(STZ+RS, n = 6), 2) recurrent hypoglycemia (STZ+RH, n = 7), and 3) recurrent hypoglycemia + metoclopramide (STZ+RH+MET, 3 mg/kg IP, n = 7). After 3 days, all rats underwent a hyperinsulinemic (50 mU/kg/min) and hypoglycemic (~45 mg/dl) clamp. In the clinical trial, adults with Type 1 diabetes (age 20–60, ≥5 years duration) were enrolled in a phase II, double-blinded, placebo-controlled trial. Awareness status was assessed via Gold score, and subjects maintained drug regimens and underwent two hyperinsulinemic-hypoglycemic clamps (where blood glucose was lowered to 100, 65, 55, and 45 mg/dl) to assess counterregulation. RESULTS/ANTICIPATED RESULTS: In the pre-clinical model, glucose infusion rates (GIR) to maintain hypoglycemia were higher in STZ+RH (27±0.9 mg/kg/min) than STZ+RS (19±0.8 mg/kg/min, p DISCUSSION/SIGNIFICANCE OF IMPACT: Metoclopramide improves glucoregulatory, sympathoadrenal, and counterregulatory responses to hypoglycemia in pre-clinical models, suggesting dopaminergic regulation. While clinical data are still blinded, increased epinephrine and growth hormone responses suggest treatment may preserve or restore counterregulation.

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## Peritoneal lesion and peri-metastatic rim classification using machine learning and image processing*

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OBJECTIVES/GOALS: The goal of this study is to investigate the peri-metastatic rims of peritoneal lesions to determine features that predict malignancy using both imaging processing techniques and machine learning. This information will subsequently be added to our existing knowledge of peritoneal lesions to improve classification accuracy as benign or malignant. METHODS/STUDY POPULATION: The study population consists of 521 imaged lesions from 163 subjects with cancers of GI-origin with biopsy results as well as the clinical subject information and follow up. All images were obtained during staging laparoscopy by the senior author (TS). On the images, the central lesion as well as the surrounding peri-metastatic rim will be segmented as regions of interest (ROIs). Image processing will be used to calculate a variety of metrics for these two regions. A general estimating equation approach will be used to determine significance of these metrics compared to the dependent outcome of malignancy determined on the pathology report as the ground truth. These ROIs and significant metrics will then be used to improve the accuracy of a machine learning model to classify these lesions as benign or malignant. RESULTS/ANTICIPATED RESULTS: Our previous research showed that experts performed this task at only a 52% accuracy rate (classifying lesions as malignant or benign based on imaging). A previous machine-learning model on a much smaller dataset was able to achieve by contrast an area under the curve of 0.78. We anticipate that by including a larger dataset in addition to including the peri-metastatic rim, we will be able to improve the accuracy of the the model in this task while uncovering significant biomarkers as well that can be used in future studies. DISCUSSION/SIGNIFICANCE OF IMPACT: Classifying peritoneal lesions determines the correct treatment for cancer patients whether chemo-radiation, definitive surgery or palliative surgery. This project aims to develop an improved model that can perform this task using nonlabeled laparoscopic imaging with a particular focus on the diagnostic value of the peri-metastatic rim.

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## Liraglutide protects against microvascular dysfunction and sepsis-mediated inflammation and organ injury

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OBJECTIVES/GOALS: • To determine the impact of liraglutide on inflammation and organ injury during sepsis. • To investigate the protective effects of liraglutide on microvascular dysfunction in a clinically relevant model of sepsis. • To provide evidence for the potential therapeutic use of GLP-1 receptor agonists in endothelial dysfunction in sepsis. METHODS/STUDY POPULATION: Sepsis was induced in mice (N = 34) by intraperitoneal injection of cecal contents (1.8 mg/g body weight) and 24-hour hyperoxia (FiO2 90–95%). Mice received saline or liraglutide (0.1 mg/kg) at 6 and 18 hours post-injection and fluids and antibiotics at 12 hours. At 24 hours, mice were euthanized for plasma, bronchoalveolar lavage (BAL), and tissue collection. Plasma inflammatory markers, organ injury markers, and BAL components were measured. In vitro, primary human lung microvascular endothelial cells (HLMVECs) were treated with saline or liraglutide for 24 hours before exposure to saline or LPS (100 ng/mL). HLMVEC barrier dysfunction was evaluated using express permeability testing (XPerT) and electric cell-substrate impedance sensing (ECIS) to measure transendothelial electrical resistance (TER). RESULTS/ANTICIPATED RESULTS: In murine sepsis, illness severity scores and lung injury were improved in mice pretreated with liraglutide (N = 10). Plasma blood urea nitrogen (BUN; P = 0.0036), alanine transaminase (ALT; P = 0.0311) and vascular inflammatory markers MCP-1 (P = 0.0172), ICAM-1 (P = 0.0356), and Pecam-1 (P = 0.0493) in plasma were reduced in mice treated with liraglutide. In HLMVECs, liraglutide (1.5 nM) significantly reduced LPS-induced barrier dysfunction measured by XPerT assay (P = 0.0030) and ECIS (P = 0.0075). DISCUSSION/SIGNIFICANCE OF IMPACT: Liraglutide reduces illness severity, vascular inflammation, and organ injury in a two-hit model of sepsis. Liraglutide has direct effects in the microvascular endothelium, limiting LPS-mediated barrier dysfunction. These findings support a protective role for GLP-1 receptor agonism in sepsis, mediated through the microvasculature.

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## Elucidating structure–function relationships driving immune aging in lymph nodes

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OBJECTIVES/GOALS: The aging population faces unique health issues, many of which are exacerbated by aging-associated immune function decline. However, the driving mechanisms behind this decline are poorly understood. We use a mouse model to study the relationship between extracellular matrix (ECM) stiffness and cell mobility within the lymph node (LN) as one potential driving mechanism. METHODS/STUDY POPULATION: We will collect

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LN from young (6 weeks old) mice and acquire LN from aged (11 month old) mice from a collaborator. We will section LN for ex vivo analysis, including quantification and localization of collagen I using immunofluorescent staining, analysis of microrheological properties using multiparticle tracking (MPT) with PEGylated fluorescent nanoparticles, and migration assays to track the movement of B and T cells. RESULTS/ANTICIPATED RESULTS: We hypothesize a positive correlation between collagen deposits and stiffness within murine LNs due to known mechanisms underlying age-related fibrosis. We also hypothesize that areas of increased stiffness (as revealed by MPT) will exhibit decreased cell migration due to physical hindrance to B and T cell mobilization. Furthermore, we hypothesize that aged murine LN will exhibit a significant increase in stiffness and resultant decreased cell mobility when compared to young murine LN, particularly in areas with increased collagen localization. DISCUSSION/SIGNIFICANCE OF IMPACT: These studies will elucidate structure–function relationships driving age-associated LN fibrosis and stiffness, and the resultant impedance to cell migration, thus clarifying some of the potential driving mechanisms behind immune aging and providing data capable of informing the development of relevant models and interventions.

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## Developing a Molecular Toolkit to define NEK functions in triple-negative breast cancer (TNBC) biology*

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OBJECTIVES/GOALS: The never in mitosis kinase (NEK) family regulates vital processes, namely cell cycle progression, but their potential as therapeutic targets in TNBC has not been fully explored. Our studies aim to develop a toolkit to investigate the functional roles of NEKs in pathologies including carcinogenesis. METHODS/STUDY POPULATION: To assess differential NEK expression in normal and tumor tissues and correlation of gene expression with patient survival, we used Gene Expression Profiling Interactive Analysis (GEPIA) and Kaplan–Meier Plotter (KMPlot) pan-cancer analysis, respectively. Basal NEK protein levels were determined by immunoblot across a panel of cell lines, including breast cancer, osteosarcoma, hepatocellular carcinoma, and non-cancerous cells, to identify appropriate systems for evaluation of NEK function. Doxycycline-inducible cell lines were generated by transduction with lentiviral stocks of NEK shRNA and overexpression constructs and antibiotic selection. Expression was analyzed by qPCR and immunoblot. RESULTS/ANTICIPATED RESULTS: Expression of NEK2, 4, 5, 6, 8, and 11 was higher in breast tumors compared to normal tissue by GEPIA analysis. Further examination using KMPlot showed a correlation between elevated NEK6 expression and decreased overall survival in patients with aggressive cancers. As an initial proof-of-concept study, we analyzed NEK6 protein expression in breast cancer cells. Levels of NEK6 were elevated in TNBC cells (MDA-MB-231) compared to hormone receptor positive (HR+) breast cancer cells (MCF7). Using complementary approaches to investigate the functional role of NEK6 in breast cancer, we depleted NEK6 expression using shRNAs in TNBC cells and expressed NEK6 in HR+ cells DISCUSSION/SIGNIFICANCE OF IMPACT: Because kinase dysregulation promotes oncogenesis and metastasis, targeting kinases is a key strategy in therapeutic development. A NEK-specific molecular toolkit allows researchers to elucidate NEK functions and contributions to carcinogenesis, promoting advancement of novel therapies.

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## Integrating Implementation Science into the Wake Forest Clinical and Translational Science Award

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OBJECTIVES/GOALS: To develop and deploy an academic learning health system (aLHS) Bridge Program to capitalize on our unique organizational strengths in Implementation Science (IS) and to overcome the gap between science and practice that threatens the success of an aLHS. The aLHS Bridges includes an IS Shared Resource, intended to advance IS to practice. METHODS/STUDY POPULATION: The new IS Shared Resource is built on our expertise in dissemination and implementation science, pragmatic, and adaptive trials and the CTSI's prior success in integrating academic and clinical missions. We also leveraged our existing experts to co-lead the aLHS Bridge including Kristie Foley, PhD, inaugural Chair of the Department of Implementation Science, and Gary Rosenthal, MD, Chair of the Department of Internal Medicine. Specifically, the new IS Shared Resource builds on the capacity of the Department of IS, comprised of 36 faculty members (19 primary and 17 secondary/adjunct appointments) with expertise in qualitative and mixed-methods research, stakeholder engagement, participatory research, digital health, and organizational theory. RESULTS/ANTICIPATED RESULTS: The IS Shared Resource is primed to aid faculty with dissemination and implementation needs, including shortening the time of intervention adoption and using implementation science to inform sustainable and effective implementation practice. The IS Shared Resource is equipped to provide consultation services to faculty members to understand their specific request and match IS faculty members who are expertly trained in specific strategies or contexts. DISCUSSION/SIGNIFICANCE OF IMPACT: Leveraging current resources and our first-of-its-kind Department of Implementation Science, our CTSI was able to stand up the IS Shared Resource to support the goals of the CTSA and our greater institution mission. Using a multidisciplinary approach was essential to the success of the IS Shared Resource.

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