

**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

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**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  $\beta$ -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

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**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\*

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**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<sup>-/-</sup>) in zebrafish by deleting the entire tp53 genomic locus using TALEN mutagenesis. tp53<sup>-/-</sup> 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<sup>-/-</sup> 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<sup>-/-</sup> 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<sup>-/-</sup> 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.

Next, we expressed either WT zebrafish or human TP53 in tp53<sup>-/-</sup> animals along with kRASG12D and both genes suppressed tumor initiation and growth. We co-expressed TP53C176F (found in two ERMS patients) and TP53P153del (identified in a patient with osteosarcoma in our clinic) in zebrafish ERMS, and find that the TP53C176F allele significantly suppressed tumor initiation with effects predominantly on enhanced apoptosis. However, the TP53P153del allele initiated tumors at similar frequency compared to tp53<sup>-/-</sup> animals but increased the initiation of tumors in the head musculature. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** Different TP53 alleles identified in patient tumors have very different effects on tumorigenesis in vivo and can respond differently to potentially therapeutic compounds. Thus, the type of precision modeling demonstrated here promises to help further define patient-specific TP53 biology and improve clinical strategies in the future.

92418

### **Molecular imaging of the tumor microenvironment to predict response to combination treatment with immunotherapy in triple negative breast cancer**

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**ABSTRACT IMPACT:** Insights from this project will provide clinical guidance in treatment of immunotherapy in triple negative breast cancer and identify early imaging biomarkers of treatment response. **OBJECTIVES/GOALS:** Significant research that addresses monitoring and predicting patient response of triple negative breast cancer (TNBC) to immunotherapy is needed. Using positron emission tomography (PET) imaging to probe the tumor microenvironment (hypoxia, T-cell activation), we aim to predict early response to immunotherapy for in mouse models of TNBC tumors. **METHODS/STUDY POPULATION:** Female Balb/c mice with 4T1-luciferase mammary carcinoma cell tumors were administered paclitaxel (PTX; 10 mg/kg), anti-PD1 (200 µg), both, or vehicle (saline) intraperitoneally. Treatment was given on days 0, 2, and 5 for cohort 1 (n=16) who underwent granzyme B specific (GZP) PET imaging (T-cell activation) and days 0, 2, 5, and 8 for cohort 2 (n=12) who underwent [18F]-fluoromisonidazole (FMISO)-PET imaging (hypoxia). Bioluminescence (BLI) imaging and caliper measurements were performed to track tumor size changes at multiple timepoints and tumors were collected for histological validation on day 20. Mean standard uptake value (SUV<sub>mean</sub>) was calculated as percent of day 0, and statistical analyses were performed with unpaired t-tests and Wilcoxon-rank sum tests. **RESULTS/ANTICIPATED RESULTS:** Non-responders to treatment had a significantly higher tumor volume compared to responders starting on day 6 (p<0.05). Although no significant differences in BLI between control and single-agent therapies were found, BLI data revealed that treatment with combination PTX and anti-PD1 significantly decreased viability signal between days 3 and 6 (p=0.04). SUV<sub>mean</sub> from GZP-PET was over 250% higher in responders compared to non-responders by day 6 (p=0.03). SUV<sub>mean</sub> from FMISO-PET was 80% less in responders compared to non-responders, indicating less tumor hypoxia (p=0.04). **DISCUSSION/SIGNIFICANCE OF FINDINGS:** Non-invasive PET imaging of the tumor microenvironment can provide data on T cell activation and hypoxic response predicting response to combination immunotherapy and chemotherapy. Utilizing advanced imaging to understand biologically distinct features of the TNBC tumor microenvironment can aid in personalizing anti-cancer therapies.

### **Team Science**

84357

### **A TL1 Team Approach to Integrating Mathematical and Biological Models to Target Myeloid-Derived Immune Cells in Glioblastoma**

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**ABSTRACT IMPACT:** Predicting therapeutic responses in GBM. **OBJECTIVES/GOALS:** The goal of this team approach is to integrate mathematical models of glioblastoma (GBM) infiltrating myeloid cells that contribute to the immunosuppressive phenotype in glioma with experimental data to predict therapeutic responses to combined chemokine receptor and immune checkpoint blockade. **METHODS/STUDY POPULATION:** Orthotopic murine KRI58-luc gliomas were established in fluorescent reporter CCR2WT/RFP CX3CR1WT/GFP mice. Subsequently, an anti-CD31 injection was administered to label the vasculature. Fluorescent imaging and quantification of anti-CD3 stained sections were performed on a range of tumor sizes to acquire vasculature, tumor, T cell, and myeloid cell densities. In parallel, a system of ordinary differential equations was formulated based on biological assumptions to evaluate the change over time of tumor cells, T cells, and infiltrating myeloid cells. The model was then refined and validated by experimental results. **RESULTS/ANTICIPATED RESULTS:** Fluorescent imaging and quantification revealed a correlation between tumor size and abundance of (CX3CR1+, CCR2-) and (CX3CR1+, CCR2+) myeloid cell populations in the tumor microenvironment. The density of these cell populations and vasculature remained constant as the tumors increased in size. Computer simulations of the mathematical model will predict tumor, myeloid, and T cell dynamics. These simulations will be particularly useful to uncover information regarding myeloid cell dynamics, such as cell entry time into the tumor microenvironment. Parameter sensitivity analysis of the model will inform us of the biological processes driving these tumor-immune cell dynamics. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** GBM is a challenge as current intervention are ineffective. This study improves the understanding of glioma infiltrating myeloid cells and their impact on tumor progression. The data will serve as a basis for quantitatively predicting therapeutic responses of a novel combination treatment.

### **Translational Science, Policy, & Health Outcomes Science**

11791

### **Gray matter volume differences in bilingual compared to monolingual children**

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**ABSTRACT IMPACT:** This study examines gray matter volume differences resulting from the bilingual experience in children and