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Line1 Derepression in Specific Retrotransposon Families in Aged Mice Leads to Cytosolic DNA and Increased Inflammation

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OBJECTIVES/GOALS: The major objective of this project is two points. First, is to repeat and confirm previous observations that there is elevated cytosolic Line1 DNA in the cytoplasm of cells derived from old mice compared to young. Second is to identify which Line1s in the genome are contributing to this free DNA and test if targeting them rescues the age-related phenotype. **METHODS/STUDY POPULATION:** This project will focus on data collected from both tissues and primary cells derived from multiple tissues. Using cellular/tissue fractionation kits, we isolate specifically from the cytoplasm. This specificity is confirmed by western blotting. Measurement of the Line1 levels is measured by quantitative PCR. Subsequently, these cytoplasmic samples are sent off for sequencing in order to quantify the length of the free DNA in the cytoplasm and to identify which Line1 genomic families the cytosolic DNA originates. Additionally, FISH is utilized to visualize Line1 DNA in the cytoplasm of aged versus young cells **RESULTS/ANTICIPATED RESULTS:** We anticipate this research to confirm the hypothesis that extranuclear Line1 DNA accumulates with age in both tissues and primary fibroblasts. Additionally, we expect to be able to determine which specific families of genomic Line1 is driving this extranuclear DNA, which would suggest the active retrotransposable elements that are directly involved in this aging related phenotype. Assuming successful identification of such families, we can then target and silence these specific elements to determine not only if cytoplasmic Line1 in aged mice decreases, but additionally if the healthspan and/or lifespan of these mice improves **DISCUSSION/SIGNIFICANCE:** Derepressed Line1s have been shown to be involved in detrimental phenotypes, including autoimmune disease, cancer, and inflammaging. Targeting retrotransposons, either directly through degradation of transcriptional product of LINE1s or indirectly by improving function of regulators, will be crucial in ablating aging phenotypes

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LRP1 as a modulator of hippocampal neurogenesis and neurodegeneration*

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OBJECTIVES/GOALS: This project aims to elucidate the mechanism by which LRP1 governs hippocampal neurogenesis with a particular focus on its relevance in brain aging and memory loss. We are specifically interested in further discerning the intricacies of a novel relationship we have discovered between LRP1 and CXCR4 in adult neural stem cells. **METHODS/STUDY POPULATION:** For the in vivo studies, we are using a triple-transgenic mouse model in which we knockout LRP1 in adult neural stem cells. This is accomplished using a nestin-driven Cre-ER system in animals with floxed LRP1 and a floxed stop codon preceding a td-tomato reporter. The reporter allows for visualization of cells with the knockout and for trafficking and differentiation assays to be easily accomplished. We study stroke recovery using the middle cerebral artery occlusion model and brain

aging by inducing the knockout and allowing the mice to age. We perform behavioral batteries and histological analysis on these mice to elucidate functional changes in neurogenesis. We also incorporate in vitro studies using primary neural stem cell cultures to mechanistically test the role of LRP1 in neural stem cell function. **RESULTS/ANTICIPATED RESULTS:** We have discovered that neural stem cell LRP1 knockout caused a 10-fold loss of CXCR4 expression in conjunction with deficits in ischemia-stimulated migration from the subventricular zone. We also found that uninjured aged mice lacking neural stem cell LRP1 displayed spatial memory deficits at 9 months of age (6 months after knockout), suggesting dysregulated hippocampal function. Given this, we hypothesize that LRP1 regulates CXCR4 in the subgranular zone NSCs to enhance hippocampal memory function. Ongoing research is testing our hypothesis via hippocampal functional tests and in vitro trafficking/expression assays. We expect our research to elucidate a previously unknown link between three independently identified effectors of neurodegenerative disease: LRP1, CXCR4, and neurogenesis. **DISCUSSION/SIGNIFICANCE:** The role of LRP1 in Alzheimers disease has long eluded clarity despite its known role in trafficking many major disease players – ApoE, amyloid beta and tau. Elucidating its role in hippocampal neurogenesis, a potential disease-modifying process, could lead to novel therapeutic approaches in diseases that cause the death of 1/3 of senior citizens.

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Activation of the Glucagon-Like Peptide-1 Pathway in Obese Pre-Diabetic Individuals Improves Endothelial Function Independently of Weight Loss

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OBJECTIVES/GOALS: We aimed to determine if GLP-1 receptor agonists exert beneficial effects on surrogate measures of cardiovascular function independently of weight loss. Our objective was to compare the outcomes between GLP-1 receptor agonist treatment versus a similar drug without cardiovascular benefit versus weight loss through diet alone. **METHODS/STUDY POPULATION:** We enrolled 88 individuals with obesity (BMI \geq 30kg/m²) and pre-diabetes and randomized them in a 2:1:1 ratio to 14 weeks of the GLP-1 receptor agonist liraglutide, the dipeptidyl peptidase-4 inhibitor sitagliptin, or hypocaloric diet. Sitagliptin blocks degradation of endogenous GLP-1 but does not cause weight loss or lower adverse cardiovascular outcomes. Treatment was double-blinded and placebo-controlled for drug, and unblinded for diet. Primary endpoints were flow-mediated dilation (FMD) to assess endothelial vasodilatory function, and plasminogen activator inhibitor-1 (PAI-1) to assess endothelial fibrinolytic function. We used a general linear model for each outcome and included gender as a covariate for FMD. Baseline characteristics were similar. Mean age was 50, with 32% men and 13% black. **RESULTS/ANTICIPATED RESULTS:** At 14 weeks, diet and liraglutide caused weight loss (diet -4.3 ± 3.2 kg, $P < 0.01$; liraglutide -2.7 ± 3.2 , $P < 0.01$), while sitagliptin did not (-0.7 ± 2.0 , $P = 0.17$). Diet did not improve FMD at 14 weeks

compared to baseline (+0.9%, 95% CI [-1.5, 3.3], $P=0.46$). FMD tended to increase after liraglutide and sitagliptin but was not significant (liraglutide +1.2 [-0.3, 2.8], $P=0.12$; sitagliptin +1.6 [-0.6, 3.8], $P=0.15$). Given that liraglutide and sitagliptin work through the same GLP-1 pathway, we combined the liraglutide and sitagliptin groups for overall effect on FMD, which was significantly improved from baseline (+1.4 [0.1, 2.8], $P=0.04$). Diet and liraglutide improved PAI-1

at 14 weeks (diet -4.4U/mL, [-8.5, -0.2], $P=0.04$; liraglutide -3.4 [-6.0, -0.7], $P=0.01$), while sitagliptin did not (-1.4 [-5.1, 2.3], $P=0.46$). DISCUSSION/SIGNIFICANCE: Activation of the GLP-1 pathway by liraglutide or sitagliptin improves FMD independent of weight loss, while PAI-1 improvement is weight-loss dependent and is only seen after liraglutide or diet. Our study suggests the cardiovascular benefit of liraglutide may be due to combined improvements in endothelial vasodilatory and fibrinolytic function.

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Comparison of Statin Use to Non-Use on Cerebral Blood Flow Velocity in Older Adults at Risk for Alzheimers Disease: Data from a Phase II Multisite Clinical Trial

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OBJECTIVES/GOALS: Reduced cerebral blood flow (CBF) along with vascular risk factors (e.g., dyslipidemia) are prevalent in Alzheimers disease (AD) and related dementias. Statins are one of the most effective pharmacologic treatments for vascular risk reduction, which may contribute to CBF in individuals with an increased risk for AD. METHODS/STUDY POPULATION: Cross-sectional analysis of 212 older adults with a family history of dementia. Heart rate via electrocardiogram, mean arterial pressure (MAP) via brachial sphygmomanometers, end-tidal CO₂ via capnograph, and CBF velocity at the middle cerebral artery (MCAv) via transcranial Doppler ultrasound were collected following 20-minutes of supine rest. Mean MCAv (cm/s) was measured within each cardiac cycle and averaged over an 8-minute duration. Cerebrovascular conductance was calculated by dividing mean MCAv by MAP. Pulsatility Index was calculated by subtracting systolic MCAv from diastolic MCAv and then dividing by mean MCAv. RESULTS/ANTICIPATED RESULTS: 125 females (68 $\hat{A}\pm$ 6 years; 49 statin) and 87 males (70 $\hat{A}\pm$ 6 years; 47 statin) were included in analyses. There were no significant differences between heart rate, MAP, or end-tidal CO₂ between statin and non-statin users. After controlling for age, sex, and low-density and high-density lipoprotein, statin use did not significantly contribute to MCAv ($p = 0.09$). However, statin use did significantly contribute to cerebrovascular conductance (MCAv/MAP; $p = 0.03$) as well as Pulsatility Index (assessment of cerebral health, $p < 0.01$). DISCUSSION/SIGNIFICANCE: Our findings suggest statin use significantly and positively contributes to resting cerebral blood flow velocity and cerebrovascular health. Further investigation is warranted into statin interventions with other components of cerebrovascular function, as differences may have implications for brain health and disease pathogenesis.

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Specific cephalosporin antibiotics deplete tumor PD-L1 to inhibit DNA damage sensing and sensitize to Chk1 inhibitors in vivo*

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OBJECTIVES/GOALS: Tumor PDL1 signals to immune cells for tumor immune evasion but has cell-intrinsic signals that promote tumor virulence. We identify novel tumor PDL1 depleting drugs (PDDs) to interrupt tumor-intrinsic PDL1 signals and sensitize tumors to targeted therapy in vitro and in vivo. METHODS/STUDY POPULATION: We screened the Prestwick and LOPAC libraries for FDA-approved drugs reducing B16 melanoma PDL1 > 2.6-fold. β -lactam antibiotics were used at 80 μ M and Chk1 inhibitor rabusertib as indicated in T24 human bladder cancer, and murine ID8agg ovarian cancer, 4T1 breast cancer and B16. Genetic PDL1 KO was by CRISPR or shRNA and re-expression by lentivirus. Viability was by MTT and protein by immunoblot. We challenged 5 NSG mice/group with 2x10⁶ T24 (SQ) cells and 5 BALB/c mice/group with 5x10⁵ 4T1 cells (mammary fat pad) and treated with cefepime (200 mg/kg), rabusertib (2.5 mg/kg), vehicle, or combo daily from day 3. RESULTS/ANTICIPATED RESULTS: Structurally-related β -lactam antibiotics cefepime and ceftazidime are tumor PDDs. Cefepime or ceftazidime reduced tumor PD-L1 and thus its cell-intrinsic signals to deplete the DNA damage sensing Chk2 protein and promote rabusertib synthetic lethality in vitro and in vivo in a tumor PDL1-dependent manner independent of immunity. Structurally distinct β -lactam antibiotics did not sensitize tumor cells to rabusertib, suggesting β -lactam antimicrobial functions did not promote PDL1 depletion or rabusertib treatment effects in vivo. Although rabusertib effects were immune-independent, both PDDs induced immunogenic tumor STING signaling, suggesting they can improve tumor immunotherapy. DISCUSSION/SIGNIFICANCE: We show a rapidly translatable way to deplete detrimental tumor-intrinsic PDL1 signals, and sensitize tumors to rabusertib. We are testing PDD structure activity relationships to improve PDD effects and testing PDD effects on other treatments, e.g., PARP inhibitors, immunotherapy.

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Considerations Mid-Translation of a Novel Extracellular Vesicle Product in Myocarditis

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OBJECTIVES/GOALS: Myocarditis is an inflammatory cardiomyopathy commonly caused by viral infections. The residual burden of this disease after guideline-based therapies is substantial, as there are no pathway-specific therapies. Our long-term goal is to find and translate treatments that reduce acute myocarditis severity and prevent progression of disease. METHODS/STUDY POPULATION: Of available therapies, extracellular vesicles (EVs) are ideally suited to the task of simultaneous, specific reprogramming of multiple