Department of Medicine

Evans Days

October 19-20, 2023

WILKINS VISITING PROFESSOR LECTURE

Barbara Kahn, MD

George R. Minot Professor of Medicine,
Harvard Medical School
Vice-Chair for Research Strategy,
Department of Medicine
Senior Faculty and Former Chief,
Division of Endocrinology, Diabetes, & Metabolism,
Beth Israel Deaconess Medical Center
Institute Member, Broad Institute of MIT & Harvard

"A Novel Class of Signaling Lipids with Anti-Inflammatory and Anti-Diabetic Effects"

INGELFINGER VISITING PROFESSOR LECTURE

Gbenga Ogedegbe, MD, MPH

Dr. Adolph & Margaret Berger Professor of Population Health
Director, Institute for Excellence in Health Equity (IEHE)
NYU Grossman School of Medicine, NYU Langone Health

"Addressing Health Inequities in Hypertension: Having Impact Through Research"

BOSTON MEDICAL CENTER

BOSTON UNIVERSITY

EVANS DEPARTMENT OF MEDICINE RESEARCH DAYS

SCHEDULE OF EVENTS

Thursday, October 19th

Oral Presentations
8:15am – 9:15am | L110

Basic Science Posters Presentation
9:30am – 11:00am | Hiebert Lounge

Basic Science & Clinical Research Posters Presentation
11:15am – 12:45pm | Hiebert Lounge

Dr. Hollenberg Interviews Dr. Kahn
1:00pm – 1:30pm | Keefer Auditorium

ARC & Dr. David Coleman Prize Presentations
1:30pm – 2:30pm | Keefer Auditorium

Wilkins Visiting Professor Lecture
3:00pm – 4:00pm | Keefer Auditorium

Barbara B. Kahn, MD
George R. Minot Professor of Medicine, Harvard Medical School
Vice Chair for Research Strategy, Department of Medicine
Senior Faculty, Division of Endocrinology, Diabetes and Metabolism. Beth Israel Deaconess Medical Center

"A novel class of signaling lipids with anti-inflammatory and anti-diabetic effects"

Reception & Awards Ceremony
6:00pm – 9:00pm | Hiebert Lounge

Friday, October 20th

Ingelfinger Visiting Professor
12:00pm – 1:00pm | Keefer Auditorium

Olugbenga G. Ogedegbe, MD, MPH
Dr. Adolph & Margaret Berger Professor of Population Health
Director, Institute for Excellence in Health Equity (IEHE)
NYU Grossman School of Medicine, NYU Langone Health

''Addressing Health Inequities in Hypertension: Having Impact through Research''

CHAIR AND COMMITTEE

Chair, Department of Medicine

Anthony Hollenberg, MD

Chair, Evans Days

David Salant, MD

Blue Ribbon Panel

K. Alysandratos	Lindsay Farrer	Jean Liew	David Salant
Deborah Anderson	Jessica Fetterman	Laura Lowery	Insa Schmidt
Sabrina Assoumou	Michael Fischer	Weining Lu	Angie Serrano
Rivka Ayalon	Kari Gillmeyer	Justin Lui	Francesca Seta
Kathryn Bacon	Anna Goldman	Ivan Luptak	David Sparrow
Tamar Barlam	Valerie Gouon-Evans	Shinobu Matsuura	Katie Steiling
Tracy Battaglia	Adam Gower	Sarah Mazzilli	Carl Streed Jr.
Laurence Beck	Elliot Hagedorn	Stefani Monti	S. Subramaniam
Emelia Benjamin	Miriam Harris	Gareth Morgan	Katrina Traber
Kimberly Bertrand	Christopher Heaphy	Gustavo Mostoslavsky	Kim Vanuytsel
Nicholas Bosch	Titi Ilori	George Murphy	Ashish Verma
Markus Bosmann	Matthew Jones	Mara Murray Horwitz	Sushrut Waikar
Joshua Campbell	Naomi Ko	Matthew Nayor	Andrew Wilson
Lisa Caruso	Darrell Kotton	Katya Ravid	Howard Wolpert
David Center	Gene Kwan	Ian Rifkin	
Erin Crossey	Anica Law	Eric Roseen	
Jude Deeney	Marc Lenburg	Frederick Ruberg	
Ruben Dries	Adam Lerner	Manish Sagar	

EVANS DEPARTMENT OF MEDICINE RESEARCH DAYS

HISTORY OF THE EVANS MEDICAL FOUNDATION

This year marks the 111th year of the Evans Department of Medicine. The Evans Department of Medicine began its activities in 1912. It was established by Maria Antoinette Evans, who made a series of gifts to the Massachusetts Homeopathic Hospital, now Boston Medical Center, to endow a research department of medicine, with the stipulation that research and teaching be intimately interrelated in the department. Although technically a separate research institute, the Evans Department has always functioned as an integral part of the clinical care and training programs of Boston Medical Center and the academic programs of the Department of Medicine at Boston University School of Medicine. Many of its research programs involve components at the hospital, the Medical School, and the Boston Veterans Administration Medical Center.

This year marks the 38th Evans Department of Medicine annual research celebration, which was established in 1985 to acknowledge and foster the research activities of the Evans Department of Medicine. A two-day period of academic activity will take place during which both the basic and clinical research accomplishments of the department are exhibited. In recognition of the distinguished past of the department as a training center for faculty and practitioners, we invite eminent clinical and basic scientists to share their scholarship and enlighten present trainees and faculty.

Poster presentations of ongoing research demonstrate our vigorous present and future. The two-day event features Distinguished Clinical and Basic Science Lectures (Ingelfinger Visiting Professor and Wilkins Visiting Professor respectively), which serve as touchstones of the excellence to which we all aspire.

EVANS DEPARTMENT OF MEDICINE RESEARCH DAYS
SPEAKERS 1992-2023

	Ingelfinger Visiting Professor	Wilkins Visiting Professor
1992	Lawrence G. Raisz, MD	J. Michael Bishop, MD
1993	William G. Couser, MD	Robert J. Lefkowitz, MD
1994	Sheldon Greenfield, MD	Philip Leder, MD
1995	Marcia Angell, MD	Thomas P. Stossel, MD
1996	Jeffrey Glassroth, MD	Harold Varmus, MD
1997	Lee Goldman, MD	Phillip A. Sharp, PhD
1998	Andrew I. Schafer, MD	Mark Ptashne, PhD
1999	Jerome P. Kassirer, MD	Leroy Hood, MD, PhD
2000	Harold C. Sox, Jr., MD	James Wilson, MD, PhD
2001	Edward J. Benz, Jr., MD	Andrew Wyllie, FRS
2002	Ralph Horwitz, MD	Eric N. Olson, PhD
2003	Martin J. Blaser, MD	Stuart H. Orkin, MD
2004	Robert Moellering Jr., MD	Marc Kirschner, PhD
2005	Alan Fogelman, MD	Craig C. Mello, PhD
2006	Bradford C. Berk, MD, PhD	Richard P. Lifton, MD, PhD
2007	Nicholas F. LaRusso, MD	Elizabeth G. Nabel, MD
2008	Christine K. Cassel, MD	Stephen O'Rahilly, MD, FRS
2009	Talmadge E. King Jr., MD	David A. Flockhart, MD, PhD
2010	Richard Shannon, MD	Cynthia Kenyon, PhD
2011	William Bremner, MD, PhD	Richard Mulligan, PhD
2012	Joseph Loscalzo, MD, PhD	Aram Chobanian, MD
2013	Wendy Levinson, MD	Orian Shirihai, MD, PhD
2014	Christine A. Sinsky, MD, FACP	David A. Schwartz, MD
2015	David Johnson, MD, MACP, FASCO	Jennifer Lippincott-Schwartz, PhD
2016	John M. Carethers, MD	Glenn Dranoff, MD
2017	Nancy J. Cox, PhD	Katrina Armstrong, MD
2018	Gary V. Desir, MD	Gregg L. Semenza, MD, PhD
2019	Nancy Brown, MD	Aviv Regev, PhD
2020	Nakela Cook, MD, MPH	Elaine Fuchs, PhD
2021	Dale Abel, MD, PhD	Drew Weissman, MD, PhD
2022	Kathleen Cooney, MD	William Kaelin Jr., MD
2023	Gbenga Ogedegbe, MD, MPH	Barbara Kahn, MD

CENTERS

Evans Center for Interdisciplinary Biomedical Research (ECIBR)

Boston University Interdisciplinary Biomedical Research Office (BU IBRO; www.bu.edu/research/ibro) creates opportunities for new interdisciplinary approaches to biomedical research and, in so doing, enhances the training experience at BU. It builds on the success of the Evans Center for Interdisciplinary Biomedical Research (ECIBR) at the Medical Campus, and was established under the auspices of the office of BU Vice President and Associate Provost for Research and the Department of Medicine.

Under the leadership of Katya Ravid, professor of medicine, biochemistry, biology and health sciences, IBRO and ECIBR collaborate with the Clinical & Translational Science Institute (CTSI) to facilitate interdisciplinary basic research discovery across campuses. IBRO maintains mechanisms developed by the ECIBR, including grant support of Affinity Research Collaboratives (ARCs), workshops, and seminars aimed at creating innovative, interdisciplinary team science research.

How It All Fits Together: ECIBR (www.bumc.bu.edu/evanscenteribr) began on the Medical Campus, providing the groundwork and tools to facilitate biomedical team science. IBRO expands the reach of those efforts to the Charles River Campus, encouraging more robust collaboration across the University and inspiring initiatives that are larger in scope. ECIBR focused on investigator-initiated research topics within the medical campus, while IBRO develops cross-campus programs around research strengths at BU with the potential to develop into university-wide research initiatives and programs.

Center for Integrative Transdisciplinary Epidemiology (CITE)

The vision of CTE is to harness contemporary integrative epidemiological tools to better elucidate the multilevel determinants of health and disease in diverse populations in the community, in clinical setting and at the individual level, evaluate strategies for promoting and maintaining health and preventing and treating disease and strengthen teaching and building capacity in population health and epidemiological research.

The mission of CTE is to expand, refine and innovate the epidemiological methods and quantitative analysis of health measures to improve people's lives and to reduce inequalities in health.

We hope to achieve this transformative vision and mission by developing and facilitating highly collaborative translational and interdisciplinary epidemiological research that leverages and integrates large populations and well-defined clinical studies with high dimensional data via methodological innovation and analysis, in part by combining cohort-based genomic, proteomic and other Omics research with personalized behaviorome and exposome and the phenome.

In addition, the CITE will provide education and practical hands-on training in integrative epidemiology and quantitative health sciences methods *to train the public health workforce and clinical investigators of the future* using its resident databases, clinical trials and cohort studies.

Leveraging our expertise in planning, recruiting cohorts and establishing an infrastructure for surveillance (of cohorts, registries, crowd-sourced patient groups) we hope to contribute to *new and future observational cohort studies and clinical trials at BUSM*.

The CITE will make initial investments in creating a valued resource in five thematic areas leveraging resources at BUSM and focusing on methodological innovation:

1. *Life course epidemiology creating super cohorts with extant and new data*
2. *Integrative approaches for personalized and public health epidemiology*
3. *Novel epidemiological designs and analytical methods suited for super cohorts*
4. Designing synthetic and pragmatic *clinical trials in key areas of disease burden*
5. *Health disparities research* incorporating aforementioned themes

Evans Center for Implementation and Improvement Sciences (CIIS)

The Boston University Department of Medicine Center for Implementation and Improvement Sciences (CIIS) is a methodological hub for the scientific evaluation of efforts to improve healthcare delivery. CIIS integrates key components of implementation and improvement sciences with a focus on care within safety net systems, especially including Boston Medical Center.

What are Implementation and Improvement Sciences?

- Implementation science seeks to understand the process of evidence uptake into clinical practice. Did people perform the innovation or intervention? Why or why not?
- Improvement science seeks to rigorously measure outcomes of efforts to improve healthcare delivery. Did the new intervention measurably improve desirable outcomes?

Combining Implementation and Improvement Sciences allows CIIS to assist in the development and rigorous evaluation of endeavors that seek to improve the quality of healthcare, particularly related to care for the underserved. CIIS serves as a conduit to the free flow of ideas between clinicians, administrators, and health services researchers in the Boston University community and beyond to facilitate improvement in care that not only measures internal program adherence, but also produces inferential and generalizable evaluations of healthcare delivery.

Specific objectives of CIIS are to:

1. Guide, support, and innovate design of projects that rigorously evaluate the effectiveness of efforts to implement healthcare system change.
2. Identify factors and strategies that accelerate the adoption and promote sustainability of effective healthcare interventions in safety net systems.
3. Educate faculty and trainees in implementation and Improvement Sciences.
4. For more information, please visit our website: http://sites.bu.edu/ciis/

EVANS DEPARTMENT OF MEDICINE RESEARCH DAYS ABSTRACTS

Cardiology

NEWLY IDENTIFIED ROLES FOR PIEZO1 MECHANOSENSOR IN CONTROLLING NORMAL MOUSE AND HUMAN MEGAKARYOCYTE AND PLATELET DEVELOPMENT AND IN PRIMARY MYELOFIBROSIS

Author: Iris Karkempetzaki
Additional author(s): Vittorio Abbonante, Nasi Huang, Christina Ward, Shinobu Matsuura, Alessandra Balduini, Katya Ravid

Objective: Piezo1 is a mechanosensitive cation channel activated by a stiff extracellular matrix (ECM). We identified a role of Piezo1 in normal megakaryopoiesis and in primary myelofibrosis (PMF), hallmarked by a rigid ECM.

Methods: Bone marrow MKs from C57BL/6J control and myelofibrotic mice carrying the JAK2V617F+ mutation, or MKs derived from stem cells of patients carrying the same mutation were analyzed at mRNA and protein levels for relative Piezo1 expression. Effects of Piezo activation or inhibition on MK maturation and ploidy were assessed by flow cytometry.

Results: Piezo1 pharmacological activation increased or decreased the number of immature CD41+ or mature CD41+CD42+ control, wild type (WT) MKs, respectively, and the ploidy profile was shifted to lower ploidy. Piezo1 inhibitor, GsMTx4 decreased the number of CD41+ MKs, and tended to increase the number of mature MKs. MKs derived from JAK2V617F+ mice showed highly elevated Piezo1 expression, compared to WT controls. Pharmacological modulation of Piezo in JAK2V617F+ mouse MKs significantly augmented the changes observed in WT cells. Importantly, similar effects were observed in WT vs. JAK2V617F+ MKs derived from patients with PMF. Piezo inhibition increased proplatelet formation.

Conclusion: Piezo1 might serve as break to full MK maturation and platelet formation under normal conditions, and its upregulation in PMF MKs further aggravates hallmarks of this disease.

SGLT2 INHIBITOR EMPAGLIFLOZIN IMPROVES MYOCARDIAL ENERGETICS, HYPERTROPHY, CONTRACTILE RESERVE AND DIASTOLIC FUNCTION IN HYPERTROPHIC CARDIOMYOPATHY DUE TO MYOSIN R403Q MUTATION

Author: Tomas Baka
Additional author(s): Jarrod Moore, Fuzhong Qin, Huamei He, Jordan M. Chambers, Dominique Croteau, Raghuveera Goel, Aifeng Zhang, Hunter Smith, James A. Balschi, David R. Pimentel, Christine E. Seidman, Jonathan G. Seidman, Andrew Emili, Wilson S. Colucci, Ivan Luptak

Objective: Hearts with hypertrophic cardiomyopathy (HCM) are energetically deprived as reflected by decreased phosphocreatine to ATP ratio and increased ADP. ↓ATP/ADP leads to ↓free energy of ATP hydrolysis (ΔG~ATP) and impairs cardiac relaxation and contractile reserve. We found that sodium-glucose co-transporter 2 inhibitors (SGLT2i) improve mitochondrial function in diabetic and dilated cardiomyopathies. We hypothesize that chronic treatment with empagliflozin (EMPA) improves cardiac energetic metabolism, function and LV hypertrophy in a murine model of HCM due to R403Q myosin mutation.

Methods: Mice 9 weeks of age were treated for 16 weeks ± EMPA (0.15 mg/g chow).

At 25 weeks, echocardiography in vivo and cardiac energetic measurements in isolated beating hearts at low and high workloads were measured by multinuclear NMR spectroscopy. Transcriptomic analysis was performed at 12 weeks and proteomics at 25 weeks.
Results: Compared to WT, R403Q hearts showed: 1) ↑wall thickness and diastolic dysfunction 2) ↑intracellular sodium 3) blunted energetic and contractile reserve 4) ↓ΔG~ATP 5) ↑glucose uptake 6) ↓fatty acid oxidation. EMPA treatment improved all these findings. The structural/energetic findings were supported by gene changes that preceded changes in proteomic pathway analysis.
Conclusion: These observations confirm that energetic dysfunction plays a central role in HCM, and that interventions such as SGLT2i that improve cardiac energetic metabolism may be of therapeutic value.

LONGITUDINAL ASSESSMENT OF TOBACCO PRODUCT USE PATTERNS AND TRANSITIONS IN YOUNG ADULTS USING MOBILE HEALTH PLATFORM IN CITU 2.0
Author: Madeleine Burns
Additional author(s): Erika T. Minetti, Joseph Palmisano, Aruni Bhatnagar, Rose Marie Robertson, Rachel Keith, Naomi M. Hamburg
Objective: The complexity of tobacco product use patterns necessitates longitudinal assessment. We developed a mobile platform to capture use patterns and transitions in adults.
Methods: We created a text-messaging program that assessed tobacco product(s) used monthly for 24 months in adults (18-45 years) enrolled in the CITU 2.0 study. Baseline use patterns and transitions at last follow-up are reported along with models to predict likelihood of non-use or non-combustible use at follow-up.
Results: Baseline use patterns of 282 participants were: 50 non-users, 97 sole e-cig users, 49 sole combustible cig users, and 86 dual users. Both non-users and cig users had stable use patterns over time: 2% of non-users became sole e-cig users; 22% of cig users became non-users. Sole e-cig use was also stable with a low rate of cig uptake (4% dual, 4% sole cig), and 30% became non-users. Dual users had higher transition rates: 17% becoming cig use, 34% to sole e-cig use and 16% to non-use. In logistic regression models, compared to cig users, there was no statistically significant difference in rates of transition to non-use in sole users (OR 1.5, $p=0.3$) and dual users (OR 0.7, $p=0.4$). Rates of non-combustible use were higher in both sole (OR 27.8, $p<0.0001$) and dual users (OR 2.5, $p=0.02$) at follow-up.
Conclusion: A text-message platform can longitudinally assess tobacco product use. Transitions were common particularly in dual users. Non-combustible use at follow-up was more likely in sole and dual users.

IMPACT OF A URINE SODIUM GUIDED TREATMENT PROTOCOL ON ACUTE HEART FAILURE LENGTH OF STAY: A SINGLE CENTER QUALITY IMPROVEMENT STUDY
Author: Ross Okazaki
Additional autor(s): Kyle Jones, Karishma Pareek
Objective: Inpatient management of acute decompensated heart failure (ADHF) is associated with high costs and in-hospital complications. More objective evidence-based methods of treating ADHF are needed to improve outcomes for these patients. We hypothesize a treatment algorithm using Una as an objective marker of response to loop diuretics can decrease hospital length of stay (LOS) for patients with ADHF.

Methods: 3 plan-do-study-act (PDSA) cycles were performed to study the impact of the intervention. Median hospital LOS, rates of readmission and acute kidney injury (AKI), and protocol use rates over a 5 month period from July-December 2022 were calculated.
Results: An average of 78 patients per month were admitted with ADHF. Protocol implementation rate was 58%. Patients in the first two PDSA cycles with ADHF who were managed with the UNa protocol on average had lower hospital LOS by 1 day and similar rates of acute kidney injury (AKI). Patients in the third PDSA cycle, however, did not experience any change in hospital LOS or AKI. Readmission rates were stable across all PDSA cycles.
Conclusion: Protocolizing the implementation of spot uNa to diuretic management in a large academic hospital required extensive multi-disciplinary coordination. While there was no observable impact on key quality metrics, maintaining high levels of protocol adherence remains a challenge. The impact of kidney injury and patient demographics on Una-protocol performance should be evaluated.

PHYSICAL PERFORMANCE IN BLACK AND HISPANIC OUTPATIENTS WITH HEART FAILURE: THE SCAN-MP STUDY

Author: Christopher Valente
Additional autor(s): Cody Chiuzan PhD, Rabah Alreshq MD, Tori Blot, Denise Fine, Stephen Helmke, Carlos Rodriguez, Natalia Sabogal, Sergio Teruya, Morgan Winburn, Damian Kurian MD, Farbod Raiszadeh MD, PhD, Mathew Maurer MD

Objective: Deficits of physical function are associated with poor quality of life and adverse health outcomes, but data informing the association of these assessments among Black and Hispanic outpatients with heart failure (HF) are limited.

Methods: The multicenter, prospective Screening for Cardiac Amyloidosis With Nuclear Imaging for Minority Populations (SCAN-MP) study identified Black and Hispanic subjects with stable HF, collected baseline characteristics, and took measures using the short physical performance battery. Subjects completed a Kansas City Cardiomyopathy Questionnaire (KCCQ), and the clinical outcomes of HF hospitalization and death were ascertained by telephone and review of the electronic health record.

Results: Of 320 participants, 227 (70.9%) had physical deficits, defined by a battery score of ≤ 9. Patients with severe physical deficits reported overall lower KCCQ scores compared to those with no deficits (KCCQ score of 57.0 vs 72.4, $P < 0.001$). Physical limitation was significantly associated with risk of HF hospitalization, after adjustments for age, sex, and New York Heart Association class (severe physical deficit hazard ratio, 3.61; 95% confidence interval [CI], 1.19-10.93; $P = 0.024$; mild physical deficit hazard ratio, 2.59; 95% CI, 0.86-7.75; $P = 0.090$).

Conclusion: Reduced physical performance is highly prevalent among Black and Hispanic outpatients with HF, and it is associated with overall KCCQ score, as well as an increased risk for HF hospitalization.

BETA1 INTEGRIN IS A NOVEL THERAPEUTIC TARGET IN MALIGNANT JAK2V617F HEMATOPOIETIC STEM CELLS (HSC) IN PRIMARY MYELOFIBROSIS (PMF)

Author: Shinobu Matsuura
Additional autor(s): Sophia Long MS1, Iris A. Karkempetzaki MS1,2, Aikaterini Karagianni MS, PhD1,2, Xiaosheng Yang MS1, Nasi Huang MD1, Katya Ravid Dsc1, 1. Cardiovascular Section, DOM Boston University Chobanian & Avedisian School of Medicine, Boston, MA 2. School of Medicine, University of Crete, Crete, Greece

Objective: The JAK2 inhibitor ruxolitinib is effective at symptom management in PMF due to the JAK2V617F mutation, but does not reduce the malignant JAK2V617F HSC, a driver of disease progression and leukemic transformation. Since our earlier studies showed that beta1 integrin is highly active in JAK2V617F HSC relative to wild-type (WT) HSC, here, our goal has been to validate beta1 integrin as a molecular target of malignant JAK2V617F HSC.

Methods: Numbers of HSC in bone marrow were evaluated 24 h and 7 days after anti-beta1 integrin antibody administration in mice transgenic for the human JAK2V617Fmutation. Apoptosis, cell cycle, and single cell RNAseq were evaluated in HSC to interrogate mechanisms of action of anti-beta1 integrin antibody. Effect of anti-human beta1 integrin antibody on growth of human PMF cell line SET-2 was also evaluated.

Results: Mouse anti-beta1 integrin antibody led to up to 45% reduction of JAK2V617F HSC compared to control antibody-treated animals. Effect on wild-type HSC was negligible. Induction of cell cycle, but not apoptosis, was observed as early as 4 hours after antibody administration. anti-human beta1 integrin antibody inhibited growth of the SET-2 cell line.

Conclusion: Beta1 integrin is a promising molecular candidate for drug development aiming at reducing the number of malignant JAK2V617F HSC in PMF bone marrow.

Computational Biomedicine

A BAYESIAN NETWORK-BASED APPROACH FOR MULTI-OMICS INTEGRATION TO REVEAL UNDERLYING MECHANISMS OF HEALTHY AGING

Author: Anastasia Leshchyk
Additional author(s): Stefano Monti

Objective: Previous studies of individuals who live to old age have found that centenarians experience a significant delay in the onset of age-related diseases and disabilities such as Alzheimer's and dementia compared to the general population. Genetic studies of long-lived individuals showed that carriers of the APOE e2 allele had increased odds of reaching longevity compared to the non-e2 allele carriers. The APOE e2 allele is characterized by distinct serum proteomics and metabolomics profiles that could be useful to understand the mechanism of propagation of the genetic effect of APOE to the molecular level and eventually to phenotypes.

Methods: We are developing a novel Bayesian network-based approach that integrates genetics, multi-omics, and multi-phenotypes to identify shared molecular profiles among the subjects with familial longevity that lead to healthy aging.

Results: The preliminary analysis shows that the APOE e2 allele carriers have lower sphingolipids abundance than APOE e4 carriers and better cognitive performance (for SM:18 $-1.5<FC<1.5$, in APOE e2 carriers $P = 0.32$ for a high cognitive score in contrast to APOE e4 carriers with $P= 0.08$). Sphingolipids are fatty amino alcohols that might impair the cognitive function of elderly adults affecting their aging.

Conclusion: The novel Bayesian network-based approach of multi-omics and multi-phenotype integration can be used to make various predictions and probabilistic reasoning to discover novel biomarkers of healthy aging.

CHARACTERIZING TUMOR'S AND LYMPH NODE'S IMMUNE

MICROENVIRONMENT IN EARLY NON-SMALL CELL LUNG CANCER THROUGH MULTIMODAL SINGLE-CELL ANALYSIS

Author: Zhan (Charley) Xi
Additional author(s): Yusuke Koga, Jennifer Beane, Sarah Mazzilli1, Kei Suzuki, Joshua D. Campbell

Objective: Preliminary studies have demonstrated the predictive value of analyzing the tumor-immune microenvironments in lung cancer patients to determine their outcomes. Therefore, we aim to comprehensively, and unbiasedly, describe the immune composition, cell subtypes, and cell states in early-stage lung tumors and tumor-draining lymph nodes (TDLNs) using CITE-Seq.

Methods: Needle biopsy samples were obtained from 10 early-stage lung cancer patients undergoing lung cancer resections. Tissues were collected from normal lung tissue, lung tumors, and TDLNs. A total of 76,721 cells (4,462 from normal lung, 39,019 from tumors, and 33,240 from LN) were identified.

Results: We identified 86 clusters across various immune and epithelial cells and determined the different immune composition between N1 and N2 TDLNs. We observed 8 CD4+ T cell clusters and 11 CD8+ T cell clusters, each having a naïve (LEF1+, TCF7+) cluster and displaying increased proportions in TDLNs compared to tumors. Additionally, we found 5 CD4+ T clusters that were enriched in TDLNs, and 6 CD8+ T clusters that were enriched in tumor samples.

Conclusion: Single-cell profiling of early-stage lung tumors reveals diverse immune cell clusters in TDLNs, tumors, and adjacent normal tissue. In future investigations, we aim to determine whether these immune clusters are associated with survival, recurrence, tumor aggressiveness, and predict responses to neoadjuvant treatments.

THE ROLE OF B-CELL RECEPTOR REPERTOIRE IN LUNG SQUAMOUS PREMALIGNANT LESIONS

Author(s): Darren Chiu
Additional author(s): Carter Merenstein, Marc Lenburg, Sarah Mazzilli, Jennifer Beane

Objective: B cells and plasma cells (PCs) play a vital role in the pathogenesis of lung cancer and provide prognostic predictive value. However, the role of B cells in the bronchial premalignant lesions (PMLs), the precursor of lung squamous cell carcinoma, is poorly understood. In this study, we aim to investigate the B-cell receptor (BCR) repertoire and its association with molecular signature and progression of the PML.

Methods: We performed bulk targeted BCR sequencing on 69 bronchial biopsies obtained from 29 subjects at high-risk for developing lung cancer. The Immcantation pipeline was applied to obtain the VDJ segment, clonal cluster, and mutational load for each BCR read. We then quantified the clonality diversity, isotype switch, and somatic hypermutation (SHM) rate at sample levels and investigated the association with gene signature and the progression of PMLs.

Results: The number of BCR clones, the class switch from IgG3 to IgG1, and the mutational rates of total and CDR3 regions were positively correlated with the plasma cell transcriptional signatures and the antigen processing and presentation signatures. Among the 31 proliferative subtype PMLs, regressive lesions showed higher proportions of IgG usage, higher SHM rate in CDR3 and total BCR regions, and higher frequency of class switch from IgD/M to IgG and from IgG3 to IgG1.

Conclusion: These results suggest B and PCs prevent PMLs from progressing to higher grade lesions by switching to IgG1 BCRs and increasing SHM rates.

COMPARISON AND CHARACTERIZATION OF THE TUMOR AND LYMPH NODE IMMUNE MICROENVIRONMENT IN EARLY NON-SMALL CELL LUNG CANCER THROUGH MULTIMODAL SINGLE CELL SEQUENCING

Author: Zhan Hao Xi
Additional author(s): Yusuke Koga, Shannon McDermott, Jennifer E. Beane, Sarah A. Mazzilli, Kei Suzuki, Joshua D. Campbell

Objective: Differences in the immune microenvironment between lymph node (LN) regions in lung cancer patients remain unknown. We aim to characterize and compare cell states in the immune microenvironment within nodal regions through multi-modal profiling of the transcriptome and cell surface proteins.

Methods: Needle biopsy samples were obtained from normal lung tissue, lung tumor, a single N1 LN station and multiple N2 LN stations from 10 treatment-naive early stage lung cancer patients undergoing resections. 86,920 cells in total were generated via CITE-seq and subsequently clustered. A binary logistic regression model was applied on the cell subpopulations to identify enrichment in nodal regions.

Results: Eight cell clusters were identified including T/NK, myeloid (CD14+), B (CD19+), plasma (CD138+), neutrophil (CD16+), mast (TPSAB1+), pDC (IRF8+), and epithelial (EPCAM+) cells and were subsequently sub-clustered to generate 89 total subpopulations. Plasma cells, along with immunosuppressive mature dendritic cells (mregDCs; LAMP3+) were enriched in N1 nodal regions while naïve CD4+ T lymphocytes (CD45RA+) were significantly enriched in the N2 nodal regions (FDR < 0.05).

Conclusion: Single-cell profiling reveals diversity in immune cell populations between nodal regions in early-stage LUAD. In the future, we aim to determine if levels of these cell subpopulations are associated with recurrence and survival. In addition, we aim to validate these findings through imaging mass cytometry.

REFINING ANNOTATIONS BY IMPLEMENTING MIHC ON WHOLE SLIDE LUNG PREMALIGNANT BIOPSY IMAGES

Author: Emily Green
Additional author(s): Jennifer Beane, Eric Burks, Sarah Mazzilli, Hanqiao Liu, Emily Aniskovich (Past)

Objective: Lung cancer is the leading cause of cancer deaths worldwide. Studying premalignant lesions (PMLs) may be the key to understanding what causes the progress to malignancy. Though tumor types are clinically defined by pathologic appearance, evaluating disease by molecular signatures has become increasingly popular. Normal appearing lesions maybe undergoing molecular changes and are yet to result in pathologic histologies. mIHC is used to visualize multiple proteins simultaneously to observe cellular and immunological interaction associated with cellular histology which allow us to compare molecular profiles associated with IHC biomarkers to histology.

Methods: We explored PMLs by annotating epithelium on premalignant lung biopsy images stained with H&E and mIHC panels to test if we could better refine annotations compared to using H&E alone. We then performed LCM on corresponding slides using the annotations to locate regions to capture. Isolates are currently queued to be sequenced.

Results: Through paired two-tailed t-tests, we found we annotated significantly more unique regions when considering mIHC images compared to H&E images alone. We made an average of 0.81, and up to five, additional unique annotations when referring to

IHC stains.

Conclusion: This suggests mIHC should be performed when evaluating PMLs. We intend to locate potential biomarkers from the sequencing data to assess progression status to histologies and their corresponding mIHC staining patterns.

MOLECULAR SUBTYPING OF LUNG ADENOCARCINOMA PREMALIGNANT LESIONS IDENTIFIES FEATURES ASSOCIATED WITH AGGRESSIVE DISEASE

Author: Kelley Anderson
Additional author(s): Tran, L., Krysan, K., Wiliam, W., Fishbein, G., Green, E., Gang, L., Liu, H., Burks, E., Kane, E., Mazzilli S., Dubinett, S., Spira, A., Lenburg, M., Beane, J.Beane, J.

Objective: Lung cancer is the leading cause of cancer-related death. Adenocarcinoma (LUAD) is the most common form of lung cancer. Atypical adenomatous hyperplasia and adenocarcinoma in situ are the only known precursors in the sequence of LUAD pathogenesis, and the molecular features of aggressive premalignant lesions (PMLs) that would benefit from early interventions are poorly characterized. We hypothesized that transcriptomic changes in subsets of PMLs are linked to distinct genomic and clinicopathologic features of malignant disease.

Methods: To test this hypothesis, we performed exome sequencing and bulk RNA sequencing of laser capture microdissected tissue from tumor margins that included PMLs, tumor, and adjacent normal tissues.

Results: We discovered de novo subtypes of LUAD PMLs based on gene co-expression. One subtype had gene expression alterations similar to aggressive invasive LUAD. This molecular adenomatous PML subtype was associated with increased accumulation of known cancer driver mutations, and further characterized by altered expression of immune-related pathways.

Conclusion: Molecular signatures measured in adenomatous PML may thus enhance our understanding of pathway dysregulation and mutational heterogeneity occurring early on during LUAD carcinogenesis, and implicate immunotherapeutic strategies to prevent their progression to cancer.

BENCHMARKING SINGLE-CELL SPATIALLY RESOLVED TRANSCRIPTOMIC, PROTEOMIC, AND EPIGENOMIC ASSAYS

Author: Jason Weis
Additional author(s): Roxanna Pfefferkorn

Objective: We aim to Spatially profile premalignant, early stage lung carcinomas and metastases to the lung to reveal spatial phenotypes of the lung microenvironment facilitating tumorigenesis.

Methods: Utilizing novel, spatially resolved multi-modal assays, we seek to holistically profile various stages of cancer development within the lung. Specifically, utilizing imaging mass cytometry (Standard BioTools), we can profile the proteome; utilizing both Visium (10x Genomics) and MERFISH (Vizgen) we will profile the transcriptome; Using DBiT-Seq (AtlasXOmics), we can profile accessible chromatin. For the first time, we can holistically profile proteomic, epigenetic, and transcriptomics landscapes from a single lung cancer sample.

Results: Across pilot tests in each assay, we were able to profile multiple cancer lesions at near single cell resolution with a 20-plex protein panel, an unbiased whole transcriptome and targeted RNA panel, and accessible chromatin assays. Using novel integration methodologies, we highlight patterns of immunosuppression which facilitate

tumorigenesis across both modalities and sample types.

Conclusion: We present proof of concept data that demonstrates feasibility of profiling the transcriptome, proteome, and epigenome of a single tumor sample. We demonstrate that integration of these three modes provides complimentary information that enriches our understanding of tumorigenesis within the lung microenvironment.

ELUCIDATING AGE-SPECIFIC MECHANISMS OF HEAD AND NECK CANCER PROGRESSION AND METASTASIS THROUGH A SINGLE-CELL ATLAS

Author: Lina Kroehling
Additional author(s): N/A

Objective: Survival outcome is worse for older people with head and neck squamous cell carcinomas (HNSCC) than younger people, and the reason for this is largely unknown. By integrating publicly available single-cell RNA-sequencing data from human HNSCC tumors, we produce a high-resolution atlas of the disease through which we can identify age-specific populations and signaling events.

Methods: Five publicly available human single cell RNA-seq datasets were downloaded and integrated to produce a high-resolution atlas of HNSCC. Cells were clustered, classified, characterized by gene set enrichment analysis, and stratified by patient age to identify cell types specific to different age groups, both in the epithelial cell compartment as well in the whole tumor microenvironment (TME). Cell-cell communication analysis was performed to identify signaling events occurring between different populations, and how these signals differed in patients of different ages.

Results: Specific epithelial cell sub-populations were identified that were present largely in either old or young patients. Cell-cell communication analyses revealed age-specific signaling events between epithelial cells and cells in the TME, such as VEGF in old patients, and GRN signaling in young patients.

Conclusion: Through the creation of a high-resolution single cell atlas of human HNSCC, we are able to identify age-specific cell populations and signaling events that may lead to more aggressive cancer with older age.

ASSESSMENT OF MUTATIONAL SIGNATURE MODELS AND INFERENCE METHODS

Author: Jingwen Xu
Additional author(s): Yajima Masanao

Objective: Somatic mutations result from multiple mutational processes, which generate unique combinations of mutation types, termed “Mutational Signatures”. We developed models and inference methods to assess their effectiveness in estimating signatures.

Methods: A simulated synthetic dataset of breast cancer consisting of 214 samples and 96 single base substitution (SBS) mutation types with 8 known COSMIC signatures was used. Three versions of LDA models were tested: prodLDA, standard LDA, and Amortized LDA. For each model, inference was performed using both Monte Carlo Markov Chain (MCMC) and stochastic variational inference (SVI) methods in Pyro. We also ran scvi-tools’ Amortized LDA function which uses an autoencoding framework.

Results: All the tested LDA models inferred by SVI method ran fast but produced inaccurate duplicate signatures. In contrast, MCMC-based standard LDA and Amortized LDA models ran slower but generated more accurate estimations. Particularly, the Amortized LDA model with MCMC inference outperformed scvi-tools in terms of accuracy.

Conclusion: Overall, the MCMC inference approach showed better performance in generating accurate estimations of the true signatures for LDA models. However, when dealing with larger datasets, MCMC methods may be too slow. Therefore, for the next step, the focus will be on speeding up the MCMC process in NumPyro or investigating alternative autoencoding Bayes techniques that offer high time efficiency without compromising accuracy.

A NOVEL IN-HOUSE COMPUTATIONAL PIPELINE FOR SINGLE-CELL RNA SEQUENCING DATA ANALYSIS REVEALS A NOVEL MOLECULAR MECHANISM OF KIDNEY FIBROSIS

Author: Simon Lu
Additional author(s): Hannah Nguyen, Sayari Patel

Objective: ZEB2 is a transcription factor and ZEB2 mutations cause Mowat-Wilson Syndrome, a genetic disease with multiple congenital defects including kidney fibrosis. The objective of this study is to investigate the molecular mechanism of kidney fibrosis by evaluating the role of ZRB2 in kidney stromal progenitor knockout mice by single-cell RNA sequencing.

Methods: We generated a Zeb2-flox/flox;Foxd1Cre+ stromal progenitor conditional knockout (Zeb2 cKO) mouse. We performed 10x Genomics Chromium single-cell transcriptional profiling of kidney tissue from two wild-type and two Zeb2 cKO mice and analyzed the data using our in-house computational pipeline.

Results: We observed a significant increase in the proportion of podocytes and a decrease in macrophages in the Zeb2 cKO samples. Differential expression analysis revealed upregulation of myofibroblast markers Vimentin and Nestin in the Zeb2 cKO kidney compared to the wild-type control kidney. Trajectory and functional enrichment analysis provided insights into the potential pathways and changes in cell differentiation influenced by Zeb2 deletion. Furthermore, we integrated the gene expression profiles with L1000 drug perturbation data to predict potential drugs that could reverse the transcriptional changes caused by Zeb2 knockout.

Conclusion: We developed a novel in-house computational pipeline for single-cell RNA sequencing data analysis that reveals a novel molecular mechanism of kidney fibrosis in Zeb2 knockout mice.

CELL TYPE DECONVOLUTION ANALYSIS USING LLFS WHOLE BLOOD RNASEQ REVEALED BLOOD CELL TYPE PROPORTION CHANGES ACROSS DIFFERENT AGE GROUPS

Author: Mengze Li
Additional author(s): Tanya Karagiannis, Paola Sebastiani

Objective: As people age, their blood immune cell type proportion changes, reflecting a systematic change in their pathogen exposure and immune functionality.

Methods: Ideally, single-cell data with large sample size is the best candidate to understand the cross-sectional changes of blood cell type composition, but the cost is high. Fortunately, there are computational tools that can perform cell type deconvolution analysis to estimate cell type proportion from bulk RNAseq data. In this project, we analyzed the whole blood RNAseq data from 1,348 participants in Long Life Family Study (LLFS) using Cibersort and the cell signature matrix lm22.

Results: We identified the proportion estimates of 22 blood cell types. We then grouped participants into different age groups and compared with previous findings in a centenarians single-cell analysis of 66 participants. In both analyses, the proportions of

naive CD4 T-cells, memory CD4 T-cells and naive B-cells decreased in extreme old people compared to younger people, while CD8 T-cells, natural killer cells and monocytes were observed to increase. In addition, we observed a slight increase in memory B cells in extreme old age people, which did not reach statistical significance in the single-cell analysis.

Conclusion: These findings suggested a higher overall exposure of pathogens in older people compared to younger people. Further investigation about the analysis of cell type diversity changes across different ages is still on-going.

UNVEILING GENE MODULES ASSOCIATED WITH PROSTATE CANCER AGGRESSIVENESS: A META-ANALYSIS OF SINGLE-CELL RNA SEQUENCING DATASETS

Author: Junxiang Xu
Additional author(s): Wisdom Amigo

Objective: Prostate adenocarcinoma (PCa) is a prevalent malignancy and a leading cause of male cancer-related deaths. The limited success of recent clinical trials may be attributed, in part, to the clinical heterogeneity of prostate cancer, which encompasses clonal genomic diversity and the activation of various pro-cancer gene modules. Understanding these factors is crucial for enhancing treatment outcomes.

Methods: The raw sequencing data archives or data directly provided with the publications were used to generate a count matrix. Initial quality control (QC) assessments were conducted. Cancer cell identification involved a combination of INFERCNV, author's annotation, and gene expression against healthy prostate. The resulting expression data from cancer cells were integrated, and a Celda bi-clustering algorithm was employed for further analysis.

Results: Our meta-analysis included a total of 17 studies. Among the identified gene modules, we observed gene modules associated with lineage transformation, secretory activity, stress response, gene amplification, and metal ion metabolism across different studies, reflecting the underlying heterogeneity.

Conclusion: The identification of these intriguing gene modules provides valuable insights into the development of PCa. These findings can potentially guide future investigations using spatial transcriptomics and functional studies in both cell line and mouse models, ultimately contributing to the development of novel treatment approaches.

CHARACTERIZATION OF INTRA-TUMOR HETEROGENEITY IN MURINE ORAL TUMOR MODELS OF B-CATENIN/CBP INHIBITION

Author: Mohammed Muzamil Khan
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Objective: This study aimed to profile cell-type specific changes in oral squamous cell carcinoma (OSCC) following pharmacological blockade of Wnt/β-catenin/CBP activity using the inhibitor E7386 in a murine model.

Methods: Utilizing a tobacco-derived carcinogen, 4-nitroquinoline-1-oxide (4NQO), we established an immunocompetent mouse model mimicking human OSCC. Single-cell RNA sequencing (scRNAseq) was performed on tongue tissues from healthy mice (n=2), 4NQO-induced mouse OSCC (n=2), and E7386-treated 4NQO-induced mouse OSCC (n=2).

Results: Significant changes in cellular composition were observed between the 4NQO-

induced and E7386-treated groups. Epithelial cell proportion decreased with E7386 treatment, while endothelial and fibroblast populations increased. The immune compartment showed reduced disease-specific phenotypes, such as the late "neutrotime" sub-type, in the inhibitor-treated group. Epithelial sub-typing using known markers revealed alterations in basal (Krt5+, Krt15+; decreased in E7386 group vs. 4NQO group), cycling (Top2a, Cdc20; increased), and a distinct stress-inducing phenotype (Jun+, Fos+; decreased). Ligand-receptor interactions between epithelial-immune subtypes indicated immune response activation via H2-d1, H2-k1, H2-q4 enrichment in E7386-treated group.

Conclusion: Our findings indicate the reduction of cellular heterogeneity and plasticity profiles in 4NQO-induced tumors, along with immune response activation upon E7386 treatment in OSCC.

CLUSTERING ANALYSIS OF HISTOPATHOLOGY SCORES FROM PNEUMONIA AUTOPSY SAMPLES IDENTIFIES NOVEL HOST-RESPONSE DRIVEN PNEUMONIA SUBCLUSTERS

Author: Amulya Shastry
Additional author(s): Nicholas A. Crossland, Daniel G. Remick, Thomas G Beach, Jeet Kothari, Anna E. Tseng, Aoife K. O'Connel, Hans P. Gertje, Stefano Monti, Joseph P. Mizgerd

Objective: Host-response plays an important role in determining the severity of pneumonia. Host-response heterogeneity in pneumonia remains poorly characterized. We used multiple histopathology features to define pulmonary responses in deceased patients with pneumonia diagnosis and applied a machine learning clustering algorithm to identify novel pneumonia subclusters.

Methods: 292 H&E slides from subjects with pneumonia diagnosis at autopsy were scored based on 21 modalities such as necrosis, neutrophilic and lymphocytic infiltrate, and edema. We used Euclidean distance on scaled scores to calculate pairwise distances and consensus clustering to identify subclusters.

Results: Optimal cluster partition was determined using the consensus CDF curves, consensus scores, mClust BIC, average silhouette width, and Dunn2 index. Seven novel clusters enriched with specific histopathology features were identified using random forest and classification trees.

Conclusion: We have identified seven clusters, each of which could characterized by a set of histopathological features. Identification of novel pneumonia subclusters is expected to unlock new avenues for intervention by host-directed therapies. Potential therapeutics could focus on factors enriched in the distinct subclusters.

DETERMINING MUTATIONAL SIGNATURES OF CARCINOGENS THROUGH IN VITRO EXPOSURE

Author: Natasha Gurevich
Additional author(s): Sarah Mazzilli, Darren Chiu

Objective: The mutations found in the genomes of cancer patients are caused by various mutational processes or "mutational signatures," which are unique combinations DNA mutation types. Many signatures observed in human tumors have unknown etiology. Experimental models such as cell lines can be exposed to various carcinogens and sequenced to identify and characterize mutational signatures.

Methods: Airway epithelial cells were exposed to BaP, NTCU, or NNK in vitro, cloned, and sequenced. NMF was used to identify signatures for single base substitutions (SBS),

double base substitutions (DBS) and indels.

Results: The mutational profiles, mutational burdens, predicted signatures, and predicted exposures for the various samples exposed to carcinogens were compared to those of control samples. Patterns observed for BaP-exposed samples were consistent with SBS4, which is commonly observed in lung cancers. Distinct patterns of SBS, DBS, and indels were found in NTCU-exposed samples. NNK did not exhibit clear patterns of mutagenesis.

Conclusion: Determining the mutational signatures associated with specific exposures provides insights into the unique ways that different genotoxic agents can alter the genome and contribute to tumor development. Overall, our system can be used to systematically screen for mutagenic compounds.

SPATIALLY INFORMED PROFILING OF STAGE I LUNG ADENOCARCINOMA REVEALS AN EXTENSIVE GENE EXPRESSION SIGNATURE OF VASCULAR INVASION

Author: Dylan Steiner
Additional author(s): Lila Sultan, Travis Sullivan, Emily Green, Hanquiao Liu, Sherry Zhang, Gang Liu, Sarah Mazzilli, Kimberly Christ, Eric Burks, Jennifer Beane

Objective: Microscopic vascular invasion (VI) is predictive of recurrence in stage I lung adenocarcinoma (LUAD) but is difficult to assess via pathology and cannot be accurately predicted prior to resection. Thus, new biomarkers are needed to identify this aggressive subset of stage I LUAD tumors.

Methods: To assess molecular programs associated with VI+ LUAD we performed RNA-seq (n=171) and spatial transcriptomics (stRNA-seq) (n=16) of stage I LUAD samples from a diverse multi-institutional cohort. Four stRNA-seq capture areas included VI foci.

Results: We identified three gene expression clusters increased in VI+ stage I LUAD including genomic instability (C1), tissue remodeling (C2), and hypoxia (C3), and one increased in VI- (C4, immune surveillance). The stRNA-seq data revealed that C2 was highly expressed outside the VI focus and declined as a function of distance toward it, while C1 and C3 increased. We also identified regions of partial-EMT defined by high expression of both C2 and C3. C4 was enriched in lepidic tumor growth. Finally, we validated a bulk RNA-seq predictor of VI and established its robustness to intra-tumor heterogeneity using the stRNA-seq data.

Conclusion: Our data suggest that VI-associated gene expression is not exclusive to the VI focus but reflects a widespread molecular re-programming of the tumor toward an angioinvasive phenotype. This may enable the prediction of VI from small biopsy specimens, allowing for more tailored treatment prior to surgery.

SINGLE CELL RNA-SEQ OF THE FIELD OF INJURY ASSOCIATED WITH LUNG SQUAMOUS PREMALIGNANT LESIONS

Author: Regan Conrad
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Objective: Bronchial brushes collected from the mainstem bronchus accurately predict the presence of high-grade lung squamous premalignant lesions (PMLs). Here, we explore the shared cellular changes and gene expression programs between the normal-appearing airway and PMLs to better understand their etiology and optimize biomarkers

for PML detection.

Methods: We collected 41 bronchial brushes of normal-appearing airway and 40 endobronchial biopsies from 40 high-risk patients undergoing lung cancer screening via bronchoscopy. The cells from each sample were sorted, gated to select for live cells or on CD45- , CD45+, or EpCAM+ live cells, into 96-well plates and processed using CEL-Seq2. The celda (Cellular Latent Dirichlet Allocation) package was used to cluster cells and find modules of co-expressed genes.

Results: We observed known changes in secretory cell types associated with smoke exposure and identified basal cell populations linked to lesion histology. A high-grade basal cell population contains cells from biopsies and brushes of patients with high-grade dysplasia. An upregulated gene module in this population is associated with basal cell differentiation, inflammation, and lung cancer cell proliferation.

Conclusion: Our results support prior studies which showed airway gene expression changes linked to lung cancer development and suggest a basal cell subtype linked to severe lesion histology that may partially explain the high-grade PML signature in the accessible upper airway.

EFFECT OF MUCUS PLUGGING ON BRONCHIAL GENE EXPRESSION

Author: Whitney Souery
Additional author(s): Alejandro Diaz, Marc Lenburg on behalf of the DECAMP Investigators

Objective: Chronic obstructive pulmonary disease (COPD) is a destructive lung pathology compromising normal airway function. The quality of life of COPD patients is often worsened by mucus plugs, which can exacerbate airflow obstruction. Mucus plugging (MP) is a quantifiable feature of COPD that can be scored radiologically. As a way to better understand MP biology and identify opportunities for therapeutic intervention, we sought to identify gene expression differences in airway epithelium associated with MP.

Methods: 204 study participants from the DECAMP study were scored for MP via CT imaging by an expert lung radiologist. Each participant had RNA from airway brushings of the mainstem bronchus sequenced separately.

Results: 76 of 204 individuals had detectable MP. The expression of 960 genes was associated with the degree of MP (Spearman; $p < 0.05$). DEGs were categorized into 7 gene sets, with distinct pathway enrichments, suggesting that gene expression differences may reflect numerous MP-associated processes. Ongoing work suggests that gene expression identifies distinct patient clusters that vary significantly across clinical features such as MP score and smoking status.

Conclusion: Differences across participants may reflect numerous MP-associated processes that vary by patient phenotype. Ongoing analysis aims to better understand the interaction between MP-associated biological processes and how they contribute to the observed MP patient clusters.

PERSISTENT EFFECTS OF TOBACCO SMOKE EXPOSURE ON NASAL GENE EXPRESSION

Author: Minyi Lee
Additional author(s): Hanqiao Liu, Diane Ding, Tiffany Chan, Gang Liu, Denise Aberle

Objective: We previously identified genes in the bronchial epithelium whose expression does not return to baseline after smoking cessation. We sought to compare gene expression in nasal epithelium to validate these persistent effects of smoking and to

better understand molecular changes contributing to persistent cancer risk following smoking cessation.

Methods: Nasal RNA-seq data from 31 patients (16 former, 15 never-smokers) were analyzed using limma to identify differentially expressed genes. The relationship with previously derived irreversible smoking genes was assessed using gene set enrichment analysis (GSEA). In addition, single-cell RNA-seq data from the nasal inferior turbinate of 10 individuals (6 former, 4 never-smokers) was used to determine the cell-type specific expression of altered genes.

Results: A linear model identified 77 downregulated and 151 upregulated genes (p-value < 0.01). GSEA showed significant enrichment of bronchial persistently altered gene sets in the nasal ranked list. Persistently decreased genes were primarily expressed in goblet, club cells, and the frequency of club and goblet cells was decreased in former smokers.

Conclusion: These results suggest shared smoking-cessation associated gene expression changes in bronchial and nasal epithelium. The enrichment of the persistently decreased genes in goblet and club cells, paired with their population decrease, may offer an explanation for some persistent gene expression alterations.

IMMUNOSUPPRESSIVE & INFLAMMATORY MICROENVIRONMENT SUPPORTS PREMALIGNANT PROGRESSION IN MURINE SQUAMOUS CELL CARCINOMA

Author: Táchira Pichardo
Additional author(s): Roxanna Pfefferkorn, Darren Chiu, Jennifer Beane, Sarah Mazzilli

Objective: Immune suppression and inflammation contribute to progression of lung squamous preinvasive lesions and carcinoma (LUSC) prognosis, making it clear that defining and modeling the immune changes enabling the development of LUSC will aid the development of immunomodulatory-based interception approaches.

Methods: Using the carcinogen N-Nitrosotris-(2-chloroethyl)urea (NTCU) murine model, we characterized the immune contexture of LUSC premalignancy in two mouse strains with susceptibility to NTCU-induced dysplasia. We performed immunophenotyping via imaging mass cytometry (IMC) over the course of disease initiation and progression to carcinoma.

Results: IMC confirmed an almost 3-fold decrease in both CD8+ and CD4+ T cells in dysplastic lesions compared to carcinoma in situ and invasive LUSC. Additionally, neutrophils increased as lesions worsened in histological severity. Both cell types contribute to LC prognosis: T cells associate with better survival and neutrophils associate with poorer outcomes. These data suggest that an immunosuppressive and inflammatory microenvironment may contribute to lesion progression in the preinvasive disease context.

Conclusion: Characterization of the immune landscape of premalignant lesions that progress will lead to an enhanced understanding of the mechanisms underlying LUSC tumorigenesis, with the ultimate goal of developing and deploying immunomodulatory interventions to ameliorate progression to LUSC and ultimately reduce LC mortality.

GRAPH NEURAL DIFFUSION IN SINGLE-CELL RNA-SEQ ANALYSIS

Author: Yu-Chen Liu
Additional author(s): Juexin Wang

Objective: Graph neural diffusion is a novel machine learning algorithm that was recently introduced. We aim to build a new model for single-cell RNA-seq analysis

based on graph neural diffusion, with the goal of improving the accuracy of both clustering and trajectory analysis.

Methods: We employ the graph neural diffusion model to process the raw data from single-cell RNA-seq. This model aids us in extracting biological features from vast amounts of gene expression data, which is beneficial for clustering and trajectory analysis.

Results: We applied the graph neural diffusion model to various single-cell RNA-seq data, including human blood cells, mouse embryonic stem cells, mouse cortex cells, and more. We achieved high accuracy in clustering and clear pseudo-time relationships within these datasets.

Conclusion: We found that the graph neural diffusion model works very effectively in single-cell RNA-seq analysis. It aids us in extracting precise biological features and enhances the accuracy of single-cell clustering and trajectory analysis.

LOSS OF NOTCH1 LEADS TO DEDIFFERENTIATION OF THE BRONCHIAL EPITHELIUM

Author: Roxana Pfefferkorn
Additional author(s): Darren Chiu, Divya Venkatraman, Marc Lenburg, Avrum Spira, Jennifer Beane

Objective: Carcinogen exposure creates a field of injury along the airways and introduces mutations that can remodel the bronchial epithelium. As a result, premalignant lesions (PML) can form and progress to eventually become frank carcinoma. We use bronchial biopsies from patients at high risk of developing lung cancer to understand the early stages of lung squamous dysplasia and its implications for early detection and treatment.

Methods: We analyzed bronchial biopsies in a tripartite approach: i) we used deep targeted sequencing to identify early mutations, ii) sequenced the transcriptome, and iii) interrogated epithelial shifts and immuno-epithelial interactions through highly multiplex Imaging Mass Cytometry (IMC).

Results: NOTCH1 loss-of-function is the most frequently found mutation in lung PMLs. Transcriptomic profiling of these lesions showed up regulation of peri-goblet cells, a cell type associated with smoking, and down regulation of multiple immune components. A shift towards un- and dedifferentiated epithelial cells and a new path to goblet cell differentiation were identified through IMC. Furthermore, we found alterations in immune populations and immuno-epithelial interactions in NOTCH1 mutated biopsies.

Conclusion: NOTCH1 is tightly linked to PML development by altering the epithelium´s cell composition and its ability to interact with the immune system. We are now incorporating our findings into an in vitro system to understand the epithelial driving forces of PML progression.

CReM

AN INDUCED PLURIPOTENT STEM CELL-BASED MODEL TO STUDY LUNG MESENCHYME DEVELOPMENT AND DISEASE

Author: Andrea Alber
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Objective: The lung mesenchyme plays important roles in lung development and

disease, yet little is known about the biology of lung mesenchymal progenitors or how they initiate disease. We aimed to generate lung-specific mesenchyme and mesenchymal-epithelial co-cultures from induced pluripotent stem cells (iPSCs) in order to study lung mesenchyme development and model respiratory diseases.

Methods: We generated mouse and human iPSC lines carrying a lung mesenchyme-specific reporter and used these lines to develop a protocol for the directed differentiation of iPSCs towards the lung mesenchyme.

Results: We found that iPSC-derived lung mesenchyme (iLM) is transcriptionally similar to primary embryonic lung mesenchyme. Co-cultures of engineered mouse lung epithelium with mouse iLM increased yield of epithelial cells and impacted both epithelial and mesenchymal differentiation programs, suggesting functional crosstalk. To model pulmonary fibrosis we established co-cultures of human iLM with SFTPC-mutant and corrected human iPSC-derived alveolar epithelial type 2 cells (iAT2s) and found that co-culture with mutant iAT2s significantly increases the expression of fibrotic markers in iLM, suggesting that our co-culture system can recapitulate a fibrotic phenotype.

Conclusion: Our iPSC-derived lung mesenchyme expresses key molecular and functional features of primary lung mesenchyme and provides an inexhaustible source of cells for studying lung development, modeling diseases, and developing therapeutics.

MECHANISMS OF ALVEOLAR EPITHELIAL TYPE 2 CELL SELF-RENEWAL IN LUNG INJURY AND REPAIR

Author: Jessie Huang
Additional author(s): Pushpinder Bawa, Carlos Villacorta-Martin, Ruhi Sohal, Konstantinos-Dionysios Alysandratos, Darrell N. Kotton

Objective: Alveolar epithelial type 2 cells (AT2s) are facultative progenitors normally quiescent in adult lung, but reenter cell cycle upon injury. While this is shown in animal models, it is less defined in the human lung. We hypothesize that human induced pluripotent stem cell (iPSC)-derived AT2 (iAT2) self-renewal occurs through defined signals that recapitulate in vivo injury repair.

Methods: Human iAT2s during passaging and mouse AT2s from Sftpc-CreERT2;Rosa-diphtheria toxin A mice after injury were characterized by immunostaining, RT-qPCR, and scRNA-seq. Pairwise comparisons were performed on transcriptome data in proliferative vs non-proliferative cells. Small molecule inhibitors for differentially expressed genes (DEGs) were used on iAT2s to assess proliferation and AT2 markers.

Results: RT-qPCR and scRNA-seq revealed similar kinetics with AT2 maturation inversely correlated with proliferation. 244 overlapping DEGs in proliferative (i)AT2s included E2F family targets and chromatin remodeler EZH2. EZH2 inhibition increased proliferation, reduced maturation, and upregulated transitional cell markers.

Conclusion: Our data suggest that (i)AT2 self-renewal involves an initial proliferative state followed by return to a more mature state. Analysis of human and mouse datasets identified shared upregulated transcripts in proliferating (i)AT2s, and inhibition of EZH2 resulted in increased proliferation and reduced maturation, suggesting EZH2 may be important in AT2 self-renewal.

UNCOVERING THE GENE REGULATORY NETWORK THAT MAINTAINS THE MULTIPOTENCY OF HUMAN AIRWAY BASAL CELLS

Author: Jake Le Suer
Additional author(s): Taylor Matte, Pushpinder Bawa, Scott Randell, Finn Hawkins

Objective: Basal cells (BCs) are the primary stem cell population of the human trachea and

intrapulmonary airway epithelium. BC dysfunction contributes to airway remodeling in several chronic diseases. BC subpopulations, that differ in their proliferative capacity and differentiation ability, have been described in human airways. Our goal is to uncover the core gene regulatory network that defines the BC subpopulation with the greatest stem cell potential and understand how these cells are maintained by the airway niche.

Methods: We utilized the Cell Tag lentiviral barcoding library paired with time series single-cell RNA-Sequencing to lineage trace the progeny of individual primary BC clones and overlay their transcriptomic profile with cell fate outcomes.

Results: Through the barcoding/sequencing approach, we identified 70 unique clones, 49 of which consisted of more than 2 cells. The range of clone sizes varied from 2 to 332 cells, with a median clone size of 8. The 3 largest clones contributed to 53% of all barcoded cells.

Conclusion: Our findings support functional heterogeneity within clonal populations of airway basal cells. The ability to track individual human basal cells using lentiviral barcoding, paired with single-cell RNA-Sequencing allows for the correlation of transcriptional profiling with functional outcomes as well as future in-vitro studies to investigate the role of the airway microenvironment on stem cell maintenance.

ASSESSING THE ROLES OF KMT2D DURING NEURAL CREST- AND MESODERM-DERIVED PERICYTES DIFFERENTIATION

Author: Sandeep Sreerama
Additional author(s): Maria de Los Angeles Serrano

Objective: Kabuki Syndrome (KS) is a disease predominantly caused by a mutation in KMT2D. Presentations involve craniofacial/congenital heart defects and intellectual disabilities, suggesting a Neural Crest (NC) and NC-derived pericyte component. To elucidate whether KMT2D impacts NC and pericyte specification and function, we generated pericytes (iPs) from a KMT2D KO induced pluripotent stem cell (iPSC) line through both NC and mesoderm intermediates.

Methods: We generated NC and mesoderm from KMT2D KO iPSCs using well-established protocols. To generate iPs, we plated intermediates in pericyte induction media containing FBS. To purify the NC intermediate, we positively selected for NC using magnetic-activated cell sorting prior to pericyte induction.

Results: Our results show that we can efficiently generate pericytes from both intermediates in a KMT2D KO background. Interestingly, we found that cellular phenotypes appear to differ between KMT2D KO and WT genotypes during NC induction, especially in terms of migration.

Conclusion: Preliminary morphological results indicate that iPs can be generated from both WT and KMT2D KO iPSC lines. Notably, changes in migration during NC induction may be present between the KO and WT cells. This would align with the hypothesis that altered KMT2D function impacts the NC and suggests that downstream cell types, like pericytes, may be impacted as well.

MODELING GENETIC CREUTZFELDT-JAKOB DISEASE USING HUMAN IPSC-DERIVED CEREBRAL ORGANOIDS

Author: Aldana Gojanovich
Additional author(s): Robert Mercer, Seonmi Park, Pushpinder Bawa, Feiya Wang, David Harris and Gustavo Mostoslavsky

Objective: Develop a stable 3D model of the human cerebral cortex employing patient-specific iPSC with the E200K mutation causing genetic Creutzfeldt-Jakob disease to

study the mechanisms by which prions cause neuronal damage.

Methods: In order to study prion mediated neural disease, we established a CJD E200K-specific iPSC library, including carriers and non-carriers, and tested their differentiation towards cerebral organoids (COs). Here we describe the generation of COs cultures from nine individuals, five carriers for the E200K mutation and four non-carriers.

Results: The COs revealed variations in organoids size in which the mutant organoids were significantly smaller compared with the non-mutants. We then performed single-cell RNA sequencing (scRNA-seq) of 6 months old COs. The transcriptomic profile from E200K carriers revealed several differentially expressed gene signatures relevant to neuronal and astrocytic function that may be involved in the inflammatory process. Complementary histologic analysis of the COs together with neuronal and astrocytic cultures will provide a comprehensive cellular and molecular characterization of E200K PrP-mediated pathogenicity.

Conclusion: Our study shows that reproducible hiPSC-derived cerebral organoids expressing endogenous levels of mutant PrP can model certain aspects of human prion disease, offering an extra dimension and a powerful platform for investigating subtype pathologies and testing alleged therapeutic compounds.

SPLIT INTEIN-MEDIATED GENE THERAPY FOR ABCA3-ASSOCIATED CHILDHOOD INTERSTITIAL LUNG DISEASE (CHILD)

Author: Erin Hennessey
Additional author(s): N/A

Objective: ABCA3-associated childhood interstitial lung disease (chILD) is a rare group of complex diseases for which no treatment currently exists. This disease prevents alveolar epithelial type 2 (AT2) cells from producing pulmonary surfactant, which are necessary for maintaining lung structure. Gene therapy is being considered for the treatment of this monogenic disease, however, existing viral delivery vehicles either A) are unable to transduce AT2 cells, or B) are too small to fit the 5.1 kilobase ABCA3 coding sequence. To circumvent the limited cargo capacity of adeno-associated virus (AAV), we propose an alternative delivery method in which the ABCA3 coding sequence will be split in half and fused to split inteins.

Methods: 4 different split sites for ABCA3 are being tested. Days after plasmids are transfected into 293T cells, protein is extracted for western blot analysis to determine the most efficient split site. This site is selected for viral packaging so that the vectors can be delivered to A549 and iPSC-derived AT2 cells harboring ABCA3 mutations.

Results: Western blot analysis shows that ABCA3 split before aa S828 is most efficient at recombination into full protein. Split ABCA3 functional assays are ongoing.

Conclusion: We hypothesize that when delivered to AT2 cells, split inteins will allow for the reconstitution of the functional full-length ABCA3 protein, which will rescue the aberrant cells by enabling them to produce pulmonary surfactant.

DURABLE ALVEOLAR ENGRAFTMENT OF PSC-DERIVED LUNG TIP-LIKE CELLS INTO IMMUNOCOMPETENT MICE

Author: Michael Herriges
Additional author(s): Bibek R. Thapa, Jonathan Lindstrom-Vautrin, Feiya Wang, Carlos Villacorta-Martin

Objective: Recent work suggests cultured embryonic lung epithelial tip cells can engraft in injured immunocompromised mouse lungs, providing a method for cell-based therapy of lung injury. However, the use of embryonic cells and immunocompromised recipients

limits the clinical applicability of this approach. Directed differentiation of pluripotent stem cells (PSCs) offers an alternative source of donor cells which can overcome these limitations.

Methods: Here we describe an important step toward human PSC-based lung cell therapy with the development of a protocol for the directed differentiation of murine PSCs into distal tip-like cells.

Results: The resulting cells are transcriptionally and morphologically similar to cultured primary tip cells. These PSC-derived tip-like cells can be transplanted into syngeneic and immunocompetent mouse lungs, where they gave rise to both AT2-like and AT1-like cells that persisted for up to 6 months post transplantation. Furthermore, donor-derived cells demonstrate the lamellar body organelles and facultative progenitor capacity of AT2 cells. This suggests that donor-derived AT2-like cells are functionally similar to endogenous AT2 cells.

Conclusion: Together this work provides evidence of successful functional engraftment of PSC-derived cells into immunocompetent mouse lungs. Further characterization of this system will provide important information for the development of PSC-derived cell therapy of human pulmonary diseases.

DETERMINATION OF THE MECHANISMS THROUGH WHICH DESMOPLAKIN REGULATES AT2 CELLULAR PHENOTYPES AND THEREBY CONTRIBUTES TO THE PATHOGENESIS OF PULMONARY FIBROSIS

Author: Méline Homps-Legrand
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Objective: The genetic variant rs2076295, identified as the causal variant at the 6p24 locus by genome-wide association studies in COPD and pulmonary fibrosis (PF), strongly affects the expression of the desmosomal protein desmoplakin (DSP) in lung but not other tissues. In PF, DSP expression is downregulated only in a subset of lung epithelial cells including alveolar type 2 cells (AT2s). The objective is to determine the mechanisms through which DSP regulates AT2 maturation, proliferation, and thereby contributes to the pathogenesis of COPD and PF, especially through the modulation of the Wnt/Tcf signaling pathway.

Methods: Induced pluripotent stem cells (iPSCs) are differentiated into AT2s - called after iAT2s. DSP and its cell junction's partners (plakoglobin and p120 catenin) are knocked-down in the iAT2 using CRISPRi, to study the effects on Wnt/Tcf signaling.

Results: DSP knockdown (DSP-kd) of iAT2s results in the loss of desmosomes and disordered intermediate filaments in iAT2s with the dysregulation of adherens junctions. DSP-kd also induces both proliferation and maturation of iAT2s, and modulation of Wnt/Tcf signaling.

Conclusion: Downregulation of DSP disrupts cell junctions, alters AT2 phenotypes, and modulates Wnt/Tcf signaling that is known to play an important role in the maintenance of AT2 identity. Understanding the mechanisms through which DSP regulates these processes might provide insight into PF pathogenesis and allow for the development of novel treatment approaches.

DECIPHERING MOLECULAR MECHANISMS OF VEGFA INDUCED BILIARY EPITHELIAL CELL-TO-HEPATOCYTE CONVERSION IN LIVER REGENERATION

Author: Jasbir Singh Dalal
Additional author(s): Fatima Rizvi, Ying Tam, Norbert Pardi, Drew Weissman

Objective: End stage liver disease (ESLD) is the 12th most common cause of death in the United States. Currently, there is no therapy to prevent it. Liver transplantation remains the sole treatment of ESLD, hampered by shortage of liver donors. In our previous work we have demonstrated that biliary epithelial cells (BEC)-driven liver regeneration is driven by VEGFA mRNA-LNP delivery and could be used as an alternative to liver transplantation. Our goal is now to investigate the molecular and cellular mechanisms that drive this BEC-to-hepatocyte conversion.

Methods: BECs contribute 2% of total liver cells surrounded by laminin-rich matrix making them difficult to isolate for molecular and cellular studies. To overcome this, we have genetically tagged BEC nuclei and ribosomes and using Nuclear Tagging and Translating Ribosome Affinity Purification (NuTRAP) combined with single nuclei ATAC and RNA sequencing we will generate transcriptomic and epigenomic profiles of BECs and their progeny hepatocytes.

Results: Transient expression of VEGFA promotes BEC-to-hepatocyte conversion and restores liver function. We have generated a comprehensive molecular profile of BECs during their VEGFA-induced conversion to hepatocytes.

Conclusion: Our results may therefore have clinical implications revealing novel therapeutic targets to mitigate acute and chronic liver disease by exploiting and optimizing the alternative intrinsic regenerative ability of the liver via BEC-driven liver regeneration.

ELUCIDATING THE ROLE OF TRANSCRIPTION FACTOR NKX2-1 IN DEVELOPMENT AND DISEASE

Author: Taylor Matte
Additional author(s): Mary Lou Beermann, JC Jean, Anat Kohn, Michael Herriges, Carlos Villacorta Martin, Jake LeSuer, Andrew Berical, Robin Deterding, Darrell Kotton

Objective: NKX2-1 is a critically important transcription factor in lung development, with all lung epithelia being derived from an NKX2-1+ progenitor pool. Nkx2-1 knockout mice have hypoplastic lungs, and humans with NKX2-1 mutations often have respiratory insufficiency in a disease known as brain-lung-thyroid syndrome. Despite its demonstrated importance, NKX2-1's developmental role is not fully defined.

Methods: iPSCs were generated from a patient with a frameshift mutation in exon 3 of NKX2-1, with expected haploinsufficiency. This cell line was CRISPR corrected, inserting a GFP reporter, allowing for the study of two isogenic lines that differ only at the NKX2-1 locus. Cells were sorted and scRNA sequenced at three developmental timepoints in airway and alveolar differentiations. To confirm findings, blastocyst complementation experiments were performed with heterozygous NKX2-1-GFP cells.

Results: RNA-seq analysis revealed gene expression differences that increasingly diverged between mutant, corrected cells over time, with more differences in alveolar differentiation. We observed downregulation of alveolar type II markers and increase in non-lung endoderm markers in mutant alveolospheres. Blastocyst complementation experiments show clear outcompetition of NKX2-1 heterozygous cells in alveolar space but not airway space, confirming functional deficits in alveolar development.

Conclusion: These experiments provide insight into the developmental consequences of aberrant NKX2-1 activity.

GENOTYPE-PHENOTYPE ANALYSIS IN KABUKI SYNDROME ZEBRAFISH MODELS: HIGH-THROUGHPUT

Author: Saylor Williams

Additional author(s): M Emilia Serrano

Objective: Mutations in the epigenetic regulator KMT2D cause Kabuki Syndrome (KS): a rare multisystemic autosomal dominant condition. Patients with KS show high phenotypic variability in cardiovascular defects. KMT2D is assumed to function through its SET domain as a histone methyltransferase. However, there are several other domains in KMT2D, some of which are hotspots for mutation in KS, and it is unknown how these contribute to cardiovascular phenotypes in KS. Using our KS zebrafish models, we aim to determine whether different Kmt2d variants will have a distinct effect on cardiovascular development.

Methods: We use 8 mutant lines with missense or frame-shift mutations in 4 domains of Kmt2d: Atrophin1, FYRN-C, HMG, and SET. Early phenotype evaluation was performed by confocal microscopy of explanted embryonic hearts at 2 and 3 days post fertilization (dpf) in homozygous kmt2d mutants and wild-type siblings. Explanted heart at 2 and 3 dpf were cultured on HD-MEA chips overnight and electrophysiological measurements were performed on BioCam DupleX.

Results: We show that kmt2d homozygous mutant embryos have a transient cardiovascular developmental delay at 3dpf. Lines with homozygous or heterozygous mutations in Atrophin-1, FYRN-C, HMG or SET domains showed variable electrophysiological signatures in explanted hearts.

Conclusion: Our results suggest that mutations in different domains of Kmt2d have distinct phenotypic outcomes.

VEGFA MRNA-LNP PROMOTES BILIARY EPITHELIAL CELL-TO-HEPATOCYTE CONVERSION IN ACUTE AND CHRONIC LIVER INJURY

Author: Fatima Rizvi

Additional author(s): Ricardo Diaz-Aragon, Rodrigo M. Florentino, Anna R. Smith, Susan Wu, Elissa Everton, Ying Tam, Norbert Pardi, Drew Weissman, Alejandro Soto-Gutierrez, and Valerie Gouon-Evans

Objective: Biliary epithelial cell-derived progenitors or liver progenitor cells (LPCs), are thought to give rise to de novo hepatocytes during injury. Our work using embryonic stem cell (ESC) differentiation system revealed that KDR+ fetal hepatic progenitors are bona fide hepatoblast precursors and that KDR activation is required for their hepatic specification. We hypothesize that LPCs in injured adult livers express KDR and that its ligand VEGFA harnesses their conversion to hepatocytes.

Methods: The hypothesis was tested in acute (acetaminophen), and a chronic (CDE diet) liver injury models. Krt19-CreERT2; R26-STOPFl/Fl-tdTomato mice were used to fate trace BECs and Kdr-2A-CreERT2-2A-eYFP, R26LSL tdTomato mice to trace KDR-expressing cells. AAV8-Tbg-p21 viruses were administered to mimic hepatocyte senescence. VEGFA was delivered twice via IV injection of mRNA-LNPs. Human cirrhotic liver tissues were assessed for identification of KDR-expressing BECs.

Results: Delivery of VEGFA induced robust BEC-to-hepatocyte conversion and reversion of steatosis and fibrosis. In human and murine diseased livers, we identified KDR-expressing BECs associated with KDR-expressing-cell-derived hepatocytes. Fate-tracing of KDR+ cells demonstrated emergence of large clusters of hepatocytes being differentiated from KDR-expressing cells in injured mice treated with VEGFA mRNA-LNP.

Conclusion: The study reveals novel therapeutic benefit of VEGFA for harnessing BEC-driven repair to potentially treat liver diseases.

A NOVEL MURINE INTRAPULMONARY AIRWAY INJURY MODEL FOR CELL TRANSPLANTATION

Author: Anat Kohn
Additional author(s): Michael Herriges, Liang (Martin) Ma, Bibek Thapa, Darrell Kotton, Finn Hawkins

Objective: There are no therapies able to reverse airway damage from monogenic diseases such as cystic fibrosis and primary ciliary dyskinesia. Replacement of defective airway epithelial cells with autologous, gene-corrected airway cells could be curative. We successfully transplanted mouse primary or pluripotent stem cell derived basal cells (BCs) into the tracheas of mice post-polidocanol (PDOC) injury. These cells engraft, differentiate to airway lineages, and maintain a BC pool. Minimal injury and few donor cells are found in the intrapulmonary airways (IPAs). To directly study repair and cell transplantation in the IPAs, a micro-bronchoscope (MB) is used deliver fluids or cells to the IPAs.

Methods: For MB, mice are sedated and positioned prior to advancement into the airways. See fig for design of repair & cell transplantation experiments.

Results: IPA PDOC results in patchy loss of airway epithelium, similar to previously reported post tracheal injury. However, the repair is slower, with decellularized IPAs remain 24 hours post injury. Further, loss of AT1 but not AT2 cells is seen at 5-hours post-injury. Finally, donor BCs survive in the IPAs 3 & 6 wks post transplantation and differentiate into airway lineages.

Conclusion: PDOC injury to the IPAs results in robust loss of airway epithelium and transplant of mouse primary BCs into the IPAs is possible. These studies reveal the feasibility of MB delivered PDOC as a targeted murine model for the study of IPA repair and cell transplantation.

DECIPHERING HUMAN LUNG SQUAMOUS CELL CARCINOMA ONCOGENESIS IN-VITRO

Author: Hirofumi Kiyokawa
Additional author(s): Jessie Huang, Joshua D. Campbell, Jennifer E. Beane-Ebel, Ehab Billatos, Sarah Mazzilli, Marc Lenburg, Darrell Kotton

Objective: Lung oncogenesis is a complex process that involves acquisition of genetic mutations resulting in dysregulation of epithelial cell proliferation and differentiation. We sought to understand the stepwise process of lung squamous cell carcinoma (LuSCC) oncogenesis by introducing critical gene mutations into human airway basal cells, the origin of LuSCC.

Method/Results: First, we compared gene mutation datasets from premalignant to carcinoma-in-situ and invasive carcinoma to identify candidate mutations associated with each step. Then, we knocked out candidate genes in iPSC-derived human basal cells (iBCs) by CRISPR/Cas9 gene editing. Gene mutation analyses revealed that TP53 mutations are the most frequently detected gene mutations at the LuSCC initiation stage and chromosome 3q26 amplicon is a common abnormality found in invasive carcinoma We established TP53 mutated iBCs using CRISPR/Cas9 gene editing, which resulted in higher basal cell yield. We also developed lentiviruses to overexpress the candidate genes in Chr3q26. SOX2 overexpression (OE) results in higher LuSCC-associated gene expression, such as KRT13 and KRT6A.

Conclusion: TP53 mutations in iBCs increased cell yield in vitro. In addition, SOX2 OE enhanced LuSSC-associated gene expression, suggesting that Chr3q26 amplification is

involved in LuSSC oncogenesis. In future experiments, we will test whether or not TP53 mutation in combination with SOX2 OE is sufficient to establish cancer program in vitro.

IPSC-BASED MODELING OF RESILIENCY AND EXCEPTIONAL LONGEVITY

Author: Todd Dowrey
Additional author(s): Yvonne Lok, Pushpinder Bawa, Feiya Wang, Marianne James, Jeyoung Bang, Sang-Goo Lee, Paola Sebastiani, Thomas Perls, Vadim Gladyshev

Objective: Rare individuals with exceptional longevity (EL) suggest that we have within us the potential for longer, more healthful lives. Interestingly, it has been demonstrated that exposure to stress can accelerate the accumulation of aging-related biomarkers. We hypothesize that individuals with EL have a unique molecular profile of stress response which improves cellular function and viability under stress.

Methods: We have generated a novel bank of induced pluripotent stem cells (iPSCs) from individuals with EL. This iPSC bank was harnessed to develop a model of resiliency in which iPSCs and iPSC-derived cell types were exposed to ER stressor thapsigargin. Next, we developed transcriptomic and epigenetic signatures of stress response using bulk RNA sequencing and methylation assays respectively.

Results: We identified differentially expressed genes that suggest that EL subjects may contend with stress more productively. EL-derived neurons upregulated protein processing genes under stress. Methylation data were used to estimate biological age using epigenetic clocks to identify differences between cell types and under stress. We performed functional assessment including quantification of neurite length to assess differences in functional outcomes between EL and non-EL derived neurons following stress.

Conclusion: Our model of resiliency synergizes genomic and epigenomic-based discovery with the flexibility of iPSC-based systems to improve our understanding of resiliency mechanisms.

HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED HEPATOCYTES AS A UNIQUE APPROACH TO INVESTIGATE THYROID HORMONE ACTION AND SIGNALING

Author: Lorraine Soares De Oliveira
Additional author(s): Nora Lee1, Anne Van Der Spek2, Joseph Kaserman1, Andrew Wilson1, Anthony N. Hollenberg1

Objective: Thyroid hormone (TH) action is mediated by the thyroid hormone receptor (TR) isoforms and their coregulators. We hypothesized that human hepatocytes (iHeps) derived from induced pluripotent stem cells (iPSCs) would be a unique model to study TH action.

Methods: We have developed a serum free human iPSCs hepatic differentiation protocol with sequential exposure to growth factors to mimic human development. To demonstrate the role of TRs in TH action in iHeps we disrupted TRB1, which is the main TR isoform expressed in liver using CRISPR Cas9 technology. iHeps were treated with PBS (vehicle) or T3 (100nM) for 24h.

Results: Flow cytometry analysis demonstrate increased level of AFP and FoxA1 protein in both lines, and RT-qPCR analysis show upregulation of hepatic marker genes ALB, TTR, AFP, HNF4A, and AAT in the WT and TRBKO iHeps suggesting that iPSCs differentiated normally to hepatocytes after gene editing. RNA sequencing analysis show T3 regulated several genes involved in metabolism on WT while this

response is lost on TRBKO proposing this receptor is crucial for T3 effect in numerous genes. We also observed a few genes being regulated by T3 on TRBKO line indicating they are potentially TRalpha targets.

Conclusion: These results show the iHep model as a unique platform to study the action and signaling of TH in human hepatocytes. Furthermore, they can be a novel approach to determine therapeutic intervention for TH related diseases.

ENDOTHELIAL CELL BASED THERAPY FOR PULMONARY VASCULAR DISEASE USING INDUCED PLURIPOTENT STEM CELLS

Author: Alexander Holtz
Additional author(s): Pushpinder Bawa

Objective: To determine whether human induced pluripotent stem cell (hiPSC)-derived endothelial cells (hiEndos) require pre-patterning towards a lung-specific cell fate to enable engraftment in the mouse lung vasculature. This tool will be utilized for disease modeling and cell based therapies for genetic forms of pulmonary vascular disease, such as FOXF1-related alveolar capillary dysplasia.

Methods: I have developed a novel directed differentiation protocol to generate and maintain hiEndos over serial passages. I utilize hyperoxia-associated acute lung injury to damage the native mouse lung microvasculature prior to retroorbital injection of hiEndos. Engrafted cells are analyzed via flow cytometry and immunofluorescence.

Results: I have found that patterning of hiEndos with BMP9 induces a lung endothelial molecular profile including induction of the lung-specific endothelial cell marker, TMEM100. This patterning is required for successful hiEndo engraftment into the damaged mouse lung microvasculature. Transplanted cells engraft in continuity with the native lung microvasculture and persist for at least 1 month.

Conclusion: Patterning of hiEndos towards a lung-specific molecular fate using BMP9 is essential for successful cellular engraftment into the mouse lung microvasculature. I am currently exploring the differentiation potential and progenitor function of engrafted cells.

EARLY NOTCH INDUCTIN MODULATES THE EMERGENCE OF NASCENT MESODERM FROM HIPSC CELLS

Author: MengWei Yang
Additional author(s): Dar Heinze

Objective: We aim to identify early developmental gene markers that are upregulated during the emergence of the nascent mesoderm formation using a platform of iPSCs differentiation towards T and NK cells. We used a doxycycline-inducible iPSC line to induce Notch 1, which was crucial in the emergence of definitive hematopoiesis. Identification of this early gene signature can help identify and isolate the earliest hematopoietic stem cell and provide insight into the T cell differentiation protocol.

Methods: Cells collected at staggering times from days 0-4, were used to map the gene signatures during the early stages of T cell differentiation via 10x genomics single-cell sequencing and Flow cytometry. Selected genes were validated via qRT-PCR.

Results: Induction of the Notch 1 pathway immediately after exit from pluripotency yields robust mesodermal formation with potential for T and NK differentiation. As the cells leave pluripotency and enter the primitive streak, we found that both dox-induced iPSCs and non-induced iPSCs diverge into 2 populations for 24 hrs on day 2 which converge together from day 4 onward. This lateral mesoderm formation is associated

with the expression of a specific gene signature that resembles human embryonic development.

Conclusion: Taking advantage of a novel protocol for the differentiation of human iPSC into T and NK cells, we have identified a gene set signature associated with the earliest emergence of nascent mesoderm with definitive hematopoietic potential.

GENOTYPE-PHENOTYPE ASSESSMENT OF CRISPR-CAS9 DELETION OF MULTICILLIN IN INDUCED BASAL CELLS FOR RAPID IN VITRO ASSESSMENT OF PRIMARY CILIARY DYSKINESIA

Author: Daniel Wallman

Additional author(s): Andrew Berical, MD, Mary Lou Bermann, Anjali Jacob, MD PhD, Hirofumi Kiyokawa, MD, PhD, Darrell Kotton, MD

Objective: Thousands of variants of unknown significance (VUS) have been identified in genes known to cause Primary Ciliary Dyskinesia (PCD) and confirmatory testing is currently a bottleneck. We aim to develop a high-throughput pipeline using the human induced basal cell (iBC) system to determine the pathogenicity of VUS implicated in PCD. Here, we exemplify our progress in using CRISPR-Cas9 to delete Multicillin (MCIDAS) in iBCs and use apical out airway organoids (AOAOs) to develop rapid genotype-to-phenotype assessments.

Methods: We previously developed methods to derive iBCs and airway epithelium from human induced pluripotent stem cells (iPSCs), genetically edit iBCs using CRISPR-Cas9 and create AOAOs. Here we deleted exons 2 and 3 of MCIDAS in iBCs. These were then differentiated into ciliated cells in either air-liquid interface (ALI) culture or the new AOAO culturing process that allows for rapid differentiation of ciliated cells.

Results: Gel electrophoresis and Sanger sequencing demonstrate exon deletions in MCIDAS. RT-qPCR was performed to assess for expression of downstream markers of ciliogenesis. Immunostaining further interrogates ciliated cells in both ALI cultures and AOAOs. Cilia motility is assessed with high-speed videography.

Conclusion: MCIDAS was deleted in human iBCs. Culturing iBCs via the AOAO process provides rapid cilia phenotypes. Future work includes using this platform to interrogate the pathogenicity of a missense MCIADS VUS identified in a woman with PCD.

ALPHA-1 ANTITRYPSIN INDUCED PROTEOTOXICITY IN TYPE 2 ALVEOLAR EPITHELIAL CELLS

Author: Carly Merritt

Additional author(s): Kristy Abo, Pushpinder Bawa, Feiya Wang, Maria Basil, Ed Morrisey, Derek Byers

Objective: Alpha-1 antitrypsin deficiency (AATD) is a protein misfolding disease that leads to both fibrotic liver disease and emphysema. AATD emphysema is classically attributed to reduced circulating AAT levels and resulting protease/antiprotease imbalance in the lungs. However, there is evidence for ZAAT-driven proteotoxicity in cells that express the gene encoding for AAT. Although it has recently been shown that type 2 alveolar epithelial cells (AT2s) express this gene, the consequences of ZAAT protein expression in AATD patient AT2s has not been examined. We hypothesize that ZAAT accumulates intracellularly in AATD AT2s, inducing proteotoxicity that contributes to emphysema.

Methods: Explant lung tissue from patients with AATD, as well as healthy and COPD control tissue, was stained for AT2s and AAT and analyzed via fluorescence microscopy.

Additionally, AATD patient-derived iPSCs and syngeneic controls were differentiated into AT2- like cells (iAT2s) and analyzed via single cell RNA sequencing, flow cytometry, and immunofluorescent microscopy.

Results: We identified co-localization of AAT and AT2s in AATD but not in healthy lung tissue and increased intracellular AAT accumulation in AATD iAT2s. Enrichment of hallmark gene sets indicated cellular stress in AATD iAT2s compared to controls.

Conclusion: Overall, our findings identify heterogeneous AT2 intracellular AAT protein in AATD explant lung tissue and iAT2s, and transcriptional evidence of cellular stress in AATD iAT2s.

GENERATION OF KMT2D-/- IPSCS-DERIVED ENDOTHELIAL CELLS TO STUDY CARDIOVASCULAR DEVELOPMENT

Author: Sandra Sulser Ponce de Leon
Additional author(s): Alexander Holtz, Carly Golden, Saylor Williams

Objective: In the present work we seek to generate KMT2D-/- iPSCs-derived ECs to identify the molecular hallmarks that promote an abnormal endothelial patterning during development.

Methods: We performed a 3-phase protocol to differentiate KMT2D-/- iPSCs into ECs followed by their characterization by flow cytometry using two established ECs surface markers, CD31 and CD144.

Results: Our flow cytometry results indicate that WT KMT2D and KMT2D-/- ECs express the surface markers CD31 and CD144 (WT 90.9% and KMT2D-/- 89.9%), indicative of a successful differentiation. Additionally, KMT2D-/- display a higher proliferation rate compared to WT counterparts.

Conclusion: We conclude that KMT2D-/- iPSCs can be used as a research model to study vascular development. Further functional studies will establish the molecular targets involved in the abnormal cell proliferation profile of ECs lacking KMT2D.

MODELING INTESTINAL FILOVIRUS INFECTION USING HUMAN IPSC-DERIVED INTESTINAL ORGANOIDS

Author: Elizabeth Yvonne Flores
Additional author(s): Adam Hume, Judith Olejnik, Elke Mühlberger, Gustavo Mostoslavsky

Objective: Affected Ebola virus disease (EVD) patients lose copious amounts of fluids in a matter of days, rapidly deteriorating into hypovolemic shock and death. At present, available animal models insufficiently recapitulate the gastrointestinal symptoms of EVD patients. To fill this gap, we have established an induced pluripotent stem cell (iPSC)-derived human intestinal organoid (HIO) model to study the effects of filovirus infection on intestinal epithelial integrity.

Methods: The generation of a hiPSC CDX2-GFP reporter line highlights the emergence of hindgut intestinal progenitors during our differentiation protocol. The model was characterized by scRNAseq, IFA, qRT-PCR and used for BSL4 filovirus infections.

Results: Characterization of the HIOs revealed a significant number of CDX2 and Villin 1 expressing cells, transcriptional changes in cell identity during the stages of differentiation, and the different cell types that physiologically resemble the human intestinal epithelium. Successful robust EBOV and MARV infections of the HIOs, affecting mostly epithelial CDX2+ enterocytes was achieved. Transcriptomic analysis indicated the modulation of cell junction pathways and a set of ion transporters known to play a role in the induction of diarrhea.

Conclusion: These data suggest that EBOV and MARV compromise barrier integrity of the intestinal epithelium and cause abnormal ion flux as the basis for gastrointestinal dysfunction and diarrhea.

DEVELOPING CELL THERAPY FOR BRONCHIOLITIS OBLITERANS SYNDROME (BOS)

Author: Kevin Chen
Additional author(s): N/A

Objective: BOS is the prototypic form of chronic lung allograft failure characterized by extensive immune reactivity leading to fibroproliferative infiltrates in the bronchiolar tree resulting in end stage obstruction and parenchymal damage. The etiology remains unclear, with some evidence indicating the lack of or aberrant functioning of the airway basal cell in context of severe injury. We are developing in vivo models to test the hypothesis of basal cell dropout and the feasibility of transplanting syngeneic basal cells to ameliorate disease.

Methods: We used a model of serial polidocanol (PDOC) injury to denude the airway epithelium with histological injury characterization. We are developing other injury models to deplete airway basal cells such as PDOC injury followed by total-body irradiation, chlorine gas exposure, and orthotopic single lung transplant.

Results: We observed that serial administration of PDOC more robustly denudes the murine airway epithelium compared to single dosing with 30% of mice developing labored breathing 10 days post injury. Airway epithelium changes include tracheal squamous metaplasia and airway thickening, however, we observed hyperplasia and aberrant organization of Krt5+ basal cells.

Conclusion: Serial PDOC results in acute injury that does not phenocopy BOS-like lesions and does not result in basal cell dropout. Other approaches such as orthotopic lung transplantation can be a more clinically relevant injury model to test basal cell therapy.

OPTIMIZATION OF THE THERAPEUTIC WINDOW OF REGENERATIVE FACTORS DELIVERED WITH LIPID-NANOPARTICLE-ENCAPSULATED MRNA TO TREAT ACETAMINOPHEN OVERDOSE-INDUCED LIVER INTOXICATION

Author: Kexin Dong
Additional author(s): Amman Bhatti, Elissa Everton, Fatima Rizvi, Anna Smith, Ying Tam, Norbert Pardi, Drew Weissman, Valerie Gouon-Evans

Objective: Overdose of acetaminophen (APAP), the most common pain reliever consumed in the US, is the leading cause of acute liver failure. Currently the only available treatment is N-acetyl cysteine (NAC), whose short window of effectiveness (~10h) ends while organ toxicity is frequently still asymptomatic, leading to liver failure and liver transplant. Thus, therapeutic alternatives to treat APAP overdose are urgently needed. We hypothesize that the activation of 2 pathways in hepatocytes, hepatocyte growth factor (HGF)/HGF receptor and epidermal growth factor (EGF)/EGF receptor, is effective in accelerating liver repair for patients who present late at the ER.

Methods: A single dose of APAP was administered to induce acute liver injury in male mice after mice fasted for 14h. These mice were then given a single injection of HGF/EGF mRNA-LNP at 24h or 48h after APAP administration and were analyzed at 48h or 72h after APAP injection respectively.

Results: HGF/EGF injected at 24h and 48h after APAP administration significantly reduced liver damage in APAP-overdose mice, as assessed by histology and alanine

transaminase (ALT) level. The beneficial effects of HGF/EGF mRNA-LNP administered at 24h were also observed in males injected with a higher dose of APAP.

Conclusion: HGF/EGF mRNA-LNP shows a promising alternative therapeutic treatment with a significantly longer therapeutic window than the standard-of-care NAC in patients who are overdosed with APAP and who come late to the clinic.

GENERATION OF 3RD PHARYNGEAL POUCH CELLS FROM MOUSE EMBRYONIC STEM CELLS

Author: Lauren Ayers
Additional author(s): Martin Ma

Objective: The parathyroid glands are responsible for maintaining serum calcium levels via secretion of parathyroid hormone. There is no cure for hypoparathyroidism, a disorder which results in low calcium levels. The ability to generate parathyroid-like cells from pluripotent stem cells by directed differentiation would create opportunity to study development and disorders of the parathyroid in vitro, but there is no known protocol to create cells of this fate. During mouse embryogenesis, parathyroid cells derive from 3rd pharyngeal pouch progenitors, which in turn derive from the anterior foregut endoderm (AFE). We sought to derive cells resembling the 3rd pharyngeal pouch from murine ESC-derived AFE cells via directed differentiation.

Methods: After differentiating ESCs into AFE-like cells via established protocols, we cultured cells in media supplemented with agonists and antagonists of BMP and retinoic acid signaling as candidates to derive cells with a similar gene expression profile to the 3rd pharyngeal pouch.

Results: Media supplemented with BMP and/or RA signaling inhibitors resulted in expression of 3rd pouch markers Tbx1, Pax1, and Pax9 as measured by qPCR.

Conclusion: BMP4 and retinoic acid signaling inhibition promote a 3rd pharyngeal pouch fate from mESC-derived AFE. Future studies will include single cell RNA sequencing of cells subject to these conditions to confirm that Tbx1, Pax1, and Pax9 are expressed in the same cell population.

DEFINING THE MOLECULAR UNDERPINNINGS RESPONSIBLE FOR CD4 T CELL SPECIFICATION USING HUMAN OFF-THE-SHELF UNIVERSAL IPSC

Author: Julian Amirault
Additional author(s): Dar Heinze, Mengwei Yang

Objective: Many T cell-based therapies have been developed; however, they are time consuming and difficult as they require T cells sourced from patients being treated. Previous work resulted in a robust protocol to differentiate induced pluripotent stem cells (iPSCs) to CD4/CD8 double positive (DP) cells. There are currently challenges in prompting the emergence of CD4 single positive T cells. We aim to understand molecular cues responsible for CD4/8 lineage specification during maturation of DP T cells to single positive T cells and refine a hypoimmunogenic iPSC to decrease reliance of autologous cells for therapies.

Methods: iPSCs are differentiated to CD4/8 DP cells in a feeder-free system. Using cytokine and T cell receptor (TCR) signaling we generated a final population with both CD8 and CD4 SP T cells. To lower immunogenicity an iPSC line was created with modifications preventing expression of MHC-I while expressing HLA-E, which inhibits NK attack. Modification knocking out MHC-II and expressing CD47 would further prevent iPSC derived cells from eliciting an immune response.

Results: Cells are modified using CRISPR then screened for successful edits. A phenotyping flow cytometry panel has been developed to interrogate changes in iPSC derived T cells.

Conclusion: Increasing IL7 and TCR signaling while decreasing notch signaling allows for emergence of a mixed population of CD4 SP and CD8 SP T cells. Effective knockout of MHC-I/MHC-II and expression of CD47 and HLAE will be evaluated.

HOST PRECONDITIONING AND TRANSIENT MITOGEN EXPRESSION VIA mRNA-LNP LEAD TO ROBUST PRIMARY HUMAN HEPATOCYTE ENGRAFTMENT AND iPSC-DERIVED HEPATOCYTE-LIKE CELL SURVIVAL IN MICE

Author: Anna Smith
Additional author(s): Anna R. Smith, Fatima Rizvi, Elissa Everton, Anisah Adeagbo, Hua Liu, Ying Tam, Norbert Pardi, Drew Weissman, and Valerie Gouon-Evans

Objective: Donor livers for transplantation are scarce. Instead, transplantation of hepatocytes could restore liver function – either primary human hepatocytes (PHH) or induced pluripotent stem cell derived hepatocyte-like cells (HLC). PHH transplantation is safe in human, yet efficiency and benefit are low. HLC transplantation remains preclinical, limitations include poor survival, proliferation, and maturation of transplanted cells. We hypothesize that stimulating key regenerative pathways in transplanted hepatocytes using hepatocyte growth factor (HGF) and epidermal growth factor (EGF) and preconditioning with p21 to halt host hepatocyte proliferation will improve the engraftment of PHHs and HLCs in vivo.

Methods: We established safe, transient HGF+EGF expression in the liver via nonintegrative nucleoside modified mRNA in lipid nanoparticles. AAV8-TBG-p21 preconditions the host hepatocytes with long term p21 expression. We use NSG-PiZ mice, a model of AATD liver disease.

Results: Both p21 and HGF+EGF treatments enhance PHH survival and proliferation in vivo, shown by histology and human serum albumin levels. These strategies boost liver repopulation with functional PHHs (~30%), alleviate disease, and improve liver function. Importantly, these treatments also enhance HLC survival in vivo.

Conclusion: Blocking host hepatocyte proliferation and promoting survival and proliferation in transplanted hepatocytes enhances PHH and HLC engraftment, showing promise in liver disease treatment.

UNRAVELING THE REGULATORY INTERPLAY BETWEEN KMT2D AND WNT SIGNALING IN NEURODEVELOPMENT

Author: Carly Golden
Additional author(s): Pushpinder Bawa

Objective: While Wnt gradients are essential for cortical patterning, how KMT2D interacts with Wnt signaling and their synergistic contributions to neurodevelopment remain unknown.

Methods: Here, we utilize human iPSC-derived cerebral organoids from both wildtype and KMT2D-null backgrounds.

Results: Through single-cell transcriptomic analysis and confocal microscopy, we identify a link between KMT2D and Wnt signaling that maintains early cortical tissue integrity in vitro by regulating glial lineage differentiation.

Conclusion: Investigating these mechanisms will reveal previously unexplored

epigenetic modulation of neurogenic signaling pathways, which may be relevant to various neurodevelopmental disorders.

ALPHA-1 ANTITRYPSIN DEFICIENCY COHORT: LONGITUDINAL BIOMARKER STUDY OF DISEASE (A1BC)

Author: Mark Dodge
Additional author(s): Marissa Gallagher, Joseph E. Kaserman, Jeanine M. D'Armiento, Charlie Strange, Monica P. Goldklang, Andrew A. Wilson

Objective: Alpha-1 antitrypsin deficiency (AATD) is a monogenic disease with lung and liver disease presentations, resulting from the homozygous inheritance of a single base pair mutation in the SERPINA1 gene. Progression of lung disease in patients homozygous for the mutant "Z" allele AATD is variable and while some patients may have stable lung function over many years, some progress and deteriorate rapidly. Currently there are no predictors that would help identify patients at risk for rapid deterioration.

Methods: We have joined a multi-center observational cohort study to recruit ZZ participants and follow them over time. Study participants at each site are followed annually for a total of 3 years through a combination of medical/medication history, blood draw, pulmonary function tests, induced sputum, completion of questionnaires and CT Chest scan. All procedures will be performed on enrollment and repeated at 18 months/36 months, with the exception of monthly Alpha-net exacerbation questionnaires.

Results: We're currently enrolling participants for baseline study visits. Participant demographic and clinical data is entered into a database and linked to biospecimens, including serum, plasma, RNA/DNA and reprogrammable blood cells for iPSC generation. A total of 146 participants have been enrolled in the study including 11 participants at BU.

Conclusion: We're reviewing all collected study data with our Observation Study Monitoring Board and performing spirometry quality control.

MODELING MECHANISMS OF METABOLIC DYSREGULATION IN AATD ASSOCIATED LIVER DISEASE WITH PATIENT DERIVED IPSCS

Author: Joseph Kaserman
Additional author(s): Rhiannon Werder, Feiya Wang, Jonathan Lindstrom-Vautrin, Anne Hinds, Esther Bullitt, Xu Shi, Robert E Gerszten, Nicola Brunetti-Pierri, Marc Liesa, Carlos Villacorta-Martin, Anthony Hollenberg, Darrell N Kotton

Objective: Individuals homozygous for the "Z" mutation in alpha-1 antitrypsin deficiency (AATD) accumulate misfolded ZAAT within the ER of hepatocytes resulting in cellular dysfunction. A potential link between AATD and hepatic steatosis has previously been raised by both cohort studies and AATD mouse models. We hypothesize that ZAAT driven ER stress leads to impaired cellular metabolism and steatosis predisposing to the associated injury observed clinically.

Methods: We selected induced pluripotent stem cells (iPSCs) from ZZ AATD patients and that have undergone CRISPR based correction generating syngeneic MZ and MM iPSCs. Syngeneic iPSCs were differentiated to the hepatic stage and assessed for alterations in metabolic function.

Results: ZZ and MZ iPSC-derived hepatocytes (iHeps) had downregulation of master transcription factors associated with lipid metabolism and thyroid hormone signaling together with impaired oxidative phosphorylation. They further had transcriptomic

heterogeneity characterized by branch specific activation of the unfolded protein response (UPR) with specific cellular subsets associated with a pro-fibrotic profile.

Conclusion: ZZ and MZ patient specific iHeps demonstrate impaired lipid metabolism and mitochondrial function associated with branch specific UPR activation. Given the lack of clinically available therapies gaining further insight into the mechanisms contributing to metabolic dysfunction in AATD could inform future therapeutic discovery for this population.

Endocrinology

THYROID HORMONE RECEPTOR BETA PROMOTES NOVEL PATHWAYS IN THE RESOLUTION OF LIVER FIBROSIS

Author: Arturo Mendoza
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Abstract: Thyroid hormone receptors (TRs) are ligand-dependent transcription factors that transduce thyroid hormone signaling through their interaction with multiple histone-modifying multiprotein complexes. Among them, TRB1 is unique in that interaction with the nuclear receptor corepressors 1 (NCoR1) is required to engage its regulatory activity. However, the pleiotropic action of T3 results in both activation and repression of transcription, the mechanism of which is currently unclear. Here, we report that during the resolution of liver fibrosis promoted by T3-treatment, TRB1 downregulates multiple pathways mediating the pathology of fibrosis including the NF-KB, TGFB1 and ErbB signaling pathways. In addition, multiple pathways regulating metabolism including that of lipids and cholesterol were upregulated by TRB1. Our findings show that TRB1 promotes the resolution of liver fibrosis by leveraging hepatocyte metabolic activity and its previously unknown ability regulate novel pathways regulating the extracellular matrix synthesis and inflammation in the liver.

UTILIZATION OF HEMAGLUTTIN TAG TO IMPROVE ISOLATION OF NUCLEAR RECEPTOR COREPRESSOR SMRT

Author: John Csehill
Additional author(s): Izuki Amano, Victor Rodrigues, Megan Ritter, Anthony Hollenberg

Objective: Thyroid hormone (TH) acts via the thyroid hormone receptor (TR), a nuclear receptor. TH gene expression occurs in the presence of its ligand, TH. In the absence of TH, corepressors are recruited to TR to inhibit TH gene expression.The silencing mediator of retinoic acid and thyroid receptors (SMRT) binds to TR to form a corepressor complex that inhibits TH gene expression. The molecular mechanisms of SMRT remain unknown. We aim to elucidate how the corepressor complex changes across varying TH levels by using a novel mouse model developed in our lab.

Methods: We used CRISPR/Cas9 to add a hemagluttin tag plus a BLRP sequence on the C-terminal end of SMRT to generate SMRT-HA tag mice, which were bred with birA mice to further help with isolation and identification of SMRT given the limitations of current antibodies targeting SMRT. We studied wild type mice, birA mice, homozygous (HO) SMRT HA tag mice, and HO SMRT tag x birA mice to determine differences between genotypes.

Results: Our preliminary analysis shows genetic modification of SMRT does not impact phenotype. We found no difference between body weight, liver weight, TSH or gene expression between groups. We have been able to improve our isolation of SMRT for

future studies.

Conclusion: Utilizing CRISPR/Cas9 to genetically modify SMRT has improved our ability to isolate SMRT. SMRT HA-tag birA mice can be used for further study in states of hyper- and hypothyroidism, along with SMRT itself in any tissue.

IMPACT OF MEAL BOLUS TIMING AND BEDTIME SNACKING ON GLYCEMIC CONTROL IN HOSPITALIZED PATIENTS

Author: Sara Alexanian

Additional author(s): Michael Cheney, Jennifer C Bello Ramos, Nicole Spartano, Howard A Wolpert, Devin W Steenkamp

Objective: Basal-bolus insulin is the standard of care to treat hyperglycemia in hospitalized patients in the non-ICU setting. Matching insulin with food is a barrier to achieving blood glucose (BG) goals. The glycemic impact of a snack at bedtime without prandial insulin and delayed meal-time bolus in the hospital has not been previously explored.

Methods: The InFi study is an ongoing randomized controlled open-label trial comparing Fiasp vs aspart in hospitalized patients using the Dexcom G6 PRO CGM to assess postmeal glycemic outcomes. We performed an interim analysis of 100 subjects to assess the impact of bedtime snacks on fasting POC glucose (FG) and the impact of delayed meal bolus on postprandial sensor glucose (PPSG) time in range (TIR) 70-180 mg/dL. Meal intake was determined by a CGM heuristic, insulin bolus time by barcoded EMR.

Results: Four hour post-meal TIR was 54% for on-time boluses (n=119) vs 25% with bolus delay of >5 minutes (n=86), p <0.001. Evening snacking (9pm-12am) was associated with higher FG levels, 201 vs 158 mg/dL (N=30, 50), p = 0.03.

Conclusion: Delayed meal-time insulin bolusing is common in hospitalized patients and results in a significant reduction in post-meal TIR. Evening snacking is a significant cause of overnight hyperglycemia. Given the importance of BG control, hospitals should work to optimize meal insulin delivery and develop strategies to address bedtime food intake.

Gastroenterology

ANALYSIS OF DEMOGRAPHIC FEATURES AND SURVIVAL OF PATIENTS ACROSS SITE-SPECIFIC NEUROENDOCRINE TUMORS

Author: Ramya Radhakrishnan

Additional author(s): Grace Kim, Isa Jacoba, Natalie Sun, Sofia Shaikh, Qing Zhao, and Howard Cabral

Objective: There is a significant knowledge gap regarding epidemiology and survival rates in patients with neuroendocrine tumors (NETs) across racial/ethnic groups. The objective of this study is to determine epidemiologic trends and survival in BMC patients with NETs.

Methods: Unique patients with pathology proven NETs (N=321) diagnosed between 2001 and 2022 at BMC were enrolled. Data was collected via chart review and differences were deemed significant for p-value<0.05. Overall survival (OS) was estimated by Kaplan Meier statistics.

Results: Of 321 subjects total, most NETs localized to the GI tract (64.8%), followed by lung (18.7%) and liver (14.3%). Median age at diagnosis was 58 (range: 14 - 88). The demographic distribution of the entire sample shows 41% African American, 54%

female. When comparing organ sites in the entire cohort, significant differences were seen in gender ($p = 0.04$) and age at diagnosis ($p < 0.001$), but not race ($p=0.07$). A 3.6-fold increase in incidence rates of GI NET was also seen over the study period. OS of patients with rectal or duodenal NETs (dNETs) did not differ based on race, while age > 65 was associated with shortened OS in dNETs.

Conclusion: This study is novel to identify demographic disparities in BMC NET patients not reported in other cohorts, whereby age but not race shortened OS. Detailed clinic-pathological characteristics need to be examined to determine risk factors for survival.

YIELD OF TERMINAL ILEAL INTUBATION IN EVALUATING IRON DEFICIENCY ANEMIA

Author: Samantha Chua
Additional author(s): Max Rosenthaler, Marcel Yibirin-Wakim, Enoch Chung, Christopher Huang, James Connolly

Objective: We investigated the diagnostic yield of terminal ileum (TI) intubation during endoscopic evaluation of iron deficiency anemia (IDA) within a safety-net hospital population.

Methods: We performed a single-center, retrospective cohort study of patients who underwent TI intubation for IDA evaluation between 2012 and 2022. Demographic, clinical, endoscopic and histopathological characteristics were identified and examined in bivariate analyses.

Results: Among 2225 patients who underwent colonoscopy for IDA during the study period, 586 patients (mean age 53.2, 62.5% women) completed TI intubation (26.3%). The study cohort was predominantly English-speaking (59.6%) and had public insurance (72.9%). Twenty-one patients (3.6%) had abnormal findings in the TI. The most common findings were aphthous ulcers and erosions. No malignancy was found in any patient. Patients with abnormal TI findings were more likely to have IBD (14.3% vs 1.4%, $p = 0.005$), history of gastrointestinal (GI) cancer (19% vs 3.0%, $p = 0.005$), history of GI surgery (47.6% vs 9.0%, $p < 0.001$), prior ileocecal valve resection (42.9% vs 2.5%, $p < 0.001$), and anticoagulation use (19% vs 6.2%, $p = 0.044$).

Conclusion: Potential etiologies of IDA were found in the TI in approximately 4% of patients, suggesting an incremental benefit to performing TI intubation routinely during colonoscopy for IDA evaluation. Having IBD, history of GI cancer, prior GI surgery and anticoagulation use may predict an increased diagnostic yield.

General Internal Medicine

A NOVEL METHOD FOR IDENTIFYING LGBTQ+ VETERANS WHO PARTICIPATED IN THE PRIDE IN ALL WHO SERVED HEALTH PROMOTION GROUP USING THE VETERANS AFFAIRS DATA

Author: Christina Jefferson
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Objective: Pride in All Who Served (PRIDE) is a 10-week health promotion group created in 2016 that focuses on reducing health disparities among LGBTQ+ veterans. PRIDE has served over 800 veterans across 54 Veteran Affairs (VA) sites. To evaluate the impact of PRIDE on healthcare utilization and outcomes, we are currently identifying a PRIDE cohort using clinical notes in VA data. The objective of this study is to present

the methods for identifying PRIDE participants in the first VA site where PRIDE was delivered, as an example site.

Methods: We extracted the clinical notes of potential PRIDE participants at the Hampton Virginia VA using three data elements: 1) local note title, 2) names of PRIDE facilitators, and 3) PRIDE delivery dates. PRIDE-related terms (e.g., PRIDE group) were then searched in the clinical notes of these potential participants. Note chunks with evidence of PRIDE were extracted and reviewed by the PRIDE team for confirmation of PRIDE participation.

Results: Using the combination of the three data elements, we identified 3,734 veterans at the Hampton Virginia VA. An automated search for PRIDE keywords in the clinical notes of these 3,734 veterans resulted in 107 veterans. We further validated the note chunks of these 107 patients to confirm PRIDE participation.

Conclusion: This data-driven approach for identifying PRIDE participants may inform program evaluations of other affirmative care services across healthcare systems.

ENGAGING PEOPLE WITH LIVED EXPERIENCE IN PHOTOVOICE TO UNDERSTAND CONTEXTUAL DRIVERS OF THE OPIOID EPIDEMIC AND PROMOTE COMMUNITY ACTIVISM

Author: Peter Balvanz
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Objective: To implement a Photovoice project in Ware, Massachusetts with people with lived experience (PWLE) to understand the localized context of drug use and to elicit actions to mediate the impact of opioid use disorder.

Methods: We recruited eight PWLE to participate in a project that included an orientation, four Photovoice sessions, and an action planning session. In Photovoice sessions participants 1) developed a photo-topic, 2) took and shared representative photos, and 3) participated in a facilitated discussion. Discussions concluded with a brainstorm of action items.

Results: Several themes emerged: economic decline and lack of recreation has eroded community connectivity, providing fertile ground for drug use, lack of transportation severely limits economic opportunities, recreation, and recovery options, having a purpose and responsibility are protective against drug use, and recreation is key to replacing drugs. Participants developed several actions to address the impact of the opioid epidemic: community events to share project insights, park clean-up day led by people in recovery, and advocacy for detox or methadone clinic.

Conclusion: Photovoice provided an excellent venue for PWLE to have conversations about the opioid epidemic in their community, and suggest actions to curb drug use.

LIFE'S ESSENTIAL 8 CARDIOVASCULAR HEALTH SCORE AND CARDIORESPIRATORY FITNESS IN THE COMMUNITY

Author: Sandhiya Ravichandran
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Objective: Cardiorespiratory fitness (CRF) is a key indicator of cardiovascular health; we aimed to evaluate the cross-sectional relations of AHA's Life's Essential 8 (LE8) metrics with CRF in the community.

Methods: LE8 scores were constructed by averaging all LE8 components (0-100). CRF, measured by cardiopulmonary testing in Framingham study, was related with total LE8 score, individual LE8 components as three-level variables (optimal/suboptimal/poor),

and changes in LE8 score over an ≈8 year interval.

Results: In 1838 FHS participants (age 54±9 years, 54% women, BMI 28±5 kg/m2), mean LE8 score was 76±12. In age- and sex-adjusted models, a higher LE8 score was associated with higher peak VO2, better ventilatory efficiency, lower resting heart rate, and favorable blood pressure response to exercise ($p<0.0001$ for all) without effect modification by age, sex or CVD status. A 5-point higher LE8 score was associated with a 6.0% greater peak VO2. The eight LE8 score components were statistically significantly associated with peak VO2 in models adjusted for age and sex, but blood lipids, diet, and sleep health were no longer statistically significant after adjustment for all other LE8 components (Figure). Over an ≈8-year interval, a 5-unit increase in LE8 score was associated with a 3.7% higher peak VO2 ($p<0.0001$).

Conclusion: Higher LE8 score is associated with greater CRF in the community, highlighting the importance of CVH and LE8 metrics in maintaining and promoting CRF.

THE SUSTAINABILITY OF AN OPT-OUT ELECTRONIC-HEALTH RECORD-BASED TOBACCO TREATMENT CONSULT SERVICE AT A LARGE SAFETY-NET HOSPITAL AFTER 6 YEARS

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Objective: In July 2016, our safety-net hospital implemented a hospital-based tobacco treatment intervention in response to a state-level incentive program via an “opt-out” Electronic Health Record (EHR)-based Best Practice Alert (BPA)+order-set, triggering consultation to an inpatient Tobacco Treatment Consult service (TTC) for patients who smoke cigarettes. We report the program's sustainability 6 years later.

Methods: We analyzed data between July 2016-June 2022 of patients reported as having ‘current smoking’ status in the EHR. We compared clinician acceptance of the TTC order-set and consults completed by the TTC across the 6 years. We compared receipt of combination nicotine replacement therapy (NRT) between patients seen and not seen by the TTC.

Results: Among all adult admissions, 23.2% (39,558/170,347) had a “current smoking” status in the EHR, triggering the BPA. Clinicians accepted the TTC order-set on 50.4% (19,932/39,558) of admissions for whom the BPA fired. The TTC provided consultation to 34% (6779/19,932) of those with a consult ordered. Consultations completed remained stable over the six-years ($r= -.16$, $p=0.75$). Patients seen by the TTC were more likely to be prescribed combination NRT (37.6% vs. 22.8%, <0.01).

Conclusion: Our safety-net hospital's "opt-out" EHR-triggered TTC service is sustainable in delivering evidence-based tobacco treatment. Prioritizing and sustaining hospital-based tobacco treatment programs is crucial to expand reach to underserved populations.

EVALUATING THE FIDELITY OF COMMUNITY ENGAGED MENTAL HEALTH RESEARCH IN GHANA THROUGH THE LENS OF COMMUNITY BASED PARTICIPATORY RESEARCH (CBPR) PRINCIPLES

Author: Alex Werekuu
Additional author(s): Margaret Lombe, Judith Azumah, Phyllis Tawiah, Nana Kwame Ayisi-Boateng, Elijah Paintsil, Samuel Ofoli Mensah, Alexis Kiyanda

Objective: Community Based Participatory Research (CBPR) is an approach where academic and non-academic groups engage equally to conduct research. Prior work identified eight CBPR principles. Our aim was to assess how these principles were incorporated in CBPR mental health studies in Ghana.

Methods: We searched PubMed and identified 329 CBPR manuscripts done in Ghana; five focused on mental health. Search terms used were Ghana and concepts of CBPR. Reviewers documented the usage of CBPR principles using Indicators proposed in prior work.

Results: All five papers identified communities as the unit of research, and strengthened existing community resources (principle #1,2). Three papers considered equitable involvement, promoted co-learning, and addressed health from positive and ecological perspectives (principle #3,5,7 respectively). 2 papers integrated action for the mutual benefit of all partners (principle #4), and continual community involvement (principle #6) was seen in 1 paper.Community dissemination (principle #8) plans were absent in all but 1 paper, and 1 paper included nonacademic partners as co-authors.

Conclusion: Knowledge dissemination plans of CBPR mental health studies in Ghana are rarely documented. This could limit integration of knowledge for mutual benefit of partners (principle #4), limit continual community involvement (principle #6), and limit ability to improve on work done. Future CBPR studies on mental health should expand consideration for principles 4,6 and 8.

EXERCISE HEMODYNAMICS UNMASKS STAGE C HEART FAILURE WITH PRESERVED EJECTION FRACTION AND IMPAIRED PERIPHERAL OXYGEN EXTRACTION IN STAGE A/B PATIENTS IN A SAFETY-NET CENTER

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Objective: There is a gap in research evaluating hemodynamics and impaired peripheral oxygen extraction in diverse Stage A/B HFpEF groups. We sought to characterize the prevalence of impaired cardiac/peripheral profiles in Stage A/B HFpEF. We hypothesize both abnormal cardiac and peripheral profiles will be unmasked in exercising Stage A/B HFpEF patients.

Methods: Invasive cardiopulmonary exercise testing (iCPET) was performed in 30 individuals with Stage A/B HFpEF. Criteria for an impaired cardiac profile, with reclassification to Stage C, included exercise pulmonary capillary wedge pressure (PCWP) $\geq$ 25 mmHg or ΔPCWP/ΔCO slope > 2 mmHg/L/min. An impaired peripheral profile was defined as O2 extraction ratio < 50%.

Results: Cohort clinical data shown in Table with 47% non-white and 57% female patients. Exercise unmasked Stage C HFpEF in 23 (77%) of cases; 17% had an impaired peripheral profile (O2 extraction ratio 43 +/- 7) and 10% (3 out of 30 patients) had concomitant impaired cardiac and peripheral profiles. Both cardiac and peripheral metrics correlated to metabolic parameters.

Conclusion: In our diverse cohort, a significant proportion of Stage A/B patients reclassified to Stage C HFpEF via invasive exercise testing; peripheral impairments were also noted. Impaired cardiac profile metrics were related to metabolic parameters. Broader implementation of iCPET in early disease stages will guide further HFpEF research in novel mechanisms and therapeutics.

LET IT PASS: A CASE OF TOXIC MEGACOLON CAUSED BY SHIGELA FLEXNERI

Author: Tatyana Nguyen
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Objective: gellosis causes an estimated 80 million cases of diarrhea and 700,000 deaths annually. Extensively drug-resistant (XDR) Shigella strains are increasingly common without treatment guidelines. Here, we present a case of XDR Shigella flexneri that led to toxic megacolon despite broad-spectrum antibiotics.

Methods: Case report

Results: A 44 years-old man presented with weeks of watery diarrhea and abdominal pain. A stool PCR detected Shigella species without Shiga toxins 1 and 2. He was initially treated with 3 days of azithromycin, then 3 days of ceftriaxone before stool culture-confirmed XDR Shigella flexneri. He received 3 days of ertapenem and was discharged with 4 days of fosfomycin. Alongside antibiotics, he received loperamide 16mg/day for symptom control which he took liberally. He returned within a week with worsening abdominal pain, distension and was found to have toxic megacolon. Obstipating agents were discontinued, a rectal tube was placed, and he received 5 days of ertapenem with good effect. The toxic megacolon was thought to result from the use of antimotility agents that reduced bacterial clearance, allowing it to invade the colonic mucosa, particularly before he received effective antibiotics.

Conclusion: Given the increase in XDR Shigella species and the potential for severe complications, testing for drug resistance through culture-based measures is important. Further, even in toxin-negative species of Shigella, antimotility agents should be used sparingly.

PHARMACY-LED OPTIMIZATION OF GUIDELINE-DIRECTED MEDICAL THERAPY (GDMT) IN HEART FAILURE WITH REDUCED EJECTION FRACTION PROGRAM IMPROVES HEART FAILURE METRICS IN A SAFETY-NET HOSPITAL

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Objective: Quad-therapy with GDMT improves HFrEF outcomes but is challenging to implement in safety-net centers. We expanded an outpatient pharmacy-led medication titration program (OPTIMAL-HF) to improve HF care.

Methods: From a Boston Medical Center hospital discharge or ambulatory visit, 122 patients were referred from 2/2022 – 2/2023. A clinical pharmacist conducted either in-person/telemedicine visits. HF GDMT was titrated until goal doses/maximally tolerated doses were achieved signaling graduation. Chart review was performed with an approved IRB.

Results: Of the 28 graduates, 76% were Black/Hispanic, 71% resided in an area deprivation index ≥ 5 (scale, 1-10, higher score signifying greater neighborhood disadvantage). The median time to graduation was 22 weeks (interquartile range (IQR) 8, 29 weeks) with median of 6 visits (IQR 3, 8 visits). In the 3 months prior to enrollment, there were 13 hospitalizations (8 due to HF); in the 3 months after graduation (n=20), only 3 hospitalizations (0 due to HF) were noted. At graduation, 64% were on quad-

therapy with ≥ 71% on beta-blockers/ace-inhibitors/angiotensin-receptor blocker/angiotensin-receptor-neprilysin inhibitor/sodium-glucose cotransporter-2 inhibitors/mineralocorticoid receptor antagonists. An increased number of OPTIMAL-HF graduates reached maximum-tolerated/goal doses of GDMT with significant improvements in LVEF (Figure).

Conclusion: OPTIMAL-HF improved HF metrics supporting an effective model in a safety-net center.

PIONEERING A REMOTE PULMONARY ARTERY PRESSURE MONITORING PROGRAM: A CASE SERIES FROM AN URBAN SAFETY NET HOSPITAL WITH A DIVERSE POPULATION

Author: Sheikh Moinul
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Objective: To describe characteristics of HF patients that underwent CM implantation in a safety net hospital and compare their HF-related emergency department (ED) visits and HFH rates pre- and post-implantation.

Methods: Retrospective case series of adults that underwent CM implantation from January 2020 to March 2023 at Boston Medical Center. Data was obtained from Electronic Medical Record. Descriptive statistics summarized findings. Paired t-tests were used to calculate statistical significance of pre- and post-implantation data (p-value of ≤0.05).

Results: Our study consisted of 27 subjects who received a CM implant, 18 of whom completed 1-year follow-up. Most were African American females with high Area Deprivation Index, non-ischemic cardiomyopathy and preserved ejection fraction. There were no statistically significant differences in HF-related ED visits or HFH pre- and post-implantation, but trends suggested efficacy.

Conclusion: This case series shows the initial experience of the CM program in a diverse patient population at a safety net hospital. Future research should evaluate long-term impact on outcomes and socioeconomic factor influence.

GOING TO THE SOURCE: DISCUSSIONS WITH EARLY AND MID-CAREER FACULTY FROM GROUPS UNDERREPRESENTED IN BIOMEDICAL RESEARCH TO DEVELOP AND ENHANCE CFAR SERVICES

Author: Joseph Delamerced and Leslie Ramirez
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Objective: To discern the needs of early and mid-career faculty from underrepresented groups who are interested in HIV research.

Methods: We conducted focus groups and interviews with 15 faculty at Providence/Boston CFAR institutions. The discussion was guided using the domains of an Asset Bundle Model encompassing scientific human capital, social capital, and financial capital.

Results: Participants' identities, including their race, ethnicity, gender, sexual orientation and being a parent impacted their vision of themselves as scientists. Participants reported confusion or limited training on or access to resources for professional development, hiring staff, meeting NIH reporting requirements, international research, support for working parents, sabbaticals, and addressing workplace conflict or unsupportive work environments. Some described feeling like they were a burden on their mentors who appeared overextended. They identified attributes of effective mentors. They described a

need for additional education and support pre and post research grant award management.

Conclusion: To learn how to equitably serve all interested in HIV research, CFARs should engage and include perspectives from scientists that have historically been excluded from biomedical research. Our future work will test, implement and disseminate the ideas generated by these focus group discussions.

TRANSCRIPTOMIC ANALYSIS OF SCLERODERMA MONOCYTES IDENTIFIES DISTINCT CLINICALLY RELEVANT CLUSTERS AND NOVEL GENES ASSOCIATED WITH DISEASE COMPLICATIONS

Author: Mehmed Dinc
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Objective: Monocytes play a critical role in SSc, but their contribution to disease pathogenesis remains unclear. The aim is to investigate the changes in gene expression in monocytes and their relationship with clinical characteristics in Systemic Sclerosis (SSc).

Methods: Monocytes were isolated from 48 SSc patients and 15 controls. RNA sequencing was performed and differentially expressed genes (DEGs) identified. Hierarchical clustering categorized samples by transcriptional trends. Functional enrichment, pathways, and correlation analysis were conducted.

Results: 460 DEGs were identified in SSc versus controls, including interferon-related genes (IFI27, IFIT5, IFI44L, SIGLEC) and previously unlinked genes NUAK2 and LGALS3BP. Clustering revealed three SSc groups: “inflammatory-like” (associated with pulmonary hypertension), “T cell-like” (enriched in T cell activation pathways), and “normal-like” (resembling controls). Patients with PH were found to be primarily clustered in inflammatory-like clusters, whereas those on immunosuppressive therapy were mainly clustered in T cell-like clusters. DEGs correlated with clinical features; notably, XPR1 gene correlated with mRSS (r=0.72, padj 0.006).

Conclusion: SSc patients exhibit distinct monocyte transcriptomic profiles associated with clinical characteristics, implicating novel genes and pathways, and highlighting potential therapeutic targets.

PREVALENCE OF CARDIAC ARRHYTHMIAS IN TRANSGENDER AND NONBINARY ADULT COMMUNITY HEALTH CENTER PATIENTS

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Objective: Sex differences in arrhythmias are well-established and partly due to sex hormones. We aimed to explore arrhythmia prevalence in transgender and nonbinary (TGNB) adults.

Methods: This cross-sectional study used medical records from a community healthcare center specialized in TGNB care. Adults > 17 years with > 1 visit in 2010-2021 were categorized into self-reported gender groups as TGNB (TG-man[TGM], TG-woman [TGW], NB-assigned male at birth, NB-assigned female at birth), cisgender man (CisM), or cisgender woman (CisW). We categorized arrhythmias as atrial, ventricular, or other. Arrhythmia prevalence was reported by gender. Age- and race-adjusted regression models compared TGNB patients to CisM and CisW. Arrhythmia prevalence was described in TGNB with a gender-affirming hormone therapy (GAHT) prescription.

Results: Among 49862 adults, 7121 (14%) identified as TGNB persons. Participants were young (median age 28 years). Arrhythmia prevalence was low (0.7-1.4% in NB persons, 1.4-1.7% in TG persons). TGW and TGM had similar odds of arrhythmias compared to CisM (TGW: OR=0.89 (0.63-1.24) p=0.52 ; TGM: OR=1.17(0.82-1.62) p=0.37), but higher odds compared to CisW (TGW: OR=1.65(1.13-2.34), p=0.001 ; TGM: OR=2.15 (1.48-3.04),p<0.001). Arrhythmia prevalence appeared similar among TGNB adults who used GAHT and those who did not (TGW: 1.2 vs 2.1% ; TGM: 1.5 vs 1.9%).

Conclusion: Further research examining arrhythmias in gender minority groups and potential impact of GAHT are warranted.

FACILITATORS AND BARRIERS ACROSS THE PREP CARE CONTINUUM IN SUBSTANCE USE BRIDGE CLINICS FOR WOMEN WHO ENGAGE IN SEX WORK AND WHO USE DRUGS

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Objective: We aimed to explore facilitators and barriers to PrEP in substance use bridge clinic settings for women who engage in sex work and use drugs (WSWUD).

Methods: Bridge clinic and affiliated harm reduction health service providers and WSWUD from Boston were recruited using active outreach between 12/9/2021-8/5/2022. Participants were invited to take part in a semi-structured phone or in-person interviews that explored HIV prevention and PrEP care experiences in general and within bridge clinic settings. Deductive and inductive codes were developed and grounded content analysis used to generate themes based on the PrEP care continuum of awareness, uptake, adherence, and retention.

Results: Fourteen providers and 26 WSWUD were enrolled. WSWUD PrEP facilitators included non-stigmatizing communication, wraparound substance use treatment and HIV services, having a PrEP routine, and outreach PrEP adherences supports. WSWUD PrEP barriers included low HIV risk perceptions, and competing survival and drug use priorities. Providers PrEP facilitators included clinical note templates prompting HIV risk assessments and comfort prescribing PrEP. Provider PrEP barriers included discomfort discussing sex work risks and a lack of a clinical PrEP adherence infrastructure.

Conclusion: WSWUD and providers favoured integrated HIV prevention and substance use services in bridge clinic settings. Interventions enhancing PrEP delivery in these settings could increase PrEP access to WSWUD.

Genetics

SINGLE NUCLEUS RNA SEQUENCING REVEALS INTERACTION BETWEEN MICROGLIA AND ENDOTHELIAL CELLS IN MIXED ALZHEIMER'S DISEASE AND VASCULAR PATHOLOGY

Author: Oluwatosin Olayinka
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Objective: Single-nucleus RNA sequencing (snRNA-seq) allows for the dissection of the cell type-specific transcriptional profile of brain tissue. This is of interest in the context of Alzheimer's disease (AD) as multiple cell types play a role in its pathogenesis. AD can also co-occur with other orthogonal forms of dementia, further

complicating the pathology of the disease. Understanding how specific cell types operate in mixed dementias will be useful in understand their pathologies.

Methods: In this study we use snRNA-seq data from the hippocampi of a total of 12 patients, 11 of which have neuropathologically defined AD with or without an orthogonal form of dementia. This data was integrated with co-expression networks from a partially overlapping set of bulk RNAseq data to find gene sets of interest within cell types.

Results: We find that in those patients with both AD and vascular dementia there is a decrease in a specific set of genes related to immune activation in microglia and a decrease in genes which negatively regulate angiogenesis in endothelial cells. An association between these two sets of genes in the aforementioned respective cell types was found globally within the data and was thoroughly replicated in an independent snRNA-seq dataset of 393 samples.

Conclusion: We find strong evidence for an interaction between endothelial cells and microglia in human hippocampi. This involved microglial activation and migration and angiogenesis in endothelial cells.

COMPARISON OF COMMONLY MEASURED PLASMA AND CEREBROSPINAL FLUID PROTEINS AND THEIR SIGNIFICANCE FOR THE CHARACTERIZATION OF COGNITIVE IMPAIRMENT STATUS

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Objective: This study sought to determine how plasma and cerebrospinal fluid (CSF) protein markers are compared in the characterization of mild cognitive impairment (MCI) and Alzheimer's disease (AD) status.

Methods: This cohort study included Alzheimer's Disease Neuroimaging Initiative (ADNI) participants who had baseline levels of 75 proteins measured commonly in plasma and CSF (257 total, 46 cognitively normal (CN), 143 MCI, and 68 AD). Logistic regression, least absolute shrinkage and selection operator (LASSO), and Random Forest (RF) methods were used to identify the protein candidates for the disease classification.

Results: We observed that six plasma proteins panel (APOE, AMBP, C3, IL16, IGFBP2, APOD) outperformed the seven CSF proteins panel (VEGFA, HGF, PRL, FABP3, FGF4, CD40, RETN) as well as AD markers (CSF p-tau and Aβ42) to distinguish the MCI from AD [area under the curve (AUC)=0.75 (plasma proteins), AUC=0.60 (CSF proteins) and AUC=0.56 (CSF p-tau and Aβ42)]. Also, these six plasma proteins performed better than the CSF proteins and were in line with CSF p-tau and Aβ42 in differentiating CN vs. MCI subjects [AUC=0.89 (plasma proteins), AUC=0.85 (CSF proteins) and AUC=0.89 (CSF p-tau and Aβ42)]. These results were adjusted for age, sex, education, and ApoE ε4 genotype.

Conclusion: This study suggests that the combination of 6 plasma proteins can serve as an effective marker for differentiating MCI from AD and CN.

PROJECT INCLUSIVE GENETICS: PROTECTING REPRODUCTIVE AUTONOMY FROM BIAS VIA PRENATAL PATIENT-CENTERED COUNSELING

Author: Apolline Jungels
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Objective: Clinician bias negatively impacts the healthcare received by marginalized communities. In this study, we explored factors that influence bias held by healthcare workers and trainees about fetal diagnoses and their impact on clinical judgment in prenatal genetic testing settings. We compare genetics specialists to their non-expert counterparts.

Methods: This web-based study included clinical vignettes, implicit association tests (IATs), and an educational module. Participants were recruited via their institution or professional society. We conducted statistical analyses, including regression models accounting for demographic characteristics, to analyze recommendation patterns and change after the module.

Results: Genetics expertise strongly correlated with appropriate testing recommendation when the patient would not consider pregnancy termination ($r=1.784$ pre-module, $r=1.502$ post-module, $p<0.01$). Factors that influenced pre-module recommendation to test include high religiosity ($r=0.525$, $p<0.05$) and personal preference for testing ($r=1.112$, $p<0.01$). 42% of non-experts who answered inappropriately before the module changed their answer to the consensus one after the module.

Conclusion: Individual bias, along with structural and institutional bias, permeates family planning encounters and significantly decreases quality of care. Anti-bias training is effective, particularly for non-expert providers and can improve the care provided to individuals with intellectual disability.

NOVEL APOE Ɛ4/Ɛ4 GENOTYPE-SPECIFIC PROTECTIVE VARIANTS AGAINST AD

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Objective: To identify variants associated with a protective effect in APOE ε4/ε4 genotype participants.

Methods: We conducted a genome-wide association study (GWAS) for AD among 1,223 non-Hispanic White APOE ε4/ε4 carriers assembled by the ADGC. We found SNP associations with expression of their nearest genes in brains from 576 ROSMAP and AD incidence and cognitive testing in 3,688 FHS participants. We compared expression between ε2/ε2 (APOE2) and ε4/ε4 (APOE4) human induced pluripotent stem cells (iPSC)-derived neurons (iNeurons) and astrocytes (iAstrocytes) in a co-culture system and evaluated associations of expression levels and traits in 208 FHS brains.

Results: We identified associations of AD with 11 suggestive SNPs ($P<1E-6$). Rs75983775 near TRIB2 had a lower AD risk (OR=0.12, P=9.4E-11), higher baseline cognition, and increased TRIB2 expression in ε4+ carriers (P=0.02). TRIB2 expression increased in APOE2 iAstrocytes (adjP=0.03) and iNeurons (adjP=0.002) and elevated the Iba1 level in brains (P=0.03). Rs56035906 in TENM3 had reduced AD risk (OR=0.30, P=4.3E-7), lower AD incidence (P=0.03) and improved cognitive progression (P=0.03). TENM3 expression decreased in APOE2 iNeurons (adjP=2.4E-4) and associated with pTau 181 (P=0.007) and C4a protein expression (P=9.6E-4).

Conclusion: We identified variants that may protect against AD risk among APOE ε4/ε4 carriers, delay disease onset, and modulate expression of nearby genes.

IFNG-INDUCED IMMUNOSUPPRESSION IN LUNG CARCINOMA IS MEDIATED BY AN ENVIRONMENTAL CHEMICAL RECEPTOR (AHR) THROUGH PD-L1 AND IDO CONTROL

Author: Megan Snyder
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Objective: Clearly state the objective of the research project We have shown that the aryl hydrocarbon receptor (AhR) is a central player in regulating immune checkpoints in lung adenocarcinoma. Determining AhR's role in immune suppression will open the door for improved immunotherapies.

Methods: Clearly state the methods used to obtain the results Wildtype mice were injected with CMT167WT or CMT167AhR-KO lung carcinoma cells. Tumor growth and survival were tracked. Tumor digests were phenotyped and single cell RNA sequencing was performed on CD45+ isolates. Malignant cell PD-L1 expression in the absence or presence of exogenous AhR agonists was also determined in vitro in both cell lines via flow cytometry and qPCR.

Results: Clearly state the results of the research AhR deletion in CMT167 cells yields partial and up to complete prevention of tumor formation in our model. AhRKO tumors have greater numbers of tumor infiltrating CD4+ and CD8+ T cells per tumor volume by 5 weeks post challenge. ScRNAseq revealed a cytotoxic T cell population with granzyme and perforin-generating ability unique to AhRKO tumors. WT tumors were found to have multiple unique CD4+ and CD8+ populations with naïve or exhausted transcriptomes.

Conclusion: Clearly state the conclusions of the research project AhRKO enables tumor immunity, leading to reduction or total elimination of tumor growth. Immunological benefits correlated with AhRKO cells suggests AhR's potential as a viable immunotherapeutic target.

IDENTIFICATION OF RARE CODING VARIANTS ASSOCIATED WITH ALZHEIMER'S DISEASE

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Objective: We combined WES and WGS data assembled by the Alzheimer Disease Sequencing Project (ADSP) to increase the power for detecting associations of Alzheimer's disease (AD) with rare variants in gene coding regions.

Methods: We developed an efficient computational pipeline to perform quality control (QC) on WGS (n=16,905) and WES (n=20,504) data. The resultant ancestries were European (EA, 9,255 AD cases & 7,838 controls), African American (AA, 1,491 AD cases & 2,862 controls), and Caribbean Hispanic (CH 1,392 AD cases & 2,796 controls). In the joint and ethnicity stratified datasets, we tested for associations of AD with 250,552 bi-allelic variants that passed our QC pipeline.

Results: In the total sample analysis, variants from several known AD genes in addition to APOE including TREM2 R47H (P=2.34x10-13) crossed our study-wide significance (SWS). Novel loci including TKTL2 (P=2.35X10-8), D2HGDH, (P=1.09X10-7), CECR1 (P=4.02X10-7), PDHA2 (P=2.76X10-7), GOLGA1 (P=1.72X10-7), CYLD (P=1.84X10-7), and RP11-243M5.4 (P=1.37X 10-7) were also found. PSEN1 missense mutation (G206A, rs63750082), previously associated with early onset AD in CH, was SWS in late onset AD (P=1.58x10-13). In ethnic stratified analysis, FAM171A (P=7.20x10-7) in EA and TLR4 (P=4.58x10-7) in CH were SWS other than APOE.

Novel gene associations in LRRN4 (P=9.16X10-6) in EA and PIWIL3 (P=6.05X10-5) in CH were found.

Conclusion: Increasing genetic power by merging WGS and WES can help find novel rare coding variants.

PREDICTING NEUROIMAGING BIOMARKERS AND CLINICAL CONVERSION TO ALZHEIMER'S DISEASE IN CELL-BASED GENETIC SUBTYPES

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Objective: The complex etiology of AD obfuscates patient subtypes adversely impacting clinical trials. We developed a procedure to stratify at risk individuals for AD using cell-type specific polygenic risk scores.

Methods: We computed cbPRS in 8,481 Framingham Heart Study participants using the same genetic markers used in the Alzheimer's Disease Neuroimaging Initiative (Sahelijo et al. 2022). Low- and high-risk subtypes were defined using the 1st and the 4th quartiles of the cbPRS distributions. We conducted association tests with cognitive tests and neuroimaging biomarkers using subtype risk status as a binary outcome in ADNI. We compared conversion rates to AD using a Cox proportional hazard regression in ADNI and FHS, and atrophy patterns using brain-wide gray matter volume maps with voxel-based morphometry in ADNI.

Results: Genetic subtypes for astrocytes (Ast-M2) and oligodendrocytes (Oli-M45) were associated with memory and executive function impairment, decreased hippocampal volume, increased amyloid deposition, and decreased glucose metabolism in ADNI. Conversion rates for Ast-M2 and Oli-M45 were significantly different in both ADNI and FHS. We identified significant atrophy in bilateral hippocampi, entorhinal cortex, and amygdala for Ast-M2 and Oli-M45 (P<1E-3).

Conclusion: Polygenic risk scores informed by cell type specific brain transcriptomic networks can stratify at risk subjects with AD related changes in imaging biomarkers and higher chance of progression to AD.

LACTATE-INDUCED LOWER BRAIN PH MAY LEAD TO EPIGENETIC UPREGULATION OF TGFB2 WHICH ORCHESTRATES DYSREGULATION OF GENES IN SCHIZOPHRENIA (SCZ) AND BIPOLAR DISORDER (BD)

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Objective: Brain lactate is the main determinant of reduced brain pH in SCZ & BD. In adipose tissue lactate increases TGFB2 expression and anti-lactate drugs inhibit this effect. In expression microarray analysis of SCZ & BD postmortem brains vs controls, we found that increased TGFB2 expression correlates with a synchronized increased expression of ¾ of dysregulated genes. As TGFB2 increased expression was confirmed in qRT-PCR of 35 samples/group, TGFB2 promoter DNA was hypomethylated in SCZ & BD. While brain pH was lower in SCZ & BD (p=0.03 & p=0.01), TGFB2 expression was correlated with brain pH level, in general (r= -0.54, p=0.002) and in SCZ/BD (r= -0.54, p=0.01).

Methods: We altered pH of culture media of 5 cell lines, including of two lines of iPSC derived NSC, neurons and astrocytes using lactate or HCL.

Results: In astrocytes with 3-fold higher TGFB2 expression vs. neurons, there was a linear increase in TGFB2 expression (>100%) associated with 32% decrease in DNA methylation and 30% increase in 5-hmC level after one week culture in low vs. control pH (6.4,6.6 vs 6.8). A similar trend was observed in fibroblasts, HEK 293, MCF10A, but not in 2 cancer cell lines.

Conclusion: Lactate-induced low brain pH may lead to a synchronized gene dysregulation mediated by epigenetic upregulation of TGFB2 in SCZ & BD in astrocytes consisting of ~50% of brain cells. Thus, adjusting brain pH/lactate, or targeting key genes affected by or affect lactate/pH maybe novel strategies to treat SCZ & BD.

A GENOME-WIDE SEARCH FOR PLEIOTROPY IN MORE THAN 100,000 HARMONIZED LONGITUDINAL COGNITIVE DOMAIN SCORES

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Objective: More than 75 loci account for only a portion of the AD heritability. A more complete understanding of the genetic basis of AD can be deduced by exploring associations with AD-related endophenotypes.

Methods: We conducted genome-wide scans for 103,796 harmonized longitudinal cognitive domain scores from 23,066 subjects of community-based (FHS, ACT, ROSMAP) and clinic-based (ADRC, ADNI) cohorts using GLMM, including SNP, age, SNP×age, sex, education, and five PC. Results across cohorts were combined using inverse-variance meta-analyses. To determine significance, we applied a joint test of the main SNP and SNP×age interaction effects. Pleiotropy GWAS for each domain pair was performed using PLACO.

Results: GWAS revealed GWS associations with five established AD-related loci (BIN1, CR1, GRN, MS4A6A, APOE) and four novel loci (ULK2, CDK14, LINC02712, PURG). GWS pleiotropy was observed for language and memory with LOC107984373 (rs73005629, P=3.12×10-8) in the clinic-based cohorts, and with NCALD (rs56162098, P=1.23×10-9) and PTPRD (rs145989094, P=8.34×10-9) in the community-based cohorts. GWS pleiotropy was also found for executive function and memory with OSGIN1 (rs12447050, P=4.09×10-8) and PTPRD (rs145989094, P=3.85×10-8) in the community-based cohorts.

Conclusion: Our results provide some insight into biological pathways underlying processes leading to domain-specific cognitive impairment and AD, as well as a conduit toward a syndrome-specific precision medicine approach to AD.

CHARACTERIZATION OF LONG NON-CODING RNA AND CIRCULAR RNA IN THE AGING HUMAN HIPPOCAMPUS ACROSS THREE LIBRARY TYPES

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Objective: Ribosomal RNA depletion (ribo-) is commonly used for RNA sequencing to capture poly(A)+ mRNA, non-coding RNAs (ncRNAs), or protein-coding mRNAs that are not polyadenylated. The poly(A)-selected (poly(A)+) protocol enriches poly(A)+

transcripts including mRNAs and ncRNAs. However, many long ncRNA transcripts (>200 nt) are known to lack poly(A) tails.

Methods: In order to enrich these poly(A)- transcripts and estimate their function in aging brains, we first applied a poly(A)- protocol to generate RNAseq data for 128 human hippocampus samples. For the same samples, ribo- and poly(A)+ RNAseq data were also generated in a similar manner. We then systematically assessed the expression of linear ncRNA, detected circRNA, and compared their expression levels across the three library types.

Results: Our results show that both ribo- and poly(A)- sequencing perform well for linear ncRNA profiling, with poly(A)- libraries generally having a slightly higher number of detected expressing genes compared to ribo- libraries. The number of detected circRNA is only slightly higher in poly(A)- libraries compared to the ribo- libraries. These results suggest that both strategies are effective for studying ncRNA in human tissue.

Conclusion: Our study offers a rich source of data for exploring the poly(A)- landscape of transcriptome in the human aging brain, but also suggests the importance of understanding the potential pitfalls of each sequencing strategy, especially when studying ncRNAs.

CHRONIC INTERMITTENT ETHANOL EXPOSURE-INDUCED M6A MODIFICATIONS AROUND MRNA STOP CODONS OF OPIOID RECEPTOR GENES

Author: Huiping Zhang
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Objective: This research aimed to investigate whether chronic alcohol consumption could alter mRNA methylation levels of addiction or reward-related genes, thus increasing the risk of alcohol use disorder (AUD).

Methods: We used neuron-like (SH-SY5Y) and non-neuronal (SW620) cells as models to examine chronic intermittent ethanol (CIE) exposure-induced global m6A RNA methylation changes as well as m6A mRNA methylation changes around the stop codon of three opioid receptor genes (OPRM1, OPRD1, and OPRK1), which are known to regulate pain, reward, and addiction behaviors.

Results: CIE exposure increased global RNA methylation levels in both SH-SY5Y ($t=3.98$, $P=0.007$) and SW620 ($t=2.24$, $P=0.067$) cells. However, CIE exposure resulted in hypomethylated m6A sites around mRNA stop codon regions of OPRM1 and OPRD1 in both cell lines [OPRM1 (SY-SY5Y): $t=-5.05$, $P=0.0005$; OPRM1 (SW620): $t=-3.19$, $P=0.013$; OPRD1 (SY-SY5Y): $t=-13.43$, $P<0.00001$; OPRD1 (SW620): $t=-4.00$, $P=0.003$]. Additionally, CIE exposure downregulated OPRM1, OPRD1, and OPRK1 expression in both cell lines.

Conclusion: The present study demonstrated that chronic ethanol exposure could lead to increased global m6A RNA methylation levels but decreased mRNA methylation and expression levels of opioid receptor genes. Our findings suggested a potential epitranscriptomic mechanism by which chronic alcohol consumption remodels the expression of reward-related or alcohol responsive genes in the brain, resulting in alcohol-induced neuroadaptations.

IMPACT OF XMAP215 ON NEURAL CREST CELL MIGRATION AND CRANIOFACIAL DEVELOPMENT IN XENOPUS LAEVIS

Author: Olivia Perry
Additional author(s): Chiedza Sibanda, Burcu Erdogan

Objective: We have previously demonstrated that the critical cytoskeletal regulator, XMAP215, plays an important role in promoting axon outgrowth and guidance. However, much is unknown about how XMAP215 might regulate other types of cell migration. Since we recently showed that the XMAP215 partner, TACC3, is important for neural crest cell (NCC) migration during craniofacial (CF) development, we sought to determine whether XMAP215 also plays a role in NCC migration.

Methods: Xenopus laevis embryos were injected at the two-to-four cell stage with morpholino antisense oligonucleotides to reduce endogenous XMAP215 levels, fixed at stage 42, then imaged using ZEN software. Images were analyzed with ImageJ using previously validated metrics for quantifying CF development. Additionally, NCCs harvested from embryos were plated and analyzed to determine if XMAP215 affects cell migration in vitro. In situ hybridizations were used to monitor pharyngeal arches to determine if NCC migration was affected in vivo.

Results: We have found that XMAP215 knockdown results in statistically significant facial abnormalities, as well as changes in NCC motility patterns, both in vivo and in vitro.

Conclusion: Our results identify a role for XMAP215 in CF development. The data shows that one key mechanism of XMAP215 impacting CF development is through regulating NCC migration, in which knockdown of XMAP215 may result in disturbances to the cytoskeleton that impact cell migration and cause CF abnormalities.

PROFILING DIABETES-INDUCED CHANGES IN IMMUNE EXHAUSTION IN BREAST CANCER

Author: Christina Ennis
Additional author(s): Gerald Denis

Objective: Type II diabetes (T2D), the most common metabolic disorder, both increases breast cancer incidence and decreases survival in postmenopausal women. Despite this clear link, the molecular impacts of the T2D immune phenotype on cancer remain poorly understood. Here, we investigate immune exhaustion in diabetic tumor-infiltrating lymphocytes (TILs).

Methods: Primary human breast cancer samples were collected from patients undergoing surgical resection. Tumor samples were digested and sorted for CD4+ and CD8+ T cell subsets. RNA sequencing and differential expression analysis identified variations in immune exhaustion signatures with and without T2D.

Results: We observed increases in CD4/CD8 ratio and immune exhaustion expression in TILs from diabetic samples compared to nondiabetic. These findings suggest that T2D compromises the immune landscape within the tumor microenvironment, thwarting anti-tumor immune responses to promote tumor progression.

Conclusion: We highlight the link between T2D and perturbed immune profiles of TILs, providing insights into the potential role of metabolic status in inducing immune exhaustion that promotes pro-oncogenic pathways within breast tumors. Our observations hold particular significance for safety net hospitals, where T2D is highly

prevalent and the standard of care recommendations may not adequately meet the needs of this understudied population.

INVESTIGATING THE ROLE OF CKAP5 DOMAINS IN MEDIATING MICROTUBULE AND ACTIN INTERACTION IN XENOPUS LAEVIS GROWTH CONES

Author: Burcu Erdogan
Additional author(s): Ainsley Hutschison, Garrett Cammarata

Objective: Microtubules (MTs) and actin filaments (F-actin) are cytoskeletal components that regulate axon outgrowth and guidance. CKAP5 is a MT plus-end binding protein studied for its role in regulating MT polymerization. We identified a novel function for CKAP5 and showed that CKAP5 can directly interact with F-actin and mediates MT / F-actin alignment within growth cones. Here, we used in vitro and in vivo approaches to investigate the role of different domains of CKAP5 in mediating MT/ F-actin interaction.

Methods: To test the interaction between CKAP5 domains and F-actin, we created CKAP5 domain mutants and performed biochemical assays using purified proteins. We also used Xenopus laevis embryonic neural tube cultures to monitor how MT and F-actin alignment is affected in the presence of CKAP5 domain mutants by performing high-resolution microscopy imaging.

Results: We found that the CKAP TOG5 domain is important for mediating MT/F-actin interaction and MT/ F-actin misalignment that occurs from CKAP5 knockdown could be rescued by adding the CKAP5 TOG3-C-terminal domain back into the system.

Conclusion: Our work identifies a novel role for MT plus-end binding protein, CKAP5, in mediating MT / F-actin interaction both in vitro and in vivo and dissects the role of different domains in mediating this interaction during neural development. The consequences of this interaction during important events such as axon outgrowth and guidance remain to be investigated in the future.

LINKING STRESS TO CELLULAR SENESCENCE IN THE PROSTATE CANCER MICROENVIRONMENT

Author: Joakin Mori
Additional author(s): Koushik Gadhachanda, Natalia Feced Garcia, Christopher Heaphy

Objective: Evaluate the correlation between stress-related gene expression and a senescent tumor microenvironment (TME), an emerging hallmark of cancer linked to disparities in prostate cancer (PCa) outcome.

Methods: Using a 125-gene signature that predicts cellular senescence in vivo (SenMayo) and stress-related genes, we analyzed gene expressions in existing PCa datasets (TCGA) and computed protein-protein interaction.

Results: Hierarchical clustering revealed a gene cluster enriched for the SenMayo signature that stratified samples into three clusters and inversely correlated with tumor purity, suggesting that senescence-related genes are expressed higher in stromal cells than cancer cells. Clusters enriched for SenMayo signature were also enriched for samples expressing higher levels of stress-related genes: NR3C1, PNMT, and EPAS1. Like the SenMayo signature, NR3C1, PNMT, and EPAS1 expression negatively correlated with tumor purity (Spearman: -0.63, -0.39, and -0.65, respectively; $p < 0.001$). Protein-protein interaction analysis revealed interactions of the NR3C1 protein with EPAS1 and senescent-related proteins, including HMGB1, JUN, TNF, and EGFR. In addition to NR3C1, EPAS1 interacts with CTNNB1 and VEGF.

Conclusion: Stress-related gene expression likely contributes to a senescent stromal component of the TME. Next, we plan to characterize the spatial distribution of senescent cells and immune landscape in a diverse patient cohort of men with PCa treated at Boston Medical Center.

SCAVENGER RECEPTORS IN THE TRANSENDOTHELIAL MIGRATION OF BLOOD STEM CELLS

Author: Gwendolyn Beacham
Additional author(s): Khaliun Enkhbayar

Objective: Hematopoietic stem cell (HSC) transplantation is a potentially curative treatment for blood and immune disorders, but remains risky and inefficient. Understanding how HSCs migrate into the niche could guide new strategies to accelerate HSC homing. We identified a conserved gene expression signature for HSC niche endothelial cells that includes the scavenger receptors, stab1/2, and specific machinery for endocytosis. Our aim was to investigate the function of these factors in the niche.

Methods: To evaluate endocytosis, we injected fluorescent ligands into the circulation of zebrafish embryos. We used high-resolution microscopy to analyze endocytosis and HSC migration into the niche.

Results: The ligands were internalized by HSC niche endothelial cells into vesicle clusters that localized asymmetrically around the nucleus. At these sites we observed blood cells initiate transendothelial migration. Injection of a scavenger receptor inhibitor blocked ligand uptake and impaired HSC migration into the niche. To identify the required receptor, we used morpholinos against stab1 and stab2. Knockdown of stab2 selectively decreased ligand uptake. A similar result was observed in stab2 mutants and in embryos with mutations in dab2, an endocytic cargo sorting protein.

Conclusion: These data are consistent with a model where scavenger receptor-driven endocytosis guides HSC migration into the niche. Future work will define the set of receptors, machinery, and cargo required for HSC migration.

SR-18292 INDUCES FETAL HEMOGLOBIN SYNTHESIS AND REDUCES DISEASE PATHOLOGY IN SICKLE CELL MICE

Author: Yanan Sun
Additional author(s): Hajar Benmhammed

Objective: Sickle cell disease (SCD) is a common inherited blood disorder, which affects millions around the world. Reactivation of fetal hemoglobin (HbF) synthesis in adult erythroid cells can diminish severity of many clinical features of SCD. Up-regulation of PGC-1α has been shown to induce HbF levels in human CD34+ cells. Here we reported the in vitro and in vivo effects of SR-18292 which targets PGC-1α.

Methods: We performed RT-qPCR and western blot to measure the mRNA and protein levels of γ-globin and PGC-1α in human CD34+ cells and in SCD mice. The percentage of HbF positive cells (F-cells) was determined by flow cytometric analysis. We also performed complete blood cell count (CBC) and measured spleen (size and weight) and the ratio of sickle cells in SCD mice.

Results: We found that the protein and mRNA levels of PGC-1α and γ-globin were significantly increased in treated cells and in treated SCD mice. And the F-cells were also increased in treated cells and in treated SCD mice. And we also found that the indices of RBCs are improved in treated mice and consequently reduced hemolysis and higher fraction of circulating mature erythrocytes in treated SCD mice. There wasn't serious adverse effect on other hematopoietic cell lineages.

Conclusion: These results suggest that SR-18292 is might be a promising therapeutic drug and also indicate that modulating PGC-1α activity or the signaling pathways that it regulates might benefit therapeutically patients with SCD.

GIOTTO SUITE: A MULTI-SCALE AND TECHNOLOGY-AGNOSTIC SPATIAL OMICS ANALYSIS FRAMEWORK

Author: Jiaji Chen
Additional author(s): Joselyn Chávez, Matthew O'Brien, Irzam Sarfraz, Eddie Ruiz, Pratishtha Guckhool, Guo-Cheng Yuan, Ruben Dries

Objective: Spatial-omics technologies allow interrogation of the role of tissue architecture in specific biological processes, such as the establishment of cellular phenotypes or the inner workings of tissues. They can profile different molecular analytes such as RNA and protein: different, but interconnected, layers of the cell regulatory network. With recent advancements in spatial multiplex imaging-based platforms, the range of spatial resolutions and data has widened. From genome-wide data in coarse grain arrays down to the acquisition of individual transcript coordinates at subcellular resolution. Together, these technologies offer complementary insights for both discovery and validation research. However, integrating multiple modalities or operating with different spatial resolutions, is currently a major challenge.

Methods: Giotto Suite is a comprehensive update to our open-source R package Giotto that continues our emphasis on a technology agnostic framework for spatial data representation and provide integrative solutions from data processing to visualization. Giotto Suite disentangles morphology, spatial and feature information to create a responsive and flexible framework to analyze spatial data at multiple scales.

Results: We are able to demonstrate the flexibility and use of this new framework on several state-of-the-art spatial technologies.

Conclusion: Giotto Suite provides solutions for the analysis and representation of spatial-omic data of multiple resolutions and modalities.

EXPLORING THE LINK BETWEEN TYPE 2 DIABETES AND OBESITY ON PLASMA EXOSOMES IN TRIPLE NEGATIVE BREAST CANCER PROGRESSION AND METASTASIS

Author: Pablo Llevenes
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Objective: Investigating the impact of obesity and type 2 diabetes (T2D) on systemic metabolic changes in triple negative breast cancer (TNBC) progression. Assessing the role of plasma exosomes as mediators of these changes. As the main hypothesis, T2D and obesity alter plasma exosome content, influencing the tumor microenvironment and promoting cancer progression.

Methods: High-fat diet (HFD) fed C57BL/6J mice were studied. Plasma exosomes isolated and used to treat E0771 cells for in vitro and in vivo experiments. Gene expression analyzed using EMT array and qPCR. Migration assay and tumor growth analysis performed.

Results: HFD-derived exosomes reprogrammed EMT gene expression, leading to a pro-metastatic phenotype and upregulated metastasis-related pathways. PD-L1 receptors were also upregulated. In vivo, increased metastatic burden observed in lung and brain.

Conclusion: Plasma exosomes from HFD fed mice influence gene expression and impact TNBC metastasis.

DISSECTING THE FUNCTIONAL REQUIREMENT OF THE FETAL BLOOD STEM CELL NICHE USING A NOVEL ZEBRAFISH MODEL

Author: Zewde Ingram
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Objective: The fetal hematopoietic stem cell (HSC) niche provides a supportive microenvironment for HSCs. In zebrafish, this niche lies in a vascular plexus in the tail called the caudal hematopoietic tissue (CHT). Our objective was to dissect the functional requirement of the fetal blood stem cell niche using a novel zebrafish model.

Methods: We found that zebrafish survive surgical removal of the early embryonic tail, allowing us to study HSC development in the absence of the CHT. A combination of live-cell imaging and flow cytometry was used to evaluate HSC dynamics under steady-state and stress hematopoiesis. Bulk RNA-seq was used to transcriptionally profile HSCs from tailless fish compared to controls.

Results: Although HSC formation was normal in the tailless embryos, ~50% died before adulthood. Flow cytometric analysis revealed fewer HSCs suggesting a defect in HSC expansion. Adult tailless fish had fewer HSC clones, but exhibited normal lineage output during steady-state hematopoiesis and transplantation. Bacterial infections revealed tailless fish are more susceptible to infection. Bulk RNA-seq analysis of HSCs from tailless embryos and controls revealed significant changes in gene expression.

Conclusion: These results are consistent with a model where the fetal niche is required for embryonic maintenance of HSCs, but less consequential for adult hematopoiesis. Ultimately, this work could inform strategies for expanding HSCs for the treatment of blood and immune disorders.

GIOTTODB: A SCALABLE BACKEND FOR LARGE SPATIAL OMICS AND SINGLE CELL EXPRESSION DATA

Author: Edward Ruiz
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Objective: Spatial omics is an emerging research field focusing on the broad profiling of biological molecules (e.g. proteins, genes, etc) while retaining spatial context. The most sensitive of these technologies require only a simple tissue section while providing detailed information at the single cell or even subcellular level, making them capable of handily generating enormous datasets with tens of millions of cells. While these technologies offer many biological insights, there is a scarcity of computational methods that can deal with this data.

Methods: GiottoDB is a package written in the R statistical programming language that provides support for handling large spatial omics and single cell datasets using established database technologies. The software structure of GiottoDB is designed to work seamlessly with the accompanying spatial analysis package (Giotto Suite), but is generalizable to other packages.

Results: We have performed benchmarks using GiottoDB and the open source DuckDB database format to analyze large spatial omics datasets. Our results demonstrate that GiottoDB significantly outperforms existing methods in standard matrix operations and analysis workflows, while utilizing significantly less RAM.

Conclusion: Emerging database technologies, such as DuckDB, can effectively address the challenges associated with representing and analyzing large spatial omics and single cell datasets.

CORIN AS AN EPIGENETIC MODULATOR POTENTIALLY CHANGES THE DIRECT MACROPHAGE-CANCER INTERACTIONS WITHIN TRIPLE-NEGATIVE BREAST CANCER LANDSCAPE

Author: Sophia Murray
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Objective: Triple-negative breast cancer (TNBC) is notable for high levels of heterogeneity and increased infiltration of macrophages. Macrophages can influence prognostics by reshaping the tumor microenvironment (TME). Corin, a synthetic dual inhibitor of HDAC and LSD1, has shown promising results in melanoma. Our study aims to further investigate the anti-tumor properties of Corin on the TNBC microenvironment systematically capturing the complexity of TNBC-macrophage interactions.

Methods: We have designed a highly reproducible 3D experimental system to culture TNBC-macrophage spheroids. TNBC cell lines representing different subtypes are cultured with macrophages and then polarized into M0, M1, and M2. Thus, this model recapitulates a spectrum of immune responses seen in breast tumors. We then perturbed the spheroids with Corin. Furthermore, by implementing an in-house computational pipeline, we imaged the spatial transcriptomic profile of individual spheroids in situ using MERSCOPE.

Results: Our protocol enables stable integration of TNBC cell lines and polarized macrophages in 3D thereby mimicking in vivo tumor structure and cell-to-cell interactions. In a high-throughput fashion, the spatially resolved transcriptional landscape of the spheroids was analyzed.

Conclusion: We have built an innovative screening tool that has probed the potential impact of an antitumor drug - Corin. We have observed the drug's effects on TNBC and macrophage fractions within the heterogeneous TNBC TME.

Infectious Diseases

ATTRIBUTABLE FRACTIONS FOR UNFAVORABLE TREATMENT OUTCOMES AMONG ADULTS WITH DRUG-SENSITIVE PULMONARY TUBERCULOSIS IN INDIA

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Objective: We calculated attributable fractions (AFs) to estimate the proportion of unfavorable pulmonary tuberculosis (PTB) treatment outcomes that can be attributed to modifiable risk-factors in India.

Methods: Adults with drug-sensitive PTB were enrolled at treatment initiation and prospectively evaluated for 2 years. The primary outcome was a composite for unfavorable treatment, including failure, recurrence, and all-cause mortality. We estimated unadjusted AFs using Levine's formula and adjusted AFs as (O–E)/O where O = observed # of outcomes and E= expected # of outcomes in a hypothetical scenario if the exposure were removed and confounders unchanged.

Results: Of 2931 participants, 129 failed treatment, 80 had recurrence, and 101 died. Of these, 1132 (39.1%) were undernourished (BMI<18.5kg/m2), 1182 (40.4%) smoked, 956 (32.8%) had diabetes (HbA1c>6.5% or prior diagnosis), 437 (14.9%) reported alcohol

misuse (AUDIT>10 points), and 65 (2.2%) were HIV+. After adjusting for confounders, undernourishment, alcohol misuse, and HIV accounted for 25.1% (95%CI 17.1-32.3), 7.4% (95%CI 2.3-12.2) and 2.1% (95%CI 0.9-3.4) of unfavorable treatment outcomes, respectively. Findings were consistent in a sex-stratified analysis.

Conclusion: Undernourishment, alcohol misuse, and HIV accounted for nearly a third of all unfavorable treatment outcomes among adult PTB cases in India, presenting high-yield targets for intervention.

BENEFITS OF DIAGNOSING SUBCLINICAL TB DISEASE AMONG PEOPLE WHO SMOKE DRUGS

Author: Victoria Overbeck
Additional author(s): Tara Carney, Samantha Malatesta, Danie Theron, Tara C. Bouton, Nandi Niemand, Sarah E. Weber, Charles R. Horsburgh, Laura F. White, Rob M. Warren, Karen R. Jacobson

Objective: We compare M. tuberculosis bacterial burden and linkage to tuberculosis (TB) care among people who smoke drugs (PWSD) found through active (ACF) versus passive case finding (PCF) approaches in a rural community in South Africa.

Methods: We analyzed data from 187 individuals with TB from Worcester, South Africa. 57 individuals were recruited using ACF approach and 130 were diagnosed by self-referral in clinic (PCF). All had microbiologically confirmed TB and screened urine positive for methamphetamine or methaqualone. We obtained adjusted associations of ACF and PCF with bacterial burden using negative binomial regression for TTP and logistic regression for cavitation and smear positivity.

Results: Median (IQR) TTP for ACF was 12 (7,17) days compared to 7 (5,10) for PCF. 31 (54.4%) ACF participants were asymptomatic compared to 129 (99.2%) PCF participants reporting ≥1 symptom. 14 (24.6%) ACF participants were smear positive compared to 96 (73.8%) PCF participants (Table 1). PCF participants were more likely to be smear positive (OR:9.1, 95%CI:4.3,20.2), have cavitary disease (OR:2.4, 95%CI:1.2, 4.9) and a shorter TTP (RR:0.5, 95%CI:0.5,0.7), after adjusting for age, sex, HIV, and tobacco use.

Conclusion: We found lower bacterial burden and substantial subclinical TB disease among PWSD through ACF compared to PCF approach. Diagnosing and linking to care PWSD early in disease progression when infectivity is low has strong potential to reduce transmission and improve outcomes.

T CELL RESPONSES AGAINST NON-STRUCTURAL ANTIGENS FROM PRIOR SARS-COV-2 INFECTION ASSOCIATE WITH LOWER INCIDENCE OF SYMPTOMATIC ENDEMIC CORONAVIRUS INFECTION

Author: David Bean
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Objective: It is unknown whether SARS-CoV-2 immunity will protect against future novel coronaviruses (CoV). We examined the incidence of and immunity against the endemic CoV (eCoV) as a proxy for response against a future CoV among those with SARS-CoV-2 infection, COVID-19 vaccination, or neither exposure.

Methods: We conducted a retrospective cohort study of individuals from Boston Medical Center with respiratory infections in 2021. The individuals were grouped based on 1) a prior SARS-CoV-2 infection; 2) prior COVID-19 vaccine but no SARS-CoV-2 infection; or 3) no prior SARS-CoV-2 infection or vaccination. A subset in each group

was assessed for CoV specific immune responses, via pseudovirus neutralization and T cell stimulation assays.

Results: Incidence of symptomatic eCoV infection was lower in those individuals with a prior SARS-CoV-2 infection compared to the individuals with only a COVID-19 vaccination or no prior SARS-CoV-2 exposure ($p = 0.01$). Individuals with a prior SARS-CoV-2 infection or a COVID-19 vaccination had lower neutralization responses against OC43 pseudovirus ($p = 0.03$). The three groups had similar levels of T cells stimulated by eCoV peptides from spike and nucleocapsid, while those with a prior SARS-CoV-2 infection had elevated CD8+ T cell responses to eCoV nsp12-nsp13 peptides ($p = 0.02$).

Conclusion: Our observations suggest that incorporation of non-structural viral antigens in a future pan-CoV vaccine may improve protection against future heterologous CoV infections.

IMPACT OF CIGARETTE SMOKING ON SYSTEMIC AND AIRWAY HIV-1 RESERVOIR AND INFLAMMATION IN PEOPLE LIVING WITH HIV (PLWH)

Author: Alex Olson

Additional author(s): Archana Asundi

Objective: Cigarette smoking and HIV infection are both independent risk factors for cardiovascular and pulmonary diseases. We aimed to understand the effect of smoking and smoking cessation on systemic and airway inflammation and HIV reservoir dynamics in PLWH.

Methods: We recruited non-smokers (NS) and cigarette smokers (CS) aviremic PLWH on stable antiretroviral regimens for bronchioalveolar lavage (BAL) and blood collection. CS were enrolled in a 10-week smoking cessation (SC) program and returned for a follow up BAL and blood collection. We characterized the HIV reservoir in cells isolated from BAL and blood using droplet digital PCR and measured plasma inflammatory markers using ELISA/Luminex assays.

Results: Total HIV DNA and intracellular HIV RNA in peripheral mononuclear cells (PBMCs) and total HIV DNA in BAL was similar between NS, CS at baseline, and after SC. CS as compared to NS had greater expression of specific HIV RNA (gag and envelope) transcripts. Finally, plasma IL-6 was significantly elevated ($p=0.036$) in CS compared to NS and was not different at follow up.

Conclusion: Overall, smoking and cessation do not have significant impact HIV DNA reservoir characteristics in the peripheral blood or BAL in PLWH. However, smoking associates with altered viral RNA species, suggesting it may promote aberrant HIV transcription. Future directions include assessing inflammation and HIV-1 RNA profiles in the airway between NS and CS and the impact of cessation.

PREDICTORS OF SARS-COV-2 CULTURE CONVERSION IN A UNIVERSITY-BASED COHORTAUTHOR: GENEVIEVE DUPUIS

Additional author(s): Jacquelyn Turcinovic, Cole Sher-Jan, Laura White, Lynn Doucette-Stamm, Judy Platt, Hannah E. Landsberg, Davidson H. Hamer, Catherine Klapperich, Karen R. Jacobson, John H. Connor, Tara C. Bouton

Objective: Since March 2022, US-CDC recommends isolation for 5 days after initial COVID-19 symptoms, but little is known about predictors of individual culture-conversion by the end of isolation.

Methods: During a Boston University longitudinal study, we collected daily symptoms and anterior nasal swabs for viral culture for 10 days from participants with RT-PCR positive

SARS-CoV-2 Omicron infections. We define culture-conversion as two negative cultures, with no subsequent positive cultures. We analyzed odds of culture-conversion by day 6, based on symptoms, vaccinations/infections, viral load, and known exposures.

Results: Of 105 RT-PCR+ participants, 76% culture-converted by day 6. Seventy-five percent of participants remained symptomatic after day 5, of which 70% had culture-converted. Controlling for demographics, known exposure, previous infections, vaccinations, max symptom, viral load, and medical history, participants who were symptom-free after day 5 were significantly more likely to culture-convert (OR=14.0, 95% CI: 2.0, 306.9).

Conclusion: Most participants culture-converted by the end of the isolation period, though most had symptoms beyond day 5. Current guidance of including symptoms in determining duration of isolation is supported - those free of residual symptoms were significantly more likely to have culture-converted by day 6. Further, absence of cough, stuffy nose, or runny nose may be helpful in determining likelihood of culture negativity beyond the isolation period.

MORTALITY AND ECONOMIC IMPACT OF A POLICE SWEEP ON PEOPLE WHO ARE UNSHELTERED AND WHO USE OPIOIDS

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Objective: Many communities have growing numbers of people experiencing homelessness (PEH), many of whom use opioids and live in tent encampments. One approach to encampments is a street sweep, disrupting established services for opioid use disorder without addressing underlying problems. This study aims to: estimate the impact of a sweep on mortality among emerging adults (age 18-29) experiencing homelessness with high-risk opioid use and estimate the impact of alternative approaches.

Methods: We used a state transition model to simulate a two-year experience of a closed cohort (status-quo strategy). At baseline the simulated cohort had a mean age of 26.1 and 30% engagement with MOUD. After one year of simulation, we introduced a sweep in which individuals are either mandated civil commitment, stay on treatment, or are dispersed (sweep strategy). We additionally simulated two housing strategies, one with MOUD engagement required for entry (HWM) and one without (choice). Outcomes estimated included all-cause mortality, fatal overdose rates, and costs.

Results: Compared to status quo, the sweeps strategy resulted in 1 additional death per 400 individuals. Costs increased by 9% in the sweep strategy. HWM resulted in one fewer death compared to the sweep. Choice resulted in 2 fewer and had the highest number of person-weeks in housing and MOUD.

Conclusion: Sweeps increase overall cost and fatal overdose rates while also disrupting the health of PEH. Housing could be a life-saving alternative.

FACTORS INFLUENCING HIV-1 ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY BROAD POTENCY AND SUSCEPTIBILITY

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Asundi

Objective: Both neutralizing antibodies (nAbs) and antibodies that mediate antibody dependent cellular cytotoxicity (ADCC) are important for preventing HIV-1 transmission. Here, we aim to quantify differences in the degree of neutralizing and ADCC mediating antibodies as well as HIV-1 envelope glycoprotein (Env) sensitivity to ADCC from viremic and aviremic individuals.

Methods: Antibody responses against a panel of diverse HIV-1 Env were examined in both antiretroviral therapy (ART) naïve and ART suppressed individuals.

Results: Aviremic individuals had a 0.17-unit lower ADCC BP score compared to those with viremia, but higher than that of individuals without HIV-1. A specific Env was not consistently the most ADCC susceptible or resistant, rather the sensitivity ranking varied among the different plasma cohorts and antibody sets. There was a significant negative correlation between ADCC and neutralizing susceptibility. We observed some which broadly potent ADCC in the absence of strong nAbs. ADCC sensitivity to HIVIg did not strongly correlate with the amount of HIVIg binding or CD4 downregulation of infected cells.

Conclusion: Broadly potent ADCC responses diminish with suppressed viral levels but remain higher than HIV-1 naïve plasma, independent of ART duration. Understanding the generation of neutralizing and ADCC antibodies has implications for halting HIV-1 transmission. One future direction will be exploring the impact of this finding on the latent reservoir in these individuals.

BARRIERS IN RECRUITMENT OF AN OBSERVATIONAL SARS-COV-2 EMERGENCY DEPARTMENT COHORT AT BOSTON MEDICAL CENTER

Author: Sarah J. Thomson

Additional author(s): Roxanne Mistry, Henry Bayly, Manish Sagar, Elissa M. Schechter-Perkins, Laura F. White, Karen R. Jacobson, Tara C. Bouton

Objective: Successful recruitment is a challenging component of research, and recruitment barriers are amplified in safety-net hospital settings. It is increasingly important to engage historically underrepresented groups in research to improve health disparities and outcomes. We summarize the challenges recruiting a cohort of patients with SARS-CoV-2 from the emergency department (ED), actions to improve inclusivity, and implementation hurdles in a safety-net setting.

Methods: We conducted an observational study at the largest safety-net hospital in New England and recruited patients with COVID-19 from the ED. Investigators prioritized inclusivity through study material language translations, transport and travel reimbursement, flexible sample delivery options, compensation, and clinical staff engagement.

Results: Recruitment and retention efforts were largely unsuccessful in this setting (n=4 enrolled of n=113 eligible by chart review). We identified major impediments to recruitment success including clinical teams' perception of good candidacy, persistent language barriers, limited consent capacity, burden of participation, and ED discharge logistics.

Conclusion: Despite efforts to improve research access to underrepresented groups, safety-net hospital EDs present unique challenges for study recruitment. Study teams should prioritize continued community and clinical staff engagement efforts to improve research outcomes in these settings.

INCARCERATION AND TUBERCULOSIS DISEASE HISTORY AMONG PEOPLE WHO SMOKE ILLICIT DRUGS IN WORCESTER, SOUTH AFRICA

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Objective: To investigate the association between incarceration history and TB disease among people who smoke illicit drugs (PWSD) in a high TB burden setting.

Methods: We analyzed data from the "Transmission of Tuberculosis Among illicit drug use linkages" study, which recruits PWSD and tests for TB and HIV. Participants use methamphetamine and/or methaqualone, confirmed by urine drug screening. We compared sociodemographic characteristics and TB disease history by previous incarceration, defined as spending at least one night in a jail or prison.

Results: Of 674 participants, 438 (65.0%) reported ever being incarcerated (Table 1). Participants ever incarcerated were more often older (35 vs. 30 years), male (80.6% vs. 55.9%), and more likely to report previous TB (40.0% vs. 25.8%). Of participants with previous TB, those ever incarcerated more often reported multiple disease episodes (33.8% vs. 10.7%). At study enrollment, more participants ever incarcerated were Mycobacterium tuberculosis culture positive (10.1% vs. 2.6%) and had MTB detected on Xpert Ultra (10.1% vs. 2.6%).

Conclusion: In this PWSD cohort, a high proportion report previous incarceration, and those ever incarcerated are more likely to have previous and current TB disease. These findings may reflect drug use criminalization's impact on incarceration and incarceration's impact on TB disease occurrence. Active case finding among formerly incarcerated PWSD can improve individual outcomes and potentially avoid TB spread.

IMPACT OF PREMORBID NUTRITIONAL STATUS ON TUBERCULOSIS SEVERITY IN INDIA: A MULTICENTER PROSPECTIVE COHORT ANALYSIS

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Objective: Undernutrition is a key driver of the tuberculosis (TB) pandemic. We assessed the impact of undernutrition prior to TB onset on markers of TB severity.

Methods: We analyzed prospectively collected data for adults with TB from five sites in India. We built multivariable models to assess relationships between premorbid undernutrition (body mass index [BMI]<17 kg/m2) and markers of severity. We used logistic models for lung cavitation and high-grade sputum smear positivity, and linear models for percentage of lung affected and time to positivity of liquid culture (weeks). The models included age, sex, symptom duration, and variables with $p<0.2$ in univariate analysis.

Results: We calculated premorbid BMI for 1587 participants by adding reported weight loss due to TB from weight at treatment initiation. Altogether, 226 (14.24%) of participants had premorbid undernutrition. From multivariable models, premorbid undernutrition was associated with increased odds of lung cavitation (aOR 1.79, 95% confidence interval [CI]: 1.14, 2.86), increased odds of high bacillary sputum grade (aOR 1.05, 95% CI: 0.75, 1.48), an average 4.66% (95%CI: 0.85, 8.47) more lung affected, and an average 0.31 (95%CI: -0.61, 0.0064) shorter time to liquid culture positivity.

Conclusion: Our findings indicate that undernutrition prior to TB disease onset is associated with more severe disease. Our study provides a rationale for population-scale interventions to reduce undernutrition in regions with high TB rates.

AVF EXPLANTS FROM SECONDARY FAILURE REVEALS SECRETORY SMOOTH MUSCLE CELLS, VERSICAN, AND COLLAGEN CONSTITUTING STENOTIC REGIONS

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Objective: Arteriovenous fistulas (AVF) are the lifeline of patients on hemodialysis. In the US, 50% of AVFs fail to mature (primary failure), whereas ~40-50% of matured AVFs experience complications. No current studies investigate the wall components of mature or secondary-failed AVFs.

Methods: A morphological analysis of 21 AVF explants was performed using specialized stains along with IHC to detect components including Glypican, Agrin, Perlecan, Syndecan, Versikine, Versican and vascular smooth muscle cells (vSMCs) characteristics. Machine learning and AI-based quantification were used.

Results: Most AVF samples were from brachiocephalic veins. Explanted AVFs included aneurysms (16/21), pseudoaneurysms (2/21), and cosmetic (3/21). Of the first 18 patients, 15 had stenosis on previous venography. The AVF wall consisted of collagen, vSMCs, thrombi, blood and microcalcification. In the stenotic lesions, collagen was organized concentrically. Of different proteoglycans, Perlecan and Versican exhibited highest expression within hyperplastic intima. vSMCs showed increase in MYH11, Calponin, and SMA (secretory markers) compared to Ki67 (proliferative marker). Ki67 was highest within a 50–100-micron from the sub-endothelium.

Conclusion: AVF wall shows secretory vSMCs, Versican, and collagen in the stenotic areas. Proliferative vSMCs away from the lumen has translational significance for drug-coated balloons.

SPATIOTEMPORAL TRANSCRIPTOMIC ANALYSIS OF A NOVEL CENTRAL VENOUS STENOSIS RODENT MODEL REVEALS TNF AS A TARGETABLE PATHWAY

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Objective: Hemodialysis (HD) is the predominant modality of therapy for end-stage kidney disease. Nearly 50% of ESKD patients in the US initiate HD via central venous catheters (CVCs). CVCs result in central venous stenosis (CVS), a moribund condition with high recurrence rate. Despite its profound clinical significance, CVS pathogenesis is poorly understood.

Methods: We conducted a whole-genome transcriptomic analysis in the venous endothelium and smooth muscle cells in a rodent model. A group of 8–12-week-old Sprague Dawley rats were exposed to adenine diet (CKD group) or normal chow (non-CKD control group) followed by a guide wire damage to the right internal jugular veins (IJV). Rats were harvested on days 0, 5, and 14 following the injury.

Results: We observed thrombus followed by eccentric hypertrophy and prominent perivenular fibrosis in CKD rats compared to the injured IJV in control rats. On day five, the injured endothelium of CKD rats showed upregulation of Caspase, Wnt Signaling, Cytokine and TNF pathways. VSMCs showed significant upregulation of TNF in the injured IJV compared to the controls. Increase in TNF was validated in the injured IJV

using IF, which showed a 3-fold upregulation of TNF compared to controls ($P< 0.05$).

Conclusion: Leveraging a novel model of CVS in rats, spatiotemporal analysis has uncovered differentially regulated pathways within the endothelium and vSMCs of CKD rats. This data provides a robust foundation for mechanistic probing of CVS.

NON-INVASIVE IDENTIFICATION OF ACUTE TUBULAR INJURY USING PLASMA PROTEOMICS

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Objective: Biomarkers for the non-invasive assessment of acute tubular injury (ATI) are needed in patients with kidney disease.

Methods: Using the SomaScan proteomics platform, we measured 6592 circulating plasma proteins in 434 individuals with biopsy-confirmed kidney diseases and pathologist-adjudicated semi-quantitative assessments of histopathologic ATI. We identified proteomic correlates of ATI severity. For the proteins with the strongest associations with ATI, we evaluated cell-specific gene expression in patients with AKI in the Kidney Precision Medicine Project (KPMP).

Results: After multivariable adjustment and correction for multiple testing, 170 proteins were associated with ATI. The proteins with the strongest associations with greater ATI severity were osteopontin (p=9.8E-18), macrophage mannose receptor 1 (p=2.2E-16), and tenascin (p=1.4E-14) (Figure 1). The top proteins with inverse associations with ATI were plasma serine protease inhibitor (p= 6.1E-11), cholinesterase (p= 1.3E-10), and neuropeptide S (p=1.4E-10). In KPMP snRNA sequencing data, SPP1 (the gene encoding osteopontin) was primarily expressed in thick ascending limb (TAL) and proximal tubular (PT) cell clusters (p=4.2E-141 and 5.2E-108, comparing the expression in TAL and PT with all other cell clusters, respectively).

Conclusion: Plasma proteomic approaches may identify novel biomarkers to non-invasively identify biopsy-proven ATI.

ASSESSING THE ACCURACY OF IVUS OVER VENOGRAPHY USING AN EX VIVO MODEL SYSTEM

Author: James Decker
Additional author(s): Curtis HonShideler, Saran Lotfollahzadeh, Suvranu Ganguli, Tarek Shazly, Vipul Chitalia

Objective: Arteriovenous fistulas (AVFs) are the most preferable mode of dialysis access for ESKD patients. However, more than 50% of these patients experience various complications, primarily driven by stenosis of the outflow vein. Our previous work has demonstrated the superiority of intravenous ultrasound (IVUS) over venography in detecting outflow vein stenosis in AVFs. However, the accuracy of IVUS was not established. In this study, we set out to examine the accuracy of IVUS over venography using a fully defined geometry where prefabricated dimensions were used as a ground truth.

Methods: Using computer aided design (CAD) software, 15 vascular conduits were created with parameters extrapolated from fistulogram studies. To simulate stenosis, both regular and irregular narrowing was randomly introduced in 3 dimensions. These conduits were 3D printed using stereolithography (SLA), the most accurate type of printing with a maximum error of 0.10mm. The prints were performed at BU's Engineering Product Innovation Center

(EPIC).

Results: Several different permutations and combinations of internal and external diameters were used to generate the three-dimensional architecture of vascular conduits. Fabricated conduits simulating AVF outflow veins were successfully modeled and printed.

Conclusion: For the first time, we successfully generated a set of fabricated vascular conduits emulating stenosis in outflow veins. These conduits will be subjected to IVUS and venography to assess accuracy.

AHR INHIBITION REDUCES PERITONEAL FIBROSIS ASSOCIATED WITH PROLONGED PERITONEAL DIALYSIS EXPOSURE IN A NOVEL MURINE MODEL

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Additional author(s): A. Vazirani, S Lotfollahzadeh, I. Sellinger, J Francis1, L. Stern, and V Chitalia

Objective: Prolonged peritoneal dialysis (PD) results in fibrosis of the peritoneal membrane forcing patients to switch to hemodialysis. Despite its profound clinical importance, the specific mechanisms mediating this phenomenon remains elusive.

Methods: 12–14-week-old C57BL/6J mice received 0.2% adenine diet to induce CKD followed by PD catheter implantation. PD fluid was injected once a day for five days/week for 3 weeks along with either 10 mg/kg IP CH223191 (Aryl hydrocarbon receptor -AHR inhibitor) or a vehicle (control group, N=7/group). After 3 weeks, peritoneal membrane was examined. The PD fluid was collected analyzed using multiplex ELSA.

Results: Vehicle-treated mice showed fibrosis, inflammation and nuclear AHR in the capillaries of subperitoneal space. CH223191-treated mice showed a 5-fold decrease in subperitoneal space (P=0.011), a 9-fold decrease in fibrosis and a 7-fold reduction in AHR expression in the endothelial cells compared to mice treated with PD and vehicle alone. CH223191-treated mice also showed significant reduction in profibrotic cytokines, such as MCP1, MIP1 and IL-6 in the peritoneal fluid.

Conclusion: This study suggests that exposure to peritoneal fluid results in fibrosis and inflammation in subperitoneal space, both of which can be abrogated by AHR inhibitor. This is the first study examining the role of AHR signaling in PD-induced peritoneal fibrosis and supporting AHR inhibitors for therapeutic purposes.

FOOD INSECURITY AND HIGH BLOOD PRESSURE AMONG INDIVIDUALS WITH CHRONIC KIDNEY DISEASES IN NIGERIA AND GHANA

Author: Raghavee Neupane

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Objective: Based on the Diet, CKD, & ApolipoproteinL1 (DCA) study data, this cross-sectional study examines the relationship of food insecurity with estimated glomerular filtration rate (eGFR) and systolic & diastolic blood pressure (SBP&DBP). Few studies have analyzed these associations in sub-Saharan Africa. Understanding food insecurity is crucial in CKD patient care.

Methods: We recruited 570 participants with CKD (eGFR <60mL/min/1.73m^2, or albuminuria >30mg/g) from centers in the H3Africa Kidney Disease study. Food insecurity was measured by asking, “Did you cut meal size/skip meals due to insufficient money during the past year?” We used regression models with centers as random intercepts. Outcomes were eGFR, SBP, & DBP. We analyzed factors associated with food insecurity

using regression and a random intercept.

Results: The population's mean age was 48.7 (SD=17.5) and 47% female. The prevalence of food insecurity was 28%, highest in Southeast Nigeria (69%, p<0.0001). CKD patients in stages 3-5 had the lowest food insecurity (25%). The population had no significant association of food insecurity with eGFR, SBP, or DBP. Higher levels of BMI (OR:0.90–0.98,p<0.01), education (OR:0.25–1,p=0.05), & income (OR:0.003–0.38,p=0.01) were associated with lower odds of food insecurity.

Conclusion: Our study shows higher prevalence of food insecurity in Southeast Nigeria and lower in patients with advanced CKD. Investigating the effect of food security on kidney function or CVD in Africa merits more study.

SPATIOTEMPORAL TRANSCRIPTOMICS ANALYSIS OF CENTRAL VENOUS STENOSIS SUGGESTS POTENTIAL THERAPEUTIC TARGETS IN CKD RATS

Author: Saran Lotfollahzadeh
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Objective: VTE in patients with cancer is the second most common cause of non-cancer-related deaths. Black patients are at a 3-fold higher risk of cancer-associated VTE. Several metabolites from tryptophan (Trp) contribute to venous thrombosis in a murine cancer model. Black patients consume more Trp in their diet, and we examined the influence of dietary Trp on the cancer-associated VTE model.

Methods: A syngeneic MC-38 xenograft model was subjected to inferior vena cava (IVC) ligation. The mice were randomly assigned to 0.2%, 0, and 1.2 % Trp for five days after the xenograft reached 500 mm3. Histology of the tumor, IVC, and clots were performed. The sera were analyzed for Trp metabolomics.

Results: Clot weights were strongly correlated with plasma kynurenine levels. (R2=0.500, P:0.001) No significant differences in the xenograft weights were noted among the three groups (One-way ANOVA P = 0.091). Mice exposed to a high Trp diet with xenograft had higher kynurenine levels compared to mice without xenograft and were exposed to a high Trp diet. (ANOVA P-value: 0.0861) (***P-value: 0.0579)The mice with xenograft exposed to a high tryptophan diet had a higher TF and PAI-1 expression than those exposed to a 0.2 % Tryptophan diet. (*P =0.0585, ***P <0.0001).

Conclusion: This is the first report demonstrating the diet effect on cancer-associated VTE. This study paves the way to systematically assess health disparity-related parameters in the preclinical models of cross-organ pathologies.

ZEB2 SIGNALING IS ESSENTIAL FOR URETERAL SMOOTH MUSCLE CELL DIFFERENTIATION AND MAINTENANCE

Author: Sudhir Kumar
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Objective: Mowat-Wilson Syndrome (MWS) is an autosomal dominant complex disorder caused by mutations in the ZEB2. Congenital anomalies of the kidney and urinary tract (CAKUT) have been reported in MWS patients. However, the role of ZEB2 in urinary tract development remains unknown.

Methods: We performed ZEB2 protein expression analysis in the developing mouse ureter. We generated Zeb2 ureteral mesenchyme-specific conditional knockout mice and analyzed the urinary tract phenotypes in Zeb2 cKO mice and control mice. Ureteral cellular and molecular phenotypes were studied using cell-specific markers.

Results: ZEB2 is expressed in TBX18+ ureteral mesenchymal cells. Deletion of Zeb2 in

ureteral mesenchymal cells causes hydroureter and hydronephrosis phenotypes, leading to obstructive uropathy and early mortality. Cellular and molecular marker analyses showed that the TAGLN+ACTA2+ ureteral smooth muscle cells (SMCs) layer is not formed in Zeb2 cKO mice, but the FOXD1+POSTN+ tunica adventitia cells layer is significantly expanded compared to wild-type controls. Mechanistically, we found that Zeb2 cKO mice have significantly decreased TBX18 expression but an increased SOX9 expression in the developing ureter.

Conclusion: ZEB2 is essential for ureteral mesenchymal cell differentiation into normal ureteral SMCs during ureter development. Our study also shed new light on the pathological mechanism underlying the developmental abnormalities of the urinary tract and CAKUT phenotype in MWS patients.

SPATIAL TRANSCRIPTOMIC ANALYSIS OF KIDNEYS OF SICKLE CELL DISEASE MICE REVEALS PERTURBATION OF VEGF AND WNT SIGNALING PATHWAYS IN THE RENAL MEDULLA

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Objective: Sickle cell disease (SCD), characterised by a point mutation in the human β globin gene, manifests as hemolytic anemia and vaso-occlusive crisis and end-organ damage. Around 40% of SCD patients develop CKD and 11% develop ESKD. Peritubular capillaries surrounding the collecting duct (CD) and distal collecting tubules (DCT) are mainly affected. Although endothelial cell (ECs) damage is the inciting event, the genetic perturbations in them remain elusive.

Methods: GeoMx Mouse Whole Transcriptome analysis was performed on the kidneys of 6-8 weeks old female Townes SCD transgenic mice. CD 31, Pancadherin and Calbindin 1 were used as cell markers. Segmented ROI strategy targeted endothelium around glomerulus, DCT and CD. Differentially regulated genes were analysed and FDR $P < 0.05$ was considered as significant.

Results: The kidneys of SCD revealed glomerular hypertrophy with no tubular damage. ECs surrounding CD and DCT revealed significant upregulation of VEGF, Thrombin signaling and porphyrin pathways. DCT and CD epithelial cells revealed significant upregulation of VEGFR2, canonical and non-canonical Wnt signaling and hypoxia inducible pathways, all $P<0.05$.

Conclusion: This is the first comprehensive analysis of sickle cell nephropathy of early SCD kidneys, that has uncovered several novel pathways perturbed in the ECs and epithelium in the renal medulla. Further mechanistic studies are needed to develop deeper understanding of the pathogenesis of SCD and novel therapeutic targets.

TYMP-TRANS IL-6 SIGNALING-TISSUE FACTOR AXIS ACTIVATED IN THE DERMAL MICROVASCULATURE IN CALCIPHYLAXIS, AN ORPHAN DISEASE IN ESKD PATIENTS

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Objective: Calciphylaxis is characterized by ulceration of the skin due to thrombosis and calcification of subdermal microvasculature. Despite its fatal nature, little is known about its pathogenesis. We set out to examine genetic and molecular perturbations in calciphylaxis.

Methods: Sera and skin biopsies of calciphylaxis patients were analyzed using a spatial transcriptomics approach. O-linked protein analysis, multiplex ELISA, tissue factor activity, knock-out and knock-in assays were performed in human primary dermal endothelial cells (ECs).

Results: Compared to controls, sera from calciphylaxis increased IL-6 and PAR-1, a receptor for tissue factor (TF), in ECs. IL-6 and soluble IL-6 receptors levels were higher in the media of EC treated with calciphylaxis sera compared to controls. Thymidine phosphorylase (TYMP) was consistently upregulated in microvasculature, adipocytes and eccrine glands of skin biopsies from calciphylaxis. TYMP upregulated IL-6 in ECs, which in turn activated TF, a highly prothrombotic protein. IL-6 expression strongly correlated with TYMP and TF expression in skin biopsies of calciphylaxis patients. IL-6R antibody (Tocilizumab) abrogated IL-6 secretion and TF activation in ECs treated with calciphylaxis sera.

Conclusion: While linking IL-6 to metabolic genes and advancing IL-6 biology, this study uncovers a TYMP-trans IL-6-TF axis in calciphylaxis, which can be explored as a target in calciphylaxis, for which there is dismal therapeutic option.

SPECIFYING THE EXPRESSION OF REPULSIVE GUIDANCE CUE SLITS AND THEIR RECEPTOR ROBOS IN THE KIDNEY BY RNASCOPE IN-SITU HYBRIDIZATION

Author: Xueping Fan
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Objective: The guidance cue SLIT2 and its receptor ROBO2 are required for kidney development and podocyte function. Blocking SLIT2-ROBO2 signaling in adults can protect the kidney from glomerular injury. Our objective is to specify the cells expressing SLITs and ROBOs in kidneys.

Methods: We applied in-situ hybridization technique RNAscope to co-localize the mRNAs of SLITs and their receptor ROBOs with cell type specific markers in the mouse kidney.

Results: We found that SLIT1 is barely detectable in the kidney. SLIT2 is expressed by renal tubules with its highest expression in macula densa cells since its mRNA is co-localized with macula densa cell markers NOS1, NKCC2, PAPPA2, and PTGS2. SLIT2 expression in macula densa cells was also confirmed by co-immunostaining of SLIT2 and NOS1. SLIT3 mRNA is co-expressed with endothelial cell markers EMCN and PECAM1. Meanwhile, ROBO1 mRNA is co-localizing with EMCN and PECAM1, whiles ROBO2 mRNA is co-expressed with NOS1, NKCC2, PAPPA2, PTGS2, and the podocyte marker NEPHRIN. ROBO3 is undetectable in the kidney, but ROBO4 is expressed throughout the kidney and is co-localized with EMCN and PECAM1.

Conclusion: SLIT2 and SLIT3 are expressed in the mouse kidney but not SLIT1. ROBO1, 2, 4 are also expressed but not ROBO3. SLIT2 is highly expressed in macula densa cells and SLIT3 is likely in endothelial cells. ROBO1 and ROBO4 are probably expressed in endothelial cells whiles ROBO2 is predominantly expressed in podocytes and macula densa cells.

Pulmonary

EXPERIMENTAL MODELS TO CHARACTERIZE PNEUMONIA SUB-PHENOTYPES

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Objective: Pulmonary infections induce heterogeneous sub-phenotypes of immune responses in the lung. Pneumonia sub-phenotypes are difficult to identify in patients and require different treatments, and mouse models partially capture these sub-phenotypes. Characterizing lung pathology sub-phenotypes in humans and testing which are captured in mouse models are major research priorities.

Methods: Rapid autopsy lung samples from hundreds of elderly patients with lethal pneumonia were scored for 23 histopathoglogical features and computationally clustered into distinct sub-phenotypes. Lethal pneumococcal (Sp3) and influenza (IAV) infections in mice were evaluated for their ability to recapitualte features of human pneumonia sub-phenotypes.

Results: We observed heterogeneous pathologies across human lung samples, including differences in neutrophil infiltration. Increased fibrin in the alveolar space positively correlated with neutrophilic influx, suggesting a link between fibrin and neutrophil recruitment or activity. Lethal Sp3 and IAV infections in mice caused different histopathologies. Sp3 infection was dominated by neutrophils and elevated fibrin in the lung parenchyma, reflecting the fibrin-neutrophil association observed in human samples.

Conclusion: We demonstrate that some sub-phenotypes of human pneumonia can be captured in mouse models, but optimization is needed in order to match these more faithfully. Also, new animal models are needed to capture additional pneumonia sub-phenotypes.

VARIATION IN HOSPITAL SCREENING PATTERNS FOR SEPSIS-ASSOCIATED DIC

Author: Brandon Pang
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Objective: Disseminated intravascular coagulation (DIC) is a complication of sepsis and a risk factor for mortality. We assessed practice patterns for early DIC testing in critically ill patients with sepsis across the USA. We hypothesized there would be wide variation in screening practice due to the lack of guideline recommendations, and that hospital-level variation would have a stronger influence compared to patient-level characteristics.

Methods: Using the Premier Healthcare enhanced claims database, we identified adults admitted to ICU or stepdown with a diagnosis of sepsis. Exposure of interest was hospital of admission, with outcome of interest being early DIC testing. Using mixed-effects logistic regression with hospital of admission as a random intercept, we calculated the average risk-adjusted probability of early DIC testing per hospital, intraclass correlation coefficient (ICC) and the median odds ratio (MOR).

Results: Of the 1,633,557 patients in the initial cohort, 28,312 met criteria for early DIC testing. The average risk-adjusted hospital rate of early DIC testing was 1.69%. ICC was 0.167, indicating 16.7% of variation in testing was due to the hospital of admission alone.

Conclusion: Early testing for DIC was a rare occurrence and there was significant variation between hospitals. Further study is needed to see if this variation affects patient outcomes.

UPTAKE OF ANTIFIBROTICS FOR PATIENTS WITH IDIOPATHIC PULMONARY FIBROSIS: 2016-2022

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Objective: In 2014, two antifibrotics – nintedanib and pirfenidone – were approved by the FDA for the treatment of idiopathic pulmonary fibrosis (IPF),), a chronic lung disease. We evaluated adoption of antifibrotic medications for patients with IPF.

Methods: We used the TriNetX Network, which contains claims-based data for 108 million patients at 76 US healthcare organizations. We identified incident cases of IPF (ICD-10 J84.112) from 2016-2022. We calculated annual incidence rate of antifibrotics by assessing the number of antifibrotic prescriptions divided by the total person-time accumulated within each year. We also used multivariable regression analysis to assess demographics, comorbidities, testing, and prior healthcare encounters associated with receipt of antifibrotics within one-year of diagnosis.

Results: Of 5, 867 included patients, 895 (15.2%) were started on an antifibrotic after initial diagnosis. Baseline characteristics are seen in the Figure. Annual rates of antifibrotic uptake remained steady from 2016-2019; after 2019, there was an increase in rate of antifibrotic use, particularly in use of nintedanib compared to pirfenidone. Factors associated with receipt of antifibrotics are seen in the Figure.

Conclusion: Anti-fibrotic uptake in an incident IPF population is low, but increasing in recent years. Future work must focus on addressing potential gender bias, structural racism, and cost barriers to improve equitable uptake of these medications in eligible populations.

LUNG INTERSTITIAL MACROPHAGE DYNAMICS AND PHENOTYPES AFTER PNEUMOCOCCAL RESPIRATORY INFECTION

Author: Elise Armstrong
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Objective: To date, two subpopulations of lung interstitial macrophages (CD206+ IMs and CD206- IMs) are identified in mouse lungs. Recovery from prior respiratory infections remodels alveolar macrophage (AM) phenotype and function, but it is unclear whether IM subpopulations undergo any such changes. We hypothesized IMs accumulate within the lung and undergo phenotypic alterations in pneumococcus-experienced mice compared to naïve mice.

Methods: Mice received intratracheal instillations of Streptococcus pneumoniae serotype 19F ("experienced") or saline ("naïve") into the left lobe at day 0 and day 7. At day 35, the left lobes were collected and digested to characterize the level of recruitment and median fluorescence intensity of key IM markers by flow cytometry.

Results: In experienced lungs, CD206+ and CD206- IMs doubled in abundance compared to naïve mice, suggesting that IMs accumulate in lungs with prior pneumococcal exposures. In addition, CD206- IM abundance peaked after the first infection, whereas CD206+ IMs peaked after the second infection, suggesting differential mechanisms of accumulation. Lastly, CD206+ IMs showed altered surface marker expression in experienced lungs, which differed from both CD206- IMs and AMs.

Conclusion: These results reveal that IMs in pneumococcus-experienced mice are different compared to their naïve counterparts, warranting further investigation into their immunological roles as well as how such roles are influenced by prior infection history.

INVESTIGATING THE ROLE OF TACI AS A KEY RHEOSTAT FOR BAFF SIGNALING IN NON-INFECTIOUS COMPLICATIONS OF COMMON VARIABLE IMMUNODEFICIENCY

Author: Erik Matson
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Objective: We investigated the incidence of non-infectious complications among CVID subjects stratified according to plasma ratio of BAFF and soluble TACI and characterized phenotypic and transcriptional signatures of specific naïve human B cell subsets that are prominent in a subset of CVID patients associated with non-infectious complications.

Methods: Plasma samples from controls and CVID subjects were assayed via ELISA to detect BAFF and TACI. HEK293T cells were transduced to express GFP-tagged wild-type or mutant TACI protein. Human peripheral blood mononuclear cells were stained and analyzed on the Cytek Aurora and the BD FACSAria II. The 10X Chromium controller and NextSeq2000 systems were used to process cell and sequence libraries in the single cell RNA sequencing workflow.

Results: CVID subjects with BAFF elevations and low soluble TACI have greater incidence of autoimmune cytopenia and interstitial lung disease compared to those with low plasma BAFF or plasma elevations of both BAFF and TACI. We observe expanded activated naïve B cell subsets in CVID subjects with complications.

Conclusion: We identified a subset of CVID patients with greater incidence of complications in association with their plasma ratio of BAFF to soluble TACI, supporting TACI ‘s role as a soluble neutralizing receptor for BAFF in CVID. We explored transcriptional identities of naïve B cell subpopulations in CVID that may be contributing to pathogenesis of autoimmune and lymphoproliferative complications.

SINUSITIS AND RHINITIS FOLLOWING DEPLOYMENT-RELATED OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES AMONG US VETERANS

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Objective: Veterans deployed post 9/11 were exposed to environmental and occupational pollutants. There are limited data assessing associations of these exposures with sinusitis or rhinitis.

Methods: Potential participants had land-based deployment in Afghanistan and Southwest Asia, were recruited between 2018-2020 and were randomly selected using Defense Manpower Data Center data. An interviewer-administered questionnaire assessed rhinitis/sinusitis first reported during or post-deployment and included a 32-item exposure history. Using a priori groupings, and supported by a confirmatory factor analysis, exposures were grouped into 5 categories (see table). Ordinal coding was used for each item within an exposure category: 0, none; 1, ≤median person-days per item; 2, >median; scores within each category were summed and scaled to 100. We used Generalized Linear Models to estimate odds ratios for sinusitis and rhinitis separately, expressed per 20-point exposure score and CIs were generated by cluster bootstrapping.

Results: 1960 Veterans were included in analyses. Mean age (SD) was 40.7 (±9.7) years

and 88.5% were male. The odds of rhinitis and sinusitis were elevated for all exposures except burn pits, although only the association between toxicants and rhinitis reached statistical significance.

Conclusion: These findings suggest that Veterans are at increased risk of sinusitis and rhinitis due to deployment exposures. These data highlight the need for screening and prevention efforts.

A POLYGENIC SCORE FOR BODY MASS INDEX AND MORTALITY IN COPD

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Objective: Low BMI is associated with increased COPD mortality, but the underlying mechanisms are unclear. We hypothesized that genetically regulated BMI is associated with COPD mortality and has differential effects on respiratory and cardiovascular death.

Methods: To obtain a summative measure of genetic determinants of BMI, we developed a polygenic score (PGS) for BMI using lassosum based on large GWASs. We used a Cox proportional hazards model to examine the association between the PGS and all-cause mortality and tested for a potential non-linear effect of the PGS using penalized spline terms. For respiratory and cardiovascular mortality, we used cause-specific hazard models to account for competing risks. We performed meta-analyses to combine estimates among cohorts.

Results: We included 2811 COPDGene NHW, 753 COPDGene AA, 1708 ECLIPSE, and 751 FHS participants who had COPD. We did not observe a significant non-linear association between the PGS and mortality. For all cohorts combined, a one standard deviation increase of the PGS was associated with a higher risk for cardiovascular death (HR=1.30, p=0.0006), but not with respiratory death or all-cause mortality. We did not observe significant heterogeneity in the associations of the PGS among cohorts.

Conclusion: In individuals with COPD from smoker-enriched cohorts and a population-based cohort, higher genetically determined BMI is associated with higher cardiovascular mortality, but not with respiratory mortality.

INVESTIGATING THE ROLE OF BACTERIAL LONG-CHAIN POLYPHOSPHATES IN MACROPHAGES DURING LEGIONELLA PNEUMOPHILA INFECTION

Author: Kara Vasilew
Additional author(s): Johannes Platten, Kevin Brueck, Archana Jayaraman, Arjun Sharma

Objective: Up to 10% of community-acquired pneumonia cases are identified as Legionnaires' Disease, caused by the pathogen Legionella pneumophila. L. pneumophila primarily reside in human alveolar macrophages, replicating via the Legionella-containing vacuole. Polyphosphates are highly conserved molecules found in essentially every cell performing essential functions. We have previously found that long-chain polyphosphates (L-PolyP) produced by E. coli interfere with the host innate response against infection. Here, we investigate whether L. pneumophila co-infected with L-PolyP also interfere with the host innate response.

Methods: We utilized a mouse model for in vitro infection of L. pneumophila and L-PolyP in bone-marrow-derived macrophages, with analysis performed via flow cytometry, RNA-sequencing and ELISA assays.

Results: Here, we show that the infection of L. pneumophila and synthetic bacterial L-

PolyPs interfere with the inflammatory response in macrophages. The in vitro infection of L. pneumophila in bone-marrow-derived macrophages show a modulated inflammatory cytokine response and cell death. Additionally, the addition of S. cerevisiae derived exopolyphosphatase, which degrades L-PolyP, decreases inflammation in vitro.

Conclusion: L. pneumophila co-infected with L-PolyP increases cell death and promotes inflammation during infection in vitro. These results show that L-PolyP may help L. pneumophila evade the macrophage phagocytosis pathway.

IMPACTS OF CVID NFKB1 VARIANT 1375DELT ON MACROPHAGE FUNCTION

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Objective: Common Variable Immune Deficiency (CVID) patients are at an increased risk for comorbidities like lung disease and gastritis (CVIDc). Our work addresses the need for translational models and is focused on understanding the pathogenic role of CVID associated NFKB1 mutations. Monocytes are not only elevated in CVIDc, but their production of CVIDc associated cytokines in response to infection is critically mediated by NFkB signaling, thus making them a cell type likely susceptible to pathogenic NFKB1 variants.

Methods: To provide a rigorous evaluation of how a CVIDc associated NFKB1 variant (1375delT) impacts the inflammatory response of monocyte derived macrophages (MDMs), our approach combines primary and induced pluripotent stem cell (iPSC) models. Using WT and 1375delT samples, we stimulated iPSC derived macrophages (iMACs), and peripheral blood mononuclear cells (PBMCs) with bacterial LPS.

Results: By RNA-seq, 1375delT PBMCs adopt an inflammatory transcriptional profile that includes the inflammasome pathway components IL1B and NLRP3. By luminex panel, 1375delT iMACs also showed elevated secretion of IL1b. These signatures parallel those seen in the plasma of CVID patients.

Conclusion: These results suggest that the inflammasome may be a target of NKFB1 mutations in CVID. Next we will quantify the activity of this response in our primary and iPSC derived cells, and use CRISPR/cas9 gene editing to test whether 1375delT is necessary or sufficient for inducing this phenotype in iPSCs of different genetic backgrounds.

CAN A DECISION TOOL IMPROVE GUIDELINE CONCORDANCE? A QUALITATIVE STUDY OF LUNG CANCER SCREENING WHEN LIFE EXPECTANCY IS LIMITED

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Objective: Lung cancer screening (LCS) reduces lung cancer deaths but can cause harm, especially in those with limited life expectancy (LLE). Counter to guidelines, patients with LLE often undergo LCS. We explored provider beliefs about LLE in relation to LCS and acceptability of a decision support tool.

Methods: We interviewed 48 clinicians from 6 Veterans Affairs facilities. Using grounded thematic analysis, we explored how clinicians incorporate life expectancy estimates into LCS decisions, and receptivity to a decision support tool. Identified themes were mapped to the Cabana framework which identifies barriers that lead to guideline non-adherence, and

maps each barrier to clinician knowledge, attitudes, and behavior.

Results: We found that clinicians offer LCS to people with LLE due to limited knowledge of the life expectancy threshold at which there is little benefit of LCS; discomfort estimating life expectancy; prioritization of other factors; and fear of missing cancer (Table). Clinicians are receptive to decision support to guide and discuss LCS decision-making if it is easy to use and integrated into clinical workflow.

Conclusion: Knowledge gaps and attitudes may drive decisions to offer LCS despite LLE, a behavior at odds with guidelines. Integrating LCS decision support that predicts LCS benefit, accounting for life expectancy, into the electronic health record may improve patient selection and guideline concordance.

THE ROLE OF PROTON-ACTIVATED CHLORIDE CHANNEL 1 (PACC1) IN BACTERIAL IMMUNITY

Author: Lucien Garo
Additional author(s): Kevin Brueck, Sarah Walachowski, Marcel Strueve, Markus Bosmann

Objective: Bacterial infections remain a leading cause of global mortality despite currently available interventions. Tissue-resident macrophages coordinate initial host defense against invading pathogens. Phagocytosis and phagosomal acidification by these macrophages and other myeloid cells are critical to combat infection. PACC1, a recently discovered acid-sensitive chloride channel, is highly expressed in macrophages. Our lab has uncovered a novel role for PACC1 in macrophages, and our objective is to evaluate PACC1 function in bacterial immunity.

Methods: To study PACC1 in vivo, we directed the generation of a PACC1 knockout mouse using CRISPR/Cas9. Infection responses were investigated with models of Escherichia coli intraperitoneal sepsis and pneumococcal pneumonia, while immunologic responses were studied by flow cytometry and ELISA. Phagocytosis was assessed via acid-sensitive particles.

Results: PACC1-/- mice (compared to wild type controls) presented with higher bacterial loads and decreased survival following infection. This was associated with hyperinflammation, including excessive cytokine/chemokine release and influx of myeloid cells that failed to control bacterial burden. PACC1-/- myeloid cells showed impaired phagolysosomal acidification.

Conclusion: PACC1 loss results in susceptibility to infection, dysregulated hyperinflammation, and impaired phagocytosis. In summary, our findings suggest PACC1 is essential for protective innate host defense against bacteria.

EFFECTS OF ENVIRONMENTAL EXPOSURES ON HUMAN ALVEOLAR TYPE II CELLS

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Objective: Environmental exposures such as air pollutants comprising of particulate matter less than 2.5 um (PM2.5) can enter the pulmonary alveoli and contribute to respiratory and cardiopulmonary pathology. Preliminary data demonstrates that IL-1B is one of the key factors associated with air pollution exposure and disease. We hypothesize that PM2.5-induced expression of IL-1B dysregulates type 2 alveolar epithelial cells (AT2s) in the distal lung through activation of NFkB.

Methods: We derived AT2s from human induced pluripotent stem cell (iPSCs), referred to

as “iAT2s”. iAT2s were exposed to increasing concentrations of IL-1B and harvested for analysis. Cytokine expression was quantified by qPCR and NFkB activation by an NFkB luciferase assay. We likewise characterized the effects of IL-1B exposure on cellular phenotypes including proliferation by Edu uptake and colony forming efficiency (CFE).

Results: We observed increased expression of CXCL5, CXCL8, and TNFa mRNA iAT2s in the context of increasing IL-1B exposures. NFkB-driven luciferase expression was likewise induced by IL-1B. No differences in EdU incorporation or CFE were observed.

Conclusion: Exposure of iAT2s to IL-1B induces expression of NFkB and NFkB-dependent inflammatory cytokines in a key lung progenitor cell. Future experiments will test the direct effects of PM2.5 exposure on iAT2s cultured at an air-liquid interface to determine whether this pollutant alters AT2 biology and through what specific mechanisms.

TRANSTHORACIC ECHOCARDIOGRAPHIC CAN HELP TO PREDICT MORTALITY IN SICKLE CELL DISEASE

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Objective: Sickle cell disease (SCD) is a genetic hemoglobinopathy characterized by hypercoagulability and accelerated mortality. Transthoracic echocardiography (TTE) can reveal mortality risk factors including an elevated tricuspid regurgitant jet velocity (TRV) and diastolic dysfunction. We hypothesized that subtle differences in echocardiographic right and left heart function might predict mortality risk in SCD.

Methods: We conducted a retrospective review of 402 consecutive SCD patients treated at our institution. Clinical echocardiograms were re-analyzed with extensive coding of left ventricular (LV) and right ventricular (RV) systolic and diastolic function. Data were recorded at time of first contact or prior to VTE if applicable.

Results: 62% were HbSS/HbSβ0, 56% were female, and 19% had VTE. Prior history of acute chest syndrome, stroke, splenectomy, and avascular necrosis was more common in the VTE group ($p<0.01$ for each comparison). The mortality rate was significantly higher in the VTE group (13% v 6%, $p=0.04$). Age-adjusted logistic regression for mortality demonstrated that TRV and pulmonary artery systolic pressure, mitral valve E wave velocity, PASV, right atrial end-systole size and right ventricular base dimension were associated with mortality (Table 1).

Conclusion: Patients with SCD and VTE have increased mortality compared to those without VTE. Diastolic dysfunction of the LV, RV systolic dysfunction, and elevated pulmonary pressures were associated with mortality.

MURINE OC43 CORONAVIRUS INFECTION INDUCES OC43-SPECIFIC LUNG RESIDENT MEMORY

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Objective: The endemic coronaviruses, while generally mild in most patients, are a leading cause of viral pneumonia but remain poorly understood. Preliminary evidence from humans suggests heterotypic protection against SARS-CoV-2 after recent eCoV infection, increasing the need for eCoV models.

Methods: Experiments used a mouse model we developed of 2 infections with OC43, 7 days apart, and 4 weeks later lungs collected for flow cytometry and serum collected for Luminex assay. In some experiments, infections were combined with cyclic-di-GMP (CDG) to increase acute inflammation. T cells were stimulated ex vivo using peptide pools.

Results: Recovery from OC43 induced significant extravascular lung-resident memory (RM) T cells and high serum IgG against OC43 spike protein. CDG with infection significantly increased CD4+ TRMs and BRMs, and the BRMs had a significantly larger fraction that were class-switched and displayed additional memory markers. After stimulation with OC43 peptides, subsets of CD4+ TRMs increased Th1, Th2, and Th17 cytokines. CDG increased the fraction of antigen-specific TRMs and polarized the cells towards Th1/Th17.

Conclusion: Murine OC43 infection induces antigen-specific lung-resident memory and systemic humoral immunity. Increasing inflammation during infection significantly increases lung-resident memory, and importantly the antigen-specific fraction of TRMs. Ongoing experiments are examining heterotypic immunity against a mouse-adapted SARS-CoV-2 in the NEIDL.

CD200R MAY DIRECTLY AFFECT NEUTROPHIL FUNCTION DURING BACTERIAL PNEUMONIA

Author: Riley Pihl
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Objective: Despite effective pathogen-specific antimicrobial therapeutics, morbidity and mortality from pneumonia remains high. The vast breadth of pathogens that can cause pneumonia suggests a shared point of immune dysfunction. Neutrophils are highly inflammatory immune cells that respond quickly to bacterial infection and frequently cause tissue damage at sites of infection. Our objective is to identify and determine the impact of potential regulators of neutrophil function during different bacterial pneumonias.

Methods: Mice were intratracheally instilled with E. coli or S. pneumoniae (SP3) for 6, 24 or 48h and neutrophils were isolated from either bronchioloalveolar lavage fluid (BALF) or peripheral blood. Neutrophil surface marker expression (SME) was analyzed via 25-color panel run on a Cytek Aurora spectral flow cytometer (SFC). For bacterial clearance assays, bone marrow neutrophils were isolated via Percoll, then incubated with surface marker modulators, including blocking antibodies against CD200R. Neutrophils were then cocultured with X14 (luminescent E. coli) and luminescence was measured in real time to detect bacterial killing.

Results/Conclusion: Using SFC, we identified pathogen- and timepoint-specific differences in SME of IRs. Blocking CD200R in vitro enhances neutrophil bacterial killing compared to isotype. These data suggest that neutrophils react in a highly pathogen-specific manner, and that CD200R may play a role in regulating neutrophils during pneumonia.

EXPLORING THE CAUSAL EFFECTS OF CIRCULATING GALECTIN-3 AND SRAGE ON INTERSTITIAL LUNG ABNORMALITIES RISK: A MENDELIAN RANDOMIZATION STUDY

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Objective: Interstitial lung abnormalities (ILA) share risk factors and outcomes with interstitial lung disease, providing an opportunity to investigate early processes that may

lead to pulmonary fibrosis. Galectin-3 (Gal-3) and soluble receptor for advanced glycation end products (sRAGE) levels are significantly associated with presence of ILD in cohort studies. We aimed to explore the causal relationship of Gal-3 and sRAGE with the risk of ILA.

Methods: Two-Sample Mendelian Randomization (MR) was conducted to obtain causal estimates of Gal-3 and sRAGE on risk of ILA. Genetic instruments for Gal-3 and sRAGE were selected from publicly available GWAS summary statistics based on strict criteria of MAF > 0.05, LD (r2 < 0.001), and association with biomarkers (P<5x10-8). ILA GWAS summary statistics were calculated in 1,445 participants of the Framingham Heart Study (FHS) and replicated in an available meta-analysis. Inverse-variance weighted (IVW) approach was used for primary analysis.

Results: We identified 6 SNPs of Gal-3 and 5 SNPs of sRAGE as instrumental variables. These explained 41% and 18% of measured Gal-3 and sRAGE levels, respectively, in FHS participants. IVW analysis indicated that genetically predicted Gal-3 and sRAGE levels were not significantly associated with risk of ILA (OR of 1.04 95% CI: [0.83 - 1.31]; 1.15 95% CI: [0.72 -1.82], respectively).

Conclusion: This MR study showed no significant causal association between Gal-3 or sRAGE levels on the occurrence of ILA.

PULMONARY LYMPHATIC REMODELING IN RESPONSE TO INFLUENZA-INDUCED INFLAMMATION

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Objective: Our aim is to characterize pulmonary lymphatic endothelial cell (LEC) remodeling in a mouse model of severe influenza infection.

Methods: We performed immunohistochemical staining (IHC) for PROX1, the master transcriptional regulator of LEC fate, then counted LEC nuclei at 3, 7 and 21 days post influenza infection (dpi). We also measured EdU incorporation by dividing LECs and performed lineage tracing to determine the origin of new LECs during influenza. To isolate LECs and LEC nuclei for sequencing, we attempted fluorescence-activated cell and nuclei sorting (FACS and FANS), as well as magnetic bead enrichment.

Results: IHC for PROX1 shows a notable rise in LEC numbers by 7 dpi, with further growth by 21 dpi, after viral clearance. EdU labeling and lineage tracing suggest that LEC proliferation is the main impetus for this. FANS has emerged as the most promising strategy for obtaining samples for sequencing. We have submitted FANS-sorted LEC nuclei from healthy lungs for single nuclei RNA sequencing (snRNAseq), and are collecting 7dpi lung tissue for future nuclei sequencing. We have also submitted lysates from LECs isolated via FACS at 3dpi, when LEC numbers have not yet risen, for bulk RNA sequencing.

Conclusion: During influenza, LEC proliferation increases and pulmonary lymphangiogenesis occurs. We anticipate that our sequencing results will reveal LEC heterogeneity in origin and cooperation with immune and parenchymal cells.

ONCOSTATIN M, A MULTIFUNCTIONAL IL-6 FAMILY CYTOKINE, IS A CRITICAL COMPONENT OF THE HOST RESPONSE DURING BACTERIAL PNEUMONIA

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Objective: To combat pneumonia, there is a need to better understand pneumonic immune dysregulation. Oncostatin M (OSM), a pleiotropic interleukin-6 family cytokine, is upregulated in lungs during bacterial pneumonia. We hypothesize that changes in OSM level modify the immune response during bacterial pneumonia and thus, influence pneumonia outcome.

Methods: We used murine pneumonia models to investigate OSM-mediated changes in the host immune response. Specifically, we infected OSM neutralized or whole body OSM receptor (OSMrβ) knock-out mice by intratracheally instilling E. coli into the lungs. Outcomes include changes in bacterial burden and survival, bronchoalveolar lavage fluid (BALF) cytokines and leukocyte recruitment into the lungs.

Results: Our results show that changes in OSM level affect pneumonia outcome. During bacterial pneumonia, neutralization of OSM decreases survival but OSMrβ knock-out increases survival which may be due to the observed increase in BALF OSM in OSMrβ knock-out mice during pneumonia. We also observed changes in leukocyte recruitment but no difference in total BALF protein.

Conclusion: Our study indicates that OSM is a critical component of the host response during bacterial pneumonia and changes in the OSM level influence various immune activities. Further understanding of OSM signaling and regulation may provide important information for the development of new and effective pneumonia treatment strategies.

Preventive Medicine

EGGS, EGG-RICH NUTRIENTS, AND NONALCOHOLIC FATTY LIVER DISEASE IN THE FRAMINGHAM HEART STUDY

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Objective: While eggs have historically been considered unhealthy, they are rich in anti-inflammatory and lipid lowering nutrients such as lutein, zeaxanthin, and choline, nutrients that may prevent nonalcoholic fatty liver disease (NAFLD). We evaluated the prospective associations between eggs and their key nutrients and NAFLD in the Framingham Offspring and Third Generation.

Methods: Over 2,000 participants underwent two CT scans to secondarily assess liver fat six years apart. Incident NAFLD was defined as a liver phantom ratio ≤0.33 on follow-up after excluding prevalence. Egg and nutrient intakes were derived from food frequency questionnaires. Poisson regression models were adjusted for demographic, lifestyle, and anthropometric factors.

Results: Consuming ≥2 eggs/week (vs. <1) as part of a healthy diet was associated with 25%

lower NAFLD risk (95% CI: 0.55-1.02). Higher choline intake (vs. lower) was associated with a

31% lower NAFLD risk (95% CI: 0.49-0.96) in men but not women. Neither lutein nor zeaxanthin was associated with NAFLD. Finally, egg intake was not associated with incident NAFLD risk in women or men among those with current dyslipidemia or prevalent diabetes.

Conclusion: We found no adverse association between egg intake and NAFLD risk. In contrast, eggs as part of a healthy diet seemed beneficial. Higher choline intake was associated with lower NAFLD risk in men. This research may provide important evidence to be used in

updating dietary guidelines for NAFLD prevention.

LIVER FAT, CARDIORESPIRATORY FITNESS, AND METABOLIC MEDIATORS IN THE COMMUNITY

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Objective: We sought to determine if higher liver fat is associated with lower cardiorespiratory fitness (CRF) and if certain metabolites mediate this association.

Methods: Framingham Heart Study participants underwent vibration-controlled transient elastography for assessment of controlled attenuation parameter (CAP; a measure of liver fat) and maximal cardiopulmonary exercise testing (CPET). In individuals with available metabolomic data, we evaluated whether blood metabolites mediate the association of CAP and peak CRF (via peak oxygen uptake [VO2]).

Results: In the full study sample (N=2722, mean age 54±9 years, 53% women, mean BMI 28.0±5.3 kg/m2), higher CAP was associated with poorer peak VO2 adjusted for age, sex, alcohol use, smoking, systolic blood pressure, hypertensive medication use, diabetes, and prevalent cardiovascular disease. In 1346 participants with metabolite data, using multivariable models also adjusted for BMI, 59 metabolites were significantly associated with CAP, 58 metabolites were significantly associated with peak VO2, and 18 metabolites were significantly associated with CAP and peak VO2 at FDR of 5%. Of these metabolites, 16 significantly mediated the association, with dimethylguanidino valeric acid demonstrating the highest proportion mediated (37%; Figure).

Conclusion: Liver fat is associated with impaired CRF in the community and this association is partly mediated by circulating metabolites with known and unknown functions in metabolic health.

Rheumatology

ASSOCIATION OF TUMOR NECROSIS FACTOR INHIBITORS AND DISEASE-MODIFYING ANTIRHEUMATIC DRUGS ON THE INCIDENCE OF HIP FRACTURES AMONG PATIENTS WITH AXIAL SPONDYLOARTHRITIS

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Objective: People with axial spondyloarthritis (axSpA) have an increased risk for fracture versus the general population. In our study, we assessed the impact of treatment with tumor necrosis factor inhibitors (TNFi) or non-biologic disease-modifying antirheumatic drugs (DMARDs) on incident hip fractures in axSpA versus nonsteroidal anti-inflammatory drugs (NSAIDs).

Methods: We conducted a nested case-control study using 2006-2021 data from the MarketScan Database. We included adults 18-65 years old with ≥1 inpatient or ≥2 outpatient axSpA ICD-9 or 10 diagnosis codes separated by ≥7 days. The outcome was closed hip fracture, operationally defined by ICD 9 or 10 inpatient diagnosis codes or outpatient diagnosis codes with procedure codes, excluding high impact trauma. We evaluated medication exposure (TNFi, DMARDs, or NSAIDs) using pharmacy claims and CPT codes. We assessed the association of medication exposure with incident hip fracture risk using conditional logistic regression.

Results: We included 53,398 individuals with axSpA, with 89 cases of hip fracture. Cases had higher frequency of risk factors for fracture, including falls, osteoporosis, and

tobacco use, compared to controls. In adjusted analyses, there was no statistically significant association between any medication group and hip fracture (Table 1).

Conclusion: Using a large US insurance claims database, we found no evidence for a protective effect of TNFi or non-biologic DMARDs on hip fracture risk in axSpA.

SPATIAL FREQUENCY DOMAIN IMAGING AS A NOVEL METHOD TO QUANTIFY LONGITUDINAL SKIN CHANGES IN SCLERODERMA

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Objective: Systemic sclerosis (SSc) is an autoimmune disease with excessive collagen deposition in the skin and internal organs. The modified Rodnan skin score (mRSS) is the gold standard for evaluating skin fibrosis in SSc. but has significant limitations. Spatial Frequency Domain Imaging (SFDI) is a non-invasive technique that can evaluate tissue optical properties, including reduced scattering coefficient (μs'), which we recently showed is highly correlated with mRSS. However, the sensitivity of SFDI to longitudinal changes has yet to be examined.

Methods: mRSS and SFDI data were collected from the forearms of 6 diffuse SSc patients and 2 healthy controls at two different time points, under an IRB approved protocol and after informed consent. Correlation between changes in SFDI parameters (μs') and mRSS was analyzed.

Results: Among SSc patients, 3 showed improvement in mRSS (4 points), 1 had modest improvement (1 point), and 2 remained stable. Patients with stable mRSS also had stable μs', similar to controls (0.01 ± 0.11 mm-1 vs 0.05 ± 0.06 mm-1). When mRSS improved, there was a proportional change in the μs', and statistically significant correlation between ΔmRSS and Δμs' was observed ($r = -0.74$, $p < 0.01$).

Conclusion: Our preliminary study indicates that SFDI is sensitive to longitudinal changes in the skin of SSc patients, further supporting this method as a highly promising new tool for scleroderma skin assessment and monitoring. Future studies are required to confirm these findings.

ASSOCIATION OF PHYSICAL ACTIVITY LEVELS ON CHRONIC OPIOID USE IN ANKYLOSING SPONDYLITIS PATIENTS

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Objective: Whether exercises reduces chronic opioid use in people with ankylosing spondylitis (AS) is unknown. We assessed the association of physical activity levels with chronic opioid use in a prospective AS cohort.

Methods: We conducted a cross-sectional analysis of adults with AS in a prospective, longitudinal cohort (2003-2018), which collected clinical information every 6 months. The outcome was chronic opioid use for ≥ 6 months; the exposure was patient-reported physical activity (low or moderate/high per the International Physical Activity Questionnaire). We used multivariable logistic regression to calculate odds of chronic opioid use comparing moderate/high versus low activity, adjusting for confounders.

Results: We included 829 patients (median age 42 years, 74% male, 81% White). Low physical activity was reported by 57%; this group had higher AS disease activity and comorbidity prevalence (Table 1). Chronic opioid use was reported by 46 participants (5.5%). Those with moderate/high activity (versus low activity) had 57% lower odds

(OR 0.43, 95% CI 0.20-0.89) of chronic opioid use accounting for demographics and symptom duration. This association was attenuated after further adjustment for comorbidities, disease activity, and medications (OR 0.62, 95% CI 0.27-1.31).

Conclusion: In this cross-sectional analysis of an AS cohort, higher physical activity levels were associated with lower burden of chronic opioid use.

Vascular Biology

REDOX DYSREGULATION OF VASCULAR SMOOTH MUSCLE SIRTUIN-1 IN THORACIC AORTIC ANEURYSM IN MARFAN SYNDROME

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Objective: Thoracic aortic aneurysms and dissections (TAAD) are a life-threatening cardiovascular complication of Marfan syndrome (MFS). We investigated whether redox dysregulation of proteins, due to oxidative stress, contributes to TAAD in MFS.

Methods/Results: Reversible oxidative post-translational modifications (rOPTM) of protein cysteines, mainly S-glutathionylation, were dramatically increased in aortas of MFS individuals and fibrillin-1 hypomorphic mice (Fbn1mgR/mgR), an established model of MFS prone to TAAD. rOPTM of sirtuin-1 (Sirt1), a deacetylase we showed protects the aorta from dissections, inhibited Sirt1 activity, measured as levels of acetylated p53 and histone 3, and increased MMP2/9 activity, measured by in gel-zymography and in situ, in vascular smooth muscle cells (VSMCs) and aortas. Deletion of glutaredoxin-1 (Glrx), a specific de-glutathionylation enzyme, increased rOPTM of Sirt1, rOPTM-mediated inhibition of Sirt1 activity, and MMP2/9 activity in MFS VSMCs. On the contrary, overexpression of Glrx or of an oxidation-resistant Sirt1 mutant with an AAV prevented these changes in VSMCs. Lastly, VSMC-specific deletion of Sirt1 in Fbn1mgR/mgR mice significantly worsened TAAD progression leading to 50% increased incidence of aortic rupture compared to controls.

Conclusion: Our results strongly suggest that preventing rOPTM of Sirt1 may be a novel therapeutic strategy to prevent TAAD in individuals with MFS, for which currently no targeted therapies are available.

EMERGING USE OF CLEAR FLAVORED ELECTRONIC CIGARETTES IN MASSACHUSETTS FOLLOWING THE STATEWIDE FLAVOR BAN

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Objective: After the Massachusetts ban on flavored tobacco products in 2020, a new product class of electronic cigarettes (e-cig) with e-liquid labeled clear has emerged. We evaluated the cardiovascular health effects of clear products in young adult e-cig users compared to non-users.

Methods: We studied healthy young adults (18-45 yrs) measuring systolic and diastolic blood pressure and heart rate before and after a structured 10-min use of their own e-cig product (classified as clear or flavored) or non-use.

Results: We studied 142 e-cig users (age 22±4, 45% women) and 73 non-users (age 26±6, 55% women). Prior to the ban 93% of e-cig users used a flavored e-cig whereas after 70% used flavored and 20% used clear e-cigs. Acute use increased SBP, DBP, and

HR in clear users (8±8mmHg, 9±7mmHg, 9±7bpm) to a greater degree compared to non-use (0.03±4mmHg, 0.4±3mmHg, -1.5±3bpm, all P<0.001) and to a similar degree as flavored e-cig users (6±7mmHg, 6±6mmHg, 6±7bpm, all P=NS).

Conclusion: Following the ban, there has been a shift from flavored to clear e-cig use in young adults in Massachusetts. Both e-cig product types induce acute changes in cardiovascular health measures compared to non-use. Our findings have important implications for the regulation of e-cig products to reduce youth use.

THE ROLE OF INFLAMMATION AND AORTIC STIFFNESS ON AGING-INDUCED THALAMIC MICROBLEEDS AND MILD COGNITIVE IMPAIRMENT

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Objective: We investigated the role of inflammation on short-term memory and thalamic microbleeds using old mice to model mild cognitive impairment.

Methods: We treated young (3 mo), middle-aged (16-18 mo), and old (24-25 mo) WT mice with one of two anti-inflammatories: minocycline (10 mg/kg body wt, 30 days) or Etanercept (100 uL/mouse, 1 wk). We evaluated memory using the Novel Object Recognition test, then sectioned brains and quantified thalamic microbleeds by Prussian Blue staining. We measured microglial activation using Sholl analysis on Iba-1-labeled microglia as an indicator of inflammation. We assessed aortic pulse wave velocity, an index of arterial stiffness thought to contribute to cognitive decline with aging, using echocardiography in control and treated old mice.

Results: Anti-inflammatory treatment significantly attenuated aging-induced losses in short-term memory, aging-induced increases in thalamic microbleeds, and aging-induced thalamic inflammation. Treatment did not reverse aging-induced increases in aortic stiffness.

Conclusion: Anti-inflammatory treatment improved short-term memory in old mice. The concurrent decreases in thalamic microbleeds and in activation state of microglia, suggest a possible mechanism for improvements in memory. Short-term treatment with anti-inflammatories did not impact aging-induced aortic stiffness, suggesting that aging-associated inflammation differentially impacts aortic stiffness, thalamic microbleeds and mild cognitive impairment.

MIRNA-409-3P REGULATES INSULIN ACTION IN HUMAN ENDOTHELIAL CELLS

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Objective: Non-coding RNAs including microRNA (miRNA) regulate metabolic pathways that are relevant to endothelial dysfunction in patients with T2DM; thus, we sought to evaluate miRNA levels and their functional relevance in EC from non-diabetic (ND) and T2DM patients.

Methods: We have collected venous EC from patients with T2DM and ND (n=20/group), performed miRNA sequencing and validated results by qPCR. Phosphorylation of endothelial nitric oxide synthase (peNOS) assessment was used to demonstrate EC function and insulin resistance.

Results: Comparing patients with T2DM and controls we found 37 upregulated and 21 downregulated miRNAs (P<0.05, L2FC>1). Validation experiments in human aortic EC

(HAEC) found significant increase expression of miRNA-409-3p (P<0.05, L2FC>1.7) following high glucose palmitate (HGPAL) treatment. Insulin induced peNOS was decreased in HGPAL treatment while, inhibition of miRNA-409-3p restores peNOS (18% to 20%) as compared to controls (21%) by improving insulin action in EC. Ingenuity Pathway Analysis shows miRNA-409-3p is involved in the regulation of inflammation, insulin resistance and stress related signaling pathways. Ongoing studies are investigating the miRNA-409-3p transcriptional targets and their functional implications in T2DM patients and cultured EC.

Conclusion: Our findings provide early support for altered EC miRNA repertoire in patients with T2DM and the role of miRNA-409-3p in insulin resistance and endothelial cell function.

Other

DISPARITIES IN THE HEPATITIS C VIRUS CASCADE OF CARE AMONG REPRODUCTIVE AGE WOMEN WITH OPIOID USE DISORDER

Author: Breanne Biondi
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Objective: To analyze racial and ethnic disparities within steps of the Hepatitis C Virus (HCV) Cascade of Care (CoC) among women of reproductive age with opioid use disorder (OUD) in the United States.

Methods: We analyzed a cohort of women with OUD aged 15-44 years captured in TriNetX, a national network of electronic health records, from 2014-2022. We calculated the percent who completed each CoC step: HCV antibody (Ab) testing, HCV Ab positive, HCV RNA positive, linkage to care, treatment with direct acting antivirals, and HCV cure (negative RNA testing 12 weeks post-treatment). We stratified by race and ethnicity and conducted unadjusted and adjusted logistic regression models.

Results: Of 103,556 women with OUD in the cohort, 48,294 (47%) had HCV Ab testing between 2010-2022. Over half of the tested cohort (N=27,173; 56%) were HCV seropositive and 20,767 ever had chronic HCV infection, of which 62% linked to care. Compared to White individuals, American Indian/Alaskan Native individuals had 48% greater odds of Ab testing (OR=1.48, 95%CI 1.03-2.15), and Asian and Black individuals had 44% and 38% lower odds of Ab testing (OR=0.56, 95%CI =0.39-0.80; OR=0.62, 95%CI 0.45-0.86) in adjusted analyses.

Conclusion: Few women of reproductive age with OUD are HCV tested or treated, with disparities by race and ethnicity. Interventions are needed to improve equity in HCV screening and treatment for reproductive age women.

ALCOHOL CONSUMPTION TRAJECTORIES DURING TUBERCULOSIS (TB) TREATMENT: A LONGITUDINAL COHORT IN THE WESTERN CAPE, SOUTH AFRICA

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Objective: Alcohol consumption is associated with increased TB incidence and poor treatment response. Little is known about alcohol consumption trajectories for those with TB, especially in high-burden settings. We aimed to assess drinking trajectory differences among participants in a longitudinal TB treatment cohort in the Western Cape.

Methods: The sample consisted of 303 participants. Alcohol timeline follow-back was used to measure absolute amount of alcohol consumed over the previous 2 weeks at study visits. Alcohol use trajectory groups were identified using Group Based Trajectory Modeling. We also explored which variables characterized participants in each trajectory group.

Results: Three distinct groups with different alcohol trajectories were identified. Group 1 consisted of 186 individuals with consistent minimal alcohol consumption, Group 2 consisted of 101 participants who reduced alcohol consumption early in treatment but resumed moderate use, and Group 3 consisted of 16 participants who initially reduced alcohol use but reverted to heavy use. Employment status, tobacco use, smoked drug use, baseline AUDIT and baseline Phosphatidylethanol scores were associated with trajectory group category.

Conclusion: Alcohol trajectories differed across groups and were associated with baseline substance use. Since two groups increased their alcohol use during early TB treatment, early intervention during TB treatment may be time to address alcohol use when absolute alcohol use is lower.

BLOOD-BASED BIOMARKER IDENTIFIED FROM PLASMA AND BRAIN PROTEOME DATA WITH CONNECTIONS TO COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE NEUROPATHOLOGY

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Objective: Identification of blood-based biomarkers that are indicative of Alzheimer's disease (AD) pathology may help with early detection.

Methods: We generated proteome data in both antemortem plasma and postmortem brain tissue from participants of the Boston University Alzheimer's Disease Research Center. Dementia severity was measured by global cognitive dementia rating (CDR) scores, and pathological AD diagnosis was established using NIA Reagan criteria. We conducted association tests with dementia (CDR >= 1 for dementia, CDR < 1 for control) and autopsy-confirmed AD status as binary outcomes. Plasma proteins associated with clinical or pathological AD ($P<0.05$) were tested for association with quantitative cognitive tests and neuropathological traits, adjusted by sex and either age at last exam or death.

Results: Five proteins (ACES, AURKB, CBARP, G45IP, and MMP-8) measured in plasma were significantly associated with both pathological AD and dementia. MMP-8 was the only protein significantly associated in both plasma and brain with autopsy-confirmed AD (Pplasma=0.005, Pbrain=0.001) and dementia (Pplasma=0.01, Pbrain=9e-5). ACES and AURKB levels in plasma and brain were significantly associated with dementia ($P<0.05$). Increased MMP-8 level in plasma was associated with decreased animals ($P=0.03$) and FAS scores ($P=0.04$).

Conclusion: MMP-8 is a potential blood-based biomarker for dementia and underlying AD pathology and is associated predominantly with impaired verbal fluency.

LEVERAGING BEHAVIORAL ECONOMICS TO CAPTURE PATIENT REPORTED SMOKING DATA: A RANDOMIZED TRIAL

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Objective: To compare the effectiveness of an electronic health record (EHR) portal questionnaire versus text survey and the impact of message framing on capturing complete patient reported smoking data.

Methods: We sent an EHR portal questionnaire and a text survey to English-speaking patients who were potentially eligible for lung cancer screening. The primary outcome was the response rate for each modality and framing type -"gain", "loss" and "helpfulness". We further evaluated the data for completeness and consistency with already documented structured smoking data in the electronic medical record.

Results: Participants were more likely to respond to the text survey (19.1%) as compared to the portal questionnaire (6.9%). Across all survey rounds, patients were less responsive to the "helpfulness" frame compared to the "gain" frame (OR= 0.29, $p < 0.05$) and "loss" frame (OR =0.32, $p < 0.05$), but responses to the "gain" and "loss" messages were similar (OR=0.89, p= 0.82). Both the portal questionnaire and text survey were significantly more likely to obtain the smoking history data necessary to determine lung cancer screening eligibility as compared to medical record data.

Conclusion: We found that a learning health system approach using patient-generated survey data (PGSD) is a feasible way to engage patients with their medical record and collect complete smoking histories. Patients are likely to respond to a text-based survey using "gain" or "loss" framing to report detailed smoking histories.

REFERENCE LIMITS AND CLINICAL CORRELATES OF DIGITAL COGNITIVE MEASURES FROM DEFENSE AUTOMATED NEUROCOGNITIVE ASSESSMENT: THE FRAMINGHAM HEART STUDY

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Objective: With the recent advance in digital technologies, multiple digital tools have been developed to assess cognitive health. In contrast to standard neuropsychological tests, the reference limits and clinical correlates of digital cognitive measures are largely undetermined.

Methods: We included 382 cognitively intact participants from Framingham Heart Study (FHS) who have completed three Defense Automated Neurocognitive Assessment (DANA) tasks. To develop reference limits for each digital measure, the study sample was stratified into subgroups based on sex and three age groups. We estimated 2.5th, 25th, 50th, 75th, and 97.5th quantile thresholds in men and women separately within each age group by quantile regression model. We also assessed the association of 7 clinical factors with these digital cognitive measures by a backward elimination strategy.

Results: The age- and sex-specific reference limits for digital measures of three DANA tasks were generated. For example, median (2.5th percentile; 97.5th percentile) average response time for code substitution task was 1938 ms (1424; 2833) in men and 1893 ms (1465; 2615) in women (Table 1). With the age increase, the performance of both men and women decreases. Besides, digital measures are associated with multiple clinical factors.

Conclusion: The findings of this study could potentially aid in the interpretation of digital cognitive measures of DANA tasks in future clinical research and practice.

SERUM PROTEIN-BASED INDICES FOR THE PROGRESSION OF FRACTURE HEALING AND NONUNION

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Objective: We aimed to define a serum protein-based diagnostic for the progression and failure of fracture healing in 7 METRC civilian centers and to show a specific correlation with later radiological and clinical signs used to define delayed healing and non-union.

Methods: Patients with isolated closed extra-articular fractures of the humeral shaft underwent serial blood collections during follow-up visits of non-operative treatment. Collection timepoints were chosen to reflect critical points in fracture healing based on the current literature. Samples were processed and later assessed via somalogic assay and RNA-based assay of over 7000 different serum proteins.

Results: Preliminary data reflects 9 patients through 6 months of fracture healing including 7 healers (union) and 2 non-healers (non-union). Statistical cutoffs for the preliminary data were $p \leq 0.01$ and $Fq \leq 0.125$. 611 proteins were identified that change over the course of healing; 455 proteins were associated with non-progression of fracture healing; 100 total proteins were identified as overlapping between the two groups. Proteins of interest in the overlap group include VEGFD, MMP13, Activin A, BMP-1,4,5, CO5A1, and VCAM-1.

Conclusion: These preliminary results suggest that serum protein-based diagnostics show promise for the early identification of non-union. NABA-MATRISOME associated proteins were the group most strongly associated with change over time course of healing and non-progression of fracture healing.

DIETARY PATTERNS, BLOOD PRESSURE AND PROTEINURIA IN A WEST AFRICAN CHRONIC KIDNEY DISEASE POPULATION

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Objective: There is little known about the impact of dietary patterns on blood pressure (BP) in West Africa. Our study aims to identify associations of dietary patterns from sub-Saharan African diets with BP and proteinuria, and is the first study to do so in a well-phenotyped West African CKD cohort.

Methods: We performed a cross-sectional analysis of 24-hour dietary recalls in 583 participants from the Diet, Apolipoprotein L1, and CKD study; an ancillary study under the H3 Africa Network. The first three dietary patterns were derived via principal component analysis. We measured proteinuria by 24-hour urine samples. We used mixed-effect linear regressions to estimate the coefficients and 95% confidence interval for the quartiles of the dietary patterns after adjusting for covariates.

Results: The mean age was 49±17 years with 51% males and the mean eGFR (2009 CKD-EPI) was 68±39 mL/min/1.73m2. The median 24-hour urine protein was 0.31g (IQR=0.13-1.07). We identified the Dried fish, Oils and vegetables; Poultry and Cereal; and the Fruit and Cereal dietary patterns. Higher quartiles of Poultry and Cereal dietary intake were associated with lower systolic BP and diastolic BP. Higher Poultry and Cereal dietary consumption was associated with lower 24hr urine protein (p for trend: 0.05). Compared to Q1, Q4 of this pattern was associated with reduced odds of proteinuria.

Conclusion: Consuming a Poultry and Cereal dietary pattern may benefit BP and proteinuria in West African CKD patients.

ALCOHOL'S EFFECT ON TUBERCULOSIS TREATMENT RESPONSE: A COHORT STUDY IN THE WESTERN CAPE, SOUTH AFRICA

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Objective: We analyze data from a prospective rifampicin-susceptible tuberculosis cohort in the Western Cape, South Africa. We test for the association between alcohol use and poor treatment response, measured as delayed culture conversion at 10 weeks, adjusting for treatment adherence and other confounders.

Methods: 305 participants initiated rifampicin-susceptible TB treatment. Participants provided weekly sputum specimens for 12 weeks and had 5-weekdays of directly observed therapy. Our outcome was time-to-culture conversion (TCC), defined as two consecutive weeks with no Mycobacterium tuberculosis growth. We categorized alcohol exposure into low, moderate, high using the Alcohol use disorders identification test, two-week Timeline follow-back, and Phosphatidylethanol biomarker. We defined adherence as a time varying weekly percentage. We fit a cox proportional hazards model predicting TCC from alcohol exposure, adjusting for weekly adherence, age, sex, HIV, isoniazid resistance, smoked drug use, unemployment, and baseline culture time to positivity.

Results: Median TCC was 7 (IQR: 6,8) weeks. 72.2% (95%CI: 71.3%, 73.2%) converted by week 10. Time to culture conversion was not different for the moderate (HR:1.04, 95%CI: 0.75, 1.43) or high alcohol group (HR:1.15, 95%CI:0.77, 1.73) compared to the low alcohol group in adjusted analysis.

Conclusion: Alcohol did not impact sterilization, indicating that PWUA can respond successfully to TB therapy when medication adherence is high.

NUTRITIONAL SERINE PROMOTES CELL PLASTICITY IN ORAL CANCER

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Objective: Cancer of the oral cavity typically presents as oral squamous cell carcinoma (OSCC), a devastating malignancy with few treatments available. OSCC is driven by transformation of cell identities into more stem cell-like and mesenchymal cell states, defined as cell plasticity, leading to metastasis and therapy resistance. Given that changes in cellular metabolism have been shown to impact tumor growth and given that serine metabolism affects cell phenotypes, we aimed to decode the relationship between serine metabolism and cell plasticity in OSCC.

Methods: Using a panel of human OSCC cell lines, we interrogated the effect of serine starvation on OSCC cell identity in vitro. Cell lines cultured in either complete or serine starvation (-Ser) media were evaluated for proliferation, metabolomics, markers of differentiation and stemness, and global transcriptome by RNAseq.

Results: Under -Ser conditions, cells displayed reduced proliferation and greater serine synthesis pathway mRNA and enzyme levels. This coincided with an increase in alpha-ketoglutarate (aKG), a co-substrate for histone modifying enzymes, and reduction in markers of stemness. Additionally, RNAseq analysis showed that increased aKG was associated with a decrease in epithelial-mesenchymal transition gene expression.

Conclusion: Our findings suggest that limitation of nutritional serine induces endogenous serine biosynthesis concomitant with a loss of cell plasticity.

IDENTIFICATION OF PROTECTIVE GENOME-WIDE DNA METHYLATION PROFILES FOR ASYMPTOMATIC ALZHEIMER'S DISEASE IN POSTMORTEM BRAINS

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Objective: We aimed to analyze differential DNA methylation (DNAm) patterns in postmortem brain tissue between asymptomatic AD (AsymAD) and symptomatic AD (SymAD).

Methods: Genome-wide differential methylation between 185 AsymAD and 254 SymAD brain donors of the Religious Orders Study and Rush Memory and Aging Project (ROSMAP) was analyzed. Next, we evaluated differential expression of genes within 50 kb of significantly differentially methylated CpGs. For significantly differentially expressed genes, association tests of DNAm at their corresponding CpG site and expression level was performed. To identify shared methylated CpG sites accounting for protection on AsymAD and changes in gene expression, we investigated colocalization of DNAm for both traits.

Results: We identified 18 differentially methylated CpG sites between AsymAD and SymAD. Top-ranked findings include CpG site cg19832721(logFC=-0.020, P=5.4x10-8) in the MAPT-KANSL1 region and cg20510285 (logFC=0.012, P=8.7x10-7) in NEDD9. MAPT, MAPT-AS1, KANSL1, KANAL-AS1, and NEDD9 genes were differentially expressed between AsymAD and SymAD. Methylation of cg19832721 site was associated with KANSL1-AS1 (β=-4.81, P=0.008) Colocalization revealed that methylation of cg20510285 was a common epigenetic variation for clinical symptoms of AD and expression level of NEDD9 (β=4.95, P=9.6x10-4).

Conclusion: DNAm profiles in the MAPT-KANSL1 region may explain resilience to clinical manifestations of AD by modulating expression of genes in this region.