University of Minnesota
Department of Medicine

22ND ROBERT P. HEBBEL RESEARCH DAY

2025

April 2nd
McNamara Alumni Center

UNIVERSITY OF MINNESOTA

Twin Cities Campus

Department of Medicine
Medical School
University of Minnesota

Variety Club Research Center
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April 2. 2025

Dear Colleagues:

Welcome to the 22nd Department of Medicine's Robert P. Hebbel Research Day. This event will showcase 88 abstracts illustrating the best research from our outstanding learners, staff and faculty throughout the Twin Cities.

The collective mission of the Department of Medicine is to improve the health and well-being of individuals and communities, now and in the future, by integrating scientific discovery with exemplary medical care and training the next generation of physicians in the science and art of medicine. The success of our Department is critically dependent on the interrelationships driving this mission.

The creation of new knowledge and the translation of that knowledge into new clinical approaches, healthcare disparities reduction, and educational innovation will be presented at Research Day 2025 and highlight the Department's role in making the University of Minnesota a preeminent research medical school. Over the last several years, the Department has enjoyed considerable growth and expanded its collaborations with various Centers, Institutes, and Schools – all aspects we promote on Research Day. The dialogue among researchers, educators, clinicians, and trainees at this event is the essence of our great Department of Medicine.

We appreciate the efforts and contributions of many who enabled the success of this event. We are grateful to be able to highlight Dr. Michael J. Gale, Jr., PhD, Professor and Head of the Department of Microbiology and Immunology as our keynote speaker this year on Wednesday April 2nd.

We hope you enjoy the 22nd Robert P. Hebbel Research Day and thank you for your participation and support.

Sincerely,

Elizabeth R Seaquist, MD
Nesbitt Chair and Head
Department of Medicine
University of Minnesota Medical School

Christine Wendt, MD
Vice Chair for Research
Department of Medicine
University of Minnesota Medical School

Research Day Agenda

Wednesday April 2nd, 2025

McNamara Alumni Center

2:00 - 3:00 PM | Opening Remarks & Poster Session 1 (odd numbers)

3:00 - 4:00 PM | Keynote
"The First Responders to Epidemic and Pandemic Viruses"
Michael Gale, Jr., PhD
Director, Institute on Infectious Diseases
Professor and Head, Department of Microbiology and Immunology, University of Minnesota

4:00 - 5:00 PM | Poster Session 2 (even numbers)

5:00 - 6:00 PM | Awards Presentation & Closing Comments

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Public Health Research

Community Engagement to Inform Syphilis and Congenital Syphilis Prevention Among American Indian Populations in Northern Minnesota

Presenter: Alice Lehman
Division: Infectious Disease & International Medicine
Email: lehma154@umn.edu
Authors: Kayla Rigdon, Tyler Moose, Micheala Sanger, Nehemiah Olson, ZhaaZhaa Greensky, Mary Owen, Alice Lehman

Purpose
Syphilis and congenital syphilis cases in Minnesota increased >500% over the past decade, with disproportionate increases among women and men who have sex with women. Rates of syphilis among American Indian (AI) persons in Minnesota is 16.5 times higher than non-Hispanic white persons. National data suggest that up to 50% of women with syphilis in the Midwest use drugs (injectable or smoking) and have sex while intoxicated or high. Prevention of congenital syphilis relies on timely detection and treatment in pregnant persons and reducing AI community rates of syphilis. This community engagement study sought to understand the facilitators and barriers to syphilis prevention among AI populations in northern MN.

Methods
We held focus groups in partnership with community organizations in northern Minnesota. Topic guides covered perspectives on sexually transmitted infections(STIs), substance use and access to sexual health and prenatal services. Participants were recruited from community clinics and organizations with fliers and word of mouth with the assistance of community partner organizations. Participants were eligible to participate in the focus groups if aged 18 years or greater and self-identified as AI. Thematic analysis was applied to analyze the data using grounded theory and a framework for social-ecologic health.

Outcomes
We held 3 focus groups in 2 counties in northern Minnesota with high incidence of syphilis (St. Louis County 26/100,000 – Beltrami 43/100,000) and in proximity to reservations (Red Lake, White Earth, Leech Lake, and Fond du Lac) and urban centers (Bemidji, Duluth) where AI persons live. A total of 9 persons participated, with 89% of participants females. Most participants knew the term syphilis, however, 30% knew syphilis could cause congenital syphilis. The data was coded into four primary themes: 1) culture of sexually transmitted infections; 2) access to preventive services; 3) gender roles and 4) community knowledge. Each theme was further divided into barriers, facilitators, and recommendations.

Conclusions
Two participants knew syphilis or congenital syphilis was increasing in their communities. Differences existed between women and men's perceptions of STIs, including on shame, stigma, and openness of conversations. Healthcare providers rarely discussed STIs. Participants only received screening when something was wrong. Persons who use drugs face environmental, structural and racism barriers to accessing sexual and perinatal healthcare. Generational tribal knowledge and lived experience informs strong peer support models. Expanding access to syphilis screening will require increasing opportunities to person who used drugs in partnership with AI community informed by tribal knowledge and de-stigmatizing STIs.

Chronic Inflammatory-Related Disease and Associations with Coronary Artery Calcification in the Multi-Ethnic Study of Atherosclerosis

Presenter: Evan Manning
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Authors: Evan Manning, David Jacobs Jr, Daniel Duprez

Background

Inflammation plays a role in the development of cardiovascular disease (CVD). We defined Chronic Inflammatory-Related Disease (ChrIRD) as the collective of various non-cardiovascular and non-cancer conditions, both infectious and non-infectious, with a common basis of inflammation (Figure). ChrIRD is associated with baseline inflammatory marker levels and has a bidirectional association with CVD. Coronary artery calcification (CAC) is an inflammatory process mediated by cytokines and promoted by systemic inflammatory conditions. As both CAC and ChrIRD have inflammatory associations, we hypothesize that that baseline CAC predicts future ChrIRD.

Methods

We studied MESA participants free of overt CVD at baseline. At study entry, 45% of participants reported non-CVD conditions related to ChrIRD. ChrIRD was determined based on review of ICD codes for hospitalizations and deaths. Incident CVD was adjudicated by review of medical records. All participants underwent baseline ECG-gated non-contrast CT with Agatston scoring for CAC quantification. We performed proportional hazards regression to identify associations between baseline CAC and ChrIRD with adjustment (Figure).

Results

Participants (n=6151) had mean age 62±10 years and 47% male gender. CAC predicted both CVD and ChrIRD (Figure).

Conclusions

Baseline CAC among individuals free of overt CVD is associated with future diagnosis of inflammatory disease after adjustment for participant characteristics and risk factors.

Integrating Medical Student Education and Community Vision Health

Presenter: Hannah Hwang
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Authors: Hannah J. Hwang,[1] BA, Jaffer, Shahidali, MD[2,3]

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2. Office of Academic Clinical Affairs: Mobile Health Initiative, University of Minnesota Twin Cities, Minneapolis, MN, United States.

3. Department of Internal Medicine, University of Minnesota Twin Cities School of Medicine, Minneapolis, MN, United States.

Background: Established in 2020, the University of Minnesota Mobile Health Initiative (MHI) consists of a multidisciplinary team of healthcare professionals working in partnership with community organizations to deliver essential healthcare services to underserved populations in Minnesota (MN). In response to a community-identified need for mobile vision care, students from the University of Minnesota Medical School adopted principles from similar programs created at other academic institutions to create a mobile platform for vision screening in 2021. This student initiative has since blossomed into an interdisciplinary student group called the Mobile Health Student Interest Group (MHSIG), which actively supports MHI year-round through student volunteering and student-led projects.

Methods: This study reports the demographics of the vision screening program and the creation of the Mobile Health Student Interest Group to support the initiative. Vision screenings, primarily led by student volunteers and optometrists or ophthalmologists, included visual acuity tests, eye exams, and free glasses provided to multiple community partners with MHI. After mobile vision events, an electronic survey was provided to medical students to provide feedback regarding their volunteer experience.

Results: From June 2021 to March 2024, 21 MHI vision clinics were hosted, with 337 patients served in 11 counties. 58.6% were female with an average age of 37 years old. 8.1% of patients seen were referred for further follow-up. In a survey provided in September to October 2024, out of seven student volunteers, five strongly agreed that volunteering with MHI was a valuable educational experience and that they would volunteer again in the future. In addition, five agreed, and two strongly agreed, that they felt prepared to perform the tasks that were asked of them at the event.

Conclusions: While this study shows the impact medical students can have on vision disparities in Minnesota through volunteering during their education, our understanding of how students feel about their experience in this student-led initiative is limited. Preliminary data suggests a positive experience, but future surveys will be needed to increase our response rate while balancing focus on patient care.

SARS-CoV-2 Drives Inflammation of Intestinal Epithelium in Immunocompromised Cancer Patients

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Authors: Emily Gammon Hawkins, Christine Luo, Katherine Ladner, Allison Makovec, Phillip Wong, Jeremy Chacon, Eamon Toye, Ella Boytim, Anthony Patregnani, Emily John, Sanyukta Padmanabhan, Akshat Sarkari, Alison Wong, Julia Stuart, Andrew C Nelson, Brian Betts, Eliseo Eugenin, Justin Hwang , Emil Lou

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Abstract:

Cancer patients now receive transplantation-based treatments, which can be curative. While these immune suppressive regimens and should in theory render these patients as immune compromised, a subset will exhibit symptoms of graft versus host disease (GVHD), consisting of inflammatory symptoms that increase risk of death. In this study we examined 3 cancer patients that exhibited severe inflammatory disorders following SARS-CoV-2 infection. To do so, we integrated high-resolution microscopy and spatial transcriptomics on pre- and post-COVID infected tissue biopsies from the gastrointestinal (GI) epithelium. Through these high-resolution approaches we examined multiple tissue foci within the GI epithelium as well as the neighboring immune cells. We found that during or up to 49 days after a positive COVID test, the duodenum epithelium still exhibited persistent COVID viral elements, up-regulation of COVID receptors, activated inflammatory genes, elevated interferon alpha and gamma activity, and scarring of local intestinal epithelium. Of the up-regulated genes, we found that *SERPINA1* was a persistent indicator of infection. Further, *SERPINA1* was also a marker of the same inflammatory genes and pathways in ~600 GI tumor samples, implicating it as an inflammation marker in cancerous GI tissue as well. We performed further high-resolution microscopy focusing on visualizing the subcellular anatomy of cell clusters, located within the regions of interest that were used for spatial transcriptomic evaluation, to identify gaps between cells that were bridged by actin-based membranous protrusions reminiscent of tunneling nanotubes (TNTs). In our imaging, we identified several examples of such protrusions connecting cells that were most prominent in the post-infection samples from our matched tissue specimens. Amidst the COVID-19 pandemic, several studies reported SARS-CoV-2 viral particle association with and transfer between cells via TNTs, filopodia, and similar protrusions Altogether, the GI epithelium is an effective COVID reservoir, which potentially contributes to inflammatory syndromes that resemble GVHD. Regardless, these viral reservoirs are potential risk factors for cancer patients that shall receive transplant treatments.

Optimization of LDL control in coronary artery disease using cardiac rehabilitation as a point of intervention

Presenter: Michael Blotner
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Authors: Michael Blotner, Jeremy Van't Hof

Introduction

The 2018 American College of Cardiology (ACC) and American Heart Associate (AHA) guidelines recommend a strict low-density lipoprotein (LDL) goal of <70mg/dL for patients who suffered an acute coronary syndrome (ACS). The European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) have an even lower goal of LDL <55mg/dL for high risk patients, hoping to decrease the incidence of future cardiac events. In addition to lipid lowering therapy, standard of care for these patients includes cardiac rehabilitation. Cardiac rehab not only improves cardiovascular fitness, but also provides an environment for patient monitoring and education.

Purpose

The purpose of this quality improvement project is to assess frequency of LDL monitoring and the proportion of cardiac rehab participants meeting LDL targets after an ACS event. We will also determine the feasibility of using the cardiac rehab team as an advocate for better LDL control.

Method

Over a 6-month period, we analyzed the cholesterol and demographic data of 55 patients enrolled in cardiac rehab after an ACS event. LDL data was collected at the initial intake session, and updated at the patients' discharge session when available. After collecting baseline data, cardiac rehab staff will begin sending reminder messages to clinicians of patients not meeting the LDL target.

Results

Of 55 patients, 21/55 (38%) were at target LDL goal of <70mg/dL prior to starting rehab. At the conclusion of the 3-month program, only 10/55 (18%) had their LDL levels rechecked. In addition, only 25/55 (45%) had their cholesterol rechecked at any point after the index ACS event. Of those patients, 14/25 (56%) were not at an LDL <70mg/dL.

Conclusion

Strict control of LDL in high-risk cardiac patients is vital to the prevention of future cardiac events and decreasing mortality. Our study not only shows a low rate of cholesterol monitoring after an ACS event, but also that a majority of patients were not at optimal LDL levels by the time they completed cardiac rehabilitation. We are continuing to gather data on the predictors of LDL monitoring and control and have started the intervention of contacting the clinicians for patient who are not at the LDL target.

Association of Left Atrial Function with Mitral Regurgitation Progression: Atherosclerosis Risk in Communities Study

Presenter: Chunxiao Zhang
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Authors: Chunxiao Zhang, Wendy Wang, Riccardo M. Inciardi, Yuekai Ji, Khaled Shelbaya, Anne Eaton, Alvaro Alonso, Elsayed Z. Soliman, Scott D. Solomon, Amil M. Shah, Lin Yee Chen

Left atrial (LA) enlargement and atrial fibrillation (AF) are associated with functional mitral regurgitation (MR). It is unknown whether lower (worse) LA function is associated with MR progression, independent of LA size, in individuals without AF.

Hypothesis

We hypothesized that lower LA function was associated with higher odds of MR progression.

Aims

To evaluate the association of echocardiographic-based LA function with MR progression in the Atherosclerosis Risk in Communities (ARIC) Study, a community-based cohort.

Methods

We included 2,444 ARIC participants (mean age, 73.8 ± 4.4 years, 42% male, 21% Black) who underwent 2D-echocardiogram at visits 5 (2011–2013) and 7 (2018–2019) and excluded those with AF, heart failure, and heart valve surgery at visit 5. Based on American College of Cardiology/American Heart Association guidelines, MR was graded as stages A-D. MR progression was defined as new onset of MR at visit 7, any increase in MR stage, or mitral valve surgery for MR between visit 5 and 7. LA function was measured using strain analysis and included reservoir, conduit, and contractile strain. Logistic regression models were used for the analysis.

Results

After mean follow up of 6.5 ± 2.4 years, MR progression occurred in 504 (21%) participants. As shown in the table, lower reservoir strain was associated with higher odds of MR progression (OR [95% CI] per SD decrement, 1.16 [1.003, 1.34]) after adjusting for demographics, clinical risk factors, LA size and LV mass and function. LA conduit and contractile strain were not significantly associated with MR progression.

Conclusion

Lower LA function is associated with higher odds of MR progression, independent of LA size, in individuals without AF. More research is needed to clarify the mechanism to identify novel prevention strategies for MR progression.

Clinical Research

Determining the Prevalence of CMV Retinitis in HIV-associated Meningitis Patients in Uganda

Presenter: Sanjana Molleti
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Authors: Sanjana Molleti, Sheillah Njuki, Iddi Ndyabawe, Tu Tran, Timothy Mugabi, Enos Kigozi, David Boulware, David Meya, Conrad Muzoora, Caleb Skipper, Simon Arunga

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Cytomegalovirus (CMV) is an opportunistic pathogen that can cause long term visual impairments in persons with advanced HIV disease, typically manifesting as retinitis. The role of CMV retinitis as a potentially reversible cause of vision loss has not been adequately studied in at-risk patients and is especially difficult to detect in resource limited settings. The purpose of this prospective observational cohort study is to determine the prevalence of CMV retinitis in people with HIV-meningitis in two Ugandan hospitals. In a longitudinal, observational cohort of hospitalized patients with HIV and suspected meningitis, we performed dilated eye exams and retinal image capture at two hospitals in Kampala and Mbarara, Uganda. All participants received a full eye and dilated fundus exam by an ophthalmologist using bedside indirect fundoscopy. Suspected CMV retinitis was recorded as a preliminary diagnosis based on exam. Additionally, all participants underwent nonmydriatic retinal imaging using the Phelcom Eyer instrument, a portable fundus camera, conducted by general medical officers. Both groups recorded data independently. Images received an interpretability grade of “Full”, “Partial” or “Failed” imaging depending on their view of the optic disc, fundus, and surrounding structures. Between April to November 2024, 192 patients received an ophthalmology exam, 165 had a medical officer exam, with a total of 132 patients receiving exams from both teams. The ophthalmology team suspected CMV retinitis in 26 patients or 13.5%. Other retinal pathologies identified in the population included cotton wool spots, toxoplasmosis, and papilledema. Overall, 353 eyes were imaged with the Phelcom Eyer instrument by the medical officer, and 63.7% were adjudicated as full images, 26.1% were partial images, and 10.2% were failed. Based on these images, retinal pathology was suspected in 20.4% (72/353) of eyes. Hospitalized persons with advanced HIV disease may have a high prevalence of CMV retinitis, supporting the implementation of enhanced imaging solutions to prevent vision loss. Also, portable non-mydriatic fundus cameras appear to be a viable tool for screening retinal pathology in a hospital setting. Further research is warranted to evaluate digital imaging effectiveness as a non-inferior alternative to bedside ophthalmology exams.

C-Reactive Protein as a Predictor of Hospitalization and Mortality in Advanced HIV Disease in Uganda

Presenter: Elizabeth Schwartz
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Background

People with advanced HIV disease (AHD) remain at high risk for opportunistic infections, hospitalization, and death, despite improved access to antiretroviral therapy (ART). We aimed to evaluate the predictive value of serum C-reactive protein (CRP) for the detection of future hospitalization or death among outpatients with AHD in Uganda.

Methods

Serum CRP values were collected prospectively from 785 participants enrolled in the ENCORE trial (NCT05085171), a community-based cluster randomized trial in adults with AHD (baseline CD4 <200 cells/μL). Participants were recruited from May 2022 to February 2024 at outpatient clinics in the Kampala and Wakiso districts of Uganda. We examined baseline CRP as both a continuous predictor and as a dichotomized predictor at a threshold of 10mg/L. We used logistic regression and Cox regression models to assess CRP as a risk factor for hospitalization or death. We calculated the area under the receiver operating characteristic curve (AUC) to evaluate CRP as an independent predictor for 30-day hospitalization-free survival.

Results

A CRP threshold of 10 mg/L was identified as a strong predictor of 30-day hospitalization or death. In our cohort, 41% had an elevated CRP value (>10mg/L). Participants with elevated CRP were significantly more likely to be hospitalized (Relative Risk=3.1, p=0.002) or die (Relative Risk=8.5, p<0.001) within 30 days of enrollment. When controlling for potential confounding variables of sex, age, weight, CD4 count, and ART status in a multivariate Cox proportional hazards model, the hazard of hospitalization or death within 30 days increased by 30% (HR=1.3, p<0.001) for each doubling of CRP. When examining the composite outcome of 30-day mortality or hospitalization, CRP has an AUC of 0.76 as a univariate continuous predictor, and the multivariate model performed significantly better when CRP was included (AUC=0.80), compared to when CRP was excluded from the model (AUC=0.73).

Conclusion

High CRP values are associated with increased risk of hospitalization and/or death within 30 days among outpatients with AHD. Prospective evaluation of point-of-care CRP testing and whether real-time measurements can be used to risk stratify populations for interventions to reduce deaths is needed.

Long Term Risk of Pancreato-biliary Events After Necrotizing Biliary Pancreatitis In Patients Without Cholecystectomy Compared to Patients With Cholecystectom: A Multi-Institutional Propensity-Matched Cohort Study

Presenter: Rahul Karna
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Authors: Rahul Karna, Himsikhar Khataniar, Stuart K Amateau

Background: Current guidelines recommend same admission cholecystectomy in patients with mild biliary pancreatitis to prevent future pancreato-biliary events. Cholecystectomy after necrotizing pancreatitis (NP) is associated with potentially increased risk of adverse events and is often delayed till resolution of collections. The catastrophic nature of illness with long recovery period after index NP episode can be associated with healthcare fatigue, loss to follow up, and potentially non-adherence to cholecystectomy recommendations. Also, many elderly patients may not be optimal surgical candidates and decision of cholecystectomy is often based on risk assessment of recurrent pancreato-biliary events and surgical risk of cholecystectomy. Overall, long term risks of pancreatobiliary events in biliary NP patients not undergoing cholecystectomy compared to patients who do undergo cholecystectomy is not well established.

Methods: We performed a retrospective cohort study to evaluate risk of pancreato-biliary events in NP patients from using the TriNetX database from 2006-2021. Our study population was divided into two cohorts: NP patients without cholecystectomy (C-) compared to patients with cholecystectomy (C+). Propensity score matching (PSM) was used and risk of biliary events (choledocholithiasis, cholecystitis, cholangitis), recurrent pancreatitis, hospital admissions and mortality at 3 years in C- cohort compared to C+ cohort were assessed. Subgroup analyses was performed for patients >65 years of age.

Results: Out of 2,626 NP patients, only 955 (36.3%) underwent cholecystectomy. After PSM, 592 NP patients were included in cohort without cholecystectomy (C-) and cohort with cholecystectomy (C+) each. C- cohort had significantly higher risk choledocholithiasis (aOR:1.65, p:0.001), recurrent pancreatitis (aOR: 1.61, p:0.001), sepsis (aOR: 2.08, p:0.001), all cause hospitalization (aOR: 1.70, p:0.001) and all-cause mortality (aOR:3.06, p:0.001) than C+. In NP patients >65 years of age, C- cohort had significantly higher risk of recurrent pancreatitis (aOR: 1.61, p:0.009), sepsis (aOR: 1.61, p:0.015), all cause hospitalization (aOR: 1.56, p:0.014) and all-cause mortality (aOR:2.37, p:0.001) than C+.

Conclusions: Our study shows that only 36% of patients with biliary NP undergo cholecystectomy despite a high risk of choledocholithiasis, recurrent pancreatitis, sepsis and 3 times risk of all-cause mortality without cholecystectomy. Our study highlights need to increase adherence to cholecystectomy to prevent recurrent pancreatobiliary events and prevent mortality after biliary NP.

Preoperative Colchicine and Heart Block after the First Trans-catheter Aortic Valve Implantation: a U.S. Nationwide Analysis

Presenter: Ahmed Almahdy Mohamed
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Background

Heart block (HB) is a frequent complication following transcatheter aortic valve intervention (TAVI), partially driven by inflammation. The anti-inflammatory role of colchicine on HB post-TAVI has not been studied yet. Therefore, we investigated the association between preoperative clinical use and HB outcomes after the first TAVI in a U.S. nationwide sample.

Methods

Using the TriNetX platform, patients receiving any colchicine prescription within three months before their first TAVI were compared to matched patients never exposed to colchicine up to one year after the procedure. Primary outcomes included post-operative any new-onset or worsening atrioventricular or left bundle branch block (AV/LBB-B) and HB. Outcomes were assessed within one and six months after TAVI with an E-sensitivity analysis assessing the impact of unmeasured confounders.

Results

Of 52,860 patients, 705 took preoperative colchicine. After matching, each group included 702 patients. Colchicine was associated with a statistically significant reduction of AV/LBB-B and HB post-TAVI at one month (RR: 0.867, 95% CI [0.756, 0.994], P = 0.041*; RR: 0.887, 95% CI [0.788, 0.999], P = 0.047*, respectively) but not at six months (RR: 0.886, 95% CI [0.779, 1.077], P = 0.064; *RR: 0.887, 95% CI [0.788, 0.999], P = 0.047,* respectively). The E-value was >1.5 for the statistically significant outcomes.

Conclusion

Preoperative colchicine was associated with a lower incidence of AV/LBB-B and HB after the first TAVI at one month. However, no significant difference was observed at six month. Future prospective trials are needed to confirm these observations.

Serum C-Reactive Protein Levels Are Associated with Increased Mortality Risk in Pulmonary Arterial Hypertension Patients

Presenter: Truc Ho
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Authors: Truc Ho, BS, Madeline Chopp, BS, Madelyn Blake, BS, Sasha Z. Prisco, MD, PhD, Thenappan Thenappan, MD

Lillehei Heart Institute, Cardiovascular Division, Department of Medicine, University of Minnesota, Minneapolis, MN

RATIONALE: Inflammation is key mediator of pulmonary arterial hypertension (PAH) pathogenesis. Serum C-reactive protein (CRP) is a systemic biomarker of inflammation. Few studies have investigated whether CRP levels associate with PAH severity or outcomes. We hypothesized that serum CRP levels at the time of diagnosis correlate with PAH severity, poor response to therapy, and increased mortality.

METHODS: We studied 323 PAH patients with serum CRP levels at the time of diagnosis from the Minnesota Pulmonary Hypertension Repository. The cohort was stratified by normal CRP level compared to high (CRP ≤3.0mg/L vs. CRP >3.0 mg/L). We compared baseline characteristics and analyzed the relationship between serum CRP levels at time of diagnosis and PAH severity one year after PAH diagnosis. We used Kaplan-Meier survival curve and log-rank test to compare survival.

RESULTS: The mean age was 54±15 years and 74% were female. The median CRP level was 6.0 mg/L. Compared to patients with normal serum CRP, patients with high serum CRP levels had significantly greater BMI (30.7±9.3 *vs.* 27.4±6.1 kg/m^2, p=0.006) and mean pulmonary artery pressure (46.6±13.3 *vs.* 43.4±13.7 mmHg, p=0.04) at baseline. There was no difference in NT-proBNP at baseline (852 [IQR 248-2445] *vs.* 702 [IQR 146-2506] pg/dL, p=0.55) or at 1-year follow-up after standard of care (394 [IQR 114-1500] *vs.* 402 [123-1492] pg/dL, p=0.85). No difference was observed in REVEAL 2.0 risk score at baseline (high CRP: 8.7±3.4 *vs.* normal CRP: 8.7±3.6, p=0.84) or follow-up (7.7±3.8 *vs.* 7.9±3.8, p=0.52). Normal and high CRP cohorts had similar response to treatment defined by decreased REVEAL 2.0 risk at follow-up (35% vs. 44%, p=0.14). Over a median follow up of 5.6 years, 143 patients died or had lung transplantation. Patients with high CRP levels at baseline had significantly worse survival compared to those with a normal CRP level. Patients in the high CRP group had a 1.6-fold increased risk of mortality compared to those in the normal CRP group (hazard ratio: 1.6, 95% CI 1.1-2.3, p=0.02).

CONCLUSIONS: High serum CRP levels predict increased mortality in patients with PAH. Future studies should assess the incremental value of serum CRP to current risk prediction models in PAH. AnHIT or Miss? Evaluating the Efficacy of 4T and Modified 4T

HLA Class I Expression Shapes the Tumor Immune Microenvironment and Influences Prognosis in Prostate Cancer

Presenter: Pornlada Likasitwatanakul,
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Introduction: Human leukocyte antigen (HLA) class I comprises a family of peptide-binding proteins that regulate T-cell interactions. Here, we examined HLA class I mRNA expression and gene zygosity in prostate cancers (PCs), exploring associations with clinical outcomes, molecular features, and tumor microenvironment.

Methods: We analyzed 10,790 PC samples, of which 8,040 (75%) contained HLA transcription data, and 2,792 (26%) contained HLA genotypes in the Caris Life Sciences database. Samples were stratified into HLA-high (> 75th percentile) and -low (< 25th percentile) groups. Genomic and transcriptomic alterations were compared at an adjusted significance level of 0.05. Immune cell fractions were inferred by quanTIseq. Overall survival data was obtained from insurance claims and computed using Cox proportional hazards.

Results: Among 66 cancer types, PCs ranked 2nd, 11^{th,} and 19th lowest concerning HLA-A, HLA-B, and HLA-C expression. In PC, genes related to androgen receptor (AR) signaling, cell-surface antigens (CTLA4, PD-L1, TROP2, B7-H3, and PSMA), and the epithelial-mesenchymal transition hallmark were significantly increased in HLA-high tumors. HLA-high status was linked to higher interferon-gamma scores and a greater presence of cytotoxic and regulatory T cells, B cells, and NK cells. HLA-high tumors also exhibited a two-fold depletion in *CDK12* and *AR/FOXA1* mutations but were enriched in alterations of tumor suppressor genes (*RB1*, *PTEN*). Tumors with high HLA-A and HLA-B showed increases in TMB-H/MSI-H/dMMR status. HLA class I expression was generally lower in metastatic biopsies (Bx) compared to primary prostate Bx. When examining the zygosity of HLA alleles, metastatic Bx revealed a higher proportion of homozygous HLA-B compared to primary Bx. Last, worse overall survival (OS) was seen in prostate bx that were high in HLA-A or HLA-B (HR = 1.36, 1.21, p<0.0001, 0.008), whereas better OS was noted in prostate bx that were exclusively HLA-C high (HR = 0.70, p = 0.016).

Conclusion: In summary, HLA class I expression is lower in PC compared to other cancers, but elevated HLA levels correlate with immune cell activity, somatic alterations, and clinical outcomes. Therefore, we suggest considering HLA class I expression as a potential marker of immune activity in PC, alongside genetic mutations and ICI markers, when interpreting tumor profiling.

Investigating SOX2 as a Potential Driver of Lethal Prostate Cancer and Therapy Resistance

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Background
Prostate cancer progression and therapy resistance remain major clinical challenges. Androgen receptor (AR)-targeted therapies, such as enzalutamide and abiraterone, initially suppress tumor growth but often lead to treatment-resistant phenotypes, including neuroendocrine prostate cancer (NEPC). The transcription factor SOX2 has been implicated in this transition, yet its precise role in prostate cancer remains unclear. This study investigates SOX2's function in lethal prostate cancer and its potential as a therapeutic target.

Methods
We analyzed SOX2 expression using single-cell and bulk RNA sequencing data from prostate cancer patients. The Witte dataset, consisting of 10 patient-derived tumor samples, was used for temporal analysis, with samples collected at 4, 5, 7, 8, 10, 12, and 18 weeks to track NEPC progression. Additional datasets included 209 metastatic prostate cancer samples from SU2C and 47 patient samples (14 NEPC, 33 adenocarcinoma) from Beltran for broader comparisons. UMAP visualizations distinguished SOX2-positive and AR-positive cell populations, highlighting lineage plasticity. Chromatin immunoprecipitation sequencing (ChIP-seq) mapped SOX2 binding sites to identify regulatory interactions. CRISPR knockout (KO) of SOX2 in prostate cancer cell lines assessed its role in AR-targeted therapy resistance and tumor progression.

Results
SOX2 was highly expressed in NEPC and castration-resistant prostate cancer (CRPC), with an inverse correlation to AR expression. CRISPR knockout of SOX2 reduced cell viability and restored enzalutamide sensitivity, confirming its role in therapy resistance. Temporal analysis showed SOX2 upregulation precedes ASCL1 expression, suggesting it acts early in lineage plasticity. ASCL1, a key neuroendocrine regulator, peaks later, reinforcing the idea that SOX2 primes cells before ASCL1 fully drives differentiation. ChIP-seq identified SOX2 binding at
AR-repressed sites, supporting its role as a transcriptional regulator in therapy resistance.

Conclusion
SOX2 plays a central role in lethal prostate cancer, promoting AR-targeted therapy resistance and neuroendocrine differentiation. Targeting SOX2 or its pathways may offer new therapeutic strategies. Future studies will focus on identifying upstream regulators and indirect targeting approaches to counteract SOX2-driven tumor progression.

The Outcomes of Interval Pancreatic Cancer Following a Normal Endoscopic Ultrasound Compared to Detected Cancer: A Large Propensity Matched Observational Cohort Study

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Background: Pancreatic cancer (PC) is the third most common cancer in the United States, with overall survival depending on the stage of diagnosis. Endoscopic ultrasound (EUS) offers an opportunity to detect pre-malignant lesions in the pancreas. Despite advances in training, EUS remains an operator-dependent procedure, and interval PC can occur after a diagnostic EUS. We aim to study the proportions and outcomes of post-EUS pancreatic cancer (PEPC) as compared to detected pancreatic cancer (D-PC).

Methods: We utilized the TriNetX multi-institutional database to assess patients with PC and a preceding EUS from 2005 to 2024. EUS was identified using Current Procedural Terminology codes. Patients whose EUS occurred less than 6 months before PC diagnosis with no other EUS within the previous 18 months were categorized as having D-PC. Those who had an EUS that did not detect PC between 6 and 18 months before PC diagnosis were categorized as having PEPC. We also assessed PC in patients with associated genetic mutations. Propensity-matched cohort analysis was performed after controlling for covariates including age, race, family history of malignancy, diabetes, alcohol use disorder, tobacco use, acute pancreatitis, chronic pancreatitis, pancreatic cyst, pancreatic diseases to assess events including pancreatic surgery, chemotherapy, all-cause hospitalization and all-cause mortality. A 5-year survival analysis was performed for both cohorts.

Results: Our study found that 5.4% of PC (1127/20,828) occurred after normal EUS. The D-PC cohort included 40 patients with genetic mutations (VHL: 10; MSH2: 10; ATM:10; CDKN2A: 10), while none were found in PEPC. Proportions of PEPC out of all PC were similar in males (5.5%, 575/10429) and females (5.3%, 523/9736). Among races, PEPC had the highest proportions in Asians (11.1%, 79/714), followed by Blacks (6.3%, 135/2136) and Whites (5.1%, 788/15,450). Hispanics had higher proportions of PEPC (8.2%, 86/1043) compared to non-Hispanics (5.6%, 805/14121). After propensity matching, 1,122 D-PC and PEPC were included in analytic cohorts. PEPC had a lower rate of pancreatic surgery (D-PC=31; PEPC=13; HR: 0.41, p=0.0051), chemotherapy (D-PC=510; PEPC=371; HR: 0.63, p<0.0001), all-cause hospitalization (D-PC=782; PEPC=601; HR: 0.58, p<0.0001), and all-cause mortality (D-PC=375; PEPC=313; HR: 0.757, P=0.0003).

Conclusions: Our retrospective large database study found that 5.4% of pancreatic cancer occurred after negative EUS in the previous 6-18 months. D-PC had a higher chance of surgical resection and chemotherapy but also had higher rates of hospitalization and mortality at 5 years, suggesting tumor biology plays a role in the aggressive course of disease in D-PC compared to PEPC.

Acrolein-Induced DNA Adducts in People with Lung Cancer Who Have Never Smoked

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Introduction Lung cancer in never smokers (LCNS) is estimated to be the fifth leading cause of cancer-related death worldwide. The etiology and risk factors for most cases are not understood. Acrolein, an aldehyde found in high levels in cigarette smoke, can readily form γ-OH-Acr-dG DNA adducts, and cause *TP53* mutations mimicking those caused by carcinogenic polycyclic aromatic hydrocarbons in tobacco smoke. However, exposure can arise from other sources. This pilot study explores the hypothesis that non-tobacco-related acrolein exposure may play a role in the pathogenesis of LCNS.

Methods Patients with lung cancer who never smoked, had a light smoking history, or whose cancers had genomic characteristics common in LCNS were recruited. Participants who had undergone cytotoxic chemotherapy within the last year were excluded. Participants provided oral cavity cells and a urine sample and completed an exposure questionnaire. γ-OH-Acr-dG DNA adducts were quantified in oral cells using a validated method. Lung cancer type, mutational profile, and treatment information was obtained from the electronic medical record.

Results Thirteen patients were recruited; three were excluded from the analysis; one was a current smoker by urinary cotinine level (a marker of nicotine exposure) and urinary NNAL level (a marker of uptake of the tobacco-specific nitrosamine NNK, a potent carcinogen found only in tobacco smoke); the other two had low levels of urinary cotinine and NNAL, indicating second-hand smoke exposure or recent cessation. Of the remaining 10, 4 were female and 6 were male; 8 were never smokers. Two were former smokers whose cancers harbored EGFR mutations. One cancer had an ALK rearrangement, two had EGFR L858R mutations, seven had EGFR exon 19 deletions, and one had an EGFR L861Q mutation. Specimens were collected prior to cancer-directed therapy in 8 patients. Mean g-OH-Acr-dG DNA adduct levels was 50 (per 10^9 nucleotide; range 1-130). Mean levels in a historical cohort of 40 healthy never smokers was 7.2 (0-157.5) and 90.5 (7.3-1122) in 40 current smokers ($p<0.001$). All patients except 2 had high levels of 3-HPMA (range 374-2298 pmol/mL of urine), a marker of acrolein exposure. One patient had undetectable 3-HPMA levels despite detectable levels of g-OH-Acr-dG DNA adducts, likely a reflection of the short half-life of 3-HPMA and the persistence of DNA adducts. A urine specimen was not available for the other patient.

Conclusion Our preliminary data supports the possible role of acrolein in the pathogenesis of LCNS. The mean level of g-OH-Acr-dG DNA adducts in our patients was 50 adducts per 10^9 nucleotides, intermediate between levels in control cohorts of never smokers and current smokers, respectively. A larger study is underway. Ultimately, if our hypothesis is true, we will have identified a critical etiologic risk factor for LCNS, allowing us to identify those at highest risk, and develop prevention strategies.

Sweating the Small Stuff

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Introduction: Hot tubbing is an activity that can provide several health benefits. However, it can pose several health risks such as cardiovascular, infectious and electrolyte abnormalities. We report a patient with excessive hot tub use who presented with various electrolytes abnormalities mimicking potential renal tubulopathies.

Case presentation: Our patient is a 70 year-old male with history coronary artery disease and nephrolithiasis who presented to primary care clinic for an annual check-up. He denies new symptoms. Vitals are notable for BP 121/71mmHg, HR 57 BPM and normal temperature. Physical exam was unremarkable. Laboratory data notable for Na 137mEq/L, K 3.1mEq/L, bicarbonate 29 mEq/L, Ca 9.2mg/dl and creatinine 0.94mg/dl. CBC was normal. UA showed no hematuria or pyuria. Urine specific gravity 1.020. Urine albumin undetectable. The patient denied diuretic use, diarrhea, nausea or vomiting and his weight was stable. Repeat laboratories 2 months later showed Na 133mEq/L, K 3.1mEq/L, bicarbonate 30mEq/L, Ca 10.7mg/dl. Urinary studies showed urine potassium 80.5mmol/L, fractional excretion of potassium (FeK) 15.8%, urine sodium <20mmol/L, urine chloride <20mmol/L, urine osmolality 841mmol/kg. Renin activity 35.3ng/ml/hr, aldosterone 82.8ng/dl. He was referred to nephrology clinic due to concerns for acquired Gitelman syndrome. He denied any family history of kidney disease, volume loss or diuretics use but did report consistent hot tube use for 90 minutes per day. This raised concern for volume loss causing hypovolemia and secondary hyperaldosteronism. He was instructed to stop using the hot tub and repeat labs were obtained after 2 weeks. Laboratories showed a complete resolution of hyponatremia, hypokalemia, metabolic alkalosis and hypercalcemia.

Discussion: The patient's excessive hot tub use caused significant hypovolemia as suggested by low urine sodium, low urine chloride, elevated renin and elevated aldosterone. Hyperreninemic hyperaldosteronism in this case represents a physiological response to hypovolemia and appropriate activation of the renin angiotensin aldosterone system. This contributed to renal potassium loss and metabolic alkalosis. Hyponatremia and hypercalcemia were from hypovolemia. In addition, significant perspiration from daily excessive hot tub use led to sweat related losses of sodium, chloride, potassium and water which contributed to his hypovolemia and his electrolyte abnormalities. All abnormalities resolved with restoration of intravascular volume following cessation of hot tub use

Conclusion: General recommendation of hot tub use is between 15-45 minutes per session to avoid hypovolemia and electrolytes derangement. Individuals should stay well hydrated and avoid prolonged use especially in patients at risk.

Prevalence of Cardiac Fibrosis in Female Carriers of Dystrophinopathies: Insights from Cardiac MRI and Cardiopulmonary Exercise Testing

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Introduction: Dystrophin-deficient cardiomyopathies, including Duchenne and Becker muscular dystrophy, are X-linked recessive disorders caused by mutations in the dystrophin gene, resulting in progressive muscle weakness and near ubiquitous cardiomyopathy [1]. Female carriers of dystrophinopathies are at risk for becoming manifesting carriers due to skewed random X-inactivation in cardiac and skeletal muscle leading to variable cardiac fibrosis, LV dysfunction, and skeletal dysfunction similar to affected men [2]. Despite the significant cardiac risk for these women, there is limited research on the extent and progression of cardiac involvement in female carriers. Paired cardiac magnetic resonance (CMR) imaging and cardiopulmonary exercise (CPX) testing along with other clinical parameters can better assess dystrophinopathy carriers' cardiovascular involvement at an early stage.

Objective: To evaluate the prevalence of abnormal CMR findings and CPX data in female carriers of dystrophinopathy.

Methods: A prospective cohort of carriers of dystrophinopathies were identified through positive genetic screening, family history of carrier status, or children or siblings with Duchenne or Becker muscular dystrophy. Medical records were reviewed to extract baseline characteristics, CMR results, and CPX data. Descriptive statistics were used to analyze the data, with means and standard deviations calculated for relevant variables.

Results: A total of 23 confirmed dystrophinopathy carriers (mean age 42.9 ± 15.5 years, 100% female, 93% white) were evaluated. CMR revealed that four carriers (13%) had late gadolinium enhancement (LGE) and reduced left ventricular ejection fraction (LVEF) <50% (mean age 60.0 ± 5.48 years, mean fibrosis 8% ± 5.7). Five (16%) had LGE with preserved LVEF >50% (mean age 42.8 ± 15.02 years, mean fibrosis 4.6% ± 1.52). The remaining 16 (53%) had no LGE and preserved LVEF >50% (mean age 42.3 ± 14.68 years). CPX data revealed that carriers with LGE and reduced LVEF (<50%) exhibited the lowest peak mVO_2, estimated workload, and peak heart rate, while those with preserved LVEF (>50%) had intermediate values, and those with no LGE and preserved LVEF (>50%) had the highest exercise capacity.

Conclusion: This study reveals a prevalence of cardiac abnormalities approaching 50% in dystrophinopathy carriers. Further, CPX results suggest that the presence of LGE and reduced LVEF may be associated with diminished exercise capacity and cardiovascular performance. The differences in peak mVO_2 between groups suggest that CPX may be helpful in identifying subclinical decline before a decrease in ejection fraction is seen. These results highlight the need for earlier cardiovascular screening and more tailored therapeutic strategies for dystrophinopathy carriers, particularly those with evidence of myocardial fibrosis. This will be important to study in larger cohorts of dystrophinopathy carriers and also harness omics to gain further insights.

Exploring Disparities in Guideline-Recommended Care Delivery for Women vs. Men with Chronic Obstructive Pulmonary Disease: a National U.S. Cohort

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Rationale

- Women with chronic obstructive pulmonary disease (COPD) have worse quality of life and more exacerbations than men, but it is unclear if this is linked to care disparities.
- Objective: To compare gender differences in guideline-recommended care delivery, including spirometry for diagnosis, maintenance inhaler treatment, and tobacco cessation interventions in a national U.S. cohort.

Methods

- Retrospective analysis of individuals with COPD (≥2 ICD-10 codes for COPD in 2016-2019) using national Veterans Health Administration data.
- **Primary Outcomes**:
    - Spirometry to confirm diagnosis of COPD
    - Tobacco cessation interventions such as pharmacotherapy and/or counseling
    - LAMA and/or LAMA/LABA as maintenance inhaler treatment
- We used multivariable logistic regression models to compare receipt of guideline-based care among women vs. men, adjusting for gender, age, race, Charlson Comorbidity Index (CCI), and Social Vulnerability Index.

Results

- Of 906,547 individuals with COPD, 35,507 (3.9%) were women and 871,040 (96.1%) were men.
- Compared to men, women were younger (61.7±10.7 vs. 69.8±9.9 years), had fewer comorbidities (CCI ≥3, 22.3% vs 33.1%), and were more likely to be current smokers (56.7% vs. 49.2%).
- **Spirometry** was performed in 69.0% of women and 66.9% of men. Women had slightly higher odds of undergoing spirometry compared to men, with an adjusted odds ratio (aOR) of 1.10 (95% CI: 1.08 to 1.13) (**Figure 1**).
- **Tobacco cessation interventions** were prescribed to 38.8% of women and 35.1% of men. Women had higher odds of prescription for tobacco cessation interventions, with an aOR 1.17 (95% CI: 1.13 to 1.20).
- **LAMA or LAMA/LABA** were prescribed as maintenance inhaler therapy in 41.6% and 46.5% of men. Women had lower odds of prescription for guideline-concordant maintenance inhaler regimen compared to men, with aOR 0.82 (95% CI: 0.77 to 0.87).

Conclusion

In this national cohort of individuals with COPD, both men and women had low rates of receiving guideline-recommended COPD care. While women were more likely to receive spirometry and tobacco cessation interventions, they were less likely to be prescribed guideline-recommended maintenance inhaler therapy compared to men. Our findings highlight the need for improvement in care delivery for all patients, with priorities potentially differing by sex.

Pure Red Cell Aplasia Following SARS-CoV-2: A Case Report

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Introduction: Pure red cell aplasia (PRCA) is characterized by anemia, reticulocytopenia, and absent erythroid precursors in bone marrow[1]. We present a case of acquired PRCA following SARS-CoV-2 infection.

Case: A 55-year-old female with Crohn's disease and breast cancer developed macrocytic anemia after COVID-19 infection. Bone marrow (Images 1 and 2) showed erythroid hypoplasia with left shift, mild dyserythropoiesis, and positive parvovirus PCR (negative in peripheral blood), confirming the diagnosis of PRCA. A comprehensive evaluation was unremarkable, including nutritional studies, autoimmune serologies, viral studies, T-Cell Receptor gamma/delta studies, specialized testing (PNH, APLS, IL-6, VEGF, SPEP/UPEP), and imaging. Telomere testing revealed shortened telomeres in lymphocytes (5.1kB vs normal 6.3kB) and granulocytes (5.8kB vs normal 8.0kB). Follow-up biopsies showed trilineage hematopoiesis with negative parvovirus PCR.

Treatment: Initial IVIG courses yielded transient improvement (hemoglobin peak 10.7g/dL) before requiring transfusions. Prednisone and B12 injections were ineffective. Danazol caused intolerable side effects, and darbepoetin alfa failed. Cyclosporine improved reticulocyte count (0.008 to 0.133) despite side effects. Sirolimus subsequently normalized MCV, reduced ferritin, and stabilized hemoglobin (9.0-9.8g/dL), reducing transfusion dependency.

Discussion: This case adds to the limited literature on COVID-19-related PRCA. Similar to reports by Lee[2] and Yamazaki[3], our patient required multiple transfusions and responded to immunosuppression. The pathophysiology likely involves COVID-19-mediated immune dysregulation targeting erythrocyte precursors[4]. This case emphasizes considering viral triggers in PRCA diagnosis and demonstrates the efficacy of escalating immunosuppressive therapy when first-line treatments fail.

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Clinical Characteristics and Outcomes of Patients with Philadelphia-Negative Myeloproliferative Neoplasms with Splanchnic Vein Thrombosis: A Two-Center Retrospective Study

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Background: The Philadelphia-chromosome negative Myeloproliferative Neoplasms (MPN) are chronic leukemias with increased thrombotic risk and venous thrombosis (VT) in atypical sites, such as splanchnic vein thrombosis (SVT), defined by thrombosis in the portal, hepatic, mesenteric, or splenic veins. Guidance for optimal management of MPN patients with splanchnic vein thrombosis is limited.

Objectives: This study aims to define clinical characteristics and outcomes for individuals with MPN and SVT at two tertiary referral centers in Minnesota.

Methods: We conducted a retrospective cohort study of MPN patients with SVT from 2 tertiary referral centers. We identified adults with a World Health Organization 2016 diagnosis of an MPN (polycythemia vera [PV], essential thrombocythemia [ET], myelofibrosis [MF], prefibrotic MF, and MPN-unclassifiable [MPN-U]) and confirmed SVT from 1/1/2012-3/31/2023. Subjects were included if the first SVT occurred after or within one year before MPN diagnosis. The primary outcome was cumulative incidence of recurrent Venous Thrombosis (VT) and arterial thrombosis (AT). Secondary outcomes were SVT recanalization and overall survival, with death as a competing risk.

Results: We identified 35 MPN patients (median age at first SVT 48 years; 57.1% female). MPN diagnosis preceded SVT in 42.9% of patients (median 3 years) and occurred simultaneously or within 1 year in 57.1% (median 8 days). PV was the most common MPN subtype (42.9%), followed by ET (17.1%), MF (17.1%), prefibrotic MF (5.7%), and MPN-U (2.9%). JAK2 was the predominant driver mutation (54.3%), followed by MPL (5.7%), while 40% had no reported/detected mutation. SVT occurred in the isolated portal (11.4%), hepatic (11.4%), mesenteric (2.9%), and splenic veins (5.7%), with 48.6% involving multiple vessels. Nearly all patients (97.1%) received anticoagulation, with Direct Oral Anti-coagulants (DOACs) being the most common (58.8%), followed by Vitamin K Antagonists (VKA) (29.41%) and Low Molecular Weight Heparin (LMWH) (8.82%). Only 2.9% received antiplatelet therapy without anticoagulation. During the average follow-up of 4.2 years, the cumulative incidence of recurrent VT and AT was 19% (recurrent SVT 20%; other VT 20%; AT 17.1%). Recanalization occurred in 40%, and overall survival was 74.3% (9 deaths).

Conclusion: While anticoagulation was almost universally used, with DOACs being the preferred agent, recurrent thrombotic events remained frequent and vessel recanalization occurred in less than half of patients.

Use of Therapeutic Plasma Exchange to Reduce or Prevent Bleeding Events in Rare Acquired FXIII-Related Bleeding Disorders

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Background: Acquired factor XIII (FXIII) and alpha-2-antiplasmin (A2A) deficiencies are rare bleeding disorders. Lack of real-time diagnostic testing and guidance for management, may lead to delayed diagnosis and suboptimal management of life-threatening bleeding, respectively [1].

Objective: Discuss use of therapeutic plasma exchange (TPE) to prevent or reduce major bleeding events in acquired FXIII-related bleeding disorders [2].

Methods: Single-center case series of clinical courses of two subjects with FXIII/A2A deficiencies managed with TPE.

Results: Patient #1 71-year-old male with no prior bleeding history presented with a large atraumatic muscular hematoma. Laboratory work up showed low FXIII antigen (9% ref 75-155%), low A2A (45% ref 82-133%), low fibrinogen (<60 mg/dL ref 170-490 mg/dL) and thrombocytopenia (70x109/L ref 150-450x109/L). No A2A inhibitory antibody (ab) was detected; however, non-inhibitory ab causing rapid FXIII and/or A2A clearance could not be excluded. Over the subsequent 4 years, he received immunosuppression (IS), including steroids, IVIG, rituximab, bortezomib/dexamethasone, mycophenolate mofetil, cyclophosphamide and daratumumab. Still, he continued to develop recurrent major bleeding events requiring use of cryoprecipitate, fibrinogen concentrate, FXIII concentrate and antifibrinolytic. However, these products resulted in volume overload, prompting the decision to initiate TPE with plasma for replacement. TPE caused transient improvement in bleeding, leading to its use during subsequent bleeding and prior to invasive diagnostic procedures along with blood products (now possible from a volume standpoint). Ultimately, he was diagnosed with myelodysplastic syndrome (MDS), resulting in a prolonged hospitalization and discharge on hospice. Through an unclear mechanism, MDS may be the driver of his acquired coagulopathy.

Patient #2 71-year-old male with recurrent bleeding events including traumatic subdural hematoma, atraumatic right leg and face hematomas, procedure-induced axillary hematoma, and hematuria. He had FXIII antigen of <4%, testing suggested an inhibitor. Despite IS (steroids, rituximab, cyclophosphamide, and IVIG), along with FXIII concentrate and tranexamic acid, bleeding continued. A series of TPEs was started leading to improvement in bleeding and FXIII antigen level. At follow up, 4 months from TPE, he remains in remission and IS is being tapered.

Conclusion: In case #1, TPE likely permitted for large-volume infusion of replacement products that was challenging at baseline due to volume overload. In case #2, TPE likely reduced FXIII ab titer permitting for adequate replacement with FXIII products. Together, these two cases suggest that TPE along with concurrent IS and factor replacement is a potential treatment strategy in cases of known or suspected FXIII and/A2A inhibitors with major bleeding. Further multi-center review of TPE in these scenarios should be considered.

Association between hypotension and acute kidney injury in hospitalized patients with cirrhosis

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Background:
Acute kidney injury (AKI) is a frequent complication in patients with cirrhosis, associated with substantial morbidity and mortality. Hypotension, another common complication of cirrhosis, poses unique management challenges in hospitalized patients. Blood pressure goals for those with cirrhosis are extrapolated from other populations. Despite AKI and hypotension often coexisting in this vulnerable population, the relationship between hypotension, and the incidence of AKI remains poorly elucidated in the inpatient setting. In this study, we examine the association between hypotension, vasopressor use, and the development of AKI in patients with cirrhosis admitted to the hospital.

Methods:
We conducted a single-center, retrospective cohort study of adult patients with diagnosis of cirrhosis admitted to a tertiary medical center between Feb 2011 and Dec 2023. Patients diagnosed with and without AKI were compared. Those already on hemodialysis or peritoneal dialysis prior to admission or who had history of kidney or liver transplant were excluded. AKI was defined according to the Acute Kidney Injury Network (AKIN) criteria, while hypotension was defined as a mean arterial pressure (MAP) < 65 mmHg.

Results:
A total of 6539 patients with cirrhosis were included in the analysis. Median age at time of admission was 57.9 years [IQR 47.7-65.7] and 43.8% were female (n=2864). Median MELD-Na score was 13.54 [6.89-24.58]. 31.33% of patients were admitted to the ICU during their hospitalization (n=2049) and 13.64% required vasopressor use (n=892). Median length of total hospitalization was 5 days [3-10].

AKI occurred in 29.6% of patients (n=1935): AKIN-1 17.5% (n=1174), AKIN-2 6% (n=391), and AKIN-3 5.7% (n=370) with 8.3% (n=541) requiring hemodialysis during hospitalization. Hypotension occurred in 51.7% of patients (n=3373). Demographics and outcomes for those with and without hypotension are described in Table 1. Seven (0.1%) patients received a liver transplant during hospitalization and 609 (9.3%) received a liver transplant after hospitalization. Hypotension was strongly associated with AKI (OR 5, p<0.001). Thirty-day, ninety-day, and one-year mortality was 0.23, 0.36, and 0.53 respectively. Hypotension was associated with a higher mortality rate even when adjusted to age (OR 1.58, p<0.001).

Conclusions:
AKI is a highly prevalent complication among hospitalized patients with cirrhosis, with significant implications for morbidity, resource utilization, and mortality. The presence of hypotension strongly correlates with the development of AKI. Future prospective studies are essential to identify therapeutic targets and improve outcomes.

Esophageal Dysfunction in Patients with Subglottic Stenosis: A Descriptive Retrospective Analysis

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Introduction: Subglottic stenosis (SGS) is a complex medical condition that largely affects women between the third and fifth decades of life. SGS can be caused by trauma, systemic inflammation, or congenital anomalies. However, most cases are ultimately deemed idiopathic. Studies implicate gastroesophageal reflux disease as a potential cause of SGS; however, the diagnostic standard and pathogenesis remains unknown. We aimed to assess the esophageal function characteristics in a large SGS population to better understand possible esophageal etiologies of SGS.

Methods: We conducted a single-center, retrospective review of all non-pregnant adults diagnosed with SGS between 1/1/2014 to 6/15/2024. Patients without formal diagnosis by one of our otolaryngology SGS specialists and without esophageal function testing (esophagogastroduodenoscopy (EGD), high resolution esophageal manometry (HRM), pH testing, or barium esophagram) were excluded.

Results: Out of 441 SGS patients screened, 72 were included. 48.2% (27/56) of patients with an EGD had abnormal esophageal findings. 12.5% (7/56) had erosive esophagitis: Los Angeles grade A (5/7, 71.4%) and C (2/7, 28.6%). 7.1% (4/56) had Barrett's Esophagus: 50% long segment (≥ 3 cm). 25.0% (14/56) of patients had a hiatal hernia: Hill grade II (1/14, 7.1%), III (5/14, 35.7%), IV (3/14, 21.4%), and not reported (5/14, 35.7%). 50% (7/14) of hiatal hernias were > 2 cm. Three patients had gastroparesis, diagnosed by gastric emptying study (2/3, 66.6%) and EGD/esophagram (1/3, 33.3%). Thirteen patients had a HRM (Figure 1). Median supine integrated relaxation pressure was 21.3 (IQR 20.3-25.1) and 12.3 (IQR 0) mmHg on abnormal and normal HRM testing, respectively. 23.6% (17/72) had pH testing, 76.5% (13/17) of which were off therapy. 52.9% (9/17) of pH testing was abnormal, concerning for acid reflux. Mean acid exposure time was 9.3% (+/- 10.3%) and 4.7% (+/- 3.7%) for pH-impedance (10/17, 58.8%) and wireless pH (7/17, 41.2%) testing, respectively. 55.6% patients had a barium esophagram, 12.5% (5/40) had a timed barium esophagram (TBE). Mean height of column at one minute was 0.7 (+/- 0.99) cm. Esophageal dysmotility, intraesophageal reflux, or delay of barium tablet was seen in 62.5% (25/40) of studies.

Conclusion: This is the largest descript study of its kind on characteristics of patients with SGS as it pertains to esophageal function testing. Our results suggest there may be clinically significant abnormalities on esophageal function testing in patients with SGS. These results underscore the importance of comprehensive esophageal testing, as it may reveal underlying conditions that could contribute to the development or progression of airway stenosis. Future comparative studies will be essential to better understand the impact of reflux and esophageal dysmotility on SGS.

The Decade-long Potassium Puzzle: Betel Nut Chewing as the Etiology of Chronic Hypokalemia

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Introduction: Alternative medicines, such as herbal supplements, can have overlooked physiological effects, including drug interactions and electrolyte imbalances. Betel nut, commonly used in Asia and Africa, poses significant health risks, including metabolic disorders and addiction. This case report highlights chronic hypokalemia, hypophosphatemia, and hypercalcemia linked to betel nut chewing. Unlike previous reports focusing on hypercalcemia and metabolic alkalosis, this case highlights severe, recurrent hypokalemia as the primary symptom, underscoring the need for a thorough supplement history when evaluating unexplained electrolyte abnormalities.

Case: A 37-year-old Karen-speaking woman from Thailand with a history of type 2 diabetes, chronic hepatitis B, and depression presented in 2014 with asymptomatic hypokalemia (3.0 mmol/L; normal: 3.4-5.3). Over the next decade, she had recurrent hypokalemia (1.9-3.3 mmol/L), frequently requiring emergency department visits. Tests also showed hypercalcemia (10.2-10.8 mg/dL), hypophosphatemia (0.8-1.9 mg/dL), and metabolic alkalosis. Renal ultrasound showed medullary nephrocalcinosis. Further investigation revealed normal plasma aldosterone/renin levels and a low urine potassium-to-creatinine ratio (0.17 mEq/mmol), indicating an extrarenal source of potassium loss, and chronic betel nut chewing emerged as a potential contributor. Language barriers, low health literacy, and medication non-adherence complicated her management and delayed diagnosis. Despite counseling on its harmful effects, the patient declined to discontinue use.

Discussion: The betel nut is consumed for its purported health benefits and stress-relieving properties. However, its chronic use has been associated with numerous health risks. Hypokalemia, hypophosphatemia, hypercalcemia, and metabolic alkalosis are linked to preparing the betel nut with alkaline calcium salts to improve taste. These salts increase intestinal calcium absorption and bind phosphate, causing hypophosphatemia. The resulting hypercalcemia induces a Bartter 's-like syndrome, inhibiting the Na-K-2Cl cotransporter in the loop of Henle, mimicking a loop diuretic and causing renal potassium loss. This renal potassium loss, combined with likely gastrointestinal potassium losses, resulted in persistent hypokalemia. While this case links betel nut to hypokalemia, other herbal remedies like licorice, Diospyros rhodocalyx, and oolong tea can also cause it. This case underscores the importance of considering cultural practices and alternative medicine use when diagnosing complex electrolyte disorders.

Learning points:

1. Betel nut chewing can cause multiple electrolyte abnormalities, including hypokalemia, hypercalcemia, metabolic alkalosis, and hypophosphatemia.
2. Healthcare providers should always obtain a detailed history of supplement use and be conscious of how a patient’s cultural background may influence their disease presentation.

Kidney Dysfunction in Wild-Type Transthyretin Cardiac Amyloidosis: Prevalence and Progression Over Time

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Background:

Wild-type transthyretin amyloidosis (wtATTR) can lead to cardiac amyloidosis (ATTR-CA) and is associated with kidney involvement. While renal dysfunction is a known prognostic factor in ATTR-CA, the evolution of kidney disease (KD) *prior to and following* diagnosis is not fully characterized. Understanding the trajectory of kidney function is crucial for early identification and management.

Objective:

This study aimed to characterize the prevalence and progression of KD in patients with wtATTR-CA, focusing on changes in renal function before and after diagnosis.

Methods:

A retrospective cohort study was conducted at the University of Minnesota (2011-2024). Patients with confirmed wtATTR-CA (histological or non-biopsy criteria) were included; those with AL amyloidosis were excluded. Electronic medical records were reviewed, with the ATTR-CA diagnosis date serving as the index date. The prevalence of CKD stage ≥3b (eGFR < 45 mL/min/1.73m²) was examined at 5, 3, and 1 year(s) pre-diagnosis, at diagnosis, and 1-, 3-, and 5-years post-diagnosis using generalized linear mixed effects models with a natural cubic spline for time. The presence of comorbidities (type 2 diabetes [T2DM], hypertension [HTN], heart failure [HF]) and medication use (diuretics, SGLT2 inhibitors) were also reported.

Results:

The study included 108 patients with wtATTR-CA. 15% of the patients had T2M, 28% HTN, 33% HF, 23% were on diuretics, and 4% on SGLT2 inhibitors at the time of ATTR-CA diagnosis. Prevalence of CKD Stage ≥3b trended up from 9.7% at 5 years pre-diagnosis, to 21.3% at ATTR-CA diagnosis. The prevalence was however 16.7% at 5 years post-diagnosis for the patients that remained at follow up.

Conclusions:

Preliminary analysis demonstrates a significant prevalence of advanced CKD in patients with wtATTR-CA prior to diagnosis suggesting that KD could precede by many years cardiac involvement in these patients and likely be driven by both kidney involvement and cardiorenal syndrome with its prevalence increasing significantly up to diagnosis. These findings highlight the importance of longitudinal renal monitoring in this population, beginning well before the clinical presentation of ATTR-CA. Further analysis will explore the relationship between kidney function decline, comorbidities, medication use, and overall prognosis.

A challenging case of renal sarcoidosis presenting with severe hypercalcemia and a normal 1,25 OH vitamin D level: diagnostic and therapeutic considerations

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Introduction:

Renal sarcoidosis is a rare manifestation of systemic sarcoidosis and presents with kidney dysfunction secondary to hypercalcemia, interstitial nephritis with or without granuloma formation and nephrocalcinosis. Elevated 1,25 dihydroxy vitamin D levels are usually supportive of the diagnosis due to increased production of 1,25 dihydroxy vitamin D by activated mononuclear cells in granulomas.

Case Description:

A 53-year-old male with a past medical history significant for obesity, mild intermittent asthma, and poorly controlled hypertension, and type 2 diabetes presented for evaluation of worsening CKD. His creatinine (Cr) level was 2.73 mg/dL significantly worse than a year prior when it was 1.96 mg/dL. He also had a history of regular NSAIDs use which were discontinued. His calcium (Ca) was elevated at 10.8 mg/dL, and it had been borderline high for the prior 2 years. A hypercalcemia workup revealed a PTH level at 14 pmol/mL (15-65) , a non-significantly elevated PTH-rp level at 4.4 pmol/L (0-2.3), a normal 1,25 dihydroxy-vitamin D level at 49.6 pg/mL (19.9 - 79.3) and a low 25 hydroxy-vitamin D at 19 ng/mL (20-50). A workup for monoclonal gammopathy including a serum protein electrophoresis with immunofixation and a serum free light chain assay was also negative. His CRP level was elevated at 37 mg/L (< 5 mg/l). Given the non-diagnostic yield of the usual work up for hypercalcemia, a PET scan was ordered, and revealed bilateral hilar and mediastinal lymphadenopathy, and some mild abdominal lymphadenopathy. He underwent endobronchial ultrasound-guided lymph nodes biopsies which revealed non-caseating granuloma. As his pulmonary function tests were normal, he was not deemed candidate for treatment from a pulmonary perspective. He refused a kidney biopsy and was subsequently initiated on 40 mg of prednisone daily for empiric treatment of renal sarcoidosis. His Ca level normalized and his Cr level improved to 1.98 mg/dL. His CRP level also normalized. Due to uncontrolled diabetes, his prednisone was tapered over two months, however four months later he presented with recurrent hypercalcemia at 13.4 and severe acute kidney injury. He was reinitiated on prednisone 20 mg daily with subsequent normalization of his Ca level and improvement of his kidney function. He was thereafter switched on a steroid sparing immunosuppression regimen using mycophenolate mofetil (MMF) 1 g twice daily with stabilization of his kidney function and Ca levels.

Conclusion:

The significant improvement in calcium levels and kidney function with prednisone followed by MMF suggest the presence of significant renal sarcoidosis. Vitamin D levels were not elevated alluding to additional mechanisms contributing to hypercalcemia in renal sarcoidosis.

Opportunities to Increase Nonselective Beta-blocker Use in Compensated Advanced Chronic Liver Disease

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Background: Recent AASLD and Baveno VII guidelines recommend nonselective beta-blockers (NSBBs) for patients with compensated cirrhosis (CC), compensated advanced chronic liver disease (cACLD), and clinically significant portal hypertension (CSPH) instead of esophagogastroduodenoscopy (EGD)for variceal screening. CSPH diagnosis is guided by transient elastography (TE) and platelet count. This study aims to evaluate the potential for increasing NSBB use among patients undergoing EGD for variceal screening.

Methods: Data were extracted from the endoscopy database at the Minneapolis VA Medical Center. Patients with CC or cACLD who underwent EGD for variceal screening between October 1, 2023, and September 30,2024, were included. CSPH was determined based on TE and platelet count or evidence of portal hypertension on imaging. We assessed patient characteristics, NSBB eligibility, the presence and grade of varices, and trends in NSBB use before and after the publication of the AASLD guidance in May 2020.

Results: Among 71 patients with CC or cACLD who underwent EGD for variceal screening, the majority (77.7%) had steatotic liver disease (45.8% with metabolic dysfunction-associated steatotic liver disease [MASLD] and increased alcohol intake [MetALD], 20.8% MASLD, and 8.3% alcohol-related liver disease). CSPH was present in 78.7% of patients, but only 12.6% were diagnosed with CSPH based on TE and platelet count. Of all patients, 64.7% were eligible for NSBB therapy based on TE, platelet count, or imaging findings, while 14% had contraindications to NSBB, necessitating EGD. In 21.3% of cases, CSPH was not evident, suggesting that TE and platelet count monitoring, with NSBB initiation when indicated, could have sufficed. None of the patients undergoing EGD had varices requiring intervention. Comparisons before and after the May 2020 guidance revealed no significant changes in NSBB use (Table 1).

Conclusion & Future Directions: Approximately two-thirds of EGDs performed for variceal screening in this single-center study could have been avoided with NSBB initiation based on guideline-recommended TE and platelet count. These findings emphasize the need to optimize NSBB use in patients with cACLD and CSPH. Additionally, they highlight the importance of integrating TE into clinical practice to enhance adherence to evidence-based care and improve patient outcomes. Therefore, we will collaborate with the hepatology team to implement a standardized decision-making process and adherence to evidence-based care and develop flowcharts to streamline CSPH diagnosis and NSBB use. This will enhance patient safety, reduce costs, and improve adherence to evidence-based care.

Outcomes in Pancreatic Transplantation with or without Kidney Transplantation After Lung Transplantation

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Purpose: We present the largest known case series describing people with cystic fibrosis (PwCF) who have undergone bilateral lung transplant, followed by simultaneous pancreas-kidney transplantation (SPK) or pancreas after kidney transplantation (PAK). Recipients of SPK or PAK had ESRD, cystic fibrosis related diabetes (CFRD), and pancreatic insufficiency (PI). The case series primarily describes subsequent lung allograft function, patient survival, and liberation of endocrine and exocrine pancreatic supplementation.

Methods: Retrospective case series, using chart review and transplant database. The project involved chart review of five University of Minnesota patients who received bilateral lung transplant followed by SPK or PAK from 1/1/2006-present to compile the clinical characteristics of those cases.

Results:

Patient	Year of transplant			Most recent A1c	Clinical BOS	ISHLT BOS	PERT	Insulin	# of admits in the last year
	Lung	Pancreas	Kidney						
1	5	<1	<1	5.7	No	No	No	No	1
2	17	11	11	4.8	No	No	No	No	0
3	22	5	7	5.4	Yes	Yes	No	No	1
4	19	9	9	5.3	Yes	No	No	No	0
5	21	7	7	5.5	Yes	No	No	No	0

Clinical BOS= Decline in PFTs treated empirically for BOS

ISHLT BOS = Decline in FEV1 >20% for >3 months

Conclusion: This case series represents the largest known cohort having undergone bilateral lung transplant followed by SPK or PAK. All patients in the cohort survived. Notably, all no longer require pancreatic enzyme replacement (PERT) for cystic fibrosis related PI. None require insulin for treatment of CFRD with HbA1c which is well controlled. Although 3 patients were started on empiric treatment for BOS based on declining PFTs, only one patient met the ISHLT criteria for BOS1. PFTS stabilized in all patients. Given positive outcomes and liberation from both endocrine and exocrine exogenous pancreatic supplementation, SPK or PAK appears to be a safe and effective option for PwCF with CFRD and PI who have undergone bilateral lung transplantation and developed subsequent ESRD.

Phenotypes of Non-Ischemic Refractory Ventricular Tachycardia/Ventricular Fibrillation Out-Of-Hospital Cardiac Arrest Patients Receiving Extracorporeal Cardiopulmonary Resuscitation

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Background: Severe coronary artery disease underlies the majority of refractory ventricular tachycardia/ventricular fibrillation out-of-hospital cardiac arrest (rVT/VF OHCA).However, the causes of non-ischemic rVT/VF OHCA requiring ECPR remain unexplored.

Methods: We conducted a retrospective cohort study of all patients receiving ECPR for refractory VT/VF OHCA at our center between 2016-2023 to evaluate the etiologies of non-ischemic rVT/VF OHCA. Ischemic rVT/VF OHCA was defined by acute coronary thrombosis, MINOCA, or severe chronic coronary artery disease on admission coronary angiography. All other patients were defined as non-ischemic rVT/VF OHCA. In-hospital survival was compared between patients with ischemic and non-ischemic rVT/VF OHCA. Non-ischemic subtypes of rVT/VF OHCA were reported in survivors using descriptive statistics.

Results: Among 446 patients who received ECPR during the study period, the majority had an ischemic cause of their rVT/VF OHCA (Figure 1,Panel A). The in-hospital survival was 32.3% for ischemic rVT/VF OHCA and 33.8% for non-ischemic rVT/VF OHCA (p=0.76).Among patients that survived the hospitalization and had a non-ischemic etiology for rVT/VF OHCA, >50% had genetic cardiomyopathy or aborted sudden arrhythmic death (Figure, Panel B).

Conclusion: The causes of non-ischemic rVT/VF OHCA are diverse.

SILENT AND DEADLY: AN UNRECOGNIZED SILENT MYOCARDIAL INFARCTION WITH CATASTROPHIC CONSEQUENCES

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Introduction:
Myocardial infarctions (MIs) are commonly suspected in patients with chest discomfort or other cardiac symptoms in the setting of known coronary artery disease (CAD) or established cardiac risk factors. However, “Silent MIs” tend to present without cardinal symptoms and often go unrecognized in the outpatient setting. Consequently, people with unrecognized MIs are left untreated, which puts them at risk for further complications.

Case Presentation:
A 76-year-old male, with no prior history of CAD, was seen in the primary care clinic for his annual exam. A 12-lead electrocardiogram (ECG) was obtained during the visit, and the provider did not recognize a set of premature ventricular contractions (PVCs) revealing a new infarct pattern in the anterior precordial leads.

Twelve days later, he went to the emergency department (ED) for nausea, vomiting, and diarrhea. He was discharged with a diagnosis of gastroenteritis. Four days thereafter, his symptoms progressed in addition to new onset dyspnea and excessive fatigue.

He returned to the ED in significant respiratory distress and also found to be hypotensive. A repeat ECG showed new evidence of inferolateral ST depression and T wave inversions. High-sensitivity troponin > 30,000, and a stat echocardiogram (ECHO) showed an ejection fraction (EF) of 35% with a large wall motion abnormality involving the left anterior descending (LAD) territory.

The patient's condition rapidly deteriorated, warranting intubation. With a diagnosis of cardiogenic shock, he was sent to the cardiac catheterization lab where he was found to have severe three-vessel disease with chronic total occlusions of the LAD, second diagonal, posterior descending, posterior atrioventricular, and posterolateral arteries. There was also 95% occlusion in the second obtuse marginal suggestive of a culprit lesion. Due to a low cardiac index and elevated filling pressures, an intra-aortic balloon pump was placed, and pressors were started. Twelve days later, the patient was stable enough to be taken for open heart surgery.

He was discharged three weeks later and was fully independent with daily living activities. His prior-to-discharge ECHO demonstrated an EF of 25% with moderate mitral regurgitation.

Discussion:
This case highlights the importance of recognizing clinically silent infarct patterns on ECGs. Approximately 20-30% of acute MIs are either asymptomatic or associated with non-specific flu-like symptoms. In this case, the fortuitous occurrence of PVCs revealed a clear anterior infarct. If the ECG finding had been recognized at that clinic visit, then potentially, the nearly fatal cardiogenic shock episode, that occurred two weeks later, could have been avoided. It is important for all providers, especially primary care, to identify these infarct patterns and to seek an expert’s opinion if they are uncertain. Early recognition of “Silent MIs” allows for early intervention and lowers the risk of subsequent catastrophic events.

Synchronous mantle cell lymphoma and multiple myeloma: a case report

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Mantle cell lymphoma is a relatively rare, male-predominant, non-Hodgkin B cell lymphoma with heterogeneous natural history. Constitutive overexpression of cyclin D1 is a prominent feature, a result of a translocation that places *CCND1* under the regulation of the *IGH* gene locus. Interestingly, the t(11;14) translocation that produces this dysregulation is seen in other hematologic malignancies, most notably multiple myeloma, itself more prevalent than mantle cell lymphoma.

Here, we report the case of a previously healthy 54-year-old man presenting with lymphadenopathy and B symptoms and found to have lymphocytosis and anemia, all suggestive of a hematolymphoid malignancy.

Morphologic and immunophenotypic evaluation of nodal tissue was consistent with mantle cell lymphoma, with positivity of CD20, PAX5, CYCLIN D1, SOX11, and CD38, among others. Cytogenetics completed on the bone marrow revealed a CCND1::IGH fusion, but tissue analysis could not exclude a concurrent plasma cell neoplasm. The differential diagnosis at the time thus included mantle cell lymphoma with plasmacytic differentiation versus concurrent mantle cell lymphoma plus a plasma cell neoplasm.

Given his Ki-67 (40%) and other markers, his mantle cell lymphoma international prognostic index (MIPI) was estimated at 7.3, placing him in the highest-risk category.

After receiving treatment for high-grade mantle cell lymphoma, including rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone (RCHOP), and rituximab, dexamethasone, high-dose cytarabine, and cisplatin (RDHAP), restaging bone marrow biopsy showed a persistent kappa-restricted plasma cell clone, consistent with multiple myeloma. The patient then received myeloma-focused therapies with bortezomib and dexamethasone augmented by daratumumab for the added benefit of further controlling the mantle cell lymphoma.

Once remission from both diseases was achieved, he underwent high-dose melphalan therapy with auto stem cell transplantation rescue. Subsequently, maintenance regimen with lenalidomide and rituximab was instituted, so far without recurrence of either disease.

Diagnostic ambiguity is often a feature of clinical management. This case is informative as an example of prioritizing the more aggressive pathology when there is ambiguity, as well as a window into the biology and genesis of both mantle cell lymphoma and multiple myeloma.

A Case of Hydralazine induced Vasculitis and Lupus Nephritis

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Hydralazine is a common medication typically used in the treatment of hypertension. In patients with chronic use, cases of drug induced lupus nephritis as well as antineutrophil cytoplasmic antibodies(ANCA) associated vasculitis are known to occur. We present a unique case of a 73 year old female who presented with respiratory failure complicated by cough with hemoptysis, anemia, confusion, and worsening weakness in the setting of long term hydralazine usage. Prior to symptom onset, she had developed a petechial rash on her legs bilaterally.

Laboratory findings revealed acute kidney injury (AKI) with proteinuria. Serologic testing demonstrated a positive ANA with a titer of 1:1280 with a homogeneous pattern, an ANCA titer of 1:5120 in a P-ANCA pattern. MPO and PR3 were both seen to be positive along with the antiphospholipid (APS) labs, and direct antiglobulin test (DAT). The C3 and C4 complement levels were decreased at 69 and 5 respectively, with anti-histone antibodies found to be positive. Of note, DsDNA, anti-smith antibodies, anti-GBM, along with Ro/La antibodies were seen to be negative. Computed tomography (CT) at the time showed concern for diffuse alveolar hemorrhage. Kidney biopsy was pursued and revealed immune complex mediated glomerulonephritis with focal proliferative pattern that was consistent with lupus nephritis class 3. Immunofluorescence was seen to have a full house pattern with electron microscopy revealing mild/moderate foot process effacement. Hydralazine was stopped and the patient was started on high dose steroids and cyclophosphamide. Unfortunately, the patient's creatinine continued to rise and the decision was made to start her on rituximab. The patient subsequently showed clinical improvement and was able to be discharged with close outpatient follow up. This case highlights a rare, but potentially disastrous side effect of hydralazine in a patient who developed both Lupus nephritis as well as ANCA vasculitis after long term use of hydralazine.

RENAL OUTCOMES IN PATIENTS ON EXTRACORPOREAL MEMBRANE OXYGENATION

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Background: Extracorporeal membrane oxygenation (ECMO) is a life-saving intervention for patients with cardiac arrest. This study aims to evaluate the incidence of acute kidney injury (AKI) and renal replacement therapy (RRT) outcomes in out-of-hospital cardiac arrest patients requiring ECMO.

Methods: A retrospective chart review was conducted at the University of Minnesota, analyzing patients who received ECMO following out-of-hospital cardiac arrest. AKI was defined using KDIGO criteria. Patients surviving >48 hours were included. Incidence of AKI, RRT requirement, and successful RRT weaning were assessed.

Results: Of 495 initial patients, 225 (45%) developed AKI within 48 hours. Among these, 79 (35%) required RRT. Mortality was high (85%) in RRT patients, with 67/79 dying before discharge. Of 12 patients (15%) discharged alive, 8 were dialysis free before discharge and 3 were successfully weaned off dialysis within 3 months of discharge. At one-year follow-up, only one patient remained dialysis-dependent. Mean RRT initiation time was 4.8 days (median 2 days) post-ECMO cannulation. The incidence of AKI (45%) and severe AKI requiring RRT (36%) was lower than previously reported in meta-analyses (62.8% and 44.9%, respectively).

Conclusion: This largest single-center study of out-of-hospital cardiac arrest patients requiring ECMO reveals a lower incidence of AKI and RRT requirement than previously reported. While mortality remains high among RRT patients, survivors show promising rates of dialysis independence. These findings contribute valuable insights into renal outcomes and may inform clinical decision-making for this critical patient population.

Clinical Implications (ACC Requirement): My study will help cardiovascular clinicians to better anticipate and manage renal complications in out-of-hospital cardiac arrest patients receiving ECMO treatment.

The Relationship between Systemic Estrogen Use and Sarcopenia in Early Menopausal Women: An Analysis of the National Health and Nutrition Examination Survey (NHANES)

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Background/Objective: Sarcopenia is characterized by a loss of muscle strength and muscle mass. With aging, muscle function declines prior to muscle mass. Estrogen may offer protection against age-related muscle weakness in women, and the timing of treatment (early versus late) could be critical. Most studies on estrogen and sarcopenia include participants with a wide age range and focus primarily on muscle mass, which declines after muscle function. We conducted a study to examine the association between systemic estrogen use in early menopausal women and its effects on both muscle strength and muscle mass, using publicly available data from the NHANES 2011-2012 and 2013-2014.

Methods: Eligibility criteria included menopausal women aged 50-60 who had measurements of muscle strength and muscle mass (grip strength and skeletal muscle index). The history of systemic estrogen use was assessed using a reproductive health questionnaire. Simple linear regression analysis was used to determine the relationship between systemic estrogen use and muscle strength and muscle mass.

Results: A total of 258 early menopausal women aged 50-60 had both grip strength and skeletal muscle index (SMI) data available for analysis. Among those with data on systemic estrogen use, 24 participants used an estrogen patch or a combined patch, while 83 participants used estrogen pills or combined pills. The use of an estrogen patch was associated with improved grip strength, with estimated effects of 10.3 kg (SE 1.1, 95% CI: 8.0-12.7) for the first hand and 12.9 kg (SE 1.2, 95% CI: 10.3-15.4) for the second hand. In contrast, the use of a combined patch was associated with reduced grip strength, with estimated effects of -10.1 kg (SE 1.2, 95% CI: -12.7 to -7.6) for the first hand and -12.7 kg (SE 1.3, 95% CI: -15.4 to -9.9) for the second hand. The estrogen patch was not significantly associated with changes in SMI (estimate: -0.2 kg, SE 0.2, 95% CI: -0.6 to 0.2). Similarly, the combined patch was not significantly associated with changes in SMI (estimate: 0.1 kg, SE 0.2, 95% CI: -0.2 to 0.4).

Conclusions: Our analysis revealed that the use of an estrogen patch is associated with improved muscle strength in early postmenopausal women compared to no hormone treatment therapy. However, the use of a combined patch is associated with reduced muscle strength. The use of estrogen pills or combined pills is not associated with significant changes in either muscle strength or muscle mass.

Cellular Senescence: A Predictor of Aging and Frailty in Liver Transplant Recipients

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Purpose: Patients undergoing liver transplantation are at a higher risk of death and increased healthcare utilization the frailer they are. Previous studies associated clinical measures of frailty with post-liver transplant outcomes and demonstrated the efficacy of measuring *p16^{INK4a}* expression in peripheral blood T (CD3^{+}) cells as an indicator of aging. The purpose of this prospective study is to correlate clinical indicators of frailty with a surrogate biomarker of aging and senescence in liver transplant recipients.

Methods: Patients undergoing liver transplantation at a single center were consented and enrolled pre-transplant between 09/2022-11/2022. Clinical frailty assessments were performed in accordance with the previously established Liver Frailty Index™. CD3^{+} cells were isolated from blood samples taken at time of enrollment, pre-transplant. RT-qPCR was utilized to evaluate *p16^{INK4a}* expression. Briefly, RNA isolation was performed using the Qiagen RNeasy Mini Kit, RT via Multiscribe™, and qPCR using the TaqMan™ qPCR assay with a custom *p16^{INK4a}* probe. *18s* rRNA was used as a housekeeping control. Statistical analysis was performed in GraphPad Prism v 9.4.1 (458).

Results: Seven of ten enrolled patients (70%) presented with alcoholic steatohepatitis as an indication for liver transplant and three (30%) presented with primary biliary cholangitis. The median length of stay was 12 days (IQR 6-34). Pearson correlation analysis of baseline frailty score and baseline *p16^{INK4a}* expression demonstrates a statistically significant direct relationship ($r = 0.7356$, p-value = 0.0153). Furthermore, baseline *p16^{INK4a}* expression and patient length of stay demonstrate a statistically significant direct relationship ($r = 0.7036$, p-value = 0.0232). Interestingly, baseline *p16^{INK4a}* expression did not correlate well with Sodium Model for End-Stage Liver Disease (NaMELD) or patient age ($r = 0.2960$, p-value = 0.4063; $r = 0.0591$, p-value = 0.8713). Normalized *p16^{INK4a}* expression at 30-day post-transplant to baseline expression revealed variability.

Conclusions: It appears that *p16^{INK4a}* expression is highly correlated with baseline clinical frailty, and that frailty is manifested by a longer length of stay. Moreover, age and NaMELD demonstrated a weak, statistically insignificant correlation with *p16^{INK4a}* expression. The small sample size, lack of patient diversity, and *p16^{INK4a}* expression need greater correlation with clinical events and patient complications.

Unraveling the Link Between Autonomic Dysfunction and Long COVID: Insights from Quantitative Testing

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Introduction: The COVID-19 pandemic, caused by SARS-CoV-2, infected millions worldwide between 2020-2023. While most individuals recover within weeks, a significant proportion – estimated in the millions globally – experience persistent symptoms beyond 12 weeks, termed long COVID. Symptoms of long COVID, including fatigue, postural intolerance, and cognitive dysfunction, suggest infection-induced autonomic dysfunction (AD), but the presence, nature, and severity of AD in this setting is poorly defined.

Purpose: To characterize suspected AD in long COVID patients by comparing autonomic function measures between long COVID patients, healthy controls, and individuals with known pure autonomic failure (PAF).

Methods: This retrospective study reviewed 341 patient charts from 2019 to 2023 of individuals referred for autonomic testing due to symptoms such as palpitations, lightheadedness, syncope, and postural tachycardia. Patients were categorized into three groups: (1) long COVID (persisting symptoms ≥12 weeks post-acute infection), (2) age- and sex-matched controls (COVID-negative, normal autonomic tests), and (3) PAF (COVID-negative, multiple abnormal autonomic tests). After excluding individuals who were <12 weeks post-COVID or who had predisposing nervous system co-morbidities, the study population comprised 78 long COVID patients, 25 controls, and 38 PAF patients. Beat-to-beat heart rate and blood pressure were recorded during Active Standing (AS), Valsalva maneuver, Respiratory Sinus Arrhythmia (RSA), and Tilt Table testing (TT). Statistical significance ($p < 0.05$) was assessed using Student's t-tests, Kruskal-Wallis tests, Multilinear Regression, or Fisher's test, as appropriate.

Results: Long COVID patients (84% female, 23-50 years, 14±8.6 months post-infection) exhibited a different autonomic profile compared to controls (76% female, 21-39 years) and PAF patients (52% female, 56-74 years). Specifically, Valsalva ratio and average RSA values were lower in long COVID patients compared to controls ($p=0.02$, $p<0.001$) but were higher than in PAF ($p<0.001$ for both) [**Table 1**]. The immediate blood pressure drop during AS was greater in long COVID versus controls ($p<0.001$) but less severe than in PAF ($p=0.002$). Heart rate increase during TT was more pronounced in long COVID compared to both controls and PAF ($p=0.01$, $p=0.001$). After age-and-sex adjustment, AD severity in long COVID patients was comparable to that observed in PAF, with dysfunction persisting during follow-up for up to 40 months.

Conclusion: Long COVID patients demonstrate AD comparable in severity to PAF when adjusted for age and sex. This dysfunction is characterized primarily by impaired parasympathetic activity and abnormal cardiovascular responses to orthostatic stress and can persist for extended periods. These findings support the utility of quantitative autonomic testing in long COVID patients to better understand patient disability and guide treatment strategy.

Integrated CTC- and EV-based detection of PSMA protein and efficacy of ^{177}Lu-PSMA-617 radioligand therapy.

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Background: Blood-based predictive biomarkers of sensitivity to ^{177}Lu-PSMA-617 are lacking, and may facilitate clinical decisions. Here, we studied whether integrated PSMA protein detection in circulating tumor cells (CTCs) and extracellular vesicles (EVs) is associated with outcomes in patients receiving ^{177}Lu-PSMA therapy.

Methods: We enrolled 100 metastatic castrate-resistant prostate cancer (mCRPC) pts who were candidates for ^{177}Lu-PSMA into a prospective biomarker trial. Blood samples were collected for CTC and EV analysis at baseline, at the time of response, and at progression. Baseline characteristics included serum PSA, alkaline phosphatase (ALP), hemoglobin, albumin, and radiographic tumor burden. PSMA+ CTCs were enumerated using an AI-empowered holographic imaging platform combined with in-flow protein marker analysis (Astrin Biosciences, St. Paul, MN); PSMA protein was quantified in plasma EVs using shotgun proteomics via mass spectrometry (Arafa et al., *Cancers* 2024; 16: 4261). We assessed the impact of PSMA+ CTCs and EV-derived PSMA protein on PSA_{50} responses, PFS, and OS. Multivariable Cox regressions were used to adjust for baseline PSA, ALP, and hemoglobin. Exploratory analyses of other EV-derived proteins were also conducted.

Results: Of 100 enrolled pts, 47% had Gleason sum 9-10, 62% had > 10 bone mets, 12% had visceral mets, 72% had received ≥3 prior systemic therapies, and median PSA was 57 (range 1.5–5,000) ng/mL. High PSMA+ CTC counts (> median) were associated with shorter overall survival (OS) (HR 2.71, 95%CI 1.18–6.21, p = 0.02). PSA_{50} response rates were similar for those with high and low PSMA+ CTC counts (39% vs 42%, p = 0.8). Shotgun proteomics from plasma EV samples identified > 11 000 unique proteins, of which 12% represented the cell surfaceome. EV-PSMA protein correlated with baseline PSA, ALP, and tumor burden (all $p < 0.05$). High EV-PSMA protein (> median) was associated with worse OS (1.81, 95%CI 0.97–3.35, p = 0.06). PSA_{50} response rates were similar for those with high and low EV-PSMA protein (48% vs 42%, p = 0.5). After multivariate adjustment, nonsignificant trends for shorter OS persisted for pts with high PSMA+ CTCs (HR 1.71, 95%CI 0.72–4.05) and high EV-PSMA levels (HR 1.49, 95%CI 0.78–2.84). Worse OS was also observed in pts with high EV levels of B7-H3 (HR 2.85, 95%CI 1.58–5.14, p = 0.002), Trop-2 (HR 2.23, 95%CI 1.22–4.05, p = 0.008), and STEAP1 (HR 1.69, 95%CI 0.93–3.06, p = 0.08) proteins.

Conclusions: In mCRPC pts receiving ^{177}Lu-PSMA, high PSMA+ CTC counts and high EV-derived PSMA levels portended poor survival. PSMA protein may be a novel blood-based biomarker of ^{177}Lu-PSMA sensitivity, facilitating treatment decisions, with relevance for other PSMA-targeting strategies. The robust detection and prognostic impact of additional cell-surface proteins (*e.g.* B7-H3, Trop-2, STEAP1) may fuel the development of alternative novel therapeutics.

Machine Learning in CT Head to Predict Neurological Outcomes in ECPR Patients

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Background: Predicting neurological outcomes in patients undergoing extracorporeal cardiopulmonary resuscitation (ECPR) remains a significant challenge. Head CT scans are routinely performed in ECPR patients to identify severe brain injuries and aid in multimodal neuroprognostication. However, despite serial imaging, predicting neurological recovery remains difficult. Some patients exhibit unremarkable head CT findings yet fail to regain consciousness. We hypothesize that subtle imaging patterns that were undetectable by human CT readers, may be detected by artificial intelligence (AI) and provide prognostic value. This study uses Convolutional Neural Network (CNN), a type of machine learning, to analyze head CT scans and predict neurological outcomes in ECPR patients.

Method: This retrospective cohort study included both in-hospital cardiac arrest and out-of-hospital cardiac arrest patients from 11/2015 to 7/2023 who underwent ECPR and had at least one CT head scan during their hospitalization.

We divided patients into 2 groups based on their best neurologic evaluation during their index hospitalization; patients with good neurological outcome defined as a Cerebral Performance Category (CPC) score of 1 or 2 and poor neurological outcomes defined as a CPC score of 3 or greater. For patients who where dead at discharge (CPC 5), further stratification was performed. Those who were able to follow commands at any point during their index hospitalization post-cardiac arrest were categorized as having good neurological status. In contrast, patients with brain death, brain herniation, or refractory status epilepticus were classified as having poor neurological outcomes. Patients whose care was withdrawn due to multiorgan failure and/or who remained sedated without neurological evaluation were excluded, as their neurological recovery was insufficiently understood to be categorized. CNN was trained on head CT scans to predict neurological outcomes to evaluate the model's performance by reporting its sensitivity, specificity, and predictive values in distinguishing between good and poor neurological outcomes.

Result: A total of 530 patients were included. Among survivors, 126 had CPC 1-2, and 34 had CPC 3-4. Of 370 non-survivors, 37 had followed commands post-arrest. Among the remaining 333, 47 were brain dead, 72 had brain herniation, 9 had refractory status epilepticus, care was withdrawn for 54 due to poor prognosis based on clinical and imaging, and 151 patients were exluded due to indeterminate neurologic outcomes. An initial CNN analysis (using 12 CT scans for training and 8 for testing) yielded an accuracy of 50%.

Conclusion: This is the first study that apply CNN to head CT imaging, aims to develop a valuable tool for improving neurological prognostication in ECPR patients. The initial CNN analysis achieved 50% accuracy, which is a result expected at this stage with anticipated improvements through further model training.

Impact of Pretransplant Immunosuppression on Post-Lung Transplant Outcomes

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Purpose

Aside from increased rates of post-transplant infections, the impacts of pretransplant immunosuppression (IS) on lung transplant outcomes is unknown. This study evaluates the effect of pretransplant IS on transplant complications, mortality, and infection risk.

Methods

This retrospective cohort study included lung transplant recipients in a single center between 2016 and 2023. Using a chi-squared test, we analyzed post-transplant infections and mortality in patients who received pretransplant IS compared to those who did not. In the group of patients who received IS, 3 subgroups were analyzed: steroid-only IS regimen (S), steroid and nonsteroid IS regimen (S+I), and nonsteroid IS only (I).

Results

Of 308 patients post-lung transplantation, 165 (53.6%) had an active IS therapy plan at the time of transplant, most commonly for SRTR Class D indications (115/165, 69%). Survival is shown in the Kaplan-Meier curve comparing these groups. Although not statistically significant, bacterial infections were higher in immunosuppressed patients (100% in the I group, 89% in the S+I group) compared to those not on pretransplant IS. On subgroup analysis of patients who received pretransplant IS, 1 year survival was lowest in the S+I group (79% vs. 92% in the I group, $p<0.01$). Lung and fungal infections were more common in the I group ($p=0.05$ and $p=0.03$) compared to the S+I and S groups. There was no difference among groups for CMV requiring treatment ($p=0.09$). PGD at 72 hours, 90-day survival, and rejection episodes did not differ between the groups. Among patients receiving IS pretransplant, airway complications, wound dehiscence, length of stay, and ventilator days were higher in the immunosuppressed patients with a trend toward significance.

Conclusion

There was no difference in 1-year survival in lung transplant patients on pretransplant IS compared to those who were not. Patients on S+I had an increase in mortality compared to the other IS subgroups, while those in the I group had a greater burden of post-transplant infections.

Cardiogenic Shock During Delivery: A Two-Decade Analysis of Trends in Incidence, Mortality, and Mechanical Circulatory Support

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Abstract

Cardiogenic shock (CS) during delivery is a rare but life-threatening condition. However, data on its incidence, mortality trends, and mechanical circulatory support (MCS) utilization remain limited. For this study, we analyzed the National Inpatient Sample from 2000 to 2021 to identify deliveries complicated by CS using ICD codes. Incidence rates per 100,000 deliveries were calculated and trends were analyzed. Mortality rates in those with CS were evaluated over time. MCS utilization, including intra-aortic balloon pump (IABP), extracorporeal membrane oxygenation (ECMO), and Impella devices, was assessed. Among 87,570,766 deliveries, 2,190 cases of CS were identified (2.5 per 100,000 deliveries). The incidence of cardiogenic shock increased significantly, from 1.0 to 5.8 per 100,000 deliveries (p-trend < 0.001). The average mortality rate was 32.7%, showing a modest decline over time (p-trend < 0.001). MCS was utilized in 265 patients (12.1%), with 136 receiving IABP, 138 receiving ECMO, and 30 receiving Impella. MCS utilization remained stable throughout the study period (p-trend = 0.346). The incidence of cardiogenic shock during delivery rose significantly over the past two decades, though mortality remains high at 32.7% with only a slight decline. Despite the increase in cases, MCS utilization has remained steady. These findings highlight the need for enhanced management strategies to improve outcomes for pregnant women with cardiogenic shock.

Evaluating the Role of Cyclophosphamide in Premature Ovarian Insufficiency in Patients with SLE: Bridging Knowledge Gaps and Preliminary Data

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Background: Systemic lupus erythematosus (SLE) is a multi-system autoimmune disorder that disproportionately affects women. Severe manifestations of SLE include lupus nephritis and lupus cerebritis. Cyclophosphamide (CYC) is effective in inducing remission but has significant

gonadotoxic effect. While higher doses are linked to premature ovarian insufficiency (POI), the impact of newer low-dose regimens, such as the Euro-Lupus Protocol, is less defined. Despite its efficacy, hesitancy remains in adopting CYC into treatment.

Objective: This study aims to assess risk of POI in women with SLE receiving CYC in 2 complementary parts: Part A: an analysis of our cohort of SLE patients, to assess CYC treatment indications, gonado-protective therapies, and ovarian function. Part B: a systematic review and analysis of the impact of CYC on POI incidence rate in patients with SLE, focusing on the gaps in knowledge regarding the effects of high-dose vs. low-dose regimens.

Methods: Part 1: Cohort data are extracted from our Minnesota Multidisciplinary Vasculitis Program (M2VP)'s registry analyzing the number of patients treated with CYC, indications for treatment, and proportion of patients receiving prophylactic leuprolide for gonadal protection. Part 2: A systematic review and meta-analysis is conducted following PRISMA guidelines, synthesizing available studies and data from multiple databases (Ovid, PubMed, Embase, SCOPUS and Cochrane). The review focuses on identifying risk factors associated with the incidence of POI in patients with SLE, impact of CYC on POI, impact of gonad protective therapies, and differences in high-dose and low-dose CYC.

Results: Part A: Data was extracted from our cohort of 212 female patients, of whom 31 received CYC. Median age at first dose was 26 years old. Lupus nephritis classifications were: Class V(n=4), Class IV(n=5), Class V+ IV(n=5), Class III (n=3), and Class II(n=1). Racial distribution included Asian (n=5), Black (n=13), White (n=12), and American Indian (n=1). Indications for CYC were lupus nephritis (n=21), lupus cerebritis (n=4), and severe SLE flares (n=6). The GnRH agonist leuprolide was administered to 14 patients. Of the 31 participants, 23 reported no history of irregular menses and 8 reported irregular menses. Part B: 643 studies underwent abstract review of which 240 were identified for full text review.

Conclusion: This study highlights the importance of evaluating the impact of CYC treatment in patients with SLE. The hesitancy in CYC use and inconsistency in Leuprolide prophylaxis emphasize the need for clearer insights into high- and low-dose regimens to guide fertility preservation in SLE management.

Score in Detecting Heparin-Induced Thrombocytopenia in VA-ECMO Post-ECPR

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Background
Heparin-induced thrombocytopenia (HIT) is an exaggerated and potentially fatal immune response to heparin, leading to decreased platelet counts and increased risk of thrombotic events. The 4T score and modified 4T score (m4T) have been designed to standardize the screening process for patients suspected of having HIT. While the 4T score centers on typical features of HIT including thrombocytopenia, timing of platelet count fall, thrombosis or other sequelae, and other causes of thrombocytopenia, the m4T includes more detailed medical history and hospitalization criteria, as well as a more refined assessment of thrombosis and thrombocytopenia. Neither score has been well studied or compared in VA ECMO patients post-extracorporeal cardiopulmonary resuscitation (ECPR). The purpose of this study was to evaluate both the prevalence of HIT in this patient population, as well as the predictive values of the standard compared to modified 4T scores, using measures of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Methods
This was a single center retrospective analysis of medical records of 1439 patients treated with VA-ECMO following cardiac arrest. Patients included were those aged 18 years or older, anticoagulated with heparin or low molecular weight heparin (LMWH), and had a HIT screening test ordered within the first 10 days of heparin exposure. Exclusion criteria included use of non-heparin anticoagulants, baseline platelet count less than 40,000, prior HIT diagnosis, or allergy to heparin. Both 4T score and m4T score were calculated for each patient. Data analysis included bivariate analysis for assessment of score effectiveness, and multivariate logistic regression models to compare 4T and m4T score performance, as well as control of confounding variables.

Results
76 patients were eligible for the study after application of inclusion and exclusion criteria. The standard 4T score showed PPV of 25.9%, a NPV of 91.8%, and a specificity of 69.2%. The Modified 4T Score showed a PPV of 18.18%, a NPV of 90.6% and a specificity of 44.6%.

Conclusions
In this cohort of patients on VA-ECMO post ECPR, both the 4T and m4T scores demonstrated high NPV, reinforcing their utility as effective screening tools for HIT. Screening using the m4T score demonstrated a superior sensitivity over the classic 4T score. While both scoring systems provide effective screening, the m4T score may be better suited for use in the VA ECMO post ECPR population due to its increased sensitivity, however further prospective studies are warranted to evaluate the optimal screening approach in this high-risk population.

Pilot Testing the Treatment Burden Screening Questionnaire in Type 2 Diabetes Care

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Context: Nearly all patients with type 2 diabetes have comorbid chronic conditions, adding complexity to self-management. A tool to more efficiently identify and communicate points of patient-perceived treatment burden during a primary care visit that addresses self-management complexity may lead to enhanced patient-centered care plans and improve outcomes.

Objective: To test the acceptability and usability of the Treatment Burden Screening in Diabetes (TBSD) questionnaire, a novel brief tool designed to identify domains of high treatment burden in patients with diabetes. The completed TBSD can be used as a conversation tool in a primary care clinician visit to focus discussion and address domains of high burden.

Study Design and Analysis: Single-arm pilot trial of adults with type 2 diabetes. Descriptive statistics of baseline demographics and patient and clinician post-encounter surveys. Qualitative analysis of post-encounter survey open-ended item responses.

Setting: Two urban primary care clinics in the Twin Cities metro of Minnesota.

Population: 50 English-speaking adults with type 2 diabetes engaged in a participating primary care visit and clinicians participating in the intervention clinic visit.

Intervention: Participating patients complete the 5-item TBSD questionnaire prior to their scheduled clinician visit and show it to their primary care clinician to facilitate a patient-centered discussion and care plan. Patients and clinicians complete a post encounter survey to assess their impressions and impact and comfort in using the TBSD tool.

Outcome Measures: Acceptability and usability: 1) To what extent does the TBSD help to inform the patient care plan, What changes, if any, were made. 2) Comfort with using the questionnaire tool, desire to use again, and would recommend to others.

Results: Of 50 patient participants, 50% were male and were on average 54 years old. Patients selected applicable burden domains on the TBSD. Patients felt TBSD questions were easy to understand (98%), focused the visit on their biggest concerns (77%), and felt comfortable discussing issues with their clinician (84%). Clinicians reviewed the TBSD in 82% of encounters. The 13 clinician participants felt comfortable discussing topics (92%), felt the TBSD revealed new information (44%), focused patient conversations (44%), and uncovered issues that don't typically arise (32%). The TBSD prompted action in the majority of encounters (64%), specifically with clinicians modifying treatment regimens, placing referrals, scheduling appointments, and documenting in the chart. Clinicians would use the TBSD during the patient's next visit (72%) and would recommend it to colleagues (84%).

Conclusions: In pilot testing, the TBSD identifies patient-perceived treatment burden and contributes to shaping care plans in primary care visits with patients with diabetes. Patient and clinicians find the TBSD acceptable and useful to bring focus to patient-perceived treatment burdens.

The Differences In The Inpatient Outcomes And Utilization Of Temporary Mechanical Support In Cardiogenic Shock Patients With Anterior And Inferior Wall Myocardial Infarction: A Retrospective Analysis

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Introduction: Cardiogenic shock (CS) is a critical complication of acute myocardial infarction (MI), contributing significantly to morbidity and mortality despite advances in revascularization and supportive therapies. MIs are classified based on anatomical location, anterior wall MI, typically caused by ischemia in the left anterior descending artery (LAD), and inferior wall MI, usually due to occlusion of the right coronary artery. Mechanical circulatory support (MCS), such as intra-aortic balloon pumps (IABP), percutaneous left ventricular assist devices (pLVAD), and extracorporeal membrane oxygenation (ECMO), is essential for stabilizing hemodynamics and enhancing cardiac function in CS patients. However, research comparing outcomes and management strategies for anterior versus inferior STEMI in CS remains limited.

Methods: We conducted a retrospective analysis using the National Inpatient Sample databases from January 2016 to December 2020. We identified all patients aged ≥18 years who were admitted with cardiogenic shock, and then further identified patients with concomitant diagnoses of anterior and inferior STEMI. We compared the rates of MCS utilization and inpatient outcomes between these patient groups.

Results: A total of 96,515 admissions for cardiogenic shock related to anterior and inferior myocardial infarctions were analyzed. Over the period there was a slight increase in the incidence of both Anterior and inferior STEMI as indicated by the trend lines in Figure 1. The use of mechanical circulatory support (MCS) in CS patients with inferior STEMI was lower than in those with anterior STEMI (42.5% vs. 60.5%; adjusted odds ratio [aOR] 0.51, $p < 0.001$) (Figure 1). After adjusting for demographics and comorbidities, the anterior STEMI group exhibited a higher inpatient death rate compared to the inferior STEMI group (31.6% vs 29.9%, $p<0.0114$). There was also a higher incidence of acute kidney injury (AKI) (5.9% vs. 4.3%, $p<0.0001$) and lower discharge to home in the anterior STEMI group compared to the inferior STEMI group (36.9% vs 31.6%, $p<0.0001$).

Conclusion: In conclusion, our study reveals that the utilization of MCS in the contemporary era is lower in patients with inferior MI compared to those with anterior MI. Additionally, patients with anterior MI who develop CS face higher mortality rates compared to those with inferior MI. These findings underscore the importance of considering the specific characteristics and management strategies for different types of STEMI-related CS.

Pharmacy Access Varies by Age and income in Both Rural and Urban Areas in Minnesota

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Background: Cardiovascular disease (CVD) management often requires long-term medications, but adherence is low for many risk factors like diabetes and hypertension. While pharmacy accessibility may influence adherence, it varies by sociodemographic factors in urban areas and less is known about rural access.

Methods: Using Rural-Urban Commuting Area codes, all census tracts in Minnesota (MN) were assigned 1 of 3 designations: urban, large rural city/town, small and isolated rural town. Census tract demographic and socioeconomic data were obtained from the 2015-2019 American Community Survey. Using this information, census tracts were stratified into tertiles by the proportion of residents >65 years old. We created a second dataset stratifying by the proportion of residents with income below the federal poverty line. A list of pharmacies in Minnesota (MN) from 2020 was obtained from the MN Board of Pharmacy and geocoded. Each pharmacy was designated as a chain, supermarket-based, community, or associated with a health system. We then evaluated pharmacy type, hours, count, and density for each age and income based tertile.

Results: Census tracts with higher proportions of adults over 65 were predominantly white, less educated, and more rural. Tracts with higher poverty rates had more Black and American Indian residents, lower education levels and greater rurality. In 2020, there were 916 pharmacies. In small rural and urban regions, pharmacy density was higher in the oldest tertiles with no difference by age in large rural towns. Generally, as poverty decreased, pharmacy density decreased (Figure). Urban areas offered better access outside regular hours (>70% extended hours, >82% open weekends), while small rural areas had limited access (<40% extended hours, >75% open weekends). Supermarket pharmacies had the best access (>95% open after 6 PM and on weekends). In comparison, chain pharmacies were more accessible in urban areas but less in large rural cities (65% extended hours, 87% open weekends) and small towns (23% extended hours, 66% open weekends).

Conclusion: Pharmacy density was greater in regions with more remote rural populations, a higher percentage of residents over 65 years old, and higher poverty levels. Access to pharmacies outside regular business hours was more limited in small rural communities. Additional data on travel distance and time to nearby pharmacies is necessary to better assess access.

Basic and Preclinical Research

Overcoming CD16 shedding with ADAM-17 blockade in a novel multifunctional killer engager complex improves NK cell anti-tumor efficacy

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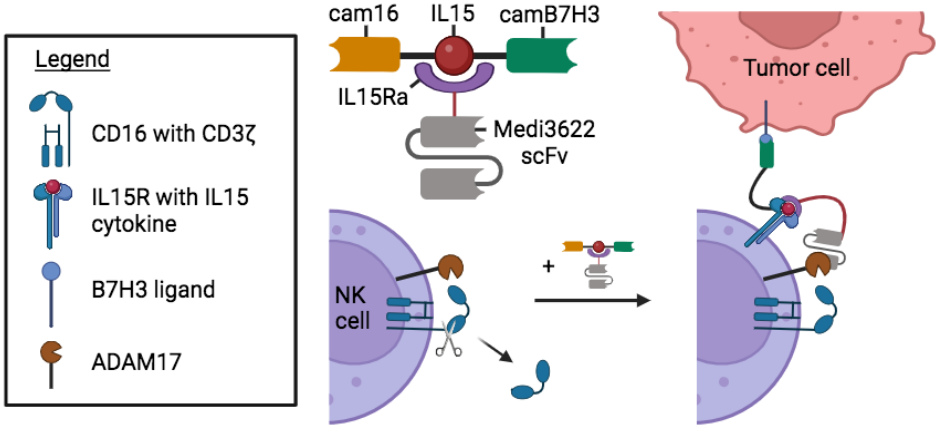
Natural killer (NK) cells mediate antibody-dependent cellular cytotoxicity (ADCC) through their Fc receptor, CD16, the only receptor capable of independently triggering NK cell activation without additional co-stimulatory signals. However, clipping of CD16 from NK cells by a disintegrin and metalloprotease-17 (ADAM-17) upon NK cell activation can undermine the efficacy of ADCC-based immunotherapies and limit tumor control across various cancer types. To address the need for a pan-cancer immunotherapy that preserves CD16 expression and sustains ADCC capability while providing activation and proliferation signals to the NK cells, we built upon the previously described Tri-specific Killer Engager (TriKE) platform which showed strong preclinical efficacy. The TriKE consists of a nanobody arm that engages CD16, an interleukin (IL)-15 moiety, and a nanobody arm binding B7H3, an antigen highly expressed on various solid tumors. Here, we incorporated a Poly-Antigen Cytokine Complex (PACC) as a “backpack” to the TriKE. The PACC consists of an ADAM-17-blocking antibody fragment arm linked to an interleukin-15 receptor alpha (IL-15Rα) to noncovalently bind IL-15 in the TriKE, forming the self-assembled TriKE-PACC molecule. Upon tumor or PMA stimulation, the TriKE-PACC retained CD16 expression on the NK cell surface more effectively when compared to the TriKE alone. The IL-15-IL-15Rα component in the TriKE-PACC induced higher pSTAT5 signaling and together with the anti-ADAM-17 component, robustly increased NK cell proliferation. Furthermore, the TriKE-PACC-treated NK cells showed markedly enhanced cytotoxicity when sequential challenged with tumor targets over a prolonged period of time (5-days). These *in vitro* results translated to significantly better tumor control and improved survival of xenogeneic mice engrafted with human ovarian cancer cells. This efficacy could be attributed to higher NK cell counts and CD16 retention in the intraperitoneal washes from the TriKE-PACC treated mice compared to the TriKE-treated mice. While evaluations of the TriKE-PACC in additional solid cancer models are ongoing, this innovative approach is promising for enhancing the efficacy of NK cell immunotherapies, particularly in solid tumor settings where ADCC is impaired by CD16 clipping.



Graphical abstract. To abrogate CD16 shedding from NK cells by ADAM-17, a single chain variable fragment of an ADAM17 inhibitor, MEDI3622, can be engineered onto the pre-existing TriKE molecule via an IL-15Ra ectodomain that when coupled with the IL-15 moiety, may enhance cytokine signaling. In addition to directed NK cell targeting of B7H3-expressing solid tumors, this novel TriKE-PACC molecule aims to retain CD16 on NK cells, thereby prolonging ADCC potential of NK cells. With a molecular weight that surpasses the threshold for glomerular filtration rate of the kidneys, the potentially longer serum half-life of this tetra-specific killer engager emphasizes its clinical translatability.

Co-Activating Tandem (CAT) CARs improve CAR NK cell functionality

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Chimeric antigen receptor (CAR) NK cells are a promising avenue for immunotherapy that intrinsically resolve many of the major setbacks to CAR T cell therapy, but have nonetheless failed to reach the level of consistent clinical efficacy seen with CAR T cells. CAR NK cells traditionally express a single engineered construct that utilizes one or more intracellular signaling domains to drive antigen-specific functionality, similar to the standard model of T cell activation. The activation of NK cells in situ, however, is not usually driven by the triggering of a single or pair of receptors; rather, it is governed by a suite of activating receptors working in tandem. We have therefore developed a series of novel CAR NK cells that utilize two distinct CAR constructs co-expressed by the same cell in order to better mimic natural NK cell activation. These Co-Activating Tandem (CAT)-CAR NKs contain a primary CAR optimized for cytotoxic function alongside a secondary CAR that uses a separate promoter and a different signaling domain. The intracellular domains of these secondary CARs were selected for their ability to induce NK cell proliferation and cytokine production–two features that are correlated with clinical efficacy. We tested the functionality of all possible combinations of two primary CARs and five secondary CARs against a plethora of tumor cell lines in vitro and identified several CAT-CAR NKs with significantly increased antitumor functionality compared to both the primary CARs alone and to previously-published single CAR constructs. We also compared the functionality of CAT-CAR NKs in which both the primary and secondary CARs engaged the same tumor antigen to those in which the two CARs targeted separate antigens. Our work demonstrates the success of combining multiple CAR constructs within the same cell to optimize NK cell functionality, providing a powerful new tool for NK cell-based immunotherapies.

Bruton Tyrosine Kinase Inhibitor Rilzabrutinib Reduces Vaso-Occlusion and Markers of Inflammation and Adhesion

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Abstract

Inflammation and increased leukocyte-erythrocyte-endothelial interactions, due to upregulated expression of adhesion molecules and activated endothelium, are thought to play a essential role in initiation and progression of sickle cell disease (SCD) cardinal complications, vaso-occlusive crisis and end-organ damage. We evaluated the effect of reversible Bruton tyrosine kinase (BTK) inhibitor rilzabrutinib and irreversible BTK inhibitor RA15539667 on inflammatory and adhesion molecule expression, and microvascular stasis in a murine vaso-occlusive crisis (VOC) model. Pretreated HbSS Townes SCD mice were implanted with dorsal skinfold chambers (DSFC) to examine stasis and challenged with hypoxia/reoxygenation (H/R) or infusion of hemoglobin (Hb) A prior to laboratory analysis. Rilzabrutinib or RA15539667 pretreatment significantly decreased microvascular stasis post-H/R and -Hb challenge ($P \leq 0.01$), spleen weight ($P < 0.05$), and inflammation and leukocytes ($P < 0.05$) vs vehicle control. Rilzabrutinib significantly decreased liver NF-κB phospho-p65 activation ($P < 0.0001$) and lung P-selectin and vWF mobilization after H/R ($P < 0.01$). Transcriptomic analysis showed rilzabrutinib downregulated pro-inflammatory genes associated with inflammasome, adhesion, complement, and thrombosis in the kidney and liver of SCD mice challenged with H/R or Hb. Rilzabrutinib and RA15539667 ameliorated inflammation through multiple mechanisms and prevented microvascular stasis in Townes SCD mice.

JAK2V617F MPN patients have pro-thrombotic signature with low free Protein S and increased Gas6

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Introduction: Thrombosis is a major driver of myeloproliferative neoplasm (MPN) morbidity and mortality. The *JAK2*^{V617F} kinase mutation, the most prevalent MPN driver mutation, increases thrombosis risk. *JAK2*^{V617F} CD34^{+} hematopoietic stem cells have elevated Axl and MERTK expression. Activation of Tyro3, Axl, and MERTK (TAM) receptor tyrosine kinases by Protein S and growth arrest-specific gene 6 (Gas6) proteins binding initiates a pro-inflammatory and thrombotic signaling cascade. We hypothesize that in *JAK2*^{V617F} individuals, Protein S and Gas6 activate TAM signaling to initiate vascular activation and thrombosis.

Methods: Using commercial ELISA kits, we quantified total and free Protein S, complement C4B1 binding protein, Gas6, Tyro3, Axl, and MERTK levels in plasma samples from *JAK2*^{V617F} MPN donors identified by the WHO 2016 Criteria. Age-, race-, and sex-matched controls were included. Gas6, p-STAT3, p-AKT, p-Axl, and p-MERTK protein expression was assessed by immunoblots from three separate *JAK2*^{V617F} donor-derived blood outgrowth endothelial cell lines or HUVEC endothelial cells transfected with *JAK2*$^{V617F+}$ or *JAK2*^{WT} expression vectors.

Results: Compared to non-MPN patients, *JAK2*^{V617F} MPN patients had no change in total Protein S. Protein S circulates with C4B1 binding protein, which was significantly lower in *JAK2*^{V617F} MPN patients. However, compared to non-MPN patients, *JAK2*^{V617F} MPN patients had lower free protein S. Conversely, *JAK2*^{V617F} MPN patients had significantly higher plasma Gas6 and MERTK levels. Immunoblots of *JAK2*$^{V617F+}$ endothelial cells showed elevated p-STAT3 and Gas6 protein. Lastly, compared to controls, *JAK2*$^{V617F+}$ endothelial cell conditioned media did not exhibit increased Gas6 secretion.

Conclusion: *JAK2*^{V617F} MPN patients exhibit a pro-coagulant state with low free protein S. In *JAK2*^{V617F} MPN, increased plasma Gas6 and MERTK levels patients suggest that Gas6-TAM may contribute to MPN-related vascular activation. *JAK2*^{V617F} endothelial cells do not secrete Gas6, suggesting a potential autocrine Gas6-TAM signaling mechanism. Our results suggest that in *JAK2*^{V617F} patients strategies targeting Protein S-Gas6-Axl-MERTK may reduce vascular activation and inflammation.

Ruxolitinib and Interferon therapy reduce endothelial-leukocyte interactions in *JAK2^{V617F} MPN*

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Introduction: The *JAK2*^{V617F}mutation, which occurs in up to 95% of polycythemia vera (PV) myeloproliferative neoplasm (MPN) patients, increases thrombosis risk 6-fold. The JAK-STAT inhibitors ruxolitinib and interferon are both approved and used for treatment of MPN patients. However, limited clinical or pre-clinical data exists on whether they reduce thrombosis. Overall, *JAK2*$^{V617F+}$ mouse models show inconsistent thrombosis and bleeding phenotypes, hindering pre-clinical study reproducibility. Therefore, we have developed an *in vitro* endothelialized microfluidic model to assess MPN-flow behavior. We *hypothesized* that in our microfluidic model, compared to *JAK2*$^{V617F+}$ subjects on best available therapy, *JAK2*$^{V617F+}$ subjects on ruxolitnib or interferon would have leukocytes with fewer pro-adhesive interactions. We tested this hypothesis by assessing *JAK2*$^{V617F+}$ MPN blood flow in untreated and ruxolitnib and interferon-treated subjects.

Methods: Primary human umbilical vein endothelial cells (HUVECs) were exposed to venous shear (15 dynes/cm^2) in microfluidics for a 3-day culture period. On day 3, endothelialized microfluidics were treated +/- 10 ng/mL TNF-α for 4 hours. Blood samples were obtained from consented *JAK2*^{V617F} MPN individuals aged ≥18 years with ET, PV, or MF as diagnosed by the WHO 2016 Criteria identified in the M Health Fairview Masonic Cancer Center Clinics. Healthy age, race, and sex-matched blood samples served as controls. Clinical data on patients was collected and stored in REDCAP. Platelets and leukocytes were visualized using Calcein AM, then perfused through the microfluidic under a fluorescent microscope to observe blood-endothelial interactions in real-time. Post-experiment, videos were analyzed to quantify the velocity and adhesion of platelets and leukocytes for each treatment condition.

Results: Velocity and adhesion were compared between untreated *JAK2*^{V617F} patients and patients on ruxolitinib and interferon respectively. When perfused over TNF-α activated endothelium, untreated *JAK2*^{V617F} MPN (n=9) had a significant reduction in cell velocity compared to control. Under the same conditions, ruxolitinib-treated *JAK2*^{V617F} MPN patients (n=4) had no significant change in cell velocity. There was no difference in complete blood counts between these groups. In a separate subgroup analysis, compared to untreated *JAK2*^{V617F} MPN patients (n=9), interferon-treated *JAK2*^{V617F} MPN patients (n=6) had no significant change in cell velocity when perfused over a TNF-α activated endothelium compared to control.

Conclusion: Our data shows that independent of reduction in cell counts, treatment of *JAK2*^{V617F} MPN individuals with either ruxolitinib or interferon decreases adhesion to activated endothelium compared to control. This suggests that both interferon and ruxolitinib work to reduce leukocyte-endothelial interactions in *JAK2*^{V617F} MPNs. Further studies to determine specific mechanisms by which each treatment reduces these interactions are underway.

Human CD8-iTreg: the next generation of Treg-based immunotherapies

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The development of (regulatory T-cell) Treg based cell therapies has demonstrated the power of harnessing the natural immunoregulatory function of Treg in treating immunologically complex conditions including autoimmune disease, transplant rejection and hematological malignancies. Unlike CD4 Treg, CD8 Treg remain a largely overlooked Treg subset with untapped potential. We have recently characterized a bifunctional subset of human CD8 induced Treg (hCD8-iTreg) that are phenotypically distinct and acquire potent immunosuppressive and anti-leukemic potential.

hCD8-iTreg were generated by culturing peripheral blood $CD8^{+}CD25^{neg}$ T-cells in IL-2, rapamycin, and high-concentration TGFβ with anti-CD3/28 stimulation. hCD8-iTreg demonstrated potent suppressor activity correlating increased expression of CD103, CD39, and Foxp3. Unexpectedly, in addition to an upregulation of canonical Treg-associated markers, we found comparable expression of cytolytic proteins including perforin, granzymes, and FAS in hCD8-iTreg compared to donor-matched $CD8^{+}$ cytotoxic T-lymphocytes (CTLs), corresponding with potent tumoricidal activity. Critically, in a combined model of xenogeneic graft-versus-host disease (GVHD) with refractory $CD19^{+}$ B-cell leukemia; hCD8-iTreg encoded with CAR19/4-1BB were shown to significantly reduce tumor burden and prolonging animal survival compared to CAR19/4-1BB CTL recipients (43d vs. 29d median survival; $p<0.0173$), without evidence of exacerbated GVHD.

Single cell RNA-sequencing highlighted a distinct transcriptional signature in which hCD8-iTreg were found to share several phenotypic characteristics of both suppressor and effector T-cell populations. Unbiased GSEA analysis revealed significant enrichment of CD4 and CD8 Treg gene sets, as well as CD8 effector-memory T-cell gene-set ($p<0.0001$ all comparisons). Comprehensive examination of hCD8-iTreg cytolytic mechanisms, utilizing state-of-the-art super-resolution microscopy, also revealed that potent cytolytic function was largely dependent on incorporation of perforin and multiple granzymes into thrombospondin-4^{+} cytolytic supra-molecular attack particles (SMAPs) released from hCD8-iTreg compared to donor-matched CTLs. Strikingly, when compared to their CTL counterparts, hCD8-iTreg secreted significantly less pro-inflammatory cytokines. Combining their SMAPs-mediated tumoricidal cytotoxicity and paralleled dampening of pro-inflammatory toxicities form a strong rationale for the consideration of hCD8-iTreg as the next generation of Treg-based cell immunotherapies.

IGF and Insulin system in regulating hallmarks of cancer

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The Insulin-like growth factor (IGF) system is implicated in various cancers and is known to regulate cell growth, proliferation, and metabolism. Type 1 IGF receptor (IGF-1R) targeting strategies have been unsuccessful, probably due to its characteristic to form heterotetrameric hybrids with the homologous insulin receptor (IR). This introduces multiple IGF-1 binding sites and draws focus on IR and its isoforms – IR-A and IR-B, as potential therapeutic targets. A high IR-A to IR-B ratio is observed in various tumors including breast cancer. IR-A dominates fetal growth and development while IR-B is predominantly found in liver and skeletal muscle where glucose uptake is regulated via glucose transporter GLUT4. IR's important role in maintaining physiological metabolism necessitates delineating IR isoform roles to develop efficient targeted strategies. The side effects of anti-IGF-1R therapy–hyperinsulinemia and hyperglycemia–warrant strategy development to manage the toxicity associated with non-specific IR targeting. Ideally, targeting IR-A specifically, and leaving IR-B unaffected, might be an effective IGF and insulin system targeting approach. Our group developed IR K/O MCF-7L (ER+ breast cancer cell line) and introduced IR-A or IR-B to generate isoform-specific cell lines. The real-time ATP production rates suggest IGF-1 promotes glycolytic ATP production in MCF-7L, MCF-7L IR K/O, IRB-C9, and MDA MB 231 (TNBC) but not in IR-A, while, insulin at physiological concentration doesn't affect ATP production. Therefore, IR-B may maintain the metabolic demand of ER+ breast cancer cells when activated by IGF-1. Additionally, IR inactivation and metabolic stimulation via IGF-1 suggest investigating alternative fuels. We are interested in investigating glutamine as it promotes tumorigenesis by acting as a nitrogen source for amino acid and nucleotide biosynthesis and carbon source to replenish tricarboxylic acid (TCA cycle) and lipid biosynthesis. Glutamine metabolism has pleiotropic effects including mTOR activation and reactive oxygen species (ROS) maintenance. This further supports proliferation, metastasis, and drug resistance. Preliminary experiments indicate the dependency of MCF-7L on both fuels to maintain the glycolytic phenotype when stimulated with IGF-1. IGF-1 also stimulated migration in MCF-7L and MDA MB 231 cell lines while insulin promoted this phenotype in MDA MB 231 only. Further investigation will elucidate IR isoform roles in cellular hallmarks of cancer.

Examining APOBEC3B in TCGA and ICGC Breast Cancer Datasets Reveals Altered Drug Metabolism Pathways

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Despite improvements in both screening and treatment, breast cancer mortality remains high, with nearly a third of patients diagnosed with breast cancer worldwide succumbing to the disease. APOBECs are a large family of deaminase enzymes that act endogenously to restrict viral replication. Upregulation of APOBECs has been documented in many cancers, where their conversion of cytosine to uracil leads to a unique mutation signature dominated by C-to-T transitions. Within this enzyme family, APOBEC3B (A3B) has shown increased expression levels in breast cancer. However, it remains unclear if tumors with an increased A3B mutational signature consistently display elevated expression levels across different breast cancer subtypes. Studies indicate that the A3B mutational signature is observed in approximately 30-50% of breast cancer cases, with higher prevalence in triple-negative breast cancer (TNBC) and HER2-enriched subtypes. Moreover, the pathways that may be involved in modulating A3B levels are an area of growing research interest. Using DNA and RNA sequencing data from breast cancer patients in The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), we found more than 64% of tumors with increased A3B mutational signature enrichment had an above median average A3B expression when compared to the A3B expression of non-enriched tumor samples (p-value < 0.001). Through KEGG, Gene Ontology (GO), and Reactome pathway analyses of high versus low A3B-expressing tumors, we identified several pathways related to tumor drug metabolism. Specifically, the tamoxifen regulatory pathway was altered; high levels of A3B expressing tumors had decreased expression of CYP2D6 and CYP3A10. Notably, this downregulation was consistent in both luminal-A-like and luminal-B-like samples. Previous publications showed that increased A3B expression was associated with tamoxifen resistance in mice, and these results suggest a potential relationship between high A3B expression and intratumoral tamoxifen metabolism through CYP2D6. Additionally, pathways related to uracil synthesis, namely those involving drugs such as 5-fluorouracil and 6-mercaptopurine, were also downregulated in tumors expressing high levels of A3B. Some of these downregulated pathway genes include: IMPDH1, NME1, TK1, and DPYS. The majority of these downregulated genes relate directly to pyrimidine synthesis and is consistent across luminal-A-like, luminal-B-like, and basal-like samples. Combined, this data suggests tumors with increased A3B expression may be more capable of developing resistance to treatments like tamoxifen, conversely A3B high tumors may be more susceptible to drugs such as 5-fluorouracil. The presence of elevated A3B in breast cancers could be exploited to develop A3B modulating strategies designed to enhance efficacy of existing therapies and avoid drug resistance.

Unique interactions between E-cadherin loss and Estrogen receptor drive tumorigenesis in Invasive Lobular Cancer

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Estrogen receptor (ER) expression and E-cadherin (*CDH1*) loss are the two major molecular hallmarks of invasive lobular carcinoma (ILC) of the breast; however, it is unclear how ER signaling and E-cadherin loss interact during ILC tumor formation. Furthermore, prior research suggests that ER may have a unique role in ILC initiation and progression. We hypothesize that E-cadherin loss alters global expression and ER activity in human mammary epithelial cells (HMECs) to promote ILC tumorigenesis.

To test the effect of E-cadherin loss during estrogen-driven tumorigenesis, we established a CRISPR/Cas9 approach for knocking out *CDH1 in* primary HMECs. Since primary HMECs did not show robust ER expression, we also re-expressed ER using lentiviral transduction of *ESR1*. We observed decreased ER phosphorylation at the Serine 167 site in the *CDH1* knockout (KO) cells, suggesting impaired ER signaling. We also performed RNAseq from two independent *CDH1* targeting gRNAs vs non-targeting gRNA (NTG) control (-/+ ER). When comparing the two *CDH1* gRNAs to NTG, 2882 shared genes were differentially regulated. Analysis of these shared genes showed the enrichment of estrogen response and cell cycle pathways and repression of epithelial-mesenchymal transition and glycolysis in the *CDH1* KO sample, corresponding to human ILC data. Metabolic studies showed decreased glucose uptake in the KO cells; we also observed a decrease in glycolysis and an increase in the mitochondrial ATP production rate in the KO cells compared to control in a Seahorse™ ATP rate assay.

In addition, we hope to identify mediators that drive estrogen-induced tumorigenesis using the mouse mammary intraductal (MIND) model, which better recapitulates the progression of ILC and response to estrogen *in vivo*. We injected control and *CDH1* KO cells into the mammary glands of adult female NSG mice, with estrogen supplemented in their drinking water, and assessed for engraftment using bioluminescence imaging (BLI). BLI suggests enhanced engraftment of *CDH1* KO cells compared to *CDH1* wild-type HMECs and a survival advantage for HMECs expressing *ESR1*. Notably, Cell-titre-glo™ survival assay showed increased survival with cMyc and myrAKT oncogenes in *KO* cells, suggesting oncogenic cooperation with E-cadherin loss. Understanding the unique interactions between E-cadherin loss and ER in ILC could lead to novel therapeutic approaches for the prevention and treatment of ILC.

Selective targeting of estrogen receptor positive breast cancer stem- like cells with oncolytic virus

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Oncolytic viruses are promising agents that infect and kill tumor cells, while sparing healthy cells. Our research aims to use Mammalian orthoreovirus (MRV) as an oncolytic to prevent and treat metastatic breast cancer (BC). BC is the second leading cause of cancer deaths among women in the United States. Estrogen receptor positive [ER+] BC has a more favorable prognosis; however, patients can experience cancer recurrence over 10 years after initial diagnosis. Breast cancer stem-like cells (BCSCs) are slowly proliferating cells that drive metastasis and resistance to therapies targeting rapidly proliferating tumor cells. No specific drugs target BCSCs. Therefore, our goal is to use MRV to eliminate BCSCs. Our current studies are focused on developing novel MRV variants that will target BCSCs. Toward this end, we tested several MRV strains against therapy-sensitive (MCF7) and therapy-resistant ER+ BC cells (paclitaxel resistant, TaxR). Our RNA-seq analysis indicate that therapy-sensitive and therapy-resistant BCSCs respond differently to MRV infection. Interferon signaling pathways were activated in both therapy-sensitive and therapy-resistant BCSCs, however, the timing of their activation differed. In response to MRV infection, therapy-sensitive BCSCs induced activation of these interferon signaling pathways earlier than the therapy-resistant BCSCs. Nonetheless, we find that parental strains can significantly reduce BC cell viability in 2D culture but their effect on BCSCs is modest. To identify MRV strains with enhanced capacity to infect and kill BCSCs, we serially passaged a parental MRV strain in therapy-resistant BCSC-enriched tumorspheres. We isolated several clones and found that at least two are more effective at killing BCSCs compared to parental strain. The effect of these BCSC-adapted viruses is greater in the therapy-resistant TaxR cell model compared to the therapy-sensitive MCF7 cell model. Our whole genome sequencing of BCSC-adapted viruses identified changes that will result in viral protein amino acid modifications that may correlate with enhanced potency against BCSCs. We are currently performing a series of experiments designed to compare the stages of infection and cell death induction that differ between the parental and BCSC-adapted viruses. We anticipate that this research will lead to the development of an enhanced MRV-based virotherapy as a novel strategy to treat therapy-resistant ER+ BC.

Quantifying Protein-Protein Interactions in ER+ Breast Cancer through the NanoBit Assay

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The recurrence of metastatic Breast Cancer (BC) stemming from acquired endocrine and chemotherapy resistance presents a burden to patients with Estrogen Receptor (ER) positive breast cancer. Protein overexpression and altered protein-protein interactions (PPIs) result in abnormal BC cell signaling, which can promote cancer progression and cancer stem cell (CSC) activity. The overexpression and cytoplasmic localization of the steroid-receptor coactivator protein PELP1 in ER+ BC promotes PELP1 PPIs with SRC-3, ER, Progesterone Receptor (PR), and the glycolytic isoforms 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) and PFKFB4, modulating CSC activity. Targeting this protein complex could provide a therapeutic approach for treating ER+ BCs. We aim to develop a high-throughput assay to monitor PPIs and treatments that may disrupt these PPIs. The NanoBit Assay (NB), developed by Promega, analyzes PPIs through the association of the luciferase subunits LgBit and SmBit. These subunits are fused to a protein of interest on the N or C termini. The LgB and SmB weakly associate on their own (K_d = 190 μM), but when they are brought together upon direct interaction of the two proteins of interest, the LgB and SmB will form a functional enzyme, and a strong luciferase signal will be produced. Gibson Assembly was used to create the PFKFB3, PFKFB4, and PR NB constructs necessary to quantify PELP1 PPIs. DNA sequencing and Western Blot confirmed alignment and protein expression. Transient transfection of LgB and SmB combinations was performed in HEK293T cells. After a day of hormone deprivation, cells were treated with 1nM E2 or left untreated as a negative control. NanoGlo Live Cell Reagent was added to each well, and luminescence data was collected on a BioTek Synergy2 plate reader. As a positive control, the previously modeled SRC-3:ER interaction was tested in the presence of the ER selective estrogen degrader (SERD) fulvestrant. Introducing fulvestrant decreased luminescence 100-fold relative to the vehicle. Significant increases in luminescence were demonstrated for interactions between interactions of LgN SRC-3:SmC PFKFB4, LgC PELP1:SmC PFKFB4, and LgC PELP1:SmC SRC-3 (Two-Tailed T-test, $n = 5$, $p < 0.05$). Future NB studies will elucidate PPIs between PR and other PELP1/SRC-3 complex proteins. Any significant findings will then be introduced to fulvestrant and other inhibitors shown to disrupt PELP1/SRC-3 complexes and inhibit CSC activity and BC progression.

Steroid receptor-driven cell signaling and migration in pancreatic ductal adenocarcinoma cell models

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Steroid hormone receptors (SRs) are crucial for modulating transcription in cancers such as breast, prostate, uterine, and ovarian. While SRs are well studied in these cancers, there are other cancers associated with elevated SR expression that remain understudied. One such cancer is pancreatic ductal adenocarcinoma (PDAC). PDAC is an aggressive cancer with a 5-year survival around 10% and a growing incidence. Risk-factors for PDAC include conditions that are associated with cellular stress and inflammatory signaling such as diabetes, obesity, and chronic pancreatitis. While PDAC is associated with elevated Progesterone Receptor (PR) and Glucocorticoid Receptor (GR) expression, there have not been extensive studies of GR or PR in PDAC. We hypothesize that SRs (such as PR and/or GR) contribute to PDAC progression, and that post-translational modifications, ligand binding, and/or selected cofactors modulate SR actions in the context of stress-activated signaling pathways associated with aggressive cancer phenotypes. Here, we present a study of SRs in PDAC cell culture with a focus on GR and its ligand-dependent and -independent activation. For this study we used three PDAC cell lines; PANC1, ApsC1, and Hs766T. We characterized cellular migration (via transwell migration assay) and protein expression (via western blot) in response to treatment with SR ligands, SR inhibitors and cytokines, including TGFbeta, IL-1beta, and LIF which are prevalent in the PDAC tumor microenvironment and promote PDAC progression. We observed that in response to ligand or cytokine treatment, PDAC cell lines exhibit phosphorylation of GR at Ser134, a stress-induced p38 MAP Kinase site that drives advanced cancer phenotypes in triple negative breast cancer models. This phosphorylation of GR is paired with activation of components from oncogenic signaling cascades such as p38 and AKT. In transwell migration assays, we observed that glucocorticoid or cytokine treatment induced increased migration of PANC-1 cells relative to vehicle-treated controls. Co-treatment with GR antagonists (mifepristone) reduced cytokine-induced migration. This study provides a preliminary understanding of SR integration with established signaling pathways and aggressive biology in PDAC. In the future, we hope to expand this work to include additional biologic assays, the rigorous interrogation of stress-activated signaling pathways, and transcriptional studies that define SR-dependent gene signatures in PDAC.

Mineralocorticoid receptor mediated advanced cancer phenotypes in triple-negative breast cancer models

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Glucocorticoid receptor (GR) senses a convergence of host and cellular stress signaling to orchestrate an array of advanced cancer phenotypes associated with triple-negative breast cancer (TNBC) progression. GR isoforms are activated by a class of closely-related adrenal steroid hormones collectively known as corticosteroids. The Lange lab studies the interface of steroid hormone receptors (SRs) with mitogen- and stress-activated signaling pathways. Recently, we reported p38 MAP kinase mediated phosphorylation of GR at serine 134 (Ser134) in response to tumor microenvironment-derived cytokines, such as TGFβ1. Phospho-Ser134-GR (p-Ser134-GR) upregulated gene sets that promote cancer cell survival, chemoresistance, and migratory/invasive behavior in TNBC models. In addition to acting as ligands for GR, glucocorticoids also bind to and activate another closely related SR - the mineralocorticoid receptor (MR). MR via its physiological hormone aldosterone, mediates stress-activated hypertension, inflammation and fibrosis. However, the elucidation of functional responses upon activation of MR remains untapped area in breast cancer research. Our probe into public datasets (METABRIC, and TCGA) demonstrated significantly higher expression of MR transcripts in TNBC relative to luminal breast cancer. Further, high expression of MR predicted worse overall survival in ERα-negative breast cancer patients compared to the low expressing cohort. Interestingly, MR shRNA-knockdown or MR inhibition with two competitive antagonists significantly reduced aldosterone and TGFβ1 induced migratory and stemness phenotypes of TNBC cells compared to the non-specific shRNA control (GFP-shRNA) cells. This biological phenotype observed upon MR knockdown parallels the reduced migration we observed when the Ser134 MAPK consensus site on GR was mutated to alanine in TNBC models. Preliminary data further suggest that cytoplasmic GR/MR complexes associate upon TGFβ1 treatment and nuclear GR/MR complexes form with a combination of aldosterone and dexamethasone. We are currently investigating and dissecting cytokine- and hormone mediated regulation of GR/MR signaling and transcriptional complexes and their functional role via RNA seq studies. The overarching goal of our work is to elucidate if MR is a required GR binding-partner and a potential therapeutic target in GR+ TNBC metastatic processes.

The role of ketone bodies as critical regulators in the neonatal brain development

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Neurological disorders arising from impaired neuronal development affect over 50 million people worldwide, imposing significant burdens on both individuals and society. Nutrient deficiencies, particularly in long-chain polyunsaturated fatty acids (PUFAs), confer irreversible neurological risks to offspring. While PUFA supplementation during early postnatal stages demonstrates neurodevelopmental benefits, results remain inconsistent. Our recent findings indicate that ketone bodies remodel PUFA lipid pools in an acetoacetyl-CoA synthetase (AACS)-dependent manner in the adult liver. Although neonatal brains consume approximately half of the liver's daily ketone output - a demand 70-150% greater per unit body weight than adults - the mechanisms by which ketone bodies support neuronal development remain unclear.

To address this question, we measured the expression of ketolytic enzymes in various brain regions of wild-type (WT) neonatal mice. AACS expression was highest early in development and progressively decreased across all brain regions with advancing age. In contrast, expression of succinyl-CoA:3-ketoacid CoA transferase (SCOT), responsible for ketone oxidation, increased progressively, suggesting a developmental shift from anabolic utilization of ketones toward oxidative metabolism. This adaptation parallels the brain’s known resource allocation shift from growth to maintenance and conditioning over time. Further, we assessed neuronal ketone uptake *in vitro* by culturing neuronal cells for 4-18 days, representing different developmental stages, and measuring residual ketones in culture media from 0 to 48 hours post-treatment. Both acetoacetate and beta-hydroxybutyrate utilization began at 11 days *in vitro* (DIV), coinciding with peak dendritic growth and synaptogenesis and representing early postnatal neuronal development. *In vivo* studies were conducted using WT and AACS-knockout (AACS-KO) mice from postnatal day 21 (P21) on standard chow. Ketolytic enzyme expression and energy metabolites in neonatal brains were quantified at P21 via Western blotting and mass spectrometry, respectively. AACS-KO mice exhibited gender-specific increases in acetyl-CoA and decreases in succinyl-CoA, indicative of enhanced mitochondrial ketone utilization. Upcoming lipidomics and untargeted metabolomics analyses will further determine alterations in brain metabolic profiles, specifically quantifying free and esterified PUFA levels.

This study aims to expand the understanding of ketone bodies beyond their conventional metabolic roles as an alternative energy source during glucose scarcity, directly linking ketones to developmental stage-specific functions in the developing brain.

The Enduring Metabolic Impact of Ketone Bodies During Pregnancy and Lactation on Adult Offspring

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Inadequate maternal nutrition during pregnancy affects millions in the United States, increasing the risk of cognitive, metabolic, and neurodevelopmental disorders later in life. During late gestation, maternal glucose utilization declines, and ketogenesis increases (i.e., “accelerated starvation”), while neonates exhibit high ketone turnover. Despite this, no guidelines exist for monitoring ketones during pregnancy or lactation. Contrary to the notion that all ketosis should be avoided in pregnancy, gestational physical activity, which induces mild ketosis, has been shown to enhance offspring metabolic health and cognitive function. Given the high metabolic demands of fetal and neonatal growth, ketone bodies are a regular energy source, yet the impact of physiological ketosis during early development remains unclear.

To investigate the effects of enhanced maternal ketone availability during gestation and/or lactation, breeder drinking water was supplemented with 3% 1,3-butanediol (BD), a precursor of the predominant ketone body, D-β-hydroxybutyrate (βOHB), during these two developmental stages. Maternal health was assessed by measuring body weight, blood glucose concentration, ketone concentration, and non-esterified fatty acid (NEFA) levels. BD supplementation had no significant effect on maternal weight or blood glucose levels during pregnancy. As expected, maternal ketone concentrations increased to 2.4 ± 0.69 mM in pregnant BD-supplemented dams, compared to 0.6 ± 0.07 mM in non-pregnant dams at the end of pregnancy, confirming enhanced ketone metabolism in late gestation.

Offspring development was monitored by tracking neonatal weight, ketone body concentrations, NEFA levels during lactation, and brain and heart mass at 13 weeks of age. Progeny whose mothers were administered BD only during lactation exhibited lower overall body weight gain and had increased brain and heart mass relative to body weight at weaning age, suggesting a potential shift in energy allocation or organ development. This effect was not observed in progeny whose mothers were administered BD during both pregnancy and lactation. Long-term metabolic effects of ketone exposure were evaluated via glucose tolerance tests (GTT), ketone tolerance tests (KTT), and fed/fasted serum ketone levels. Notably, 12 week-old male offspring from BD-treated mothers during both gestation and lactation displayed sex-specific impairments in glucose metabolism. This effect was not observed in progeny whose mothers were administered butanediol during lactation alone. Further analysis is required to determine the precise metabolic adaptations, including systemic energy homeostasis.

These findings indicate that while maternal ketone supplementation does not significantly impact maternal health, it alters early growth patterns and glucose regulation in offspring, with potential long-term metabolic consequences.

Western Diet Abolishes Cognitive Benefits of Ketone Body Precursor, (R,S)-1,3-Butanediol, in a Mouse Model of Tauopathy

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Neurodegenerative diseases, like Alzheimer's and related Tauopathies, pose a growing public health crisis in the United States with cases expected to triple over the next 25 years. These conditions are exacerbated by obesity and involve a progressive reduction in cerebral glucose metabolism with accumulation of neurofibrillary tangles, together leading to neuronal loss and cognitive decline. Ketogenic therapies are promising strategies that provide alternative energy sources to support brain metabolism and neuroprotection. One emerging intervention is [R,S]-1,3-butanediol (BD), a ketone body precursor that elevates both D- and L-β-hydroxybutyrate (βOHB) levels in circulation. D-βOHB is rapidly metabolized for energy while the role of L-βOHB in neuroprotection remains unknown. To investigate whether BD enhances cognitive resilience in a model of neurodegeneration, we administered 10% (v/v) BD in drinking water to male and female forebrain Tau-transgenic (rTg4510) mice and littermate controls for 20 or 30 weeks, beginning at 6 weeks of age, while consuming normal chow or high-fat, Western diet. We assessed body and tissue mass, fat composition, and blood ketone levels using gravimetry, echo-MRI, and LC-MS, respectively. Cognitive function was evaluated using the Barnes maze. We found that 10% BD rapidly induced physiological ketosis in male and female mice (1.5 mM and 3.0 mM βOHB, respectively) while maintaining euglycemia. BD treatment increased circulating L-βOHB distribution to 85% compared to negligible levels in water controls. Although BD did not significantly affect body mass, systemic glucose tolerance, or overall ketone body uptake, Tau-transgenic mice displayed a reduction in body weight and fat mass compared to littermate controls. As expected, Tau-transgenic mice showed high expression of Tau in the forebrain and displayed severe cognitive impairment at both 20- and 30-week timepoints. However, in mice treated with BD for 20 weeks while on normal chow diet, memory acquisition and recall performance in the Barnes maze improved to match non-transgenic controls, a phenomenon more pronounced in female mice than in males. Notably, these cognitive benefits were abolished in Tau-transgenic mice consuming a Western diet for 20 weeks. While BD did not prevent cognitive decline in mice treater for 30 weeks, it was associated with significant increase in survival ($p=0.0006$). These findings suggest that BD-induced ketosis preferentially elevates circulating L-βOHB and may enhance cognitive function in both time- and diet-dependent manner. BD-induced ketosis may also promote survival with potential sex-specific benefits. Ongoing studies are examining the effects of BD treatment on brain pathology and neuronal utilization of energetic substrates, which will elucidate the neuroprotective potential of ketogenic strategies and could offer new therapeutic strategies for combating neurodegenerative diseases.

Does Adenosine Deaminase Acting on RNA-1 (ADAR1) Have a Role in CSCs Maintenance in NSCLC?

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Background: Non-small cell lung cancers (NSCLCs) are the leading cause of cancer-related death worldwide. NSCLC is known for its aggressive behavior and therapeutic resistance which leads to relapse and mortality after initial response. This behavior is attributed to the presence of subpopulation of cancer cells that possess self-renewal capacity and the initiation of multiple clones within the tumor known as cancer stem cells (CSCs). ADAR enzymes are responsible for the A-I editing of self-RNA in physiological conditions to escape degradation by the immune system. Recently, ADAR was found to be highly expressed in cancer cells using the same mechanism to evade detection and destruction by the immune system. Our study investigates the role of ADAR editing activity on the maintenance of CSCs in NSCLC.

Methods: ADAR1 gene was knocked out using CRISPR-Cas9 in both human and murine cell lines A549, H2009, and LM2, respectively. Western blots were done to confirm the KO of ADAR1. The effects of ADAR1 KO were tested both in vitro and in vivo. In vitro, using CCK-8 cell proliferation assay and IFN signaling pathway western blots. In vivo, tumorigenesis was investigated using xenograft model of human NSCLC and murine immune competent and immune deficient models. To test the effects of ADAR1 KO on CSCs characteristics, tumorsphere assay, ALDH1A1 western blot, flow cytometry analysis using ALDEFLUOR assay, CD133 and CD44 surface markers were done.

Results: ADAR1 was knocked out in two human and one murine NSCLC cell lines and confirmed with western blots. Tumor formation was nearly abrogated after ADAR1 KO in both murine and human xenografts. ADAR1 KO did not affect the in vitro cell growth in the 2D cell culture in all three cell lines. ADAR1 KO did not substantially alter the type-I IFN response in A549 and H2009 cell lines. In A549 cells, ADAR1 KO cells showed near complete loss of ALDH1A1 by Western blot. Likewise, using the ALDEFLUOR assay, activity of ALDH was significantly decreased in A549 (59.8% vs 0.07%) and LM2 cells (43.6% vs 13.4%). However, the ALDEFLUOR assay did not show any changes in the ALDH level in H2009. Tumor sphere assay also confirms our results with loss of tumorsphere formation after ADAR1 KO in the three cell lines.

Conclusion: ADAR1 KO markedly reduced CSCs characteristics like in vivo tumorigenesis, tumor sphere formation and ALDH levels in NSCLC models. However, it does not have effect on in vitro cell proliferation and IFN signaling in A549, H2009 and LM2 cell lines. Thus, RNA editing of ADAR1 may mediate these effects in NSCLC models. We continue to investigate the mechanism by which ADAR1 regulates maintenance of CSC phenotype in NSCLC.

Necessity of Notch3 signaling in myofiber maturation in a pluripotent stem cell transplant model

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Abstract
Pluripotent stem cell-derived myogenic progenitors change from an embryonic to postnatal molecular signature upon satellite cell engraftment, which coincides with upregulation of Notch3. Since a role for Notch3 in skeletal muscle maturation is unknown, here we investigate whether Notch3 is required for the in vivo molecular maturation switch. Our results show that lack of Notch3 in transplanted progenitors (N3KO) does not impact engraftment, but leads to increased numbers of embryonic myofibers. Conversely, transplantation of Notch3 overexpressing (N3OE) myogenic progenitors results in lower numbers of embryonic myofibers, but diminished muscle grafts when compared to empty vector (EV) controls. Secondary transplantation studies confirmed these effects, whereby Notch3 overexpression significantly reduced secondary engraftment. Importantly, secondary grafted fibers of N3KO satellite cells had increased numbers of embryonic myofibers compared to N3OE and EV controls. Taken together, these findings demonstrate that Notch3 signaling is required for myofiber maturation, and that constant activation of Notch3 impairs muscle regeneration. Transcriptional profiles of N3OE donor-derived satellite cells suggest that dampened regeneration may be driven by inhibitory alterations in cell cycle regulation.

Human iPSC-derived iPAX7 myogenic progenitors are transcriptionally mature after incorporation into murine myofiber *in vivo*

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Abstract

Muscular dystrophies are incurable skeletal muscle degenerative disorders. Pluripotent stem cell (PSC)-based cell therapy is an attractive option to rebuild damaged muscle and improve functional output. We have shown that conditional expression of PAX7 (iPAX7) in differentiating PSCs generates large numbers of human myogenic progenitors that can not only replace damaged myofibers but seed the stem cell pool, providing long-term engraftment. Though we previously demonstrated that iPAX7 myogenic progenitors engraft and provide functional improvement in several dystrophic mouse models, it remained unknown whether donor-derived myofibers have transcriptionally matured to match the surrounding tissue. Does introduction into the muscle environment facilitate iPSC-derived myofiber maturation or is iPAX7 maturation limited to what can be achieved *in vitro*? Here, we transplanted iPAX7 myogenic progenitors into muscles of non-dystrophic ("WT") and dystrophic ("DMD") mice and compared the transcriptional landscape of human donor-derived myofibers with respective *in vitro*-differentiated iPAX7 myotubes. Pairing this bulk RNA sequencing approach with computational deconvolution of human reads, we were able to pinpoint key myogenic changes that occur during the *in vitro*-to-*in vivo* transition and demonstrate that iPAX7-derived myofibers that form in both WT and DMD models are far more adult-like in nature than iPAX7-derived myotubes that are terminally differentiated *in vitro*. Additionally, we compared iPAX7-derived myofibers against publicly-available human skeletal muscle datasets to demonstrate that iPAX7-derived myofibers formed in both WT and DMD mouse muscle in large part match the developmental stage of adult skeletal muscle. These data confirm developmental maturity, and consequently further suggest the utility of cell-based therapies.

The effects on the interferon gene expression signature following the loss of adenosine deaminase acting on RNA (ADAR1) in an ADAR1-amplified NSCLC cell line

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Introduction: Adenosine deaminase acting on RNA 1, or ADAR1, is a gene that catalyzes the post-transcriptional conversion of adenosine to inosine in RNA. This function allows ADAR1 to inhibit the type-I IFN response by reducing the pool of cytoplasmic dsRNA species that bind to dsRNA sensors in the type-I IFN signaling pathway. Previous studies have investigated the effects of ADAR1 amplification on tumorigenesis, where it was shown that the reduction of ADAR expression in ADAR1-amplified non-small cell lung cancer (NSCLC) cell lines resulted in cancer growth inhibition. The loss of ADAR1 in murine melanoma models has also been shown to result in an increased type-I IFN gene expression signature and apoptosis upon IFN-β treatment. The present study aims to integrate these insights to demonstrate the effects on the interferon gene expression signature following the loss of ADAR in NSCLC cells that amplify ADAR1.

Methods: ADAR1 was knocked out via CRISPR/Cas9 in H1975, a human NSCLC cell line. ADAR1 protein loss was confirmed through Western blot analysis. The amplification of ADAR1 in H1975 cells was assessed using quantitative real-time PCR. The H1975 cell line was specifically chosen because previous studies have conflicted over whether it amplifies the ADAR1 gene. The activation of the type-I IFN signaling pathway in H1975 wild-type (WT) and ADAR1 knockout (KO) cells was assessed through Western blot analysis. The expression of ALDH1A1, a cancer stem cell marker, was assessed through Western blot analysis to evaluate stem cell properties between H1975 WT and ADAR1 KO. The cells were analyzed via the ALDEFLUOR flow cytometry assays and tumorsphere assays to further evaluate stem cell properties.

Results: ADAR1 was knocked out in H1975 and confirmed through Western blot analysis.

Conclusion: H1975 was found to overexpress the ADAR1 protein. After ADAR1 KO, H1975 was determined to not be dependent on the ADAR1 protein for survival.

Unveiling Active Bacteria Present in Celiac Patients using Click Labeling and Anaerobic Culturing

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Celiac Disease (CeD) affects roughly 1% of the global population. However, currently the only treatment is a gluten-free diet, which is not only difficult to maintain but also has varied results. Celiac Disease is only present in people containing the DQ2 or DQ8 haplotype, which is present in about 35% of the population. What causes only some of these individuals to develop Celiac Disease is largely unknown. It is increasingly understood that people with digestive diseases have distinct microbial communities compared to healthy individuals, and Celiac Disease is no exception. Recent studies have shown differences between bacterial populations of the duodenum and stool in CeD patients compared to control groups. More specifically, lactic acid bacteria responsible for important metabolic pathways in the gut such as *Lactobacillus* are markedly decreased in CeD patients compared to control groups. However, it is still largely unknown which bacteria are active in CeD patients as a whole and therefore might contribute to celiac-related symptoms and inflammation. In this study, we collected duodenal mucus and biopsies from celiac and control patients receiving diagnostic upper endoscopies. These samples were incubated with L-AHA, an azide-containing methionine analog. L-AHA is incorporated into newly synthesized proteins of translationally active bacteria during incubation in an anaerobic chamber. We next labeled L-AHA containing bacteria with Cy5-DBCO via strain-promoted cycloaddition, allowing for fluorescence-based detection of active bacterial cells. Subsequently these fluorescently-labeled bacteria were sorted via fluorescence-activated cell sorting followed by shotgun metagenomic sequencing. A portion of the collected biopsy and mucous samples were plated on de Man Rogosa Sharpe (MRS) medium in anaerobic conditions to determine which viable lactic acid bacteria were present. By identifying differences in microbiome composition between healthy and CeD patients, certain bacterial pathways or strains can potentially be targeted for new Celiac Disease treatment.

In vivo gene therapies to prevent Fanconi anemia-associated bone marrow failure and cancer development

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Fanconi anemia (FA) is a rare genetic cancer-predisposition syndrome caused by mutations in certain DNA repair genes (i.e. FA genes). FA patients are 100- to 1000-fold more likely than the general public to develop acute myeloid leukemia and specific solid tumors, such as head and neck, esophageal, and anogenital squamous cell carcinomas. Furthermore, FA is the most common bone marrow failure (BMF) syndrome. Symptomatic loss of blood cells correlates with exacerbated DNA damage accumulation and progressive elimination of hematopoietic stem and progenitor cell (HSPC) populations. There is no curative treatment for FA, necessitating new therapeutic and prevention measures. The major focus of our research is to use genome editing technologies to correct FA causative mutations in cells throughout the body, including HSPCs, to prevent development of FA-associated cancers and BMF. To this end, we are generating a humanized FA mouse model harboring the *FANCA* Spanish founder mutation (SFM; c.295 C>T), the most common FA patient mutation in the world, and deficient in alcohol dehydrogenase 5 (Adh5) and transformation-related protein 53 (Trp53). We are testing two *in vivo* gene therapy strategies. 1. We will employ adenosine base editor ABE9-SpRY delivered by recombinant adeno-associated viruses (rAAVs) or lipid nanoparticles (LNPs) to correct the SFM back to the functional wild-type *Fanca* sequence. 2. We will insert the entire wild-type *Fanca* cDNA into the endogenous *Fanca* locus using a novel homology-mediated end-joining (HMEJ) approach. Importantly, we have demonstrated that both strategies are proficient in repair-defective FA cells. Finally, we will measure *in vivo* genetic correction in several tissue types, including bone marrow HSPCs. We hypothesize that our genome engineering approaches will drastically reduce FA-associated cancer development and BMF, and can be applied in therapeutic applications for a myriad of other premalignant disorders.

Myomesin 2 is a marker of cardiac maturation

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In recent years, studies have shown that the mouse heart can regenerate after injury in the first few days after birth. Since regeneration appears to be limited to this time period, we are interested in understanding what developmental changes cause mice (and presumably humans) to lose their regenerative ability. New cardiac myocytes can be generated from pluripotent stem cells to repair an injury, but they have one major disadvantage; stem cell derived cardiac myocytes do not mature beyond the neonatal stage of development. This leads to shortcomings in our understanding of how protein expression aids in regeneration and development. We performed mass spectrometry on cardiac proteins isolated from E15, P1, P10 or adult hearts and identified Myomesin-2 (MYOM2) as a protein that is upregulated with cardiac maturation. MYOM2 is a protein that is localized to the M-band and involved in organizing contraction. Our results show that MYOM2 increases its expression level from embryonic timepoints to adulthood. While absent during embryonic development, MYOM2 becomes more centralized to the M-band and localizes between the Z-disks of the sarcomere. To further understand its shifting localization, we have utilized isolated cardiac myocytes and immunohistochemistry staining to analyze key shifts in its expression and understand its potential role in marking and promoting cardiomyocyte maturation. Future experiments will test whether inhibition of MYOM2 in adult cardiomyocytes could enhance their regenerative potential.

Septin4 regulates cardiac fibrosis induced by pressure overload

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Introduction: In response to cardiac injury, the heart undergoes a remodeling process, which can become maladaptive and contribute to the development of heart failure. One of the most important components in cardiac remodeling is the development of fibrosis, characterized by excessive deposition of extracellular matrix (ECM) proteins by cardiac fibroblasts. The small GTPase Septin4 (Sept4) has been suggested to be involved in the regulation of myofibroblast differentiation and fibrogenesis in other organs. However, its role in cardiac remodeling remains unclear. The present study aims to investigate whether Sept4 mediates alterations associated with cardiac remodeling induced by cardiac pressure overload.

Methods: We used transgenic mouse models to genetically delete Sept4, including whole-body knockout (KO) mice and $Sept4^{fl/fl}Tcf21^{MCM/+}$ mice with specific deletion of Sept4 gene in fibroblasts. Mice underwent transverse aortic constriction (TAC) to induce cardiac pressure overload. Functional and molecular analyses on hearts were performed either at baseline, 1- or 4-weeks post-injury timepoints.

Results: After TAC injury, WT mice showed a significant reduction in cardiac function and the development of heart failure, whereas KO mice maintained normal cardiac function. KO hearts exhibited decreased levels of cardiac ECM deposition and fibrosis compared with WT hearts. Similarly, $Sept4^{fl/fl}Tcf21^{MCM/+}$ hearts were protected from increased cardiac ECM deposition and fibrosis after TAC, when compared to $Sept4^{fl/fl}$ hearts. Consistent with these findings, the differentiation of fibroblasts into myofibroblasts was impaired in KO hearts compared to WT controls, and in $Sept4^{fl/fl}Tcf21^{MCM/+}$ hearts compared to $Sept4^{fl/fl}$ hearts. Mechanistically, Sept4-mediated myofibroblast differentiation was found to be dependent on TGF-β signaling pathway, involving the Smad-dependent signaling cascade and calcineurin activation.

Conclusions: Our results identify Sept4 as a critical regulator of ECM remodeling in the heart. Sept4 deletion leads to reduced levels of cardiac fibrosis and attenuation of pressure overload-induced cardiac dysfunction. Sept4 regulates the myofibroblast differentiation and, consequently, the ECM deposition in the heart through the TGF-β-mediated signaling pathway, which involves Smad-dependent signaling cascade and calcineurin. These findings highlight Sept4 as a potential target for preventing fibrosis and improving outcomes in heart failure.

Evaluation of Novel Oxazolidinone Derivatives' Action Against *Mycobacteria*

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Due to the rise of antibiotic resistant bacteria, there is a great need to develop new therapies to combat bacterial infections. Tuberculosis (TB) is the number one infectious killer in the world, having caused 1.5 million deaths in 2023. A new class of drugs, Ozaxolidinones, are in development to treat *Mycobacterium Tuberculosis* (M. tb) strains that are resistant to first line antibiotics. Oxazolidinones are protein synthesis inhibitors that act on the ribosomal 50S subunit of the bacteria, preventing synthesis of the 70S initiation complex, a prerequisite for bacterial reproduction. Currently, there are two Oxazolidinones approved by the FDA, linezolid, a second line TB antibiotic, and tedizolid.

Although linezolid has been successful in treating TB, its toxicity has led to the need for further research into new derivatives with less off target effects. We are developing these compounds by changing the placement of an aldehyde group. Three of these compounds, EWL_19, JC2 P98B, and KRL_46, are undergoing testing by minimum inhibitory concentration (MIC) and growth assay against *Mycobacterium Smegmatis* (M. smeg), Bacillus Calmette-Guérin (BCG), and M. tb. For both assays, the compounds were diluted to concentrations ranging from 25ug/mL to 0.39ug/mL, in a serial 2-fold dilution. For the MIC assay, the bacteria was plated with the compounds alone, while for the growth assay human macrophages were infected with bacteria for 4-6hrs, the macrophages were washed to dispose of extracellular bacteria, and then the compounds were added. Depending on the strain of bacteria, fluorescence, luminescence, or OD was used to measure bacterial growth for 7 days. Controls included a positive control of bacteria alone, a vehicle control of DMSO, and a negative control antibiotic. All compounds were tested by MIC assay with all bacterias, a growth assay using THP-1 cells was performed with M. smeg, and a growth assay with monocyte derived macrophages (MDMs) isolated from blood was performed with M. tb.

JC2 P98B and ELW_19 have bactericidal activity in all three bacterias in a dose-dependent manor starting at a concentration of 0.78ug/mL for M. tb and 1.56ug/mL for BCG as seen by both the MIC and the growth assays. In both the MIC and growth assay for M. tb and BCG, KRL_46 slightly decreased bacterial growth at the highest concentration tested, 25ug/mL, but there was no significant change in the growth of M. smeg. The placement of the aldehyde group likely blocks the binding site of KRL_46 to the bacterium leading to poor functionality. JC2 P98B out performed EWL_19 by decreasing M. tb bacterial burden in MDMs at lower concentrations, but EWL_19 outperformed in decreasing bacterial growth in the MIC assay. We hypothesize that this is due to the aldehyde group changing the ability of ELW_19 to enter the macrophage, and plan to perform further tests to verify this. We also plan to test more Oxazolidinones derivatives, as well as testing them against *Mycobacterium abscessus* (M. abscessus).

Early Divergent Immune Activation of Human Alveolar Macrophages After BCG Infection

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Numerous studies suggest that the initial immune response to Mycobacteria in the lung may shape subsequent clinical outcomes across the tuberculosis spectrum. We aim to integrate cutting-edge technologies to evaluate the heterogeneous human innate immune response to respiratory infections. This will enhance our understanding of the cellular mechanisms underlying early immune phenotypes, such as resistance or establishment of infection.

We established an exvivo distal airway model of mycobacterial infection and identified 8 AM Superclusters in healthy humans. We identified that the CK and IFN AM superclusters had higher number of cells and DEGs after BCG infection. Understanding how early immune phenotypes in the lung influence resistance to or establishment of infection will enable us to identify novel therapeutic targets. Additionally, this knowledge will help us identify populations that would benefit most from preventive treatment strategies.

CRISPR-Cas9 Gene Editing for Generation of DMD Null Isogenic Lines and Large Dystrophin Gene Correction to Understand DMD Cardiomyopathy Pathophysiology

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Background: Duchenne muscular dystrophy (DMD) is a severe X-linked disorder characterized by progressive muscle degeneration, including cardiac dysfunction. Patient-derived induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) provide a physiologically relevant model to study disease mechanisms such as aberrant calcium cycling. However, genetic variability between patient and control lines can introduce confounding factors, making it challenging to attribute observed phenotypes solely to the disease mutation. Isogenic hiPSC lines, generated through gene editing, provide a genetically matched system for direct comparison, allowing precise investigation of disease-specific molecular defects.
Our objective was to generate an isogenic DMD null hiPSC line from our control hiPSC lines using CRISPR-based gene editing and correct the dystrophin mutation in a patient derived DMD hiPSC line and assess the presence of dystrophin and functional abnormalities such as calcium handling abnormalities as an indicator of disease pathophysiology.
Methods: A healthy control iPSC line was genetically modified to knockout Exon 51 of the dystrophin gene. SpCas9 was used to introduce the guide RNAs through electroporation. Upon DNA sequencing of the bulk cell population, single cell colonies were selected and grown out for further DNA sequencing to confirm the desired knockout. Additionally, these methods were utilized to correct two DMD patient lines and to correct the generated DMD null hiPSC line. Along with the control and two DMD patient cell lines, the isogenic iPSCs were differentiated into cardiomyocytes. To assess calcium handling, the iPSC-CMs were carried out to day 60, loaded with Fura-2 dye, and paced at 0.5 Hz. Utilizing an IonOptix calcium imaging system, calcium transients were recorded to evaluate differences in calcium handling dynamics between control and DMD-mutant iPSC-CMs. From the calcium transient data, we assessed the time to peak (amplitude) and signal decay (decay tau).
Results: In comparison to the control iPSC-CMs, the gene-edited DMD null iPSC-CMs exhibited altered calcium transients, including higher decay tau (prolonged decay time) and reduced peak amplitude, consistent with impaired calcium handling observed in DMD pathology. The difference in amplitude between the control and isogenic control was significant, however the difference in decay tau was not significant. A greater sample size is needed to confirm the significance of the prolonged decay time.
Conclusion: The use of isogenic lines minimizes genetic background variability, strengthening the link between the observed defects and the DMD mutation. This study establishes an isogenic iPSC-CM model for DMD. Initial studies exhibit calcium dysregulation and call for additional to confirm disease pathology. This model will provide a robust platform for investigating molecular mechanisms and testing potential therapeutic interventions for DMD-related cardiomyopathy.

S100A4 is a potential therapy target for cardiofibrosis in DMD

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Background: Duchenne muscular dystrophy (DMD) results from mutations in the dystrophin gene and inevitably leads to a deadly cardiomyopathy. Fibrosis can be detected non-invasively in patients living with DMD using gadolinium contrast enhanced cardiac magnetic resonance imaging. Cardiac fibrosis progresses and extensive pathologic cardiac remodeling due to fibrosis leads to cardiac pump failure and lethal arrhythmias. It is the major hallmark of DMD heart and other malfunctional tissues. Damaged cardiomyocytes release TGF beta which could stimulate S100A4 expression in fibroblasts, S100A4 is a protein that promotes inflammation and fibrosis and is a potential therapy target for fibrosis and cancer metastasis.

Methods: HiPS derived fibroblasts were used to observe S100A4 levels and their response to the treatment of TGF beta, S100A4 gene knockdown and S100A4 inhibitor**.** Cell viability Assay and the cell migration assay were used to study cell proliferation and migration in those fibroblast. S100A4 Gene knockdown, Western blot analysis, Real-time reverse transcription PCR, Immunohistochemistry analysis and Immunofluorescence stain were also used for this project.

Results:

1.Preparation of iPS derived normal and DMD fibroblast: Human normal and DMD iPS cells were used to differentiate cardiac fibroblast, the differentiated cells are CD29 97%, CD105 97.2%, CD31, CD45 and Troponin T <3%, consistence with human cardiac fibroblast. DMD (5169 and C604), control (PLZ and M15, and isogenic null DMD isogenic controls (EX51 knock out) hiPSC derived fibroblasts were developed and used in the following experiments. **2. TGF β1 promotes the DMD phenotype and S100A4 expression in hiPSC-fibroblasts.** Cell proliferation and migration are enhanced in HiPS derived fibroblasts when the cells were cultured with TGF β1. TGF β1 also stimulated the S100A4 and ECM protein production. **3. S100A4 knockdown reduces the DMD phenotype in hiPSC-fibroblasts.** When Knocking down S100A4 in HiPSC-derived fibroblast, the cell proliferation and migration were inhibited. Thus, Inhibiting S100A4 in fibroblasts may reduce fibrosis development. 4**. The S100A4 inhibitor niclosamide inhibits DMD phenotype and S100A4 expression in hiPSC-fibroblasts.** Niclosamide is a well known S100A4 inhibitor and is in clinical trial for fibrosis inhibition. Niclosamide inhibited cell proliferation, migration and ECM protein production in the above hiPS derived fibroblast.

Conclusion: Inhibiting S100A4 in DMD hiPSC-fibroblast, which replicates native DMD fibroblast in fibrosis development, is a possible therapy for reducing cardiomyopathy fibrosis.

Lamin A/C Patient Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes Demonstrated Altered Nuclear Structure and Reduced Focal Adhesion Complexes

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Introduction: Lamin A/C cardiomyopathy is a genetic condition affecting the heart, caused by mutations in the gene *LMNA*, which encodes a key nuclear membrane protein, Lamin A. This highly penetrant gene mutation leads to ventricular arrhythmias, sudden death, and severe dilated cardiomyopathy. Like other dilated cardiomyopathies, heart cells in Lamin A/C cardiomyopathy suffer from poor cell adhesion, suggesting defects in the formation of focal adhesions (FA), complexes of proteins that connect the cytoskeleton to the extracellular matrix. Understanding the mechanisms behind its progression is critical for advancing treatment strategies. Lamin A/C patient-derived induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) provide a valuable model for studying Lamin A/C cardiomyopathies, as they carry patient-specific genetic abnormalities. In this study, we assessed changes in nuclear morphology and focal adhesions in LMNA hiPSC-CMs as they play a role in cardiomyopathy development.

Methods: iPSC-CMs from LMNA cardiomyopathy patient (L35P) and healthy controls were immunostained for Lamin A to assess nuclear morphology. The focal adhesion complexes were visualized using fluorescent microscopy in CMs transfected with a vinculin biosensor.

Results: Immunostaining revealed significant nuclear abnormalities in LMNA cardiomyocytes, including oblong and smaller nuclei with a higher prevalence of folding and blebbing. Our analysis revealed that LMNA cells exhibited lower circularity compared to controls (0.71 VS 0.81, p = 0.0083). LMNA cells also exhibited a higher percentage of nuclei with abnormal morphology than control cells, characterized by membrane buckling, folding, and deviations from circular or elliptical shapes (78% vs 19%). Analysis of FA complexes showed that LMNA cardiomyocytes tended towards lower focal adhesion density though a larger sample size is needed to determine significance (0.015 vs 0.033 FAs/um^2, p = 0.23). FA size remained comparable between LMNA and control groups (1.46 vs 1.52 um^2, p = 0.68).

Conclusion: Structural abnormalities in LMNA cardiomyopathy extend beyond the nucleus, impacting cell surface integrity. Defects in the nuclear membrane correlate with impaired FA formation, suggesting disruptions in cytoskeletal-ECM interactions. These findings highlight the need for further research into mechanotransduction defects and the interplay between nuclear and membrane abnormalities in LMNA cardiomyopathy. Future studies will explore the mechanistic relationship between nuclear defects and FA dysfunction to better understand disease progression and potential therapeutic targets.

Elucidating Cardiometabolic Changes in Dystrophic Cardiomyopathy

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Introduction: Cardiomyopathy is ubiquitous and deadly in patients with Duchenne muscular dystrophy (DMD) and there is no effective cure yet. While there are many factors affecting cardiomyopathy progression, one under-researched aspect of DMD cardiomyopathy is cardiometabolism. The majority of DMD patients develop obesity and insulin resistance during their lives, and while the more sedentary lifestyle resulting from muscular degeneration certainly plays a role, there are likely more underlying causes driving this due to the early onset of these symptoms. Additionally, there is evidence of dysfunction in mitochondria, the metabolic powerhouse of the cell, prior to the onset of cardiac dysfunction. In addition to developing metabolic syndrome, DMD patients are often in a state of chronic adrenergic stress, which has been shown to worsen cardiac function including mitochondrial function, and thus may likely be further impairing cardiometabolic function in DMD.

Methods: Cardiomyocytes were generated using human induced pluripotent stem cells (hiPSCs-CMs) derived from DMD patients. For metabolic analysis, hiPSC-CMs were seeded into Seahorse cell culture plates, and oxygen consumption rate was measured at baseline and following injections of oligomycin, FCCP, and an Antamycin A/Rotenone mixture. For calcium imaging, hiPSC-CMs were seeded onto glass-bottom dishes, loaded with the fluorescent calcium dye Fura-2, and calcium transients were recorded using an IonOptix calcium imaging machine. Experiments were carried out with and without beta-adrenergic stress (i.e. isoproterenol treatment).

Results: DMD hiPSC-CMs exhibited reduced respiratory function relative to controls, characterized by lower oxygen consumption rate during basal respiration (60.1 vs 76.9 pmol/min/1000 cells, $p < 0.001$), as well as greatly diminished maximal respiratory capacity (47.9 vs 85.5 pmol/min/1000 cells, $p < 0.001$) and reserve capacity (8.33 vs 58.05 pmol/min/1000 cells, $p < 0.001$), indicating not only impairments at rest, but a drastically reduced ability to respond to increased energy demands. Respiratory function and capacity were further impaired in the presence of adrenergic stress. Additionally, DMD hiPSC-CMs exhibited abnormal calcium handling, characterized by irregular beating and pro-arrhythmic activity (e.g. afterdepolarizations). This aberrant phenotype was greatly exacerbated by adrenergic stress, but largely ameliorated in cells pre-treated with the beta-blocker propranolol, even in the presence of isoproterenol.

Conclusion: DMD hiPSC-CMs exhibit significantly impaired electrophysiological and metabolic function compared to control hiPSC-CMs, made worse by the addition of adrenergic stress, a clinically-relevant factor. While beta-blockers show promise at mitigating adrenergic stress in DMD cardiomyopathy, further intervention is needed to ameliorate the metabolic dysfunction and to assess the ways in which this may improve cardiac function as a whole.

Bioreactor Optimization for perfusion and loading of a 3D Bioprinted Human Chambered Muscle Pump

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Introduction: 3D-Bioprinted heart organoid models could serve as useful tools for modeling cardiac function in healthy and disease states, especially cardiomyopathies. Previously, our lab has developed such a model termed the human chambered muscle pump (hChaMP), in which induced pluripotent stem cells (iPSCs) are printed in a cardiac bioink in the shape of a miniature heart, expanded and differentiated into cardiomyocytes. Currently, the hChaMP is limited by suboptimal thickness of viable tissue due to lack of perfusion and by irreproducibility in iPSC expansion and differentiation to cardiomyocytes. Previously, we have designed a mini-bioreactor to perfuse the hChaMP during iPSC expansion and differentiation but the addition of the hChaMP to the bioreactor is still difficult and inconsistent, often resulting in damage to the fragile hChaMP or leaks within the system. Various modifications to the hChaMP and bioreactor design and setup protocol were tested with the goal of improving these outcomes.

Methods: hChaMPs were redesigned using AutoDesk Meshmixer from a previously reported design derived from an MRI of a human heart. Acellular hChaMPs were printed in 7% gelatin methacrylate, 0.5% LAP bioink via the INKREDIBLE extrusion bioprinter in a gelatin coacervate support bath and crosslinked via ultraviolet (UV) light. The bioreactor and holder were designed in SOLIDWORKS and printed with BioMed Clear resin using a Formlabs 3B printer. For bioreactor setup, the hChaMP was added to the holder and tubing was inserted into the inlet and outlet. Then the tubing from the hChaMP was connected to the inlet and outlet of the bioreactor with food dye pumped through to test for leaks.

Results: Remodeling of the hChaMP design and the addition of a customized 3D-printed holder substantially improved the ease of tube insertion into the hChaMP inlet and outlets, while reducing hChaMP damage. Addition, of Christmas tree attachments to the inlet and outlets of the bioreactor, improved ease of hChaMP insertion into the bioreactor and prevented leaks between the connections.

Conclusion: Consistent addition of the hChaMP to the bioreactor will advance our future goals of producing consistent hChaMPs with improved tissue thickness. Further work will include optimizing iPSC expansion and differentiation conditions using 3D bioprinted squares and subsequently adapting parameters within the bioreactor to optimize iPSC expansion and differentiation within the hChaMP.

Hypomagnesemia Promotes Atrial Fibrillation Risk via Inflammation, Oxidative Stress, and Sarcoplasmic Reticulum Diastolic Ca2+ Leak

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Background: Atrial fibrillation (AF) affects over 60 million individuals worldwide. Diabetes mellitus (DM) is recognized as an independent risk factor for AF development. Recent findings suggest that inflammation, mitochondrial oxidative stress, and ryanodine receptor 2 (RyR2) phosphorylation-mediated diastolic Ca^{2+} release from the sarcoplasmic reticulum (SR) play crucial roles in DM-associated AF pathogenesis. Hypomagnesemia (HypoMg), a condition that exacerbates inflammation and oxidative stress, is frequently comorbid with DM. Importantly, HypoMg and its downstream effects are reversible with magnesium repletion.

Objective: In this study, we investigated whether HypoMg increases AF predisposition and the underlying mechanisms. We also propose that magnesium repletion reverses these processes.

Methods: At 10 weeks of age, C57BL/6J mice were assigned to three groups: control mice on normal diet (2 g/kg Mg^{2+}); HypoMg mice on six weeks of low Mg^{2+} diet (15-30 mg/kg Mg^{2+}); and Mg^{2+} repletion mice on six weeks of low Mg^{2+} diet followed by six weeks of normal diet. Mg^{2+} differences were assessed with a serum Mg^{2+} assay. We used confocal imaging to quantify mitochondrial reactive oxygen species (mitoROS), Western blotting to quantify protein expression, and electrophysiology tests to determine AF inducibility.

Results: HypoMg increased mitoROS in atrial cardiomyocytes, inflammation (interleukin-1 beta levels), phosphorylated RyR2 in atrial tissue, and AF inducibility, all of which were reversed with Mg^{2+} repletion.

Conclusion: HypoMg promotes AF risk by enhancing cardiac inflammation, mitoROS, and RyR2 phosphorylation. Moreover, Mg^{2+} supplementation may reduce AF susceptibility in DM by reversing these changes, presenting a possible strategy to mitigate DM-associated AF risk.

Histone Deacetylase 6 Inhibition Increases Acetyl-Nrf2 to Promote Anti-oxidant Response to Hemin

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Introduction: In sickle cell disease (SCD), intermittent hemolysis results in hemoglobin release and free heme, which drives a pro-oxidant, pro-inflammatory environment. When free heme levels rise, it can lead to the liberation of Nrf2 from its inhibitor, Keap1, allowing Nrf2 nuclear translocation, antioxidant response element (ARE) binding, and initiation of antioxidant genes and γ-globulin transcription. Relevant antioxidant genes include *HMOX1* (HO-1), and *NQO-1.* In multiple studies, increased Nrf2 activity reduces SCD pathogenesis, in part due to increased HO-1, therefore strategies to increase Nrf2 are desirable. The class IIb histone deacetylase 6 (HDAC6) protein is unique as it provides post-transcriptional modification of proteins in the cytoplasm. We hypothesize that HDAC6 inhibition will increase Nrf2 stability through increasing Nrf2 acetylation leading to increased anti-oxidant response.

Methods: Primary human umbilical vein endothelial cells (HUVEC) between passages 1-6 were used for experiments. For immunoprecipitation, cells were exposed to 20 μM hemin plus or minus 2 μM BML-281 or the proteasome inhibitor MG-132 for 6 h. Total protein lysate was collected followed by agarose bead immunoprecipitation for Nrf2 (Cell Signaling Technologies 127121) followed by separation of 4-20% Tris-Hcl gel. Proteins were then immunoblotted for post-transcriptional modifications including total ubiquitin, acetyl-lysine, and total protein. For other studies, cell lysates were collected for either qRT-PCR, immunoblotting at 4, 6, 12, and 24 hours after treatment. For reactive oxygen species (ROS), HUVEC cells were treated with DCFDA per protocol followed by 30 min treatment with 2 μM BML-281 or vehicle. Next, 20 μM Hemin was added followed by 4 h incubation. ROS formation on fluorimeter at excitation/ emission at 485 nm / 535 nm.

Results: Compared to untreated ECs, hemin treated ECs had lower Nrf2 ubiquitination and acetyl-lysine, consistent with increased HDAC6 activity. Likewise, treatment with the proteasome inhibitor MG132 also reduced Nrf2 ubiquitination and acetyl-lysine. Treatment with BML-281 (HDAC6i), increased Nrf2 ubiquitination and acetyl-lysine modification. Treatment with hemin plus BML-281 prevented loss of Nrf2 ubiquitination and acetyl-lysine. Consistent with enhanced Nrf2 activity, compared to HUVEC treated with hemin alone, hemin plus BML-218 treatment resulted in significantly higher *NQO1* and *HMOX-1* mRNA expression at 6 and 12 hours and higher HO-1 protein expression at 24 h. Likewise, compared to hemin-treatment alone, HUVEC treated with hemin plus 281 had significantly lower reactive oxygen species production at 24 hours.

Conclusion: Collectively, our data suggest that in the presence of hemin, HDAC6i results in increased Nrf2-acetylation which increases transcription and expression of critical antioxidant proteins such as HO-1. Overall, these data support further studies of HDAC6i in SCD.

IGF1R-specific NK cell engager inhibits rapidly proliferating and quiescent hormone receptor positive breast cancers in vivo.

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Patients with hormone receptor-positive (HR+) breast cancers frequently recur with metastases 5-20 years after diagnosis and completion of chemotherapy or prolonged endocrine therapy. A potential mechanism of resistance in HR+ patients is the presence of slow dividing or dormant well-differentiated cancer cells that result in clinically detectable metastases after prolonged latency. One way to eliminate these is immunotherapy. The objective of this study is to target HR+ breast cancers using a natural killer (NK) cell based approach that targets cell surface receptors. The type I IGF receptor (IGF1R), a cell surface receptor tyrosine, is expressed on HR+ breast cancers and its expression is tightly related to estrogen receptor (ER) function. We hypothesized that a drug targeting NK cells to IGF1R expressing HR+ breast cancer cells will be effective in killing them. We generated IGF1R specific Tri specific Killer Engagers (TriKE) that leverages expression of IGF1R on HR+ cells to cause NK cell directed cell death. The IGF1R TriKE consists of a humanized single domain antibody (sd) sequence of a CD16 nanobody to engage NK cells, a humanized sd sequence of a nanobody against IGF1R to target HR+ cells, and an IL-15 molecule between to drive NK expansion and survival. We have previously shown that the IGF1R TriKE degranulated NK cells against multiple IGF1R+ HR+ breast cancer cell lines (MCF-7L, T47D and ZR-75-1).

Herein, we investigated the effect of IGF1R TriKE on tumor growth of estrogen-stimulated proliferating tumors and in a quiescence model of breast cancer. Female NSG mice bearing MCF-7L tumors supplemented with 17-beta-estradiol (E_2) were randomized to the following cohorts: control with no human NK (huNK) cells, huNK cells, huNK cells+IL-15 and huNK cells+TriKE treatments. huNK cells were infused iv weekly and IL-15 or IGF1R TriKE thrice a week for 4 weeks. IGF1R TriKE inhibited estrogen-stimulated tumor growth ($p<0.0001$) in 3 independent experiments. Further, CD56+ huNK in blood of mice treated with the TriKE persisted for 3 weeks compared to IL-15 cohort and were also detected in tumor sections of the TriKE cohort by huCD45 staining. The TriKE also inhibited growth of IGF1R+ patient-derived xenografts (PDX UCD4] but did not affect growth of IGF1R negative PDX tumor (HCI-003) *in vivo*. To test the effect in a quiescent model, female ovariectomized NSG mice were implanted with MCF-7L cells and given 17-beta-E_2. When tumors were 150mm^3, E2 was withdrawn to allow cells to become quiescent and a week later mice were randomized to the same treatment groups as above. IGF1R TriKE significantly killed quiescent tumor cells compared to control ($p<0.0001$) and IL-15 groups. Further Ki67 positivity in tumors treated with the TriKE was significantly lower than the control group.

Our data suggest that IGF1R TriKE kills both rapidly proliferating and quiescent breast cancers and could be a potential approach to eliminate slow growing breast cancer cells in HR+ patients.

RSPO2 Drives Distinct Oncogenic Features in Treatment-Resistant Prostate Cancers

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Prostate cancer is the most diagnosed cancer in men, as well as the second most lethal. While primary prostate cancer has a high 5-year survival rate (~99%), metastasis to nonlocalized sites such as bone marrow results in a much worse survival rate (34%). The standard of care for patients is androgen deprivation therapy; however, resistance to this treatment can arise and result in castration-resistant prostate cancer (CRPC), a disease state with relatively few successful treatment options. One pathway that has been implicated in metastasis and CRPC subtypes is the canonical Wnt signaling pathway, which is thought to be regulated by four R-spondin proteins, RSPO1/2/3/4. A 2015 seminal study nominated RSPO2 as a potential player in CRPCs, but no conclusive follow-up study has been conducted with regard to the oncogenic features of RSPO2. When interrogating the potential oncogenic implications of this family of proteins, we discovered that distinct from RSPO1/3/4 and known Wnt genes *CTNNB1* and *APC*, *RSPO2* alterations are more frequently observed in mPCs. Our analysis of genomic data from two cancer databases, TCGA Pan-Cancer Atlas (n = 10,967) and cBioPortal (n = 2,510), revealed that *RSPO2* has increased rates of alterations, specifically amplifications in mPCs as compared to *CTNNB1* mutations and loss of *APC* (~22% vs. ~8-9%). We also observe that *RSPO2* amplifications strongly correlate with epithelial-mesenchymal transition (EMT) transcription factors, including *ZEB1*, *SNAI1/2*, and *TWIST1/2*. Gene Set Enrichment Analysis (GSEA) was conducted from the RNA of both AR+/- RSPO2 and CTNNB1, revealing distinct gene expression profiles. *RSPO2* was enriched in pathways associated with EMT and therapy resistance, whereas *CTNNB1* was linked to canonical Wnt and androgen response. Protein structure modeling showed that RSPO2 differs from RSPO1/3/4, which may support a functional divergence from the canonical Wnt signal transduction pathway. Altogether, our findings display that *RSPO2* leads to the promotion of various oncogenic features and differs from *RSPO1/3/4* functionally. Given the uniqueness of RSPO2 from its familial proteins, as well as the robust divergence of *RSPO2* overexpression vs *CTNNB1* overexpression *in vitro*, continued perturbment of non-canonical Wnt signaling pathways and their potential effect on RSPO2 could lead to a new therapeutic avenue for CRPC patients.

Liver PSAT1 governs liver-specific and systemic glucose adaptations to sulfur amino acid restriction

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Dietary sulfur amino acid (SAA) restriction (SAAR) mitigates obesity and impaired glucose control in humans and rodents. The improved glucose homeostasis by SAAR is associated with hepatic and systemic adaptations. Serine, a non-essential AA, is necessary to produce cysteine (a proteogenic SAA) and can be generated de novo from glucose. This study tested if adaptations in the liver *de novo* serine synthesis pathway are required for SAAR to improve glucose control. Mice with a liver-specific knockout (KO) of the serine synthetic enzyme, phosphoserine aminotransferase 1 (PSAT1), and wild type (WT) littermates were fed a high-fat, SAA-sufficient (CTRL) or SAAR diet for 6 weeks. Glucose (GTT) and insulin tolerance (ITT) tests evaluated glucose homeostasis. Isotope tracing untargeted metabolomics was completed by combining an intravenous infusion of [U-^{13}C]glucose in the conscious, unrestrained mouse with LC-MS/MS untargeted metabolomics to define the *in vivo* fate of glucose within the liver. Body composition, metabolite concentrations, and molecular regulators of glucose metabolism were measured to fully interpret glucose phenotypes. SAAR lowered body weight and adiposity similarly in both genotypes. SAAR increased liver PSAT1 protein ~60-fold in WT mice. The increased PSAT1 was accompanied by higher liver serine and glycine (~1.5-2-fold) in WT-SAAR compared to WT-CTRL mice. The rise in liver serine and glycine was mitigated in KO-SAAR compared to WT-SAAR mice but was still significantly higher than KO-CTRL which suggests other sources of serine besides de-novo synthesis. On a CTRL diet, KO mice had impaired insulin tolerance compared to WT mice. Glucose tolerance was comparable between WT-CTRL and KO-CTRL mice. SAAR improved glucose tolerance and insulin tolerance in both genotypes. However, the increase in glucose tolerance was blunted in KO-SAAR compared to WT-SAAR mice. The greater glucose tolerance in WT-SAAR mice compared to KO-SAAR mice was associated with increased partitioning of glucose to serine. Specifically, WT-SAAR mice showed 15% of the liver serine to be labeled by the intravenous infusion of [U-^{13}C]glucose. In contrast, liver ^{13}C-serine labeling was completely attenuated in KO-SAAR mice. SAAR fed mice also had ~25-fold increase in the enzyme asparagine synthetase (ASNS) and a concomitant increase in liver asparagine enrichment from 0% in CTRL to ~12% in SAAR. An increase in asparagine has been shown to increase intracellular serine through an AA exchange transporter, which could explain the increase in liver serine in KO-SAAR compared to KO-CTRL. Together, these data show that SAAR promotes glucose partitioning to serine and asparagine within the liver, which is linked to improved glucose tolerance. These results also suggest an increased heightened synthesis and uptake of serine, a SAA precursor, is required for glucoregulatory adaptations to SAAR.

RNA-seq Analysis to Uncover Drivers of Cancer Cells forming Tunneling Nanotubes for Intercellular Communication

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Abstract

Background: Tunneling nanotubes (TNTs) are dynamic membranous protrusions that are critical for intercellular communication in cancer, yet their transient nature and lack of definitive molecular markers pose challenges for systematic study. To address this issue gap in knowledge, we used bulk and single-cell transcriptomic sequencing approaches to identify differentially expressed genes in ovarian cancer cells enriched for TNTs to identify molecular pathways and genes potentially implicated in TNT formation, maintenance and function.

Methods: We used edgeR and Seurat to perform differential expression analysis on ovarian cancer cells grown in conditions favoring upregulation of TNT formation, and further performed single-cell sequencing of the same cell type isolated via spatiotemporal genomic analysis (SaGA) and fluorescence-activated cell sorting (FACS).

Results: We found 917, 51 and 331 genes upregulated in bulk sequenced TNT forming SKOV3, COV and ES-2 ovarian carcinoma cell lines, respectively. Gene families that repeatedly came up across all three of the cell lines included carbonic anhydrases, matrix metalloproteinases, SERPIN (Serine Protease Inhibitor-30), and SNORA (Small Nucleolar RNAs). We narrowed down candidate genes of interest to 23 differentially expressed genes (DEGs), many of which are involved in cellular stress response, organization of the extracellular matrix or intracellular cytoskeleton, cell adhesion, and other pathways. Further analyzing the single cell sequenced ES-2 cells, we found the differential expression of KISS1 gene, a metastasis suppressor implicated in cytoskeletal organization, significantly upregulated in TNT-enriched cells, highlighting its potential role in TNT formation. Other genes, such as EPHB1, MMP3, and WNT7B, were also upregulated in TNT-enriched cells, suggesting their involvement in regulating cell adhesion, migration, and differentiation. Conversely, we observed downregulation of BGN, a gene associated with promoting metastatic potential and NFkB signaling in cancer cells, as well as SYNGR1, a gene traditionally linked to synaptic transmission but also involved in vesicle exocytosis, which may influence TNT formation.

Conclusions and Future Directions: Bulk and single cell RNA sequencing was feasible and beneficial for performing transcriptomic analysis of TNT-enriched cancer cells, and provides a novel approach to understand molecular factors that drive formation of these protrusions. The identification of sets of differentially expressed genes that serve as the molecular markers of TNT formation, maintenance and function in ovarian cancer paves the way toward understanding the role of TNTs in tumor heterogeneity and drug resistance.

Immune tolerance shape distinct fate decisions and differential pathogenicity among CD4 T cells specific for disparate hybrid insulin peptides during autoimmune diabetes

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CD4 T cells targeting hybrid insulin peptides have been found in the pancreas of both mice and humans with autoimmune diabetes. Monoclonal T cells reactive to hybrid insulin peptides derived from insulin and chromogranin A (InsC-ChgA) or islet amyloid polypeptide (InsC-IAPP) are pathogenic in NOD mice. We sought to determine how tolerance shapes the fate and pathogenic potential of polyclonal CD4 T cells responding to hybrid insulin peptides. We discovered that nearly all T cells in NOD mice responding to InsC-ChgA become activated within 3 weeks of life, whereas T cells responding to InsC-IAPP become maximally activated at 8 weeks of life, with ~30% of cells remaining naïve throughout adulthood. Within the pancreas, the InsC-ChgA-specific cells showed a strong preference towards Th1 while InsC-IAPP-specific cells were enriched for Il-10 expressing FOXP3$^-$ cells. Both populations of cells had a low frequency (<10%) of FOXP3$^+$ regulatory T cells. XCR1$^+$ dendritic cells were required for the activation of InsC-ChgA-specific cells early in life but dispensable by 10 weeks of life. T cell receptor sequencing revealed that both InsC-ChgA and InsC-IAPP-specific cells have highly restricted repertoires, including public clonotypes. Overall, these data show that central tolerance and inflammation-induced alterations in antigen processing and presentation shape the fate of hybrid insulin peptide-specific T cells, impacting their capacity to induce autoimmunity.

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$C5F_2$-hexyl-(cuban-1-yl-methyl)-biguanide ($C5F_2$-HCB) overcomes an immune excluded tumor microenvironment (TME) and suppresses tumor growth in the ovarian dependent ER+HER2- $SSM2^{ucd}$ mammary carcinoma allograft model

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Introduction:
Heme-binding biguanides inhibit cytochrome P450 monooxygenase-mediated biosynthesis of the epoxy fatty acid (EpFA) (±)14,15-EET, which promotes progression of ER+HER2- breast cancer as well as regulatory T cell (Treg) proliferation. However, there are no known strategies to exploit this vulnerability. Through structure-based design, heme binding cubane derivatives of metformin were identified, which potently inhibited (±)14,15-EET biosynthesis. Fluorination of the hexyl moiety improved pharmacokinetics (PK) and reduced toxicity. Among the fluorinated derivatives, $C5F_2$-HCB potently inhibited (±)14,15-EET biosynthesis (IC_{50}=3.1 uM for $C5F_2$-HCB vs. 5 mM for metformin). $C5F_2$-HCB and metformin are candidate agents for TME activation from “cold” to “hot” due to their inhibition of EETs biosynthesis and EET-driven oxidative phosphorylation (OXPHOS) which promotes tumor hypoxia. We then tested whether $C5F_2$-HCB or metformin could inhibit immune exclusion in the immunologically “cold” ovarian dependent STAT1 KO $SSM2^{ucd}$ mammary carcinoma model, in part, by reducing Tregs and/or increasing CD8 or CD4 tumor infiltrating T cells (TIL). We hypothesized that biguanides, by reducing EETs, would suppress Tregs and promote effector T cells in the TME; $C5F_2$-HCB would be more effective than metformin in modulating the TMEBoth biguanides were tested at their maximum tolerated dose ($C5F_2$-HCB; 18 mg/kg daily and metformin; 50 mg/kg every other day).

Results:
In the $SSM2^{ucd}$ allograft, all combination treatment groups with biguanides reduced tumor growth relative to the IgG control at the endpoint (day 63) : metformin + IgG (51.5±9.8%), $C5F_2$-HCB + IgG (53.3±12.8%), metformin + anti-PD-1 (50.4±11.5%), and $C5F_2$-HCB + anti-PD-1 (49.6±12.8%) [($P < 0.05$) for all comparisons]. In contrast, anti-PD-1 antibody alone failed to reduce tumor growth at the endpoint. Flow cytometry analysis of the TME revealed that only $C5F_2$-HCB + IgG treatment increased CD8+ tumor-infiltrating lymphocytes (TILs) compared to all other treatment groups (>2-fold; $P < 0.05$). Additionally, only $C5F_2$-HCB + anti-PD-1 reduced intratumoral Tregs (>3-fold; $P < 0.05$). $C5F_2$-HCB in combination with IgG or anti-PD-1 reduced intratumoral macrophages by $\geq$50% ($P < 0.001$) and reduced the M2/M1 macrophage ratio by 32-61% ($P < 0.05$) relative to other groups. No effects on CD4+ T cells were observed. Furthermore, $C5F_2$-HCB and IgG or anti-PD-1 increased intratumoral neutrophils by 75-120% compared to the anti-PD-1 or IgG ($P < 0.05$). (Multiple comparisons tested by ANOVA).

Conclusion:

$C5F_2$-HCB and metformin inhibited growth of the $SSM2^{ucd}$ allograft in combination with either anti-PD1 or IgG. In contrast to metformin, only $C5F_2$-HCB modulated the TME, including increased CD8+ TILs with IgG, reduced Tregs with anti-PD-1, and decreased the M2/M1 macrophage ratio with IgG and anti-PD-1. $C5F_2$-HCB, was effective in overcoming an immune excluded TME and may present a strategy for clinical translation.

Ketogenesis protects against MASLD progression and preserves liver polyunsaturated fatty acid homeostasis

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Metabolic dysfunction-associated steatotic liver disease (MASLD) affects over 30% of the global population and is the leading cause of liver disease in Western countries. Despite the FDA's approval of the first drug for treating MASLD in 2024, treatment options remain limited, emphasizing the need to understand the molecular origins of liver dysfunction. Ketogenesis, the production of ketone bodies, is a homeostatic liver pathway whose impairment has been linked to hepatic steatosis and accelerated de novo lipogenesis in MASLD. Here, we investigated the molecular mechanisms by which ketogenesis protects against MASLD. First, we measured whole-body ketone turnover in patients with histologically-diagnosed MASLD, and found a strong positive correlation between hepatic ketogenesis rates and liver injury, suggesting ketogenesis accelerates as a compensatory response to liver metabolic stress. In mice, deleting the rate-limiting step of ketogenesis – 3-hydroxymethylglutaryl-CoA synthase 2 (HMGCS2) – in hepatocytes impaired hepatic fat oxidation and increased susceptibility to steatosis. Deleting mitochondrial β-hydroxybutyrate dehydrogenase in hepatocytes also impaired hepatic fat oxidation, but unexpectedly, did not increase susceptibility to steatosis or liver injury. This suggested that preservation of ketogenesis protects against MASLD, partially through mechanisms that extend beyond overall rates of fat oxidation. To explore the role of ketone bodies in hepatic metabolism, we employed ^{13}C stable-isotope tracing, which revealed a robust ability of the liver and hepatocytes to metabolize ketone bodies to support lipid anabolism. Most notably, we discovered that ketone bodies were the preferred substrate for polyunsaturated fatty acid (PUFA) elongation, compared to other carbon sources (e.g., glucose, acetate). Lastly, using HMGCS2 and acetoacetyl-CoA synthetase knockout mice, we showed the requirement for hepatocyte-sourced ketone bodies to maintain free- and esterified-PUFA lipid pools in the liver, stimulating the hypothesis that ketogenesis-derived PUFAs confer protection against MASLD progression. These findings offer implications relevant to therapeutic interventions such as time-restricted feeding and low carbohydrate diets for the treatment of MASLD, as well as many other human diseases including diabetes, cardiovascular and neurological disorders.

The potential role of brain-specific collagen 25 in Cushing's disease

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Background/Objective: Cushing's disease (CD) is a rare endocrine disorder characterized by hypercortisolemia due to a corticotroph adenoma in the anterior pituitary, leading to unregulated cortisol secretion and a high recurrence rate. The extracellular matrix (ECM) plays a crucial role in cancer development and progression. It is also important in pituitary tumorigenesis and drug resistance. Collagen 25 alpha 1 (COL25A1), an ECM gene originally purified from Alzheimer's disease plaques, is a brain-specific collagen. The association between COL25A1 and pituitary disease has not been previously reported.

Methods: We analyzed the potential role of COL25A1 in CD using human tumor and autopsy-derived pituitary samples. Immunostaining was performed to assess protein expression, and whole transcriptome analysis was conducted using spatial genomics techniques. Additionally, an in vivo xenograft mice model was utilized to further examine COL25A1's effects.

Results: COL25A1 mRNA and protein were detected in human pituitary tissues. Spatial genomics analysis revealed significantly increased COL25A1 expression in CD adenomas compared to normal corticotrophs ($p=0.017934$). In normal corticotrophs, COL25A1 was weakly expressed in the cytoplasm, whereas in CD adenoma cells, it was strongly expressed in the nucleus, suggesting a possible nuclear translocation. In both in vitro and in vivo studies, cytoplasmic COL25A1 suppressed cell proliferation and growth while also reducing ACTH levels in the cultured medium. Investigation of nuclear COL25A1 expression *in vitro* is currently ongoing.

Conclusion: Our preliminary findings indicate that COL25A1 contributes to the CD phenotype and may serve as a potential therapeutic target for the disease.

Health Services Research

Selective refusals and selective requests: ethical challenges in the care of a patient after traumatic spinal cord injury

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Case Description:

A 31-year-old patient is admitted to the Surgical Intensive Care Unit after a high-speed motor vehicle accident. With his cervical spinal cord injury, he is dependent on the ventilator. His initial course is complicated by multiple cardiac arrests secondary to mucous plugging. He spent the majority of the next two years in the hospital, where patterns of care requests (specifically bronchoscopies) and refusals (wound cares, airway clearance) emerged. The clinical ethics teams have been involved to answer the following questions: 1) Do clinicians have an obligation to offer procedures that are not medically indicated? 2) Does a pattern of selective refusals, often resulting in critical illness, justify clinician-directed transition of code status to do not resuscitate, if his behaviors aren't consistently aligned with his stated goal of prolonging life? 3) How can hospitals best support teams through the moral distress experienced in ethically complex cases?

Impact/Discussion:

A particular complexity of this patient's care is that his selective refusals are contradictory to his stated care goals and code status. While most patients have not experienced the full gamut of critical illness and resuscitation, he has the embodied knowledge of prolonged ICU stays, suggesting a robust understanding of full code status, should he re-arrest. Multidisciplinary meetings, both for providers and with the patient, have been the cornerstone of his care, and are an important mechanism to mitigating the moral distress associated with his patterns of requests and refusals. Multidisciplinary consensus has also served as a strong foundation to support clinicians in not offering medically unnecessary treatments, the provision of which we consider to be ethically inappropriate. For a patient with decision-making capacity, it is both ethically appropriate and necessary to set clear boundaries with the patient. Understandably, the question of medical futility has been raised since the cares declined have led to recurrent episodes of critical illness by way of mucous plugging and bacteremias. A conversation has been initiated with the patient about a transition to comfort-focused cares if he continues to decline routine—but for him, life-sustaining—cares.

Conclusion:

Ethics consultation and multidisciplinary teams can provide longitudinal support to providers and patients in complex cases where goals of care and patient behaviors are seemingly discordant. Including a decisional patient in developing and enacting boundaries is an important step in providing goal-concordant and quality care.

Environmental Justice Index and Mortality in Rural Women and Men with COPD: A National U.S. Cohort Analysis

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RATIONALE

- Mortality for men and urban women with chronic obstructive pulmonary disease (COPD) has declined, while mortality for rural women is increasing.
- Previous studies suggest women may be more sensitive to environmental factors, which are often more prevalent in rural areas. Our research, using the Environmental Justice Index (EJI), found several categories where rural women face a higher burden than their urban counterparts.
- We hypothesized that environmental and social factors contribute to worse outcomes for rural women compared to rural men with COPD. To assess this, we compared associations between EJI and mortality in rural women and men with COPD.

METHODS

- Retrospective analysis of individuals with COPD (≥2 ICD codes) in 2016-2019 using national Veterans Health Administration (VHA) data.
- Rural designations were based on Rural-Urban Commuting Area codes.
- We used the EJI, a tool created by the CDC, to measure the combined impact of 36 environmental, social, and health factors, providing percentile ranking for each census tract (a higher score indicates greater burden).
- We used multivariable logistic regression models to assess the associations between EJI and mortality, comparing women and men with COPD who are living in a rural area. All models were adjusted for age, race/ethnicity, tobacco use, and Charlson Comorbidity Index.

RESULTS

- Of 370,640 individuals with COPD living in a rural area, 3% were women and 97% men. EJI percentile (mean±SD) was 56±26, with similar distributions between women and men.
- The mortality rate for was 26% for women and 31% for men. A higher EJI was associated with increased odds of mortality (**Figure 1**). Both rural men and women had similar trends in mortality by EJI.
- There were no statistical differences in EJI or subdomains including poor internet access, age >65, living with a disability, residing in mobile homes, walkability, access to parks, and prevalence of conditions such as cancer, diabetes, and hypertension, when comparing rural women vs. men.

CONCLUSIONS

- Both rural men and women with COPD exhibited similar trends in mortality by EJI, suggesting that environmental and social factors are critical contributors to health outcomes in rural populations regardless of sex. While rural women face rising mortality rates, further research is needed to explore specific gender-related vulnerabilities and develop targeted interventions to address these disparities.

Robert P Hebbel 2025 Research Day Awardees

Basic/Preclinical

Kyle Fulghum	Western Diet Abolishes Cognitive Benefits of Ketone Body Precursor, (R,S)-1,3-Butanediol, in a Mouse Model of Tauopathy
Andres Ortega	Liver PSAT1 governs liver-specific and systemic glucose adaptations to sulfur amino acid restriction
Patrick Ernst	Elucidating Cardiometabolic Changes in Dystrophic Cardiomyopathy

Fellow/ Clinical Science

Rahul Karna	Long Term Risk of Pancreato-biliary Events After Necrotizing Biliary Pancreatitis In Patients Without Cholecystectomy Compared to Patients With Cholecystectomy: A Multi-Institutional Propensity-Matched Cohort Study
Sharon Li	Impact of Pretransplant Immunosuppression on Post-Lung Transplant Outcomes

Graduate Student/ Basic Science

Chinasa Ufondu	Unique interactions between E-cadherin loss and Estrogen receptor drive tumorigenesis in Invasive Lobular Cancer.
Natasha Roman Ortiz	Selective targeting of estrogen receptor positive breast cancer stem- like cells with oncolytic virus
Eric Queathem	Ketogenesis protects against MASLD progression and preserves liver polyunsaturated fatty acid homeostasis
Joel Pardo	Examining APOBEC3B in TCGA and ICGC Breast Cancer Datasets Reveals Altered Drug Metabolism Pathways
Eli Levin	Bioreactor Optimization for perfusion and loading of a 3D Bioprinted Human Chambered Muscle Pump

Resident/ Clinical Science

Kayla Hamann	Exploring Disparities in Guideline-Recommended Care Delivery for Women vs. Men with Chronic Obstructive Pulmonary Disease: a National U.S. Cohort

Pornlada Likasitwatanakul	HLA class I expression shapes the tumor immune microenvironment and influences prognosis in prostate cancer
Dzhalal Agakishiev	Renal Outcomes in Patients on Extracorporeal Membrane Oxygenation
Elizabeth Schwartz	C-Reactive Protein as a Predictor of Hospitalization and Death Among People with Advanced HIV Disease in Uganda

Undergraduate Student: Medical Student/ Basic and Clinical Science

Connor Slette	Quantifying Protein-Protein Interactions in ER+ Breast Cancer through the NanoBit Assay
Jordan Sauve	Prevalence of Cardiac Fibrosis in Female Carriers of Dystrophinopathies: Insights from Cardiac MRI and Cardiopulmonary Exercise Testing

Staff/ Basic and Clinical Science

Ali Hammad	Does Adenosine Deaminase Acting on RNA (ADAR1) Have a Role in CSCs Maintenance in NSCLC?
Ahmed Almahdy Mohamed	Preoperative Colchicine and Heart Block after the First Trans-catheter Aortic Valve Implantation: a U.S. Nationwide Analysis