with an ATF6 knockdown. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** Due to the increasing prevalence of PARP inhibitors in the clinic, it is vital to uncover mechanisms contributing to resistance. This work has the potential to identify targetable pathways conveying resistance to PARP inhibitors that may improve ovarian cancer patient outcomes.

Precision Medicine

34646

Deficiency of Novel Adipokine Tetranectin Increases Obesity and Insulin resistance in Females

George Plasko¹, Sijia He¹, Jingjing Zhang², Fen Liu², Juli Bai¹, Lily Dong¹ and Feng Liu¹¹UTHSA and ²Central South University, Changsha, China

ABSTRACT IMPACT: Novel adipokines like tetranectin help explain why some people progress from obesity to diseases like diabetes, atherosclerosis, and dislipidemia **OBJECTIVES/GOALS:** Obesity has an established association with diabetes, dyslipidemia, and atherosclerosis.

Preventing progression from obesity to insulin resistance requires understanding of the regulatory mechanisms involved in the loss of insulin sensitivity. Adipose tissue is well known to function as an endocrine organ that produces many kinds of adipokines. **METHODS/STUDY POPULATION:** Blood sample analysis from human patients and mice was used to determine associations between tetranectin and obesity. Samples were tested with a monoclonal anti-tetranectin antibody for detection with western blot. A tetranectin mutant knock out mouse line was compared to wild type littermates on high fat diet for 4 months. Insulin tolerance tests and glucose tolerance were used to determine progression to insulin resistance and glucose intolerance. Histological analysis of metabolic tissue was used to demonstrate adipocyte hypertrophy and liver steatosis. **RESULTS/ANTICIPATED RESULTS:** In the current study, we report the identification and initial characterization of a novel adipokine tetranectin. Tetranectin, which is coded by the C-type lectin domain family 3 member B (CLEC3B) gene, is ubiquitously expressed in various mouse tissues, whereas it is highly enriched in white adipose tissue. We found that the serum level of tetranectin was much higher in both obese and diabetic patients. Knocking out the tetranectin gene in mice protected against glucose intolerance in males but reduced insulin and glucose tolerance in females, without effects on food intake and body weight for either sex. Mechanistically, tetranectin targets liver tissues and its deficiency increases lipid accumulation in hepatocytes in females. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** We have identified a novel adipokine which mediates a different metabolic crosstalk among tissues to maintain systemic glucose and lipid metabolism in different genders. Further investigation of tetranectin's function could yield a new target for precise therapeutic treatment for obesity and its associated metabolic diseases in different genders

73565

Defining tp53 tumor suppressor functions in zebrafish embryonal rhabdomyosarcoma*

Kunal Baxi, *Jiangfei Chen, Amanda Lipsitt, Nicole Hensch, Long Wang, Abhik Bandopadhyay, Aaron Sugalski, Andrea Gilbert, Eleanor Chen, Peter Houghton, Gail Tomlinson and Myron Ignatius
University of Texas Health Science Center at San Antonio

ABSTRACT IMPACT: By assessing function of mutant (patient-specific) tp53 in zebrafish embryonal rhabdomyosarcoma will inform clinicians of the severity of mutant tp53 alleles. **OBJECTIVES/GOALS:** This study aims to define loss- and gain-of-function TP53 mutations by comparing effects in tp53-null and wild-type tumors. In addition, it aims to generate a rapid in vivo analysis platform to assign function to patient specific TP53 mutations in the clinic **METHODS/STUDY POPULATION:** To define tp53 function in ERMS pathogenesis, we previously generated a new tp53-null mutant (tp53^{-/-}) in zebrafish by deleting the entire tp53 genomic locus using TALEN mutagenesis. tp53^{-/-} zebrafish spontaneously develop a spectrum of tumors including sarcomas, leukemia and germ cell tumors (Ignatius . . . Baxi et. al., eLife) reminiscent of tumors observed in Trp53-null mice. Using the tp53^{-/-} mutants to generate kRASG12D-induced ERMS, we discovered that tp53 is a potent repressor of metastases but rather surprisingly had no effect on self-renewal (Ignatius . . . Baxi et. al., eLife). Here, using tp53^{-/-} zebrafish, we assessed effects of wild-type and mutant (patient specific) tp53 on tumor initiation, proliferation and apoptosis. **RESULTS/ANTICIPATED RESULTS:** ERMS tumor initiation in the tp53^{-/-} background is observed in > 97% of animals whereas only <40% of wild-type animals develop ERMS. Additionally, tp53 is a potent suppressor of ERMS proliferation and its effect on apoptosis is minor.