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 Â± 6 years; 49 statin) and 87 males (70 Â± 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. ?-lactam antibiotics were used at 80 ?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 ?-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 ?-lactam antibiotics did not sensitize tumor cells to rabusertib, suggesting ?-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

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pathogenesis mechanisms necessary in diseases like myocarditis, without similar immunosuppressive risks as their pharmacologic counterparts. We obtained plasma from healthy men and women and isolated nanoparticles, which were analyzed for physiochemical markers of human EVs. After confirming presence of EVs, we injected these plasma-derived extracellular vesicles (PEV) into male BALB/c mice vs. PBS control intraperitoneally on days -1, 0, 1 of viral infection (day 0) in a highly translational, mouse model of myocarditis. Hearts were examined at day 10 at the peak of acute myocarditis, using standard histological and cell composition analyses. RESULTS/ANTICIPATED RESULTS: Mice treated with PEV had significantly less myocardial inflammation both histologically and by gene expression of immune markers in the heart. The immunoregulation by PEV treatment decreased many key components of innate immune response networks that are known to be upregulated during acute myocarditis: TLR4+ mast cells and macrophages and complement. These pathways drive the profibrotic gene and protein changes that lead to remodeling, fibrosis, and disease progression observed in patients. We anticipate that when we analyze later timepoints in this model (day 35 post infection) which are normally associated with development of chronic myocarditis, dilated cardiomyopathy, we will see reduction of this fibrosis and of damage-associated changes. DISCUSSION/SIGNIFICANCE: These data suggest that EVs from plasma may be a novel treatment for viral myocarditis, but translation of EVs is hindered by their heterogeneity. We demonstrate characterization both physiochemically and functionally in a well-defined model. Such practices are necessary as these contemporary products challenge current regulation standards.

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## Long non-coding RNA WNT5A-AS1 shows sex-dimorphic differences in survival for males with glioblastoma

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OBJECTIVES/GOALS: The goal of this study is to evaluate the role of WNT5A and WNT5a-AS1 in sex-differences of GBM progression. In our preliminary studies, we found that a long non-coding RNA WNT5A-AS1 is overexpressed in male GBM patients. We also found that WNT5A-AS1s expression shows a negative correlation with overall survival within male patients. METHODS/STUDY POPULATION: We will define the mechanism by which WNT5A-AS1 regulates WNT5a-mediated glioma stem cell (GSC) maintenance by assessing the effects of inhibiting WNT5A-AS1 expression on transcriptional activity and stemness in GSCs. We will determine if there are distinct Wnt-signaling patterns in male and female isogenic murine astrocytes by examining the expression of downstream proteins in the Wnt signaling pathway and how inhibition of WNT5A-AS1 alters this expression. We will then examine the impact of WNT5A-AS1 on temozolomide (TMZ) resistance in-vitro and in-vivo. We will assess the cell viability and survival of GBM PDX cells upon treatment with TMZ in vitro. Next, we will assess the capacity of knockdown of WNT5A-AS1 to increase sensitivity to TMZ-induced cell death and prolong survival in vivo in intracranial models. RESULTS/ANTICIPATED RESULTS: We hypothesize that WNT5A-AS1 targets Wnt5a and regulates its expression. We anticipate that knockdown of WNT5A-AS1 will upregulate WNT5A expression. We also expect that inhibiting WNT5A-AS1 will alter GSC stem maintenance and functional effects. We expect to see an increase in downstream Wnt5a signaling proteins in males vs females when treated with exogenous Wnt5a. We hypothesize that knockdown of both, WNT5A-AS1 and WNT5A will alter the expression of downstream proteins. We hypothesize that knockdown of WNT5A-AS1 will decrease tumor growth and therapeutic resistance to TMZ while increasing survival in patient derived xenographs in vivo and in vitro. DISCUSSION/SIGNIFICANCE: This study will provide insight into the mechanisms underlying the difference in GBM onset and progression between male and female patients, which is clinically important. We will also characterize the biological role WNT5A-AS1 which is currently unknown to date and elucidate differential role of GSCs in GBM progression between male and female.

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## Elevated Phosphate Levels Induce Lung Inflammation and Exacerbate Pulmonary Fibrosis*

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OBJECTIVES/GOALS: Using a cell culture model, we will determine the effects of phosphate on primary lung cell cultures and use our results to delineate a pathway through which these changes are carried out. Using animal models, we will determine the effects of phosphate on inflammatory and fibrotic lung injury, both in the presence and absence of CKD. METHODS/STUDY POPULATION: For our in vitro experiments, human lung fibroblasts were treated with concentrations of 1 to 5 mM sodium phosphate and FGFR inhibitors. Expression levels of interleukin (IL)-1beta, IL-6, and IL-8 were analyzed by qPCR and secretion of these cytokines was measured by ELISA. Phosphorylation of PLCy and ERK was measured by western blot. Using an in vivo approach, we placed C57Bl/6 mice on a high phosphate (3%) diet to elevate serum phosphate levels in the absence of kidney injury and administered bleomycin via oropharyngeal aspiration to generate an acute inflammatory response. Serum FGF23 levels were measured by ELISA and serum analysis for phosphate and renal function were obtained. Furthermore, expression of FGF23 pathway and inflammatory markers were analyzed in murine lung tissue using qPCR and western blotting. RESULTS/ANTICIPATED RESULTS: Augmented phosphate concentrations led to increased cytokine expression and secretion from human lung fibroblasts as well as a concomitant increase in PLCy and ERK phosphorylation. Inhibition of FGFR1 reversed the effects of phosphate on the inflammatory cytokines and PLCy/ERK phosphorylation. Serum FGF23 levels were significantly upregulated in mice on a high phosphate diet and further increased in mice subjected to a high phosphate diet with exposure to bleomycin. Both serum phosphate and creatinine levels were significantly elevated as well. Additionally, high phosphate and bleomycin increased local FGF23 expression in murine lung tissue, when compared to controls or each stimulus alone. DISCUSSION/SIGNIFICANCE: Phosphate has a significant impact on inflammation and fibrosis in the lung, indicating that the existence of pulmo-renal crosstalk exaggerates pulmonary injury and that there are biological pathways that may be targeted therapeutically to mediate these effects. These results could have a substantial impact on the quality of life for CKD patients.

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