June 14–18, 2026 | Cape Coral, FL

AASLD
The Liver Meeting®

SAVE THESE IMPORTANT DATES FOR THE LIVER MEETING®!

MAY 30
Preliminary Program available online

JULY 15
AASLD Member registration opens

JULY 22
General registration opens

AUGUST 26
Early bird registration deadline

SEPTEMBER 15-25
Late-breaking abstract submission open

OCTOBER 5
Press embargo lifts for regular abstracts

OCTOBER 7
Regular registration deadline

NOVEMBER 5
PLAN AHEAD: #TLM26
starts on Thursday
- Postgraduate Course, Part 1-4
- Basic Science Symposium, Part 1-4

NOVEMBER 6
AASLD Fellows' and Members' Reception

NOVEMBER 7
- Awards & Honors Gala
- Liver Care for Frontline Clinicians
  Invite an APP or clinician and save on your registration

ELEVATE YOUR EXPERIENCE, SCAN FOR UPDATES

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2026 Organizing Committee

Kari Nejak-Bowen, MBA, PhD (University of Pittsburgh)
Georg Halder, PhD (KU Leuven)
Robert E. Schwartz, MD, PhD (Weill Cornell Medical College)

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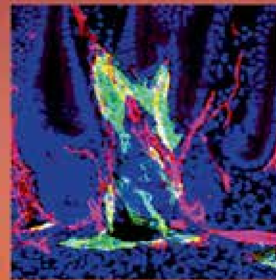

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American Association for the Study of Liver Diseases (AASLD) Junior Faculty Scholar Award Recipients

Tomasz Bednarski, PhD
University of Nebraska - Lincoln

Tianliang Sun, PhD
Icahn School of Medicine at Mount Sinai

Yilin Yang, PhD
Yale University

AASLD Trainee Travel Award Recipients

Matthew Carson, PhD
University of Pittsburgh

Michael Hu, BA
University of Pennsylvania

Kentaro Iwasawa, MD, PhD
Cincinnati Children's Hospital Medical Center

Anisha Jain, BS
University of Pittsburgh

Chang Kyung Kim, MD, PhD
University of Pennsylvania Medical Center

Noah Mac, BS
Rutgers University

Vik Meadows, PhD
University of Pittsburgh

Ekta Minocha, PhD
The University of Arizona, Tucson

Mobin Ibne Mokbul, MBBS
Mayo Clinic Rochester

Laura Molina, MD, PhD
University of Pittsburgh Medical Center

Claire Woppmann, BA
Boston University

American Society for Investigative Pathobiology (ASIP) Junior Faculty Scholar Award Recipient

Xiangyu Zhang, PhD
University of Pittsburgh

ASIP Trainee Scholar Award Recipients

Anas Al Mardini, MBBS
New York Medical College/St. Mary's General Hospital/St. Clare's Health

Dany Gould, MS
Boston University

Jinsol Han, BS
Pusan National University

Hayeong Jeong, BS
Pusan National University

Shahrbanoo Keshavarz Aziziraftar, PhD
University of California, San Francisco

Amit Kumar, PhD
University of Pittsburgh

Joseph Lee, BS
University of North Carolina

Dilnar Mahmut, MS
Boston University

Mohammad Mehraban, PhD
University of California, San Francisco

Ramasamy Selvarani, PhD
University of Oklahoma

Anubha Seth, PhD
Yale University

Tyler Yasaka, BS
University of Pittsburgh

Yingting Zhang, MS
Cleveland Clinic

Chen Zhang
University of Kansas Medical Center

MEETING PROGRAM

All sessions will be held in Tarpon Point 4

SUNDAY, JUNE 14, 2026

Badge Pick-Up
4:00-6:00 PM
Tarpon Foyer

Welcome Reception
6:00-7:30 PM
Tarpon Foyer

SESSION 001: Opening Plenary

- 7:45-8:00 PM
  Welcome and Introductions from the SLAM Organizers
  Kari Nejak-Bowen, PhD ▪ University of Pittsburgh
  Georg Halder, PhD ▪ VIB KU Leuven Center for Cancer Biology
  Robert Schwartz, PhD ▪ Weill Cornell Medical College

Keynote Lecture
8:00-9:00 PM
The Language of Metabolic Communication
Jared Rutter, PhD ▪ University of Utah School of Medicine/Howard Hughes Medical Institute

MONDAY, JUNE 15, 2026

Badge Pick-Up
7:00-8:00 AM
Tarpon Foyer

Attendee Breakfast
8:00-9:00 AM
Tarpon Point 1-2

SESSION 002: Liver Development and Regeneration
9:00-11:30 AM

Chair: Kirsten Sadler-Edepli, PhD ▪ University Abu Dhabi
Co-Chair: Kristin Knouse, MD, PhD ▪ Massachusetts Institute of Technology

- 9:00-9:25 AM
  Hepatocyte Plasticity and Reprogramming During Liver Injury
  Fernando Camargo, PhD ▪ Harvard Stem Cell Institute, Boston Children's Hospital

- 9:25-9:50 AM
  Insights into Liver Development and Regeneration
  Wolfram Goessling, MD, PhD ▪ Massachusetts General Hospital

- 9:50-10:15 AM
  Epigenetic Regulation of Regeneration in the Aging Liver
  Kirsten Sadler Edepli, PhD ▪ University Abu Dhabi

- 10:15-10:40 AM
  Beta-Catenin, Zonation, and Hepatocellular Cancer: From Bench to Bedside
  Satdarshan Paul Singh Monga, MD ▪ University of Pittsburgh School of Medicine

- 10:40-10:55 AM
  ABSTRACT 001: A Humanized Bile Acid Milieu Exacerbates Liver Injury and Biliary Tree Developmental Defects in a Mouse Model of Alagille Syndrome
  Hamed Jafar-Nejad, MD ▪ Baylor College of Medicine

- 10:55-11:10 AM
  ABSTRACT 002: Understanding How the Organism Monitors Liver Function to Properly Time Regeneration
  Kristin Knouse, MD, PhD ▪ Massachusetts Institute of Technology

- 11:10-11:25 AM
  ABSTRACT 003: Liver Regeneration CRISPR Screen Identifies E3 Ligase NEURL1B Regulates Hepatocyte Mitosis by Destabilizing Microtubule Organizing Centers
  Kirk Wangensteen, MD, PhD ▪ Mayo Clinic

Attendee Lunch
11:30 AM-12:30 PM
Tarpon Point 1-2

SESSION 003: Meet-the-Experts Luncheon I
11:30 AM-1:00 PM

Session Description: The Meet-the-expert luncheon will afford trainees and young investigators the opportunity to network with invited speakers from the meeting. While the size of this meeting will be fairly small, these meet-the-expert luncheons will ensure that the trainees and young investigators have adequate opportunities to meet the leaders in the field of liver research and begin to establish personal relationships that may benefit the research community.

Topics & Experts:

- Spatial Transcriptomics in Liver Research; Rob Schwartz, Paul Monga
- Non-Parenchymal Cells in MASH; Moritz Peiseler, Ekihiro Seki
- New Developments in Cholestatic Liver Disease; Saul Karpen, Stacey Huppert

Session 004: Liver Homeostasis and Injury
1:00-3:10 PM

Chair: Valerie Gouon-Evans, PharmD, PhD ▪ Boston University
Co-Chair: Hsuan-An Chen, PhD ▪ Rockefeller University

- 1:00-1:25 PM
  mRNA Nanomedicine for Liver Disease Treatment
  Valerie Gouon-Evans, PharmD, PhD ▪ Boston University

- 1:25-1:50 PM
  Somatic Genetics as a Discovery Engine for Liver Biology
  Hao Zhu, MD ▪ University of Texas Southwestern Medical Center

- 1:50-2:15 PM
  From Foes to Friends? Hepatic Stellate Cells in Liver Homeostasis and Regeneration
  Youngmin Lee, MD, PhD ▪ Vanderbilt University Medical Center

- 2:15-2:40 PM
  Liver Lymphatics: From Biology to Therapeutic Innovations in Liver Disease
  Yasuko Iwakiri, PhD ▪ Yale School of Medicine

- 2:40-2:55 PM
  ABSTRACT 004: Leveraging the NrHV Mouse Model to Investigate Liver Microenvironmental Remodeling Following Chronic Hepacivirus Cure
  Hsuan-An Chen, PhD ▪ Rockefeller University

- 2:55-3:10 PM
  ABSTRACT 005: Cholangiocyte–Neutrophil Crosstalk in Primary Sclerosing Cholangitis (PSC): The MIF–CD74 Axis as a Driver of Neutrophilic inflammation
  Mobin Ibne Mokbul, MBBS ▪ Mayo Clinic

Attendee Free Time - Enjoy Cape Coral
3:10-6:00 PM

Attendee Dinner
6:00-7:00 PM
Tarpon Point 1-2

Session 005: Poster Highlight Session - Lightning Fire Talks
7:00-8:00 PM

- 7:00-7:04 PM
  ABSTRACT 020: Biliary Reconstruction with B2M/CIITA Knockout Human Pluripotent Stem Cell Derived Tubular Organoids
  Kentaro Iwasawa, MD, PhD ▪ Cincinnati Children's Hospital Medical Center

- 7:04-7:08 PM
  ABSTRACT 023: Targeting Metalloprotease-Regulated BEC Plasticity for Regenerative Liver Therapy
  Soumili Sarkar MSc ▪ University of Toront

- 7:08-7:12 PM
  ABSTRACT 033: A Genetic Model of Nodular Cirrhosis Demonstrates the Reversibility of End-Stage Liver Disease
  Natasha Corbitt, MD, PhD ▪ University of Texas Southwestern Medical Center

- 7:12-7:16 PM
  ABSTRACT 047: The Post-Weaning Involutional Microenvironment Restricts β-Catenin-Driven Hepatocellular Carcinoma
  Anisha Jain, BS ▪ University of Pittsburgh

- 7:16-7:20 PM
  ABSTRACT 053: Characterizing Autoimmune Hepatitis (AIH) Using Complementary Single-Nuclei and Spatial Transcriptomics Techniques
  Nikita Sajai, BA ▪ University of California, San Francisco

- 7:20-7:24 PM
  ABSTRACT 059: Sustained Loss of ESRP2 Rewires Hepatocyte Splicing to Drive MetALD and Block Recovery
  Diptatanu Das, BS, MS ▪ University of Illinois, Urbana-Champaign

- 7:24-7:28 PM
  ABSTRACT 062: Discovering Nanoparticle Corona Ligands for Liver Macrophage Capture
  Bram Bussin, PhD ▪ Toronto General Hospital

- 7:28-7:32 PM
  ABSTRACT 064: Dissecting Hepatocyte Heterogeneity in Liver Growth to Improve In Vivo Gene Therapy
  Francesca Marabotti, MA ▪ San Raffaele Telethon Institute for Gene Therapy

- 7:32-7:36 PM
  ABSTRACT 065: Vascular Endothelial Growth Factor (VEGFA) mRNA in Lipid Nanoparticles as a Bridge Therapy for AATD Liver Disease
  Claire Woppmann, BA ▪ Boston University

- 7:36-7:40 PM
  ABSTRACT 072: Aging Disrupts Hepatic Zonation and Architecture Through Circadian Regulation of Liver Homeostasis
  Saloni Sinha, PhD ▪ Weill Cornell

- 7:40-7:44 PM
  ABSTRACT 076: Senescence Mediated Hepatocyte Injury Drives MASLD Progression
  Sadam Bhat, PhD ▪ Cedars-Sinai Medical Center

- 7:44-7:48 PM
  ABSTRACT 079: Clearance of Cholesterol-Containing Lipid Crystals Reverses Liver Stiffening and Fibrosis in a Dietary Rat Model of MASLD
  David Li, PhD MS, BS ▪ University of Pennsylvania

- 7:48-7:52 PM
  ABSTRACT 082: A Spatially Resolved Metabolic and Transcriptomic Atlas of Human Metabolic Dysfunction-Associated Steatotic Liver Disease
  Haitao Nan ▪ Chinese Academy of Sciences

- 7:52-7:56 PM
  ABSTRACT 084: LNP-Mediated Targeting of a Conserved Non-Canonical MST1/2-FOXO3 Survival Axis in Liver Myofibroblasts Reverses MASH Fibrosis
  Tobias Raabe, PhD ▪ University of Pennsylvania

- 7:56-8:00 PM
  ABSTRACT 087: Reprogramming Chronic Liver Disease Through Physiological Regeneration: Transient ZNRF3/RNF43-β-Catenin Activation Reverses MASH
  Tianliang Sun, PhD ▪ Icahn School of Medicine at Mount Sinai

Keynote Lecture

8:00-9:00 PM

Resmetirom and New Therapies for the Treatment of Patients with MASH Across the Fibrosis Spectrum - From Moderate Fibrosis to Cirrhosis

Rebecca Taub, MD ▪ Madrigal Pharmaceuticals

TUESDAY, JUNE 16, 2026

Attendee Breakfast
8:00-9:00 AM
Tarpon Point 1-2

SESSION 006: Plasticity and Transdifferentiation
9:00-11:30 AM

Chair: Stacey Huppert, PhD ▪ Cincinnati Children's Hospital Medical Center
Co-Chair: Joseph Lee, BS ▪ University of North Carolina

- 9:00-9:25 AM
  Cell Identity Conversion: Hepatocyte Generation and Cell Therapy
  Lijian Hui, PhD ▪ University of Chinese Academy of Sciences
- 9:25-9:50 AM
  Hepatocyte Plasticity and Postnatal Maturation
  Stacey Huppert, PhD ▪ Cincinnati Children's Hospital Medical Center
- 9:50-10:15 AM
  Cell Therapy for Liver Disease
  Stuart Forbes, MB CHB, FRCP(Ed), PhD ▪ The University of Edinburgh
- 10:15-10:40 AM
  Metabolic Reprogramming in Chronic Liver Disease
  Jan Tchorz, PhD ▪ University of Tübingen
- 10:40-10:55 AM
  ABSTRACT 006: Sox9 Regulates Hepatocyte Plasticity During Chronic Liver Injury
  Joseph Lee, BS ▪ University of North Carolina
- 10:55-11:10 AM
  ABSTRACT 007: Non-Canonical TFEB Activation Drives Hepatocyte Plasticity and Tumor Malignancy in TSC1 Deficient Livers
  Chen Zhang ▪ University of Kansas Medical Center
- 11:10-11:25 AM
  ABSTRACT 008: De Novo Pathological Macrophage Niches in the Injured Liver Persist Following Recovery and Sustain Prolonged Tissue Sensitivity to Secondary Insults
  Pieter Louwe, PhD ▪ Ghent University

Attendee Lunch
11:30 AM-12:30 PM
Tarpon Point 1-2

SESSION 007: Meet-the-Experts Luncheon II
11:30 AM-1:00 PM

Session Description: The Meet-the-expert luncheon will afford trainees and young investigators the opportunity to network with invited speakers from the meeting. While the size of this meeting will be fairly small, these meet-the-expert luncheons will ensure that the trainees and young investigators have adequate opportunities

to meet the leaders in the field of liver research and begin to establish personal relationships that may benefit the research community.

Topics & Experts:

- NAMS Models; Aras Mattis, Taka Takebe
- Therapeutic Advances in Liver Disease; Mathias Heikenwalder, Valerie Gouon-Evans
- Liver Metabolism and Zonation; Hao Zhu, Jan Tchorz

Attendee Free Time - Enjoy Cape Coral

1:00-6:00 PM

Attendee Dinner

6:00-7:00 PM
Tarpon Point 1-2

SESSION 008: Liver Stem Cell Biology and Organoids

7:00-8:15 PM

Chair: Ludovic Vallier, PhD ▪ Universitätsmedizin Berlin
Co-Chair: Marisa Medina, PhD ▪ University of California, San Francisco

- 7:00-7:25 PM
  Liver Organogenesis: To Bud or Not to Bud
  Ludovic Vallier, PhD ▪ Universitätsmedizin Berlin
- 7:25-7:50 PM
  Synthetic Morphogenesis of Human Liver
  Mo Ebrahimkhani, MD ▪ University of Pittsburgh School of Medicine
- 7:50-8:15 PM
  Organoid-Guided Precision Hepatology
  Takanori Takebe, MD, PhD ▪ Cincinnati Children's Hospital Medical Center

Poster Session I (ODD Numbered Posters)

8:30-10:00 PM
Tarpon Point 3

Poster Board 1

ABSTRACT 017: A Novel Nanoparticle Platform Enhances the Therapeutic Efficacy of a TSG-6–Derived Peptide in Alcohol-Related Liver Disease
Jinsol Han, BS ▪ Pusan National University

Poster Board 3

ABSTRACT 019: Selective Serotonin Reuptake Inhibitor Therapy Protects Against Cholestatic Liver Disease
Matthew Carson, PhD ▪ University of Pittsburgh

Poster Board 5

ABSTRACT 021: Dual Loss of β- and γ-Catenin from Cholangiocytes Leads to Intrahepatic Cholestasis, Intestinal Inflammation, and Microbiome Dysbiosis
Vik Meadows, PhD, MS, BS ▪ University of Pittsburgh

Poster Board 7

ABSTRACT 023: Targeting Metalloprotease-Regulated BEC Plasticity for Regenerative Liver Therapy
Soumili Sarkar MSc ▪ University of Toronto

Poster Board 9

ABSTRACT 025: Cyp2c70-Deficient Mice Develop Age-Dependent Cholestatic Liver Injury with Progressive Bile Acid Accumulation and UPR Activation

Ryan Shaw, PhD ▪ Northwestern Feinberg School of Medicine

Poster Board 11

ABSTRACT 027: The Role of Lymphatics in Primary Biliary Cholangitis

Yilin Yang, PhD ▪ Yale University

Poster Board 13

ABSTRACT 029: Acute Liver Failure in the Setting of Acute Hepatitis B and Concomitant Ashwagandha Use

Maham Shafquat, MD ▪ Memorial Healthcare System

Poster Board 15

ABSTRACT 031: System-Level Variation in Endoscopy Timing and Mortality in Cirrhosis with Acute Variceal Bleeding: A Nationwide Analysis of Practice Patterns and Outcomes

Anas Al Mardini, MBBS ▪ New York Medical College

Poster Board 17

ABSTRACT 033: A Genetic Model of Nodular Cirrhosis Demonstrates the Reversibility of End-Stage Liver Disease

Natasha Corbitt, MD, PhD ▪ University of Texas Southwestern Medical Center

Poster Board 19

ABSTRACT 035: Thrombin Induces Rapid Contraction in Hepatic Stellate Cells Driven by PAR1 Signaling

Noah Mac, BS ▪ Rutgers University

Poster Board 21

ABSTRACT 037: Perturbed Neutrophil Responses in Irf3-deficient Mice Protect from CCl4-Induced Liver Fibrosis in Mice

Yingting Zhang, MS ▪ Cleveland Clinic

Poster Board 23

ABSTRACT 039: ProtoHep: 878 Hepatocyte Differentiation Protocols Extracted from Biomedical Literature via Agentic LLM Pipeline

Aras Mattis, MD, PhD ▪ University of California, San Francisco

Poster Board 25

ABSTRACT 041: Hepatocyte Growth Factor and Epidermal Growth Factor Delivered Via mRNA in Lipid Nanoparticles Improve Engraftment of Human Primary and iPSC-Derived Hepatocytes in Mice

Dany Gould, MS ▪ Boston University

Poster Board 27

ABSTRACT 043: MASH Patient-Derived iPSC Liver Organoids Show Increased Susceptibility to Disease and Acetaminophen-Induced Toxicity

Ekta Minocha, PhD ▪ The University of Arizona, Tucson

Poster Board 29

ABSTRACT 045: Liver and Biliary Tract Cancer Incidence and Mortality in the United States, 1999–2023: A 25-Year Epidemiologic Analysis

Mohammad Amer, MD ▪ Crestwood Medical Center

Poster Board 31

ABSTRACT 047: The Post-Weaning Involutional Microenvironment Restricts β-Catenin-Driven Hepatocellular Carcinoma

Anisha Jain, BS ▪ University of Pittsburgh

Poster Board 33

ABSTRACT 049: Using Omics Technologies to Understand Hepatoblastoma Heterogeneity and Identify Tumor Cell Types and Their Microenvironment for Precision Medicine

Elise Lelou, PhD ▪ University of California, San Francisco

Poster Board 35

ABSTRACT 051: Robust Inference of Liver Zonation Reveals a Targetable PPARα Dependence in Liver Cancer

Tyler Yasaka, BS ▪ University of Pittsburgh

Poster Board 37

ABSTRACT 053: Characterizing Autoimmune Hepatitis (AIH) Using Complementary Single-Nuclei and Spatial Transcriptomics Techniques

Nikita Sajai, BA ▪ University of California, San Francisco

Poster Board 39

ABSTRACT 055: Wnt Receptor Fzd10 Marks CAR-Induced Tumor-Associated Hepatocyte Population with Cancer Stem Cell-Like Features

Elena (Yu) Sun ▪ Boston University

Poster Board 41

ABSTRACT 057: Orthotopic Xenografts of Human Hepatoblastoma Exhibit Delayed Growth in Neonatal Compared to Adult Livers of Immunodeficient Mice

Peng Wu, MD, PhD ▪ Cincinnati Children's Hospital Medical Center

Poster Board 43

ABSTRACT 059: Sustained Loss of ESRP2 Rewires Hepatocyte Splicing to Drive MetALD and Block Recovery

Diptatanu Das, BS, MS ▪ University of Illinois, Urbana-Champaign

Poster Board 45

ABSTRACT 061: Impacts of Fluid Shear Stress on NOTCH Signaling in a JAG1 KO Model of Alagille Syndrome Using Cholangiocytes

Nina Brooks, BS, MS ▪ Cornell University

Poster Board 47

ABSTRACT 063: 5’ UTRs Contain Disease-Relevant, Targetable Motifs to Treat Genetic Disease

Nicholas Hand, PhD ▪ University of Pennsylvania

Poster Board 49

ABSTRACT 065: Vascular Endothelial Growth Factor (VEGFA) mRNA in Lipid Nanoparticles as a Bridge Therapy for AATD Liver Disease

Claire Woppmann, BA ▪ Boston University

Poster Board 51

ABSTRACT 067: Temporally Controlled Expression of a Splicing Factor in Single Cells Coordinates the Metabolic and Proliferative Activities of Regenerating Livers

Nick Baker, BA ▪ University of Illinois-Urbana Champaign

Poster Board 53
ABSTRACT 069: Endothelial ARNT is Required for Wnt-Dependent Metabolic Zonation and Liver Regeneration
Chang Kyung Kim, MD, PhD ▪ University of Pennsylvania Medical Center

Poster Board 55
ABSTRACT 071: Spatial Resolution of Gene Functions in Lipid Metabolism and Liver Regeneration
Jee Won Shin, BA ▪ Mayo Clinic

Poster Board 57
ABSTRACT 073: Roles of TorsinA in Regulating Lipid Metabolism in Human Hepatocytes
Vidhyalakshmi Acharya, PhD ▪ HMH Center for Discovery and Innovation

Poster Board 59
ABSTRACT 075: The Role of Hepatic Mitochondrial Acyl-CoA Metabolism in Steatotic Hepatocytes
Tomasz Bednarski, PhD ▪ University of Nebraska, Lincoln

Poster Board 61
ABSTRACT 077: Stung by a Broken Clock: Circadian Disruption Drives cGAS-STING Activation, Pyroptosis, and Fibrogenic Remodeling in MASH
Amit Kumar, PhD ▪ University of Pittsburgh

Poster Board 63
ABSTRACT 079: Clearance of Cholesterol-Containing Lipid Crystals Reverses Liver Stiffening and Fibrosis in a Dietary Rat Model of MASLD
David Li, PhD MS, BS ▪ University of Pennsylvania

Poster Board 65
ABSTRACT 081: A SNP, a miRNA, and a Mask: Precision Rescue of TM6SF2 in Genetic Steatosis
Mohammad Mehraban, PhD ▪ University of California, San Francisco

Poster Board 67
ABSTRACT 083: Elucidating the Mechanistic Relationship Between a Genetic Variant of Interest and Increased MASLD Predisposition in Humans
Anna Peczak, BS ▪ Weill Cornell

Poster Board 69
ABSTRACT 085: Reduced ZMPSTE24 Expression Leads to Prelamin Accumulation and Development of Steatosis in MASLD Patients
Joseph Schinderle, BA ▪ University of Pittsburgh

Poster Board 71
ABSTRACT 087: Reprogramming Chronic Liver Disease Through Physiological Regeneration: Transient ZNRF3/RNF43-β-Catenin Activation Reverses MASH
Tianliang Sun, PhD ▪ Icahn School of Medicine at Mount Sinai

Poster Board 73
ABSTRACT 089: Genotype-Specific MASLD Progression in Human Liver Microphysiology Systems: Implications for Precision Medicine
Mahboubeh Varmazyad, PhD ▪ University of Pittsburgh

WEDNESDAY, JUNE 17, 2026

Attendee Breakfast
8:00-9:00 AM
Tarpon Point 1-2

SESSION 009: MASH and Fibrosis
9:00-11:30 PM
Chair: Rebecca Wells, MD ▪ University of Pennsylvania School of Medicine
Co-Chair: Yoko Yagishita, DDS, PhD ▪ Columbia University

- 9:00-9:25 AM
  Modeling MASLD Using Patient-Derived iPSCs
  Jackie Maher, MD ▪ University of California, San Francisco

- 9:25-9:50 AM
  Elevated Solid Stress and its Implications in the Cirrhotic Liver
  Rebecca Wells, MD ▪ University of Pennsylvania School of Medicine

- 9:50-10:15 AM
  Extracellular Matrix in Hepatocellular Carcinoma
  Natalie Torok, MD ▪ Stanford University School of Medicine

- 10:15-10:40 AM
  Macrophage Adaptations in Chronic Liver Disease – Lessons from Intravital Imaging
  Moritz Peiseler, MD ▪ Charite Universitatsmedizin

- 10:40-10:55 AM
  ABSTRACT 009: Dissecting the Function of Macrophage mTORC1 Signaling During the Pathogenesis of Metabolic Disease-Associated Steatohepatitis
  Xiangyu Zhang, PhD ▪ University of Pittsburgh School of Medicine

- 10:55-11:10 AM
  ABSTRACT 010: Hepatocyte Notch Drives Immune Cell Recruitment in MASH
  Yoko Yagishita, DDS, PhD ▪ Columbia University

- 11:10-11:25 AM
  ABSTRACT 011: Evaluating the Effect of MASLD Polygenic Risk in iPSC to Study Heterogeneity in Disease Progression & Resmetirom Response
  Marisa Medina, PhD ▪ University of California, San Francisco

Attendee Lunch
11:30 AM-12:30 PM
Tarpon Point 1-2

Attendee Free Time - Enjoy Cape Coral
1:00-6:00 PM

Attendee Dinner
6:00-7:00 PM
Tarpon Point 1-2

SESSION 011: Novel Technologies for Liver Research

7:00-8:15 PM

Chair: Lichun Ma, PhD ▪ National Cancer Institute, National Institutes of Health
Co-Chair: Pieter Louwe, PhD ▪ Ghent University

- 7:00-7:25PM
  Transplantation of Genetically Enhanced Allogeneic Hepatocytes Without Immune Suppression
  Markus Grompe, MD ▪ Oregon Health & Science University
- 7:25-7:50 PM
  Biliary Gene Therapy
  Holger Willenbring, MD, PhD ▪ University of California, San Francisco
- 7:50-8:15 PM
  Dissecting Spatial Intratumor Heterogeneity in Liver Cancer
  Lichun Ma, PhD ▪ National Cancer Institute, National Institutes of Health

Poster Session II (EVEN Numbered Posters)

8:30-10:00 PM
Tarpon Point 3

Poster Board 2

ABSTRACT 018: Modeling Alcohol-Induced Liver Injury Using a Human Liver-on-a-Chip Microphysiological System
TakashiTsuchiya, BS ▪ Cedars-Sinai Medical Center

Poster Board 4

ABSTRACT 020: Biliary Reconstruction with B2M/CIITA Knockout Human Pluripotent Stem Cell Derived Tubular Organoids
Kentaro Iwasawa, MD, PhD ▪ Cincinnati Children's Hospital Medical Center

Poster Board 6

ABSTRACT 022: Blood Vessel Architecture is Altered Following a Bile Duct Formation Defect in a Mouse Model of Genetic Cholestasis
Laura Molina, MD, PhD ▪ University of Pittsburgh Medical Center

Poster Board 8

ABSTRACT 024: KIF12 Deficiency Unraveled in Cholestatic Liver Disease: Linking Organelle Mispositioning to Hepatic Pathology
Anubha Seth, PhD ▪ Yale University

Poster Board 10

ABSTRACT 026: Spatial Transcriptomics Reveals a TLS-like Immune-Fibrotic Niche Driving Biliary Fibrosis in Pediatric Primary Sclerosing Cholangitis
Yunguan Wang, PhD ▪ Cincinnati Children's Hospital Medical Center

Poster Board 12

ABSTRACT 028: MRP9 is a Novel Regulator of Cholangiocyte Mitochondrial Metabolism
Chunyue Yin, PhD ▪ Cincinnati Children's Hospital Medical Center

Poster Board 14

ABSTRACT 030: Diagnostic Accuracy of Liver and Spleen Elastography for Detecting Portal Hypertension Defined by Hepatic Venous Pressure Gradient (HVPG) in Chronic Liver Disease: A Systematic Review
Mohammed Saleem Yousuf ▪ Viswabharathi Medical College

Poster Board 16
ABSTRACT 032: Pathogen-Specific Infectious Causes of Death in U.S. Liver Cirrhosis Patients: A 25-Year Population-Based Analysis
Bilal Bani Amer, MD ▪ Yarmouk University

Poster Board 18
ABSTRACT 034: Irradiated Humanized Liver Mice Develop Early Features of Radiation-Induced Liver Disease That Are Absent in Conventional Mice
Eleanna Kaffe, PhD ▪ University of Pennsylvania

Poster Board 20
ABSTRACT 036: The Impact of Mitochondrial Haplotype on Inflammation and Fibrosis in Novel OKC-HETB/W Rats
Ramasamy Selvarani, PhD ▪ University of Oklahoma

Poster Board 22
ABSTRACT 038: The Fontan Associated Liver Disease (FALD) Atlas: A Spatiotemporal Single-Cell Transcriptomics Map of Coordinated Multicellular Remodeling in the Liver
Caleb Watson ▪ University of Pittsburgh Medical Center

Poster Board 24
ABSTRACT 040: Development of Human iPSC-Derived Hepatocytes for Drug Discovery, Translational Research, and Toxicity Testing
Sabitri Ghimire, PhD ▪ Bitbio Limited

Poster Board 26
ABSTRACT 042: Upregulation of IRF9 Increases Interferon Signaling During Induced Pluripotent Stem Cell Differentiation to Hepatocytes
Ana Carolina Loyola-Machado, PhD ▪ University of Miami

Poster Board 28
ABSTRACT 044: Differential Expression of SOCS1 Increases Interferon Signaling Throughout Stem Cell Differentiation to Hepatocytes
Kelli Wysoglad ▪ University of Miami

Poster Board 30
ABSTRACT 046: Genetic Prediction of Hepatocellular Carcinoma in a Prospective Cohort of Patients with Cirrhosis
Sara Estrada, BS ▪ The University of Texas MD Anderson Cancer Center

Poster Board 32
ABSTRACT 048: Hepatocyte-Specific Loss of a Nuclear Envelope Protein LAP1 Promotes Hepatocellular Carcinoma Through Epigenetic Dysregulation
Soojin Kim, PhD ▪ HMH Center for Discovery and Innovation

Poster Board 34
ABSTRACT 050: Single-Cell Atlas of Chronic Woodchuck Hepatitis-Induced HCC Highlights Conserved Tumor-Driven Immune Exhaustion Shared with Human HCC
Yijia Liu, HBSc ▪ University Health Network

Poster Board 36
ABSTRACT 052: Alcohol Alters AhR/β-Catenin Signaling and Reshapes Liver Zonal Plasticity Leading to Immune-Metabolic Reprogramming in Metabolic-Syndrome Associated Hepatocellular Carcinoma
Liya Pi, PhD ▪ Tulane University

Poster Board 38

ABSTRACT 054: Developing a Human Precision-Cut Liver Slice Platform to Investigate the Immune Microenvironment in Hepatocellular Carcinoma
Ariya Shiwram, HBSc ▪ University of Toronto

Poster Board 40

ABSTRACT 056: β-Catenin Inhibition Delays Tumor Progression in an hMET–NRF2 Hepatocellular Carcinoma Model via Non-Canonical Signaling
Junyan Tao, PhD ▪ University of Pittsburgh

Poster Board 42

ABSTRACT 058: Zinc Finger Transcription Factor FANIN is the Upstream Regulator of Pioneer Factors Foxa1 and Foxa2 in the Liver
Irina Bochkis, PhD ▪ University of Pittsburgh

Poster Board 44

ABSTRACT 060: Pioneer Factor FOXA-Mediated DNA Demethylation in Human Hepatic Fate Programming
Makiko Iwafuchi, PhD ▪ Cincinnati Children's Hospital Medical Center

Poster Board 46

ABSTRACT 062: Discovering Nanoparticle Corona Ligands for Liver Macrophage Capture
Bram Bussin, PhD ▪ Toronto General Hospital

Poster Board 48

ABSTRACT 064: Dissecting Hepatocyte Heterogeneity in Liver Growth to Improve In Vivo Gene Therapy
Francesca Marabotti, MA ▪ San Raffaele Telethon Institute for Gene Therapy

Poster Board 50

ABSTRACT 066: A Forward Genetic Approach to Identify Modifiers of Liver Disease Risk in Alpha-1 Antitrypsin Deficiency (ATD)
Shubham Kesarwani ▪ Boston University

Poster Board 52

ABSTRACT 068: Human-Length Telomeres Delay Regeneration of the Liver Parenchyma in Mice
Michael Hu, BA ▪ University of Pennsylvania

Poster Board 54

ABSTRACT 070: Macrophage Colony-Stimulating Factor Prolongs Kupffer Cell Survival, Phenotype, and Phagocytic Potential Function In Vitro
Manmeet Sekhon, BS ▪ University of Toronto

Poster Board 56

ABSTRACT 072: Aging Disrupts Hepatic Zonation and Architecture Through Circadian Regulation of Liver Homeostasis
Saloni Sinha, PhD ▪ Weill Cornell

Poster Board 58

ABSTRACT 074: Genome Editing Highlights SUGP1 as a Key Contributor to MASLD and Lipid Accumulation
Shahrbanoo Aziziraftar, PhD ▪ University of California, San Francisco

Poster Board 60

ABSTRACT 076: Senescence Mediated Hepatocyte Injury Drives MASLD Progression
Sadam Bhat, PhD ▪ Cedars-Sinai Medical Center

Poster Board 62

ABSTRACT 078: Tropomyosin Receptor Kinase Signaling Induces mTOR Thereby Leading to Steatosis and Metabolic Reprogramming of Hepatocytes

Ting-Fang Lee, PhD ▪ Vanderbilt University Medical Center

Poster Board 64

ABSTRACT 080: Epigenetic Modifications Suppress HNF4α Expression in MASLD iPSC-Derived Hepatocytes: Effect of Vitamin C

Jiaxuan Liu, MD, PhD ▪ University of California, San Francisco

Poster Board 66

ABSTRACT 082: A Spatially Resolved Metabolic and Transcriptomic Atlas of Human Metabolic Dysfunction-Associated Steatotic Liver Disease

Haitao Nan ▪ Chinese Academy of Sciences

Poster Board 68

ABSTRACT 084: LNP-Mediated Targeting of a Conserved Non-Canonical MST1/2-FOXO3 Survival Axis in Liver Myofibroblasts Reverses MASH Fibrosis

Tobias Raabe, PhD ▪ University of Pennsylvania

Poster Board 70

ABSTRACT 086: TorsinA ATPase Activity is Essential for ApoB-Mediated VLDL Secretion in Hepatocytes

Ji-Yeon Shin, PhD ▪ Hackensack Meridian Health Center for Discovery and Innovation

Poster Board 72

ABSTRACT 088: SUGP1 Knockdown Promotes MASH in Hepatic TM6SF2 Knockout Mice

Sheila Teker, BA ▪ University of California, San Francisco

Poster Board 74

ABSTRACT 090: A PNPLA3-Deficient iPSC-Derived Hepatocyte Screen Identifies Pathways to Potentially Reduce Steatosis in Metabolic Dysfunction-Associated Fatty Liver Disease

Caren Doueiry ▪ Montefiore Einstein Medical Center

THURSDAY, JUNE 18, 2026

Attendee Breakfast

8:00-9:00 AM

Tarpon Point 1-2

SESSION 012: Liver Cancer

9:00-11:30 AM

Chair: Lars Zender, MD ▪ German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ)

Co-Chair: Marcella Steffani, MD ▪ Columbia University

- 9:00-9:25 AM
  Novel Targets and Therapeutics for Improved Systemic Therapies in Primary Liver Cancer
  Lars Zender, MD ▪ German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ)

- 9:25-9:50 AM
  Functional Genomics of Liver Cancer
  Jessica Zucman-Rossi, MD, PhD ▪ University of Paris Descartes and HEPG

- 9:50-10:15 AM
  Novel Concepts of Combinatorial Liver Cancer Therapies: From Immune-Cell Modulation to Microbe Transplantation
  Mathias Heikenwalder, PhD ▪ University of Tübingen

- 10:15-10:40 AM
  Combining Tumor Cell and Immune Targeting for Multimodal Therapy of Hepatocellular Carcinoma
  Marcella Steffani, MD ▪ Columbia University

- 10:40-10:55 AM
  ABSTRACT 012: AMPK Mediates Loser Cell Elimination During YAP-Driven Cell Competition in CCA
  Rishana Shaji, MSc ▪ KU Leuven

- 10:55-11:10 A M
  ABSTRACT 013: Zonated Signalling Pathways Shape Oncogenic Progression in Hepatocellular Carcinoma
  Alexander Raven, PhD ▪ University of Glasgow

- 11:10-11:25 AM
  ABSTRACT 014: Endothelial Cell Therapy to Repair the Liver Vascular Network and Alleviate Liver Diseases
  Dilnar Mahmut, MS ▪ Boston University

Attendee Lunch
11:30 AM-1:00 PM
Tarpon Point 1-2

SESSION 013: Metabolism and Microbiome
1:00-3:10 PM

Chair: Laura Nagy, PhD ▪ Cleveland Clinic
Co-Chair: Michael Thompson, MD, PhD ▪ Washington University School of Medicine

- 1:00-1:25 PM
  Spatiotemporal Control of Hepatic Stellate Cell Activation in MASLD Fibrosis
  Ekihiro Seki, MD, PhD ▪ Cedars-Sinai Medical Center

- 1:25-1:50 PM
  Regulation of the Fasted Response in the Liver by PPARalpha
  David Moore, PhD ▪ University of California at Berkeley

- 1:50-2:15 PM
  Cross-Organ Pathways of Fibrosis: What Makes the Liver Special?
  Laura Nagy, PhD ▪ Cleveland Clinic

- 2:15-2:40 PM
  GP130 Signaling as a Therapeutic Strategy in Alcohol-Associated Liver Disease
  Cristina Llorente, PhD ▪ University of California, San Diego

- 2:40-2:55 PM
  ABSTRACT 015: Tumor Necrosis Factor-Inducible Gene 6 Protein and its Mimetic Peptide Enhance Liver Regeneration by Modulating ASGR1 Activity
  Hayeong Jeong, BS ▪ Pusan National University

- 2:55-3:10 PM
  ABSTRACT 016: Early Bile Acid Supplementation in Mice Ameliorates Developmentally Programmed Immune Deficits and Fibroinflammatory Liver Disease
  Michael Thompson, MD, PhD ▪ Washington University School of Medicine

SLAM Awards Ceremony and Closing Remarks
3:30-4:30 PM

Attendee Free Time - Enjoy Cape Coral
4:30-6:00 PM

Attendee Dinner
6:00-7:00 PM

Meeting Adjournment
7:00 PM

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Abstract-driven Short Talks

Session 002 – Liver Development and Regeneration

Abstract 001

A Humanized Bile Acid Milieu Exacerbates Liver injury and Biliary Tree Developmental Defects in a Mouse Model of Alagille Syndrome

Hamed Jafar-Nejad[1], Yaniv Faingelernt[1,*], Duncan Fox[1], Mario F. Lopez[1], Neda Zarrin-Khameh[1], Huiping Zhou[2], Stacey S. Huppert[3], Paul A. Dawson[4], and Saul J. Karpen[2]
[1]Baylor College of Medicine, Houston, TX; [2]Virginia Commonwealth University and Richmond VA Medical Center, Richmond, VA; [3]Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [4]Emory University, Atlanta, GA; *Current address: Schneider Children's Medical Center, Petah Tikva, Israel

Background: Haploinsufficiency of *JAG1* causes Alagille syndrome (ALGS), a multisystem disorder in which liver disease is often a life-threatening feature, characterized by bile duct paucity, cholestasis, inflammation, and fibrosis. Mouse models with *Jag1* heterozygosity recapitulate ALGS-like liver phenotypes. However, since mice have a more hydrophilic bile acid pool than humans, the impact of hydrophobic bile acids on the *Jag1*$^{+/-}$ liver phenotypes remains unknown. **Methods:** To create a model with a more human-like bile acid profile, we combined *Jag1* heterozygosity with *Cyp2c70* knockout, which eliminates hydrophilic muricholic acids and shifts the bile acid pool toward a more hydrophobic human-like profile. Cohorts were analyzed by histology, biliary ink injection, serum chemistry, and liver bile acid profiling at postnatal day 30, when *Jag1*$^{+/-}$ mice exhibit robust liver phenotypes. **Results:** *Jag1*$^{+/-}$; *Cyp2c70*$^{-/-}$ animals showed decreased body weight and increased liver-to-body weight ratios compared to all other genotypes. Serum total bile acids were markedly elevated in *Jag1*$^{+/-}$; *Cyp2c70*$^{-/-}$ mice compared to their *Jag1*$^{+/-}$ and *Cyp2c70*$^{-/-}$ siblings. Hepatic bile acid profiling showed dramatic bile acid accumulation in both *Jag1*$^{+/-}$ and *Jag1*$^{+/-}$; *Cyp2c70*$^{-/-}$ animals, and a significant increase in bile acid hydrophobicity in the latter, consistent with enhanced hepatocellular stress. Histology revealed more frequent hepatocellular necrosis in *Jag1*$^{+/-}$; *Cyp2c70*$^{-/-}$ mice. Notably, although loss of *Cyp2c70* alone did not alter the bile duct-to-portal vein ratio, its combination with *Jag1* heterozygosity appeared to further reduce intrahepatic bile duct density compared to *Jag1*$^{+/-}$ animals. Ink injection studies similarly demonstrated diminished peripheral extension and density of the ink-filled intrahepatic biliary network in *Jag1*$^{+/-}$; *Cyp2c70*$^{-/-}$ mice compared to *Jag1*$^{+/-}$ mice. Liver injury was more severe in *Jag1*$^{+/-}$; *Cyp2c70*$^{-/-}$ females than males, consistent with published *Cyp2c70*$^{-/-}$ data. However, the reduction in *Jag1*$^{+/-}$; *Cyp2c70*$^{-/-}$ biliary tree complexity was comparable between sexes. **Conclusions:** Our findings indicate that a more hydrophobic, human-like bile acid profile exacerbates liver injury in the *Jag1*$^{+/-}$ ALGS mouse model and may further worsen intrahepatic biliary abnormalities. Because ink injection depends on biliary patency, the reduced biliary tree visualization may reflect either true structural loss or failure of ink to traverse obstructed ducts; however, the decreased bile duct-to-portal vein ratio supports an underlying reduction in biliary tree density. These findings suggest that hepatic bile acid composition plays an unanticipated role in *Jag1*$^{+/-}$ biliary tree formation, with implications for bile duct development and pathophysiology of childhood cholestatic diseases. **Acknowledgements:** Supported by R01DK132751 (to HJ and SSH), funds from Baylor College of Medicine (to HJ), R01DK135815 (to SJK), R01DK140485 (to PAD), Research Career Scientist Award (IK6BX004477 to HZ), and VA ShEEP grants (1 IS1 BX004777-01 to HZ). We thank Tom Gridley for the *Jag1*$^{+/-}$ strain.

Abstract 002

Understanding How the Organism Monitors Liver Function to Properly Time Regeneration

Kristin A. Knouse[1,2] and Kristina E. Lopez[1,2]
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Background: The liver has an exceptional ability to regenerate after injury which enables it to recover from diverse insults. To initiate liver regeneration, the body must first sense that liver injury has occurred and stimulate hepatocyte proliferation. Hepatocyte growth factor (HGF) plays a central role in this process. Levels of HGF protein increase in the blood after liver injury, first via the release of pre-synthesized protein stores and then via upregulation of *Hgf* mRNA. HGF signaling then stimulates hepatocyte proliferation. Despite the well-documented role for HGF in driving liver regeneration, it is unknown how the organism monitors liver function to regulate *Hgf* expression and thereby initiate regeneration. We set out to understand how the body senses liver injury to initiate

regeneration. **Methods:** We employed two orthogonal liver injury models—partial hepatectomy and acetaminophen overdose—to induce liver injury and trigger regeneration. We first used RNA FISH to determine which cell type in the liver upregulated *Hgf* after liver injury. We then used parabiosis surgeries to test whether these cells could sense liver injury via the bloodstream. We then reconstituted this signaling *ex vivo* by culturing stellate cells in mouse plasma and employed classical biochemistry approaches to determine the nature of the signal. We then performed untargeted metabolomics on mouse plasma to identify candidate signals and tested individual candidates in our *ex vivo* system. **Results:** We find that hepatic stellate cells are responsible for upregulating *Hgf* after various forms of liver injury. By combining parabiosis surgeries and *ex vivo* reconstitution, we showed that the default state of stellate cells is to express high levels of *Hgf*, but when the liver is functioning properly, a bloodborne, albumin-dependent factor suppresses *Hgf* expression. By performing untargeted metabolomics and subsequent validation experiments, we identified retinol to be this albumin-dependent signal. We find that when the liver is functional, levels of albumin-associated retinol are high and serve to suppress *Hgf* expression. Upon liver injury, retinol levels drop. Additionally, the levels of the free fatty acids palmitate and linoleate rise and compete with retinol for albumin binding. Collectively, these changes reduce the levels of albumin-associated retinol, thereby allowing stellate cells to upregulate *Hgf*. **Conclusions:** Our data support an elegant "AND" gate mechanism by which the organism monitors two orthogonal aspects of liver function—retinol metabolism and fatty acid metabolism—and is therefore able to reliably detect liver injury and initiate regeneration. Future work will explore whether manipulating this axis is sufficient to accelerate and augment *Hgf* expression and thereby improve recovery after extreme liver injury. **Acknowledgements:** This research was supported by startup funding from MIT. Kristina E. Lopez has been supported by a Ford Foundation Fellowship, David H. Koch Fellowship, and MIT School of Science Fellowship.

Abstract 003

Liver Regeneration CRISPR Screen Identifies E3 Ligase NEURL1B Regulates Hepatocyte Mitosis by Destabilizing Microtubule Organizing Centers

Dingzi Yin[1], Alexandra M. Vazquez Salgado[1,2], Chunmiao Cai[1], Amber W. Wang[2], Noam Erez[2], Markcus A. Lee II[1], Yuebo Zhang[1], Jie Chen[3], Ling Lu[4], Hsin-Yao Tang[5], and Kirk J. Wangensteen[1*]

[1]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN; [2]Perelman School of Medicine, University of Pennsylvania; Philadelphia, PA; [3]Department of Hepatobiliary Surgery, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China; [4]Department of Pathology, Shanghai Tenth People's Hospital; Shanghai, China; [5]Wistar Institute, Philadelphia; Philadelphia, PA

Background: The remarkable ability of the liver to regenerate after injury relies on hepatocyte cell division. Hepatocytes are specialized cells that are differentiated and often polyploid, with multiple copies of their chromosomes. Polyploidy is accompanied by extra centrosomes—the organelles that serve as microtubule organizing centers (MTOCs). Having supernumerary centrosomes can result in the formation of multipolar spindles during mitosis, which can cause mistakes in chromosome alignment and prolong the process of cell division. How hepatocytes successfully divide despite potential challenges related to polyploidy and supernumerary centrosomes remains unclear. **Methods:** To investigate how hepatocytes manage these mitotic challenges, we developed a robust in vivo CRISPR interference (CRISPRi) screening system in regenerating mouse livers. This approach allowed us to systematically suppress gene activity and identify which genes are specifically required for hepatocyte proliferation, as opposed to other cell types. We compared our findings with gene essentiality data from various cell lines to pinpoint genes unique to hepatocyte division. Further, we used genetically engineered knockout mice, RNA sequencing, and proteomic analyses to study the function of NEURL1B and its role in regulating hepatocyte proliferation. **Results:** Our study identified the E3 ubiquitin ligase NEURL1B as a key factor required for hepatocyte division during liver regeneration and tumor formation. Interestingly, NEURL1B is not necessary for cell division in other cell types or during normal liver development. This specific requirement is linked to a spindle pole organization pathway that is unique to hepatocytes. NEURL1B is found at centrosomal and non-centrosomal MTOCs, where it destabilizes these structures. We discovered that NUMA1, a protein involved in spindle formation, is a substrate of NEURL1B. Early in mitosis, hepatocytes display multiple MTOCs organized by NUMA1. As mitosis progresses into metaphase with dynamic remodeling of these MTOCs into two spindle poles, NEURL1B facilitates this process via centrosome inactivation, a mechanism rarely described in the literature. Without NEURL1B, hepatocytes experience delays in mitosis, improper chromosome alignment, and increased polyploidy. Data from human single nucleus RNA sequencing and cell culture experiments indicate that NEURL1B plays a similar role in human hepatocytes, suggesting a conserved mechanism across species. **Conclusion:** By studying mitosis in living organisms, our

research highlights a unique strategy employed by hepatocytes to handle extra centrosomes during cell division. We have identified NEURL1B as a critical regulator of this pathway. These findings not only advance our understanding of liver regeneration but also suggest potential targets for new therapies aimed at promoting liver repair and treating liver cancer. Acknowledgements: Arnold and Mabel Beckman Foundation, Mayo Clinic Center for Regenerative Medicine, Mayo Clinic Center for Cell Signaling in Gastroenterology (C-SiG), NIH Grant P30DK084567. **Conflict of interest:** K.J.W. has received research support from Calico Life Sciences and Pfizer. The remaining authors declare no competing interests.

Session 004 – Liver Homeostasis and Injury

Abstract 004

Leveraging the NrHV Mouse Model to Investigate Liver Microenvironmental Remodeling Following Chronic Hepacivirus Cure

Hsuan-An Chen[1], Mariana Nogueira Batista[1], Tzu-Jou Chen[1], Nataliya Prokhnevska[2], Alex Lercher[1], Leon Louis Seifert[1], Bruno Cogliati[3], Tesia Bobrowski[4], Alice O. Kamphorst[2], Brad R. Rosenberg[4], and Charles M. Rice[1]
[1]Laboratory of Virology and Infectious Disease, The Rockefeller University, New York, NY; [2]Cancer Immunology Research Program, Icahn School of Medicine at Mount Sinai, New York, NY; [3]Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York, NY; [4]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY

Background: Hepatitis C virus (HCV) infection is a major risk factor of hepatocellular carcinoma (HCC), a leading cause of cancer-related mortality worldwide. Although the advent of direct-acting antivirals (DAAs) has substantially reduced HCC incidence, patients with established liver disease remain at elevated cancer risk after viral clearance. The mechanisms underlying this persistent risk remain poorly defined, limiting effective prevention. A key unanswered question is whether viral cure after chronic infection fully reverses liver damage or instead leaves behind durable alterations in the liver environment that promote HCC. The lack of tractable animal models and clinically relevant cure strategies have limited research that enable causal and mechanistic analyses of liver remodeling following viral cure. To address this, we developed an immunocompetent mouse model of chronic infection using Norway rat hepacivirus (NrHV), which recapitulates fibrosis and spontaneous HCC. Building on this model, we have now established a clinically analogous DAA regimen that cures chronically infected mice, enabling controlled, longitudinal interrogation of liver environment remodeling as a function of infection duration and time since cure. **Methods:** The C57/bl6 male mice aged 10-12 weeks old were transiently depleted with CD4 antibody before the infection of NrHV to initiate chronic infection. To determine the effect of viral cure, NrHV- or mock-infected mice at 3 months post-infection were treated with either vehicle or a combination of two DAAs, sofosbuvir and pibrentasivir, twice a day for 28 days. The viremia was longitudinally monitored up to 3 months post-treatment and livers were harvested for histology assessment, RNA-seq and immunophenotyping. **Results:** Our DAA regimen achieved an approximately 75% cure rate in chronically infected mice. At 3 months post-cure, chronic infection was largely reversible at both the histological and transcriptomic levels in the liver. Notably, we observed a significant decrease in regulatory T cells (Tregs) and the emergence of tissue-resident CD8 T cells in the cured liver. Finally, antibody-mediated depletion studies indicate that CD8 T cells are required for successful DAA-mediated viral clearance in chronically infected mice. **Conclusion:** With the establishment of this cure regimen, we will further investigate how chronic viral infection imprints the liver environment and how the timing of viral cure (i.e., short- versus long-term infection) shapes immune surveillance, cancer risk, and liver resilience to subsequent injury. These studies aim to provide a mechanistic framework for the persistent risk of hepatocellular carcinoma following HCV cure.
Acknowledgements: We thank the funding support from F99/K00 NCI Predoctoral to Postdoctoral Fellow Transition Award, SNF Institute for Global Infectious Disease Research Grant and Rockefeller University Advancement of Translational Research Award.

Abstract 005

Cholangiocyte–Neutrophil Crosstalk in Primary Sclerosing Cholangitis (PSC): The MIF–CD74 Axis as a Driver of Neutrophilic Inflammation

Mobin Ibne Mokbul[1,2], Abid A. Anwar[1,2], Papawee Sutthirat[1,2], Maleeha Kalaiger[1,2], Zhengsheng Zhang[1,2], Usman Yaqoob[1,2], Moira Hilscher[1,2], Robert C. Huebert[1,2], and Nidhi Jalan-Sakrikar[1,2]
[1]Division of Gastroenterology & Hepatology, Mayo Clinic, Rochester, MN; [2]Centre for Cell Signaling in Gastroenterology (C-SiG), Mayo Clinic, Rochester, MN.

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Background: Primary sclerosing cholangitis (PSC) is a progressive cholangiopathy characterized by biliary inflammation and fibrosis. While immune dysregulation is central to PSC, the role of neutrophils and their interaction with bile duct epithelial cells, cholangiocytes, remain insufficiently defined. We hypothesized that cholangiocyte-derived factors engage with receptors on neutrophils to promote their pathogenic activation within the peri-biliary niche. **Methods:** Human PSC and control liver tissues were analyzed using immunofluorescence (IF) to assess neutrophil localization, and formation of neutrophil extracellular traps (NETs). Murine PSC models (Mdr2⁻/⁻ and DDC-diet) were used for mechanistic insights and in vivo functional relevance. Neutrophils were depleted using anti-Ly6G antibodies, and NET formation was inhibited using the PAD4 inhibitor, Cl-amidine. Spatial transcriptomics (NanoString GeoMx DSP) and cell–cell communication analyses were performed to identify ligand–receptor interactions for cholangiocyte-neutrophil axis on human PSC livers. **Results:** Neutrophils were markedly enriched in human and murine PSC livers. These neutrophils were localized adjacent to bile ducts, exhibiting increased NETs formation (CitH3 positivity). Peripheral neutrophil depletion reduced liver infiltration, attenuating inflammation and fibrosis. Similarly, pharmacologic inhibition of NETosis also attenuated liver enzymes and fibrosis, supporting a pathogenic role for liver neutrophils. Notably, neutrophil infiltration progressively increased in peri-biliary regions over the course of disease. This pattern suggests not only ongoing recruitment of circulating neutrophils but also the potential for enhanced survival or retention of resident neutrophils within the biliary niche. Supporting this, spatial transcriptomics and CellChat analyses identified a prominent MIF–CD74 signaling axis between cholangiocytes and neutrophils, implicating a role in neutrophil persistence and accumulation. Macrophage migration inhibitory factor (MIF) expression was significantly upregulated in PSC cholangiocytes, including patient-derived organoids, and redistributed to the cell periphery under inflammatory conditions. $CD74^+$ neutrophils were enriched in peri-biliary regions in PSC and displayed heterogeneous but prominent expression patterns. Spatial proximity analysis demonstrated non-random localization of MIF^+ cholangiocytes and $CD74^+$ neutrophils, supporting functional interaction. MIF expression was validated with IF and cytokine array in cholangiocytes exposed to TNFα. Analysis on publicly available single cell dataset of human PSC livers also revealed proinflammatory and prosurvival neutrophil signatures consistent with pathogenic activation. **Conclusions:** The MIF–CD74 axis represents a central mechanism of cholangiocyte–neutrophil crosstalk in PSC, may be by promoting neutrophil persistence, activation, and downstream tissue injury. Targeting this pathway offers a biologically plausible and translationally actionable strategy to modulate innate immune–driven fibrosis in PSC. **Acknowledgements:** The work on this project was supported by funding from AASLD Gupta Award in PSC, PSC Partners Seeking a Cure, Canada Research Award, Mayo Clinic K2R Award.

Session 006 – Plasticity and Transdifferentiation

Abstract 006
Sox9 Regulates Hepatocyte Plasticity During Chronic Liver Injury

Joseph Lee[1], Jackie Brinkman[2,3], Fransky Hantelys[4], Jasmine Sun[1], Hannah R. Hrncir[1], Kendall G. Kanakanui[1,5], Brianna Goodloe[4], Jesse R. Raab[2], and Adam D. Gracz[1,3]
[1]Cell Biology and Physiology, [2]Genetics and Molecular Biology, [3]Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC; [4]Division of Digestive Diseases, [5]Genetics and Molecular Biology, Emory University, Atlanta, GA

Background: The liver possesses striking regenerative potential. In response to chronic cholestatic injury, hepatocytes can acquire plasticity and transdifferentiate to biliary epithelial cells (BECs). In liver development, the transcription factor *Sox9* normally regulates the differentiation of bipotential hepatoblasts, committing them to a BEC lineage and forming intrahepatic bile ducts (IHBDs). Resembling biliary development, hepatocytes also express *Sox9* in response to cholestasis. However, the role of *Sox9* in hepatocyte plasticity remains unknown. **Methods:** To determine if *Sox9* is required for plasticity, we used AAV8.TBG.Cre to delete *Sox9* in all hepatocytes from $Sox9^{fl/fl}$:$R26^{LSL-tdTomato}$ mice (Sox9HepKO) and induced lineage-tracing, prior to 6 weeks of 3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC) feeding to induce cholestatic injury. We then used antibodies against BEC markers A6 and OPN and conducted gene expression and chromatin analysis through RNA-seq, ATAC-seq, and CUT&RUN. **Results:** *Sox9* deletion resulted in a decrease in lineage-traced BECs originating from hepatocyte-ductal transdifferentiation (HDT) and an increase in metaplastic hepatocytes expressing A6 and OPN. Chromatin assays and RNA-seq on Sox9+ hepatocytes isolated from uninjured livers and following 6

weeks of DDC implicated NF-κB signaling in these regenerative responses. Organoid assays further supported NF-kB signaling as a regulator of HDT. **Conclusions:** Together, these data suggest that both *Sox9* and NF-kB are necessary for hepatocyte transdifferentiation in response to chronic cholestatic injury. **Acknowledgements:** NIH/NIDDK R01DK132653 (Gracz), NIH/NIDDK F31DK134199 (Hrncir)

Abstract 007

Non-canonical TFEB Activation Drives Hepatocyte Plasticity and Tumor Malignancy in TSC1 Deficient Livers

Chen Zhang, Sha Neisha Williams, Hong-Min Ni, and Wen-Xing Ding
Department of Pharmacology, Toxicology and Therapeutics, The University of Kansas Medical Center, Kansas City, KS

Background: Hepatocellular carcinoma (HCC) is a highly heterogeneous disease, and hepatocyte plasticity is increasingly associated with poor prognosis and aggressive tumor behavior. Transcription factor EB (TFEB) is a master regulator of lysosomal biogenesis and cellular metabolic adaptation. Although Tuberous Sclerosis Complex 1 (TSC1) loss leads to mTORC1 activation, its impact on TFEB regulation and hepatocyte fate in liver tumorigenesis remains unclear. This study aimed to determine how hepatic Tsc1 deletion influences TFEB activity and hepatocyte plasticity during HCC development. **Methods**: *Tsc1* Flox/Flox and *Tfeb* Flox/Flox mice were crossed with albumin Cre mice to generate liver-specific *Tsc1* knockout (L-*Tsc1* KO) and L-*Tsc1/Tfeb* double KO (DKO) mice. Additionally, a liver-specific *Tfeb* knockin (L-*Tfeb*-KI) mouse was created. These mice were housed for various time points up to 12 months. Liver injury, tumor development, and histological changes were assessed by biochemical assays and immunohistochemistry. Human liver and HCC tissue microarrays were analyzed for TFEB and CK19 expressions. Bulk and single cell RNA-sequencing datasets from mice and human HCC were analyzed to define TFEB associated transcriptional programs. **Results**: Loss of hepatic *Tsc1* resulted in robust mTORC1 activation, as indicated by increased phosphorylation of S6 and 4EBP1. Despite this, TFEB exhibited increased nuclear localization and transcriptional activation. L-*Tsc1* KO livers showed marked hepatocyte plasticity, characterized by reduced HNF4α expression and increased YAP signaling, and developed spontaneous HCC with enrichment of SOX9 and CK19 positive biliary epithelial cell like populations. Deletion of TFEB attenuated ductular reaction, fibrosis, and progenitor-like features, and partially restored hepatocyte identity, but had limited effects on liver injury and tumor incidence. Instead, TFEB deletion altered tumor phenotype and was associated with reduced progression toward a progenitor-like state. In human HCC datasets, increased TFEB activity correlated with YAP activation, SOX9 expression, and a progenitor-like transcriptional signature associated with poor clinical outcome. **Conclusions**: Hepatic *Tsc1* loss induces non-canonical activation of TFEB despite mTORC1 hyperactivation. TFEB primarily promotes hepatocyte plasticity and the development of a malignant tumor phenotype, rather than tumor initiation. These findings identify TFEB as a key regulator of tumor heterogeneity and progression in HCC.
Acknowledgements: *R01AA031230.*

Abstract 008

De Novo Pathological Macrophage Niches in the Injured Liver Persist Following Recovery and Sustain Prolonged Tissue Sensitivity to Secondary Insults

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Background: Metabolic-associated steatotic liver disease (MASLD) represents a spectrum of disease states in which the liver undergoes profound changes ranging from asymptomatic simple steatosis to the more advanced stages of the disease, including non-alcoholic steatohepatitis (MASH) and hepatocellular carcinoma. During the earlier stages of the disease, lifestyle changes and subsequent weight loss appear to alleviate symptoms. Yet, little is known about the liver's capacity to recover following such dietary changes and subsequent weight loss. **Methods:** Mice were placed on a choline-deficient, amino acid-defined diet (CDA-HFD) with sugar water for a period of 4 weeks to induce MASLD. Mice were then switched to a conventional chow diet with regular water for 2–40 weeks to investigate liver recovery using immunofluorescence microscopy, histology, and spatial lipidomics. Using $Il34^{KO}$ $Lrat^{\Delta Csf1}$ transgenic mice we investigated the role of these cytokines in MASLD in greater detail. **Results:** Here, we show that in a murine model of diet-induced MASLD, the liver exhibits a remarkable ability to clear lipids following a dietary switch and disease resolution. However, during the early stages of MASLD, we observed that macrophages—either of embryonic or monocyte origin—give rise to highly autofluorescent structures. In later stages of the disease, these structures resemble ceroid-laden macrophages described previously. As the disease progresses, these macrophages become increasingly abundant, cluster together, and are eventually encapsulated by stellate cells to form granuloma-like structures. Notably, even after lipid clearance and clinical recovery from MASLD, these structures persist for at least 40 weeks, representing a long-term compositional change in the recovered liver. We show that these structures contain various lipids absent from the healthy liver, most notably the ganglioside GM1. These structures also form a localized niche containing $GPNMB^+$ lipid-associated macrophages (LAMs), which are uniquely dependent on local CSF1 and IL-34 produced by adjacent stellate cells. In the absence of stellate cell-derived CSF1 and IL-34, these LAM-stellate cell aggregates are disrupted and cleared from the liver. Finally, we show that the presence of these structures confers increased sensitivity of the MASLD-recovered liver to acute CCl_4-induced liver injury. Conversely, when CSF1 and IL-34 are absent—and the structures are no longer present—this heightened sensitivity is abolished. **Conclusions:** Taken together, our findings reveal significant long-term compositional change of the MASLD-recovered liver, indicating an altered post-MASLD hepatic homeostasis with consequences for secondary hepatic insults. **Acknowledgements:** The work presented here was made possible through Marie Skłodowska-Curie and FWO postdoctoral funding awarded to Pieter A. Louwe and ERC/FWO project funding awarded to Prof. Dr. Martin Guilliams and Prof. Dr. Charlotte Scott.

Session 009 – MASH and Fibrosis

Abstract 009

Dissecting the Function of Macrophage mTORC1 Signaling During the Pathogenesis of Metabolic Disease-associated Steatohepatitis

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Background: Metabolic dysfunction-associated fatty liver disease (MASLD) is one of the leading causes of liver failure in the world. Nevertheless, the mechanisms involved in MASLD development are not well understood, and there are no specific treatments beyond lifestyle modifications. Thus, there is an active effort to identify culprit cellular processes to gain mechanistic insight for developing treatment strategies. Recent studies have indicated renewed interest in the role of macrophages in MASLD. Different subsets of macrophages contribute to hepatic inflammation and fibrogenesis during MASLD progression to metabolic disease-associated steatohepatitis (MASH). However, little is known about the function of mTORC1 signaling and autophagy/lysosome-mediated lipid metabolism in hepatic macrophages during MASH. **Methods**: To explore

the role and mechanism of macrophage mTORC1 signaling in diet-induced MASH, we determined the hepatic macrophage mTORC1 signaling and autophagy during the pathogenesis in mouse and human MASH samples. In addition, we generated macrophage-specific Raptor KO mice to evaluate the protective effect of macrophage mTORC1 inactivation against steatosis and fibrosis during MASH progression. Moreover, we evaluated the expression of amino acid transporters in hepatic macrophages to investigate the mechanism regulating mTORC1 activation during MASH. **Results:** We found that hepatic macrophage mTORC1 signaling was highly elevated during the development of MASH in mouse and human, which interfered with macrophage-mediated lipid metabolism through inhibiting autophagy. Blocking macrophage mTORC1 signaling significantly restored autophagy and alleviated steatosis and fibrosis in diet-induced MASH. Furthermore, we demonstrated that the mTORC1 hyper-activation in macrophages was critically linked to amino acids, confirmed by the observation that the amino acid transporter SLC7A8 was highly expressed in hepatic macrophages while SLC7A8 expression was upregulated in macrophages and associated with mTORC1 activation during MASH. **Conclusions:** We characterize macrophage mTORC1 signaling as a marker and critical mediator of the pathogenesis of MASH through inhibiting the lysosomal-autophagy system. We further reveal that amino acids are key mTORC1 inducers in hepatic macrophages during MASH, implying the selective inhibition of amino acid-induced mTORC1 activation may work as a novel therapeutic strategy. **Acknowledgements:** Pilot and Feasibility grant from Pittsburgh Liver Research Center (5P30 DK120531-07).

Abstract 010

Hepatocyte Notch Drives Immune Cell Recruitment in MASH

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Background: The prevalence of metabolic dysfunction–associated steatotic liver disease (MASLD) continues to rise globally and is now a leading cause for liver transplantation in the U.S. Metabolic dysfunction–associated steatohepatitis (MASH), an advanced stage of MASLD, is associated with increased liver inflammation, but the molecular mechanisms underlying hepatic immune cell trafficking are incompletely understood, and no approved therapies directly target these pathways. We previously established a mouse model with hepatocyte-specific Notch activation (L-NICD), which develops diet-independent liver immune cell accumulation and fibrosis, without significant hepatocyte injury. One mechanism of increased immune cell infiltration in these mice is through increased expression of monocyte chemoattractant protein 1 (MCP1), a key chemokine known to regulate monocyte migration. However, aside from monocyte-derived macrophages, multiple immune cell populations remain elevated in L-NICD:MCP1^{HepKO} mice, suggesting hepatocyte Notch activity regulates MCP1-independent mechanisms that contribute to broader immune cell recruitment. **Methods:** Sequential RNA-seq data across three MASH mouse models and ChIP-seq data from hepatocyte cell lines were used to identify candidate Notch downstream factors regulating immune cell recruitment. Functional roles were assessed using genetically engineered mouse models. **Results:** Integrated screening identified intercellular adhesion molecule-1 (ICAM-1) as a Notch downstream factor upregulated in hepatocytes in MASH. While ICAM-1 in endothelial cells is well known for mediating leukocyte transmigration, its role in hepatocytes during MASH remains unclear. To investigate this, we generated mice with hepatocyte-specific ICAM-1 deletion in the context of Notch activation (L-NICD:ICAM1^{HepKO}). Unexpectedly, L-NICD:ICAM1^{HepKO} mice showed increased CD45^{+} immune cells as compared to either Cre- control or L-NICD mice. Not all immune cell populations were equally affected – of those tested, CD8^{+} T cells were markedly increased by ICAM deletion. Notably, increased CD45^{+} immune cells in L-NICD:ICAM1^{HepKO} mice was accompanied by significantly increased liver fibrosis as assessed by Sirius Red staining (positive area: 0.72 ± 0.48% in L-NICD vs. 2.24 ± 0.75% in L-NICD:ICAM1^{HepKO}) and fibrotic marker expression. **Conclusions:** These findings suggest a previously unrecognized protective role of hepatocyte ICAM-1 in governing hepatic immune homeostasis during MASH progression. Ongoing studies aim to define ICAM-1-sensitive immune cell populations and molecular mechanisms underlying this effect, potentially enabling novel therapeutic strategies for MASH by targeting the hepatocyte Notch–ICAM-1 axis to regulate immune cell trafficking. **Acknowledgements:** This research is supported by NIH RO1DK131169 and RO1DK119767

Abstract 011
Evaluating the Effect of MASLD Polygenic Risk in iPSC to Study Heterogeneity in Disease Progression and Resmetirom Response
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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) affects ~30% of the population, yet only a subset of individuals progress to metabolic dysfunction-associated steatohepatitis (MASH). The molecular and genetic drivers of disease progression and variability in response to resmetirom, the first FDA-approved therapy for MASH, remain poorly understood. We hypothesize that genetic variation captured by polygenic risk scores (PRS) contributes to heterogeneity in MASLD progression and therapeutic response. **Methods:** Using genomic data from the POST cohort, we calculated a weighted polygenic risk score (PRS) from 15 replicated loci identified in the trans-ancestry genome-wide association study of chronic ALT elevation in the Million Veterans Program. Induced pluripotent stem cells (iPSCs) from donors in the top and bottom 20th percentiles of the cohort (e.g., high vs. low MASLD PRS, N=12) were incubated for 24hr under conditions of metabolic stress (palmitate and/or oleate) or a BSA control, with or without 100μM resmetirom, and neural lipid accumulation, cellular oxidative stress, and mitochondrial membrane potential were quantified. Experiments were repeated after differentiation into iPSC-derived hepatocytes. **Results:** Under basal conditions, high MASLD PRS lines had reduced mitochondrial membrane potential (0.46±0.11 fold, $p<0.01$) and oxidative stress (0.19±0.04 fold, $p=0.01$) compared to low PRS lines. Exposure to 0.2mM palmitate, a fatty acid preferentially oxidized by mitochondria, significantly increased mitochondrial membrane potential (1.23±0.06 fold, $p<0.01$) and oxidative stress (4.19±0.91 fold, $p<0.01$) in the high PRS lines. These effects were partially attenuated by co-treatment with 0.4mM oleate. In contrast, low PRS lines showed no significant change in mitochondrial membrane potential and only modest increases in oxidative stress (2.23±0.07 fold, $p<0.01$), which were fully reversed with oleate supplementation, indicating greater metabolic resilience. Resmetirom treatment significantly reduced oleate-induced cellular steatosis in both groups; however, high-PRS iPSCs demonstrated a greater reduction than low-PRS iPSCs. Notably, high PRS lines displayed substantially greater inter-line variability across all cellular measures compared to low PRS lines (e.g., 78.1% vs. 10.2% CV for oxidative stress measured after palmitate + oleate treatment). Similar findings were observed after differentiation into iPSC-derived hepatocytes. **Conclusions:** MASLD polygenic risk is associated with distinct mitochondrial and stress-response phenotypes, as well as increased cellular heterogeneity under metabolic challenge. These findings suggest that PRS may help stratify disease progression and predict responsiveness to resmetirom. **Acknowledgements:** This work was supported by NIH R01 DK130391 and the UCSF Liver Center P30 DK026743.
Session 012 – Liver Cancer

Abstract 012
AMPK Mediates Loser Cell Elimination During YAP- Driven Cell Competition in CCA
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Background: Cell competition is a phenomenon in which fitter "winner" cells eliminate neighboring "loser" cells to maintain tissue homeostasis. In the liver, this process contributes to tumor progression, particularly in cholangiocarcinoma (CCA). Among the key regulators, YAP, a downstream effector of hippo signalling pathway, not only drives CCA but also shapes the competitive interactions between tumor (winner) and surrounding hepatocytes (loser). However, the mechanisms governing loser cell elimination in YAP- driven cell competition remain poorly defined. We hypothesized that energy stress in loser cells activates AMPK, promoting autophagy dependent loser cell elimination in YAP-driven cell competition. **Methods:** We employed the NICD-AKT mouse model of cholangiocarcinoma using hydrodynamic tail vein injection (HTVi) for *in vivo* construct delivery to study YAP-driven cell competition. We analyzed the localized AMP/ATP ratios and metabolic gradients at tumor boundaries using spatial metabolomics. CRISPR/Cas9-mediated knockout of AMPK and its upstream kinases was employed to test functional roles. **Results:** Peritumoral “loser” cells showed elevated AMP levels and AMPK activity at the tumor boundary. AMPK knockout impaired loser cell elimination. Surprisingly, loss of upstream

kinases did not, indicating non-canonical AMPK activation. **Conclusion:** AMPK acts as a metabolic checkpoint linking energy stress to autophagy driven elimination of loser cells in YAP-driven competition.

Abstract 013

Zonated Signaling Pathways Shape Oncogenic Progression in Hepatocellular Carcinoma

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Background: Alone, oncogenic mutations to the hepatocyte genome are poor drivers of cancer initiation. Additional signals and promoters are needed to induce oncogenic growth and lesion formation. The hepatic lobule consists of various regionalized signaling gradients that normally specify metabolic zonation and maintain liver function. We therefore explored how these compartmentalized signals influence tumor formation in the context of mutant-Wnt-driven HCC. **Methods:** Genetically engineered, conditional, oncogenic alleles were recombined using a hepatocyte specific AAV8.TBG.cre virus. A low viral titer was used to model the development of single mutant clones into early lesions and tumors in the mouse liver. Cancer models were profiled using spatial and bulk transcriptomic assays along with immuno-histochemistry techniques. Small molecule inhibitors and additional KO alleles were then used *in vivo* to validate findings from the transcriptomic assays. **Results:** Hyper activation of the Wnt signaling pathway, via an oncogenic ß-Catenin mutation, induced zone-3 differentiation, which was not permissive to cancer. Oncogenic growth required reduced Wnt signaling, MTOR activity and a proliferative translatome. Targeting MTOR and protein translation prevented mutant clone progression. Furthermore, transient MTOR suppression had a significant impact on late-stage tumor burden, reducing the number of tumors and extending survival. MAPK activation, which promotes a zone-1 phenotype, also enhanced Wnt-driven tumorigenesis by antagonizing Zone-3 differentiation. Strikingly, the level of genetic MAPK activation or ectopic MAPK activation in a precancerous, steatotic, diseased state also influenced progression of Wnt mutant clones – indicating a just right level of MAPK signaling is required for tumorigenesis. **Conclusions:** How mutations integrate with liver physiology and hepatic zonation is critical for tumor initiation. Wnt-driven cancer needs to overcome a zone-3 differentiation barrier to activate RNA translation and proliferative programs that are permissive to oncogenic growth. By understanding the early biology of disease reveals vulnerabilities, such as MTOR activation, which can be targeted. This raises the opportunity to use preventative interventions in at risk populations to prevent cancer formation. **Acknowledgements:** Cancer Research UK, Cancer Grand Challenges: Team Specificancer.

Abstract 014

Endothelial Cell Therapy to Repair the Liver Vascular Network and Alleviate Liver Diseases

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Background: Liver sinusoidal endothelial cells (LSECs) are essential for liver homeostasis, function and regeneration. Specifically, activation of the master endothelial VEGFA/KDR axis in LSECs promotes hepatocyte proliferation following hepatectomy through endothelial-derived paracrine signaling. Building on this observation, we hypothesize that VEGFA delivered via nucleoside-modified mRNA encapsulated in lipid nanoparticle (mRNA-LNP) will enhance the engraftment of human induced pluripotent stem cell (hiPSC)-derived endothelial cell (iEC), enabling reconstitution of liver vasculature and mitigation of liver injury. **Methods**: Liver EC injury was induced in immunocompromised NSG mice using monocrotaline (MCT). The model was optimized by determining the appropriate MCT dose (150 vs 200 mg/kg), transplantation window when host ECs are the most damaged, and number of iECs delivered via intrasplenic injection (1×10^6 vs 1.5×10^6). Injury kinetics were assessed at 1, 3, and 7 days post-MCT. We developed a modified differentiation protocol to generate iECs expressing key endothelial markers, including CD31, CD144, KDR, and LYVE1. iECs were engineered to express luciferase to enable longitudinal tracking of engraftment via weekly in vivo bioluminescence imaging. VEGFA mRNA-LNPs were administered via retro-orbital injections, as previously successfully performed (Rizvi et al., *Cell Stem Cell*, 2023). **Results**: RT-qPCR analysis of MCT injury (200 mg/kg) showed significant reductions in CD31, KDR, and LYVE1

expression at 24 hours. Hematoxylin and eosin staining revealed progressive hepatic hemorrhage, initiating at 24 hours and becoming widespread by 72 hours. These findings identified 24 hours post-MCT administration as the optimal time point for iEC transplantation, when host ECs are maximally damaged or dysfunctional. Following transplantation of 1.5×10^6 iECs, VEGFA mRNA-LNP treatment significantly enhanced iEC survival and engraftment, resulting in a 23.4-fold increase in bioluminescence signal at 4 weeks post-transplantation compared to controls. Immunofluorescence staining for human $CD31^+$ iECs and mouse KDR^+ LSECs confirmed integration of large iEC clusters into the host sinusoidal network. Engraftment was primarily periportal (co-localized with $EpCAM^+$ ducts), with additional pericentral localization near GS^+ hepatocytes across multiple lobules. **Conclusions**: Overall, we established an optimized MCT-induced EC injury model suitable for evaluating endothelial cell-based therapies in alleviating liver disease. We demonstrated the potent role of VEGFA mRNA-LNP to significantly improve iEC survival and engraftment for at least 4 weeks post transplantation. This approach represents a promising strategy for advancing endothelial cell-based therapies for liver disease. **Acknowledgments**: CTSI TL1 Pre-Doctoral Fellowship 5TL1TR001410-09 to DM and NIHR01DK124361-01A1 to VGE.

Session 013 – Metabolism and Microbiome

Abstract 015
Tumor Necrosis Factor-inducible Gene 6 Protein and Its Mimetic Peptide Enhance Liver Regeneration by Modulating ASGR1 Activity
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Background: Restoration of liver mass after hepatectomy is essential for postoperative recovery, yet effective strategies to enhance liver regeneration remain limited. In liver transplantation, both donors and recipients depend on sufficient regeneration of the remnant liver, and impaired regeneration can result in serious complications, including liver failure and biliary disorders. Mesenchymal stem cell–derived secreted factors have emerged as promising regenerative mediators. Among these, tumor necrosis factor-inducible gene 6 protein (TSG-6) has been reported to exert hepatoprotective effects in injured livers. However, its role in liver regeneration remains unclear. Herein, we investigated the regenerative potential of TSG-6 and its underlying mechanism in mice subjected to partial hepatectomy (PHx). **Methods:** 8-week-old male C57BL/6J mice underwent 2/3 partial hepatectomy (PHx) and received an intraperitoneal injection of either PBS, as a vehicle, recombinant TSG-6 or synthetic peptide at the time of surgery. Mice were sacrificed at 0, 12, 24, 48, or 72 hr after PHx for serum and liver collection. **Results:** In a mouse model of two-thirds PHx, TSG-6 administration accelerated the recovery of liver mass and function, accompanied by earlier hepatocyte proliferation and transient lipid accumulation compared to vehicle treatment. TSG-6 directly bound asialoglycoprotein receptor 1 (ASGR1) and suppressed ASGR1/proprotein convertase subtilisin/kexin type 9 (PCSK9) activation, resulting in the upregulation of lipid transporters, including cluster of differentiation 36 and low-density lipoprotein receptor. This, in turn, increased hepatic lipid accumulation and ATP production, supporting the energetic demands of proliferating hepatocytes. Binding specificity of TSG-6 on ASGR1 was evaluated using a synthetic peptide (peptide7) corresponding to the ASGR1-binding region of TSG-6. Peptide7 recapitulated the regenerative functions of TSG-6 in PHx mice. However, neither TSG-6 nor peptide7 was effective in promoting liver regeneration ASGR1-knockdown mice with PHx. **Conclusions:** These findings demonstrate that targeting ASGR1 with TSG-6 or peptide7 enhances liver regeneration by modulating the ASGR1/PCSK9 axis and increasing the lipid-derived energy supply needed for hepatocyte proliferation. The therapeutic potential of TSG-6 and peptide7 in liver recovery merits further investigation. **Acknowledgements:** This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) to Youngmi Jung (RS-2025-00517677 & RS-2025-02215983, Republic of Korea).

Abstract 016

Early Bile Acid Supplementation in Mice Ameliorates Developmentally Programmed Immune Deficits and Fibroinflammatory Liver Disease

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Background: Maternal obesogenic diet exposure (MODE) promotes worse fibroinflammatory liver disease and alters bile acid homeostasis in offspring. One mechanism of transmission is via vertical transfer of an altered microbiome to offspring which affects the development of critical gut immune cell populations including regulatory T cells. Bile acids (BA) may be a critical intermediate in this process as we have previously shown that MODE alters the gut microbiome and decreases secondary bile acid levels in the gut lumen of early offspring. Furthermore, supplementation with a secondary bile acid, ursodeoxycholic acid (UDCA), in the perinatal period resolves deficiencies in the gut immune cell populations in MODE offspring. We hypothesize that early shifts in offspring bile acid metabolism will also affect hepatic immune cell populations and that early secondary bile acid supplementation will ameliorate developmental programming of liver disease. **Methods:** Beginning at four weeks of age, female mice were fed either chow (CON) or high fat, fructose, cholesterol (MODE) diet for 6 weeks before being bred with lean males. A subset of MODE offspring were gavaged with UDCA daily between 2 and 3 weeks of age. Liver was collected from offspring at 3 and 4 weeks of age. BA homeostasis was assessed through measurement of BAs via mass spectrometry on liver of 3-week-old offspring. Flow cytometry was performed on liver at 4 weeks of age to assess changes in immune cell populations. A cohort of offspring were weaned to a regular chow diet and at 10 weeks of age placed on DDC diet for 2 weeks to induce cholestatic liver injury. Histological, serum, and qPCR analyses were performed following DDC diet exposure. **Results:** BA profiling on 3-week-old liver identified an increase in abundance of tauro- and glycine- conjugated primary bile acids and a decrease in abundance of the secondary bile acid UDCA in MODE offspring. Hepatic CD4 lymphocytes, γδTCR lymphocytes, and regulatory T cells were decreased in MODE offspring liver. UDCA supplementation resolved decreases in these hepatic immune cell populations. After DDC diet feeding, MODE offspring exhibit increased ductular reaction, inflammation, fibrosis, and bile infarcts. UDCA supplementation early in life in MODE offspring decreased cholestatic liver injury in offspring. **Conclusions:** MODE shifts offspring bile acid metabolism including reductions in secondary bile acids with associated decreases in critical immune cell populations. Early bile acid supplementation could mitigate this developmental programming event and protect from increased fibroinflammatory liver disease susceptibility.

Poster Sessions

Poster Session – Alcoholic Liver Disease

Abstract 017

A Novel Nanoparticle Platform Enhances the Therapeutic Efficacy of a TSG-6–Derived Peptide in Alcohol-Related Liver Disease

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Background: Alcohol-related liver disease (ALD) is a globally prevalent chronic liver disorder caused by excessive and prolonged alcohol consumption, for which effective and practical therapeutic options remain limited. Previously, we demonstrated that tumor necrosis factor-inducible gene 6 protein (TSG-6), a mesenchymal stem cell-derived cytokine, and its mimetic peptide (peptide YJ) attenuate liver fibrosis in an ALD mouse model by inhibiting hepatic stellate cell (HSC) activation through suppression of CD44 cleavage into its intracellular domain (CD44ICD). In the present study, we aimed to enhance the therapeutic applicability of peptide YJ by incorporating it into a novel nanoparticle (NP)-based delivery platform, and to evaluate its therapeutic efficacy in ALD. **Methods:** Human primary HSCs at 70% confluence were serum-starved overnight and then treated with peptide YJ-loaded NPs (YJ-NPs) at concentrations equivalent to 43.2 or 86.4 ng/mL of YJ, or with empty NPs (Blank-NPs) as controls. For in vivo studies, 7-week-old male C57BL/6 mice were fed either

an isocaloric control diet or a 5% (v/v) ethanol-containing Lieber–DeCarli liquid diet for 12 weeks. At week 9, mice received weekly intraperitoneal injections of YJ-NPs (12.5 μg/kg) or Blank-NPs for 3 weeks while continuing the diet. Mice were sacrificed at week 12 for collection of serum and liver tissues. **Results:** Both YJ-NPs and Blank-NPs were efficiently internalized into human primary HSCs within 30 minutes to 1 hour, confirming effective cellular uptake. YJ-NPs showed minimal cytotoxicity at both tested concentrations. Notably, YJ-NPs significantly suppressed HSC activation markers, including α-SMA and TGF-β, and reduced CD44ICD levels at 48 and 72 hours post-treatment. In vivo, chronic ethanol feeding induced marked hepatic steatosis, hepatocyte injury, inflammation, and fibrosis compared to control diet-fed mice. Treatment with YJ-NPs significantly reduced serum AST and ALT levels, hepatic triglyceride accumulation, and histological liver damage, particularly fibrosis, compared with Blank-NP-treated mice. Furthermore, elevated hepatic CD44ICD expression observed in ethanol-fed mice was significantly decreased following YJ-NP treatment. **Conclusions:** These findings demonstrate that peptide YJ delivered via a novel NP-based platform is effectively targeted to the liver, where it inhibits CD44 cleavage, suppresses HSC activation, and ameliorates ALD-associated fibrosis. This delivery strategy provides sustained and stable peptide bioavailability and highlights the therapeutic potential of YJ-NPs as a promising treatment approach for ALD. **Acknowledgements:** This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) to Youngmi Jung (RS-2025-00517677 & RS-2025-02215983, Republic of Korea) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education to J. H. (RS-2025-25433636, Republic of Korea)

Abstract 018

Modeling Alcohol-induced Liver Injury Using a Human Liver-on-a-chip Microphysiological System

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Background: The current experimental approaches for studying alcohol-associated liver disease (ALD) have limitations due to insufficient expression and activity of alcohol-metabolizing enzymes in hepatocytes using the standard cell culture systems and animal models. We have developed a human liver-on-a-chip using human liver cells, which express relevant levels of molecules related to hepatocyte functions and drug- and alcohol-metabolizing enzymes. Our human liver-on-a-chip system contains human hepatocytes co-cultured with liver sinusoidal endothelial cells (LSECs). We hypothesize that this liver-on-a-chip model is a valuable model for studying ALD under human-relevant conditions and, in addition to hepatocytes, LSECs play a significant role in metabolizing acetaldehyde to prevent the progression of ALD. We tested this hypothesis using our liver-on-a-chip model. **Methods:** Liver-on-a-chip cultured with primary human hepatocytes only and hepatocytes plus LSECs, and standard 2D hepatocyte culture were used. Cells were also treated with 0.2 % ethanol (~43.5 mM). We measured albumin production, MRP2 expression, cytochrome p450 and alcohol-metabolizing enzymes, and levels of transaminases, with and without ethanol treatment. We also modified aldehyde dehydrogenase activity in LSECs using siRNA or Alda-1. **Results:** The liver-on-a-chip model showed higher expression of albumin, CYP1A2, CYP3A4, ADH1, ALDH2, and CYP2E1, and more biliary canaliculi formation compared with the standard 2D culture. Disease-relevant concentration of ethanol treatment increased AST, ALT, and acetaldehyde release in liver-on-a-chip without LSECs, but not in the 2D culture system, indicating high alcohol metabolizing capacity in a liver-on-a-chip. In contrast, the chip with LSECs lowered AST, ALT, and acetaldehyde. These data suggest that, in addition to hepatocytes, LSECs play a role in alcohol/acetaldehyde metabolism and hepatocyte damage. Consistent with this, alcohol metabolizing enzymes ADH1 and CYP2E1 are exclusively expressed in hepatocytes, whereas ALDH2 is expressed in both hepatocytes and LSECs. Silencing ALDH2 in LSECs increased AST, ALT, and acetaldehyde levels whereas activating ALDH2 with Alda-1 treatment reduced AST/ALT levels in ethanol-treated liver-on-a-chip, underscoring that ALDH2 in LSECs is crucial for protecting against ethanol-induced liver damage. **Conclusions:** A human liver-on-a-chip system nicely recapitulates human-relevant liver functions in vitro and is a useful tool to study ALD. Moreover, our study showed the crucial role of LSEC's ALDH2 in ALD. **Acknowledgements:** This work is supported by the National Institutes of Health. (R21AA031173 to Ekihiro Seki)

Poster Session – Cholestatic Liver Disease

Abstract 019

Selective Serotonin Reuptake Inhibitor Therapy Protects Against Cholestatic Liver Disease

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Background: Cholestatic liver disease is characterized by disrupted bile flow and the accumulation of toxic bile in the liver. Currently, there are limited effective therapies to slow disease progression. Patients afflicted with cholestatic liver disease have increased circulating serotonin compared to healthy patients. Prior research demonstrated that serotonin signaling regulates biliary proliferation, fibrosis, inflammation, and bile acid metabolism in models of cholestasis, with disparate effects on disease progression. Selective serotonin reuptake inhibitors (SSRIs) inhibit the serotonin transporter, which increases serotonin signaling. SSRIs are commonly used to treat depression and psychiatric disorders, but have also been used to treat pruritus in patients with cholestasis. However, the role of serotonin signaling on cholestatic liver disease progression and the impact of SSRI therapy in these patients remains largely unknown. **Methods:** Patient health records were collected from the University of Pittsburgh Clinical Trial Office. Wild-type (WT) animals were fed normal chow or 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet for 4 weeks. Animals were injected with vehicle control (VEH) or sertraline (SSRI, 10 mg/kg) for one week prior to sacrifice. Liver injury was evaluated by serum biochemistry, histology, qRT-PCR, and immunohistochemistry. Whole livers were processed for RNA-seq. **Results:** Following SSRI treatment, serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total bilirubin were decreased in DDC-fed mice, suggesting that SSRI therapy reduces hepatocyte and biliary injury during cholestasis. While fibrosis and inflammation were not affected by SSRIs, ductular mass was reduced, and hepatocyte proliferation was enhanced in DDC + SSRI mice versus DDC + VEH. RNA-seq analysis showed that SSRI treatments reduced cellular stress responses, including senescence, which was validated by P21 staining. Evaluation of clinical records reveals that roughly 15% of patients afflicted with cholestatic liver disease are currently prescribed an SSRI, demonstrating the clinical relevance of these findings. **Conclusions:** Our work reveals that SSRIs may suppress cellular stress responses in cholestatic liver diseases, thereby having a beneficial therapeutic effect. Future work will evaluate whether prolonged SSRI therapy can decrease hepatic fibrosis and inflammation in preclinical models. Further, additional work is needed to discern the potential protective effects of SSRIs in patients with cholestasis. **Acknowledgements**: R01DK103775.

Abstract 020

Biliary Reconstruction with B2M/CIITA Knockout Human Pluripotent Stem Cell Derived Tubular Organoids

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Background: Severe biliary stricture is a major cause of progressive cholestatic liver disease and liver failure in children, typically managed with palliative Roux-en-Y hepaticojejunostomy and ultimately liver transplantation. These approaches do not replace native extrahepatic bile duct (EHBD) tissue and are limited by anastomotic failure, ischemic injury, and immune-mediated ductopenia. While stem cell–derived biliary models have advanced understanding of intrahepatic cholangiopathies, platforms that recapitulate the structure, cellular

diversity, and immune properties of the EHBD remain lacking. This study aimed to generate a functional, immune-evasive, pluripotent stem cell–derived EHBD suitable for disease modeling and reconstruction. **Methods:** We developed a stepwise differentiation strategy to generate human biliary organoids (HBOs) from human induced pluripotent stem cells (iPSCs). Cellular composition, regional identity, and maturation were assessed by single-nucleus RNA sequencing, immunofluorescence, flow cytometry–based immunoprofiling, and bile acid transporter assays. To enhance immunocompatibility, hypoimmune iPSCs were generated via CRISPR-mediated deletion of B2M and CIITA, eliminating MHC class I and II expression. Engraftment, immune evasion, and injury responses were evaluated in humanized mice. For translational testing, HBOs were engineered into lumenized tubular grafts and anastomosed to the gallbladder in a rat model of biliary stricture. **Results:** iPSC-derived HBOs reproducibly formed multicellular structures composed of cholangiocytes, peribiliary progenitors, fibroblasts, and smooth muscle cells, recapitulating human EHBD architecture. Transcriptomic analysis identified regionally distinct extrahepatic cholangiocyte populations with signatures distinct from intrahepatic models. Hypoimmune HBOs lacking MHC expression showed robust engraftment and persistence in humanized mice without rejection. Notably, these HBOs also exhibited resistance to hypoxia-induced injury. Lumenized HBO grafts integrated with the host biliary system after gallbladder anastomosis in rats, restoring bile flow and improving biliary patency in severe stricture models. **Conclusions:** We establish a scalable, genetically tractable platform for generating functional, immune-evasive EHBD tissue from pluripotent stem cells. This system enables mechanistic studies of cholangiopathies, modeling of post-surgical and ischemic injury, and represents a step toward bile duct replacement therapies capable of evading HLA-mediated rejection. **Acknowledgements:** We would like to acknowledge the Bio-Imaging and Analysis Facility, the Integrated Pathology Research Facility, and the Comparative Medicine Division at CCHMC for their support. This work was supported by NIH grants DP2 DK128799-01 and R01DK135478 to T.T., and by the Korea–US Collaborative Research Program to D.C. and T.T.

Abstract 021

Dual Loss of β- and γ-Catenin From Cholangiocytes Leads to Intrahepatic Cholestasis, Intestinal Inflammation, and Microbiome Dysbiosis

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Background: Wnt/β-catenin signaling is essential for normal liver function and cell structure through its role in adherens junctions (AJ). These structures are composed of β-catenin, which links E-cadherin to a-catenin and the actin cytoskeleton. Loss of β-catenin in adherens junctions is compensated by γ-catenin, a homologous desmosomal protein; however, the impact of AJ in the biliary epithelium has yet to be explored. To discern the role of AJs in intrahepatic bile ducts, we studied conditional loss of the two catenins using cre-lox approach. Since bile is secreted into and reabsorbed from the small intestine for enterohepatic circulation, we also wanted investigated the consequences of altering intrahepatic bile duct AJs on intestinal homeostasis and gut microbiome. **Methods:** We utilized Ctnnb1 fl/fl; Jup fl/fl mice with Opn-iCreERT2+/- to delete both β-catenin and γ-catenin from cholangiocytes. Littermate floxed mice without Opn-iCreERT2 were used as controls. All mice received tamoxifen (100 mg/kg) dissolved in corn oil injected intraperitoneally. Mice were randomly assigned to two groups: DKO2 received 2 doses of tamoxifen and DKO4 received 4 doses of tamoxifen. Male and female mice were used in this study. Plasma, liver, ileum, colon, feces, and cecum content were collected from all mice. **Results:** DKO2 mice display increased serum levels of ALP, AST, and ALT without loss of body weight. Fecal TBA were reduced in DKO2 two weeks after tamoxifen injection, which are restored by six weeks. DKO4 mice exhibited increased morbidity with over 20% body weight loss, along with elevated serum levels of ALP, ALT, AST, and total bilirubin and total lack of bile flow compared to control mice. DKO4 mice show reduced fecal TBA levels and circulating secondary bile acids compared to DKO2 and control mice. DKO4 mice exhibit significant intestinal inflammation, increased number of goblet cells per crypt/villus unit, an increase in lysozyme-positive cells in the ileal villi, and elevated fecal lipocalin 2 levels compared to DKO2 and control mice. Additionally, microbial sequencing indicates that DKO4 mice have an increase in pathogenic bacterial genera, such as Prevotella, compared to WT mice. **Conclusions:** Dual loss of β- and γ-catenin in cholangiocytes causes severe

intrahepatic cholestatic injury in mice, disrupting ileal epithelial cell populations. Severe impairment of bile flow in DKO4 mice results in decreased colonic mucin production, increased colonic inflammation, and a favorable environment for pathobiont expansion, suggesting that optimal bile duct integrity and, in turn, bile flow influence gut function and bacterial clearance.Understanding the role of AJs in cholangiocytes and, in turn, in the normal liver-gut communication may help in understanding diseases impacting both organs, such as IBD and PSC.
Acknowledgements: This work is supported by R01 NIDDK (PM), ALF Postdoctoral Award (VM), and BWF PDEP (VM).

Abstract 022

Blood Vessel Architecture is Altered Following a Bile Duct Formation Defect in a Mouse Model of Genetic Cholestasis

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Background: We previously showed that loss of Yes-associated protein 1 (YAP) in early liver development (Foxa3-Cre YAP KO) leads to an Alagille syndrome-like phenotype, with failure of intrahepatic bile duct development and severe cholestasis. Given the close relationship between the bile ducts and hepatic arteries, we questioned whether these mice showed defects in hepatic artery formation as well. **Methods:** We deleted *Yap1* during early liver development using the *Foxa3* promoter to drive Cre expression. We evaluated the vasculature of these mice using light microscopy, transmission electron microscopy (TEM), and RNA-sequencing. We then examined changes to the vasculature of patients with early stage pediatric cholestatic disease. **Results:** We examined WT and YAP KO liver tissue with immunohistochemistry for CD31 (general vasculature), alpha-smooth muscle actin (a-SMA; marks pericytes particularly thick around hepatic arteries), and Lyve1 (lymphatic vessels and some sinusoids). We found that most portal tracts in YAP KO mice lacked a-SMA-lined vessels and instead had visible lymphatic vessels which seemed to be dilated in contrast to those in WT mice. The liver vasculature plays a significant role in regulating zonation, and indeed we found that YAP KO mice showed an expansion of periportal (Cyp2f2) and midzonal (Ccnd1) areas, while no change was found in the pericentral area (GS, Cyp2e1). We next asked if there was a change to the ultrastructure of the liver vasculature, but TEM analysis showed there was no significant change in the fenestrations of the sinusoids and their relationship with the hepatocyte brush border. We examined RNA-sequencing data but did not find a significant enrichment in hypoxia-related genes in YAP KO liver relative to WT liver. Next, we are examining the hepatic vasculature in early biopsies from patients with pediatric cholestasis from the Children's Hospital of Pittsburgh to determine whether our murine findings show relevance in conditions like Alagille syndrome (bile duct paucity) and congenital hepatic fibrosis with Caroli syndrome (bile duct overgrowth), in contrast to hepatocyte-driven cholestasis like PFIC1 and alpha-1-antitrypsin deficiency (no direct genetic damage to bile duct formation). Despite vastly different numbers of bile ducts in these specimens, the ratio of smooth-muscle-lined hepatic arteries to portal veins remains stable across disease groups, suggesting that arterial development is not dependent on normal bile duct development. However, we do see an increase in dilated lymphatic channels around portal tracts of most disease groups, suggesting a potential role for lymphatic vessels in the liver's response to developmental injury. **Conclusions:** We show that in our YAP KO mice, hepatic artery formation is developmentally dependent on bile duct formation. These mice show altered hepatic zonation and increased lymphatic channels but mostly retained sinusoidal fenestration. Further work is ongoing to address the functional implications of this change as well as to query whether patient livers with pediatric cholestasis show distinct vascular changes that can be modeled in mice. **Acknowledgements:** Thanks to NIH grants R01DK119435 and R01DK103775 to K. Nejak-Bowen; P30DK120531 to P. Monga and the Pittsburgh Liver Research Center, including the Biorepository, Genomics, and Imaging cores; R38HL150207 and UPMC Dept. of Pathology internal funding to L. Molina; ASIP SROPP funding to A. Piedy.

Abstract 023

Targeting Metalloprotease-regulated BEC Plasticity for Regenerative Liver Therapy

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Background: Biliary diseases are a major cause of liver-related morbidity and frequently progress to end-stage failure, where liver transplantation remains the only definitive therapy. A hallmark response to biliary injury is the ductular reaction (DR), characterized by expansion of biliary epithelial cells (BECs) within a dynamically remodeled portal niche shaped by epithelial–mesenchymal interactions and extracellular matrix turnover. Tissue inhibitor of metalloproteinases-3 (TIMP3), the most broadly acting endogenous inhibitor of matrix metalloproteinases (MMPs, ADAMs and ADAMTSs) is well-positioned to regulate this microenvironment; however, its role in biliary niche homeostasis and BEC fate remains poorly defined. Interrogation of mouse and human single-cell RNA-sequencing datasets revealed progressive upregulation of TIMP3 in BECs with increasing disease severity, implicating TIMP3 as an injury-responsive regulator of biliary epithelial remodeling. **Methods:** Wild-type and *Timp3*$^{-/-}$ mice were subjected to DDC-induced cholestatic injury. DR dynamics were evaluated using histopathology, serum biochemistry, confocal imaging, and integrated multi-omics profiling. EpCAM^{+} BECs were FACS-isolated and expanded as 3D organoids to dissect cell-intrinsic effects of *Timp3*. To assess regenerative capacity, organoid-derived expanded and/or differentiated BECs were transplanted into murine models of hepatic and biliary injury. **Results:** Loss of *Timp3* markedly amplified ductular expansion across injury stages and led to aberrant enlargement of ductal structures during chronic cholestasis, accompanied by exacerbation of cholestatic serum markers. *Timp3*$^{-/-}$ EpCAM^{+} BECs displayed significantly enhanced organoid-forming efficiency and proliferative capacity, indicating that TIMP3 intrinsically restrains progenitor activation. This phenotype was associated with heightened periportal fibroblast activation, accelerated laminin remodeling, increased collagen deposition, and a striking reduction in primary cilia in both injured ducts and organoids. Multi-omics analysis revealed activation of ADAMTS-dependent pathways and enrichment of stemness-associated transcriptional programs driven by the SMAD1/5–ID2 axis in *Timp3*$^{-/-}$ BECs. Notably, these progenitors exhibited premature hepatocytic differentiation. In preliminary transplantation studies, recipients of Timp3$^{-/-}$ BECs demonstrated improved regenerative competence in both hepatic and biliary disease model. **Conclusions:** Together, this work identifies TIMP3 as a key regulator of the biliary progenitor niche during cholestatic injury. We have shown that TIMP3 deficiency promotes BEC activation, expansion, and sustained plasticity while impairing primary cilia formation, linking progenitor fate decisions to ciliary integrity with implications for biliary repair and regenerative cell therapy. **Acknowledgements:** This work was supported by CIHR (Canadian Institute of Health Research) and Triangle fellowship.

Abstract 024

KIF12 Deficiency Unraveled in Cholestatic Liver Disease: Linking Organelle Mispositioning to Hepatic Pathology

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Background: Cholestasis is a major cause of pediatric liver transplantation, yet effective treatments are scarce. Recent studies, including ours, have uncovered that bi-allelic rare loss-of-function mutations in KIF12 cause pediatric high-GGT cholestasis, fibrosis, ductular reaction, and bile duct loss. Despite being identified over 40 years ago, the roles of kinesins in the liver remain poorly understood. KIF12, a presumed microtubule-associated motor protein, has an elusive role in cholestasis pathogenesis. **Methods**: Analysis of a human liver single-cell atlas revealed predominant KIF12 expression in cholangiocytes. We developed inducible pluripotent stem cell (iPSC)-derived cholangiocyte-like cell (iCC) and 3D biliary organoid models of human KIF12 deficiency (Arg219*)

to investigate cellular and molecular pathogenesis. **Results**: Wild-type (WT) and Arg219* human iPSCs were successfully differentiated into iCCs and were validated with each stage-specific marker. High GGT activity in Arg219*iCCs was noted, consistent with the reported clinical patient phenotype. In Arg219* iCCs, there was a striking disruption in organelle distribution, with both mitochondria and lysosomes showing a perinuclear localization, in contrast to WT. These findings were corroborated through immunostaining and overexpressing organelle-specific markers. Seahorse assay revealed a significant decrease in maximal respiration, indicating impaired mitochondrial function and reduced oxidative phosphorylation capacity, highlighting impact on cellular bioenergetics. Additionally, cilia are mislocalized in Arg219* iCCs, as reported by immunostaining and cilia-specific assay, revealing a fourfold increase in bent cilia and intracellular cilia compared to WT. The organoid model further validated cilia's altered position in Arg219*, independent of membrane polarization. Importantly, over-expressing WT KIF12 in Arg219* rescues organelle mislocalization, including cilia defects seen in Arg219* iCC. **Conclusion**: KIF12 deficiency in iCC leads to increased GGT and transcriptional alterations with pronounced organelle mislocalization, suggesting impaired organelle trafficking. This evidence underscores novel insights into the cellular and molecular insights driving the pathogenesis of human cholestatic liver disease. The successful rescue of ciliary defects through KIF12 overexpression not only highlights the essential role of KIF12 but also opens new avenues for therapeutic intervention. **Acknowledgements:** American Liver Foundation Travel Award, Doris Duke Charitable Foundation, NIH, DK131033, DK099257, DK034989.

Abstract 025

Cyp2c70-deficient Mice Develop Age-dependent Cholestatic Liver Injury with Progressive Bile Acid Accumulation and UPR Activation

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Background: Cholestatic liver diseases are the leading indication for pediatric liver transplantation and lack therapies preventing disease progression. Toxic bile acid (BA) accumulation drives hepatic injury, ER stress, and the unfolded protein response (UPR). We previously showed that impaired expression of hepatic X-box binding protein 1 (XBP1) and UPR mediators contributes to liver injury in neonatal vs. adult mice. However, traditional murine cholestasis models are translationally limited by the hydrophilic, less toxic murine BA pool. We used Cyp2c70 knockout (KO) mice, which exhibit a human-like hydrophobic BA profile, to investigate age-dependent ER stress, liver injury, and UPR activation. **Methods:** Female Cyp2c70 KO mice and heterozygous controls were assessed at 10 days, 16 days, 18 days, 3 weeks, and 8 weeks of age. Serum liver chemistries were measured, and hepatic BA composition was quantified via LC-MS. Bulk hepatic RNA sequencing was performed, and key ileal targets assessed by qPCR. **Results:** At 10 days, serum ALT, ALP, and BA levels in KO mice were indistinguishable from controls. Hepatic injury emerged at 16–18 days; by 3 weeks serum ALT was dramatically elevated (3976.4 ± 1575.3 vs 37.5 ± 20.42 U/L) and improved by 8 weeks (346 ± 157 vs 42 ± 11 U/L). Serum bile acids were also elevated at 3 weeks (>700 vs <5 µmol/L) and 8 weeks (38 ± 18 vs <5 µmol/L). Hepatic BA analysis showed BA accumulation and a more hydrophobic composition in KO mice, peaking at 3 weeks and remaining elevated through 8 weeks; the Heuman hydrophobicity index rose from ~−0.4 in Het to a peak of +0.36 in KO at 3 weeks. Bulk hepatic RNA sequencing revealed a hepatic stress response emerging at 3 weeks in KO mice, with induction of the PERK/ATF4 (*Atf4*, *Atf3*, *Ddit3*) and IRE1/XBP1 (*Xbp1*, *Hspa5*, *Dnajb9*) UPR arms, downregulation of BA homeostasis gene networks, and sustained upregulation of acute-phase reactants (*Saa1*, *Saa2*) and the ductular-reaction marker *Spp1* through 8 weeks. Ileal IBABP and ASBT transporter expression showed no major age- or genotype-dependent differences. **Conclusion:** Cyp2c70 KO mice, characterized by a humanized hydrophobic BA pool, develop hepatic BA accumulation and age-dependent cholestatic liver injury most severe at 3 weeks and spontaneously improving by 8 weeks. This is accompanied by acute PERK/ATF4 and IRE1/XBP1 UPR activation and global downregulation of BA homeostasis gene networks at 3 weeks, followed by a sustained acute-phase and ductular-reaction signature through 8 weeks.

Ileal BA-handling genes were unchanged, suggesting a hepatically driven phenotype during a developmental window potentially relevant to neonatal- and infantile-onset pediatric cholestatic liver diseases.

Abstract 026

Spatial Transcriptomics Reveals a TLS-like Immune-Fibrotic Niche Driving Biliary Fibrosis in Pediatric Primary Sclerosing Cholangitis

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Background: Pediatric-onset primary sclerosing cholangitis (PSC) and the overlap variant with autoimmune hepatitis are cholestatic conditions in which peribiliary inflammation and fibrosis progressively reorganize hepatic tissue into spatially distinct domains. The intercellular signals activating hepatic stellate cell (HSC) and driving fibrogenesis across spatial domains remain poorly characterized. **Methods:** We performed spatially resolved transcriptomics on liver biopsies from 5 patients with PSC (median age 19 years, 3 males) and one healthy control. Spatial domains were identified using Novae, and cell-cell communications (CCC) were inferred using CellNest. Empirical CCC probability was estimated as the ratio of observed interactions to plausible. Differential communication was assessed using paired Wilcoxon signed-rank tests. **Results:** Novae identified four domains (D1-4) representing a continuum of disease progression. D1 occupied hepatocyte (Hep)-rich lobular regions (75% of cells) with relatively preserved architecture, D2 and D3 represented intermediate states characterized by reduced Hep (55%) and increased plasma cells, NK cells, T cells, and macrophages (MP), consistent with inflammatory infiltration at the portal-parenchymal interface. In contrast, D4 localized to portal and fibrotic periductal regions, was predominately (~85%) consisted of activated HSC, myofibroblasts, cholangiocytes, MP, and lymphoid cells. D4 emerged as the dominant fibrotic niche, with selective enrichment of TNFR2-associated signaling and tertiary lymphoid structure (TLS)-like chemokine programs, including CCL21-CCR7, CCL21-CXCR3, CXCL12-CXCR3, and CXCL12-CXCR4. Key interactions included cholangiocyte-derived SPP1–ITGB1, endothelial-derived CCL21 signaling to dendritic and T cells, and stromal CXCL12-CXCR4 signaling to plasma and T cells, consistent with a TLS-like immune-fibrotic microenvironment. D2 showed enhanced collagen-integrin and TNFSF14 signaling involving activated HSC, LSEC, MPs, and Kupffer cells, consistent with early stromal remodeling and leukocyte adhesion. D3 represented an interferon-γ-driven inflammatory niche dominated by CXCL9, CXCL10, and CXCL12 signaling through CXCR3 and CXCR4, consistent with recruitment of Th1 cells, NK cells, dendritic cells, and MPs. **Conclusion:** PSC progression is characterized by a structured spatial trajectory from disrupted matrix homeostasis to stromal remodeling, interferon-driven immune recruitment, and ultimately a cholangiocyte-centered TLS-like immune-fibrotic niche. The convergence of CCL21, CXCL12, SPP1, TNF, and LIGHT signaling implicates TLS as a central mechanism linking chronic immune activation to biliary fibrosis in pediatric PSC.

Abstract 027

The Role of Lymphatics in Primary Biliary Cholangitis

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Background: Liver lymphatic vessels (LVs) lie in close proximity to bile ducts, but their role in biliary diseases remains unclear. Primary biliary cholangitis (PBC) is an autoimmune cholestatic liver disease characterized by bile duct injury, inflammation, fibrosis, and cirrhosis. We hypothesize that hepatic LVs play a critical role in maintaining immune tolerance and that restoration of lymphatic function may mitigate PBC. **Methods:** Liver specimens from PBC patients were analyzed to assess hepatic LV changes. Single-cell RNA sequencing (scRNA-seq) datasets from healthy controls (n=9) and PBC patient livers (n=10) were analyzed (HRA008003; HRA002347). Functional and mechanistic studies used ARE-Del-/- mice, which recapitulate key features of human PBC, including female predominance and elevated serum IFN-γ, and a surgical lymphatic obstruction (LOx) model to test the direct impact of impaired hepatic lymphatic drainage. Primary human liver lymphatic endothelial cells (LyECs) were treated with IFN-γ (100 ng/mL, 24h) *in vitro*. **Results:** Human PBC livers (n=17) exhibited a 51%

reduction in LV area ($p<0.001$) compared with healthy controls (n=3), indicating hepatic lymphatic regression. scRNA-seq analysis further showed a 94% reduction in LyEC frequency in PBC livers versus controls ($p<0.001$). Consistent with human findings, ARE-Del-/- mice showed reduced LV number (52%, $p<0.05$) and LV area (43%, $p<0.01$) compared with wild-type mice. LOx-induced blockade of liver lymphatic drainage increased peri-biliary $CD45^+$ immune cell accumulation by 1.9-fold, portal fibrosis by 1.4-fold, and liver injury by 1.8-fold (all $p<0.05$), supporting a direct role for hepatic lymphatics in restraining biliary inflammation and injury. Mechanistically, LyEC-focused analysis in human PBC scRNA-seq revealed enhanced IFN-γ signaling, impaired cell adhesion, and activated apoptotic pathways; at the same time, CCL21, a key lymphatic chemokine for immune cell trafficking, was downregulated ($p<0.05$). In primary human liver LyECs, IFN-γ reduced CCL21 expression by 18% ($p<0.05$). Consistently, immunostaining of both human PBC and ARE-Del-/- mouse livers showed marked loss of CCL21 in LVs. Finally, therapeutic hepatic VEGF-C overexpression by AAV8 increased LV number by 2.65-fold ($p<0.001$), reduced liver injury by 48%, portal inflammation by 52%, and biliary fibrosis by 57% (all $p<0.05$). Together, these findings identify hepatic lymphatic dysfunction as a clinically relevant and important contributor to PBC pathogenesis. **Conclusions:** Hepatic LVs are reduced and functionally altered in PBC patients and murine models, promoting immune cell accumulation, portal inflammation, and biliary injury. Restoration of lymphatic function with VEGF-C ameliorates disease, supporting hepatic lymphatics as a clinically relevant and potential therapeutic target in PBC. **Acknowledgements:** This study was supported by NIDDK (1R01DK138584) to YI and Yale Liver Center Pilot Funding (NIDDK P30 DK034989).

Abstract 028

MRP9 is a Novel Regulator of Cholangiocyte Mitochondrial Metabolism

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Background: Cholangiopathies, characterized by cholangiocyte injury, are major causes of liver disease. Understanding cholangiocyte metabolism during homeostasis may uncover new therapeutic targets. We recently identified a biallelic frameshift variant in *ABCC12* in a patient with intrahepatic cholestasis and bile duct paucity. *ABCC12* encodes the ATP-binding cassette protein MRP9, whose function is poorly understood. Prior studies in zebrafish and mice showed that MRP9 loss sensitizes cholangiocytes to bile acid–induced cell death, but the underlying mechanism remains unclear. **Methods:** We used three complementary models to investigate MRP9 function: *Abcc12* knockout zebrafish and mice, and a human H69 cell line homozygous for the *ABCC12* variant identified in the index patient. MRP9 localization was assessed by immunostaining, and cholangiocyte ultrastructure by transmission electron microscopy. Mitochondrial function and metabolism were evaluated by Seahorse assays and global metabolomics. Transcriptomes of wild-type and MRP9-deficient cholangiocytes were compared. Confocal live imaging was used to assess mitochondrial morphology and dynamics. **Results:** MRP9 localized to mitochondria in human cholangiocytes. Mitochondrial damage was observed in cholangiocytes of *Abcc12*$^{-/-}$ larval zebrafish and neonatal mice prior to overt changes in cell morphology or number. Seahorse assays demonstrated impaired mitochondrial function in MRP9-deficient neonatal mouse cholangiocytes and H69 cells. Metabolomics revealed TCA cycle dysfunction and a shift toward glycolytic metabolism in MRP9-deficient H69 cells. Enhancing mitochondrial biogenesis via PGC1α overexpression rescued cholangiocyte loss *in vivo*. Transcriptomic analysis identified dysregulation of porphyrin metabolism, a pathway closely linked to mitochondrial function, in *abcc12*$^{-/-}$ zebrafish cholangiocytes. Consistently, ectopic accumulation of the heme intermediate protoporphyrin IX (PPIX) was observed in MRP9-deficient cholangiocytes across all models. Reducing PPIX levels through chemical and genetic approaches rescued cholangiocyte death in both H69 cells and zebrafish. **Conclusions:** Our study identifies mitochondrial dysfunction driven by disrupted porphyrin homeostasis as the mechanism for cholangiocyte injury caused by MRP9 deficiency. It unveils, for the first time, the essential contribution of porphyrin metabolism to cholangiocyte biology and its potential implications in cholangiopathies. **Acknowledgements:** R01DK140321 and James Heubi Investigator Award (to C.Y. and A. M.); R01DK095001 (to A.M.); The Peter and Tommy Colucci Award for PFIC Research and Steele Family Fund for Genetic Liver Disease (to C.Y.).

Poster Session – Clinical Liver Disease

Abstract 029

Acute Liver Failure in the Setting of Acute Hepatitis B and Concomitant Ashwagandha Use

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Background: Acute hepatitis B infection is uncommon in the United States but may progress to acute liver failure. Although most cases are mild or asymptomatic, concurrent ashwagandha use, an emerging cause of herb-induced liver injury, may exacerbate hepatic injury and worsen outcomes. **Case Presentation:** A 30-year-old woman with no significant medical history presented with headache, nausea, vomiting, myalgias, and poor oral intake after a febrile illness following travel to the Dominican Republic. Labs on admission showed (AST >15,000 U/L, ALT >11,000 U/L), hyperbilirubinemia (3.7 mg/dL), and coagulopathy (INR 2.5), consistent with acute liver injury. She was initially mentating well. History was negative for alcohol, illicit drugs, acetaminophen, or prescription medications, though further questioning revealed recent ashwagandha use. Extensive infectious and autoimmune workup was negative except for positive hepatitis B surface antigen and core IgM antibody, confirming acute hepatitis B with a viral load of 4.28 million IU/mL. Hepatitis C, HIV, and acetaminophen levels were negative. Doppler ultrasound showed patent hepatic vasculature. Over the next 48 hours, the patient developed acute encephalopathy, worsening coagulopathy, hypoglycemia, and progressive respiratory failure requiring ICU admission, endotracheal intubation, and CVVHD for acute kidney injury. Tenofovir was initiated for acute hepatitis B with high viral load, while lactulose, rifaximin, and vitamin K were administered for hepatic encephalopathy and coagulopathy. Despite aggressive supportive management, the patient remained critically ill with a predicted 21-day survival of approximately 10% without transplantation and was transferred to a liver transplant center for urgent evaluation, where she ultimately underwent deceased donor liver transplantation (DDLT). **Conclusion:** Fulminant hepatitis B is a rare, life-threatening cause of acute liver failure characterized by rapid hepatic decompensation, coagulopathy, and encephalopathy due to immune-mediated hepatocyte necrosis. Management is largely supportive, and outcomes remain poor without transplantation. Ashwagandha, an emerging cause of herb-induced liver injury, may worsen hepatic injury through oxidative stress and impaired detoxification pathways, raising concern for a multifactorial “dual-hit” process in this case. The patient initially presented with severe coagulopathy without encephalopathy, consistent with early phase of disease but rapidly deteriorated despite aggressive supportive care and ultimately required DDLT.

Abstract 030

Diagnostic Accuracy of Liver and Spleen Elastography for Detecting Portal Hypertension Defined by Hepatic Venous Pressure Gradient (HVPG) in Chronic Liver Disease: A Systematic Review

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Background: Portal hypertension is a major consequence of chronic liver disease and a key determinant of clinical outcomes, traditionally assessed using the invasive hepatic venous pressure gradient (HVPG). Given the limitations of HVPG measurement, ultrasound-based elastography has emerged as a promising noninvasive alternative. This systematic review evaluated the diagnostic accuracy of liver stiffness measurement (LSM) and spleen stiffness measurement (SSM), obtained through transient elastography (TE) and shear-wave elastography (SWE), for detecting HVPG-defined portal hypertension in adults with chronic liver disease. **Methods**: A comprehensive search of PubMed/MEDLINE, Embase, and the Cochrane Library, from database inception through December 2024, identified eight eligible studies comprising 1,636 patients. Clinically significant portal hypertension was primarily defined as an HVPG of ≥10 mmHg. **Results:** Across studies, LSM demonstrated moderate to high discriminatory performance, with area under the receiver operating characteristic curve (AUROC) values ranging from 0.74 to 0.94, depending on modality and population. SWE generally showed

superior performance compared with conventional TE, particularly when standardized reliability criteria were applied. SSM provided complementary hemodynamic information and improved risk stratification in selected cohorts, although it did not consistently outperform LSM alone. Variability in stiffness cutoffs and study design contributed to methodological heterogeneity. **Conclusions:** Overall, ultrasound-based elastography demonstrates clinically meaningful diagnostic accuracy for identifying portal hypertension and offers a practical, noninvasive approach for risk stratification, particularly in compensated cirrhosis; however, it does not fully replace invasive HVPG measurement. **Acknowledgement:** This review article did not receive any specific grant from any funding agency. We would like to thank our authors for their valuable time and contributions

Poster Session – Fibrotic Liver Disease

Abstract 031

System-Level Variation in Endoscopy Timing and Mortality in Cirrhosis with Acute Variceal Bleeding: A Nationwide Analysis of Practice Patterns and Outcomes

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Background: Timely endoscopy is central to the management of acute variceal bleeding in cirrhosis, yet real-world delivery remains heterogeneous. The impact of system-level factors, including hospital teaching status, on endoscopy timing and outcomes has not been fully characterized at a national level. **Methods:** We conducted a retrospective study using the National Inpatient Sample, identifying adult hospitalizations with cirrhosis and acute esophageal or gastroesophageal variceal bleeding. Endoscopy timing was categorized as none, early (hospital day 0–1), or delayed (≥2). The primary outcome was in-hospital mortality; secondary outcomes included length of stay (LOS) and total charges. Survey-weighted multivariable regression models adjusted for demographic, clinical, and hospital factors. Interaction between endoscopy timing and teaching status was assessed. **Results:** The weighted cohort included ~179,820 hospitalizations: 30.6% no endoscopy, 50.7% early, and 18.8% delayed; 78.8% occurred at teaching hospitals. Unadjusted mortality was highest without endoscopy (12.0%) versus early (7.1%) and delayed (9.0%). After adjustment, both early and delayed endoscopy were associated with significantly lower mortality compared with no endoscopy. Teaching hospitals demonstrated slightly lower odds of early endoscopy. A significant interaction between endoscopy timing and teaching status was observed, with greater relative mortality reduction in non-teaching hospitals. Delayed endoscopy was associated with increased LOS and higher charges. Among patients undergoing endoscopy, delayed timing was associated with lower adjusted mortality compared with early endoscopy but with substantially greater resource utilization. **Conclusions:** Inpatient endoscopy is associated with improved survival in cirrhosis hospitalizations with acute variceal bleeding, with outcomes influenced by system-level factors. The observed variation by teaching status suggests disparities in care delivery and resource utilization. While delayed endoscopy may reflect survivorship bias and patient selection, these findings highlight critical opportunities to optimize timing strategies and standardize care pathways nationally.

Abstract 032

Pathogen-Specific Infectious Causes of Death in U.S. Liver Cirrhosis Patients: A 25-Year Population-Based Analysis

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Background: Liver cirrhosis impairs innate and adaptive immunity through reduced complement activity, impaired neutrophil function, and disrupted gut barrier integrity, rendering patients highly susceptible to fatal infections. While infections are recognized drivers of hepatic decompensation and mortality, population-level data on pathogen-specific infectious causes of death and their temporal trends remain scarce. Identifying responsible organisms and how their burden shifts over time is critical for surveillance, prevention, and policy. **Methods:** U.S. Multiple Cause of Death records from CDC WONDER (1999–2024) were analyzed for decedents with liver cirrhosis. ICD-10 codes A00–B99 identified infectious underlying causes. Non-pathogen-specific diagnoses—unspecified septicaemia (A41.9), unspecified bacterial infection (A49.9), unspecified gastroenteritis

(A09.9), and similar non-etiologic codes—were excluded from primary analysis as they do not identify a causative organism; these are reported separately. The focused analysis included viral hepatitis (HCV, HBV), HIV, *Clostridioides difficile* (CDI), and organism-named bacteraemia (*Staphylococcus aureus*, Gram-negative organisms, *Candida* spp.). Temporal trends were assessed over the full 25-year period. **Results:** Among 1,383,217 cirrhosis-associated deaths, 120,955 (8.7%) listed an infectious underlying cause. Non-pathogen-specific codes accounted for 20,340 deaths (16.8% of infectious) and were analyzed separately. Hepatitis C virus (HCV; B18.2/B17.1) dominated with 75,596 deaths (62.5%). HCV mortality rose 2.2-fold from 2,049/year (1999) to a peak of 4,497/year (2014), then declined 69% to 1,381/year by 2024, coinciding with direct-acting antiviral (DAA) approval. Hepatitis B virus (HBV; B16.9/B18.1/B94.2) accounted for 13,337 deaths (11.0%), declining from 611/year (1999) to 212/year (2024); a transient 2002–2005 spike reflects B94.2 sequelae reclassification. HIV (B20–B24) contributed 7,421 deaths (6.1%), declining steadily from 388 to 138/year (−64%) over the study period. In contrast, *Clostridioides difficile* (A04.7) emerged as a rising threat, increasing from near-absent before 2002 to 167 deaths/year by 2015, sustaining at 127/year in 2024. Organism-named bacteraemia (*S. aureus* A41.0, Gram-negative A41.5, candidal B37.7) accounted for 1,665 deaths (1.4%), with modest increases in recent years. **Conclusions:** HCV remains the dominant pathogen-specific infectious cause of death in U.S. cirrhosis patients, with a steep post-DAA decline. HBV and HIV deaths have decreased alongside therapeutic advances. Against this backdrop, *C. difficile* has emerged as a rising infectious threat in this immunocompromised population. These findings document the shifting pathogen-specific mortality landscape in cirrhosis and underscore the need for continued etiologic surveillance and preventive strategies targeting emerging bacterial pathogens. **Acknowledgements:** Data sourced from the CDC Wide-ranging ONline Data for Epidemiologic Research (CDC WONDER) Multiple Cause of Death database, 1999–2024. Supporting figures and supplementary tables are provided separately from this abstract.

Abstract 033

A Genetic Model of Nodular Cirrhosis Demonstrates the Reversibility of End-stage Liver Disease

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Background: Cirrhosis, characterized by extensive fibrosis and liver dysfunction, is the leading cause of liver-related death globally. Currently, the only definitive treatment is liver transplantation, but reversing advanced liver fibrosis remains a tantalizing possibility. The factors that regulate cirrhosis development and reversal are understudied in part because of a lack of tractable mouse genetic models. Loss-of-function mutations in the *Doublecortin domain containing 2a* (*Dcdc2a*) gene are associated with congenital liver fibrosis, and could provide critical genetic insights into end-stage liver fibrogenesis. **Methods:** We used mouse genetics, histology, RNA-seq, and gene expression assays to determine if deletion of *Dcdc2a* might lead to liver dysfunction and fibrosis. We used AAV approaches to model gene replacement therapies for *Dcdc2a*. **Results:** While whole-body *Dcdc2a* knockout (KO) mice were embryonic lethal, whole-body heterozygous mice exhibited mild liver fibrosis. However, liver-specific *Alb-Cre; Dcdc2a$^{fl/fl}$* (LKO) mice developed severe liver fibrosis that rapidly progressed to nodular cirrhosis with portal-portal fibrous septa by 4 weeks of age. In addition, LKO mice exhibited signs of end-stage liver decompensation including portal hypertension, splenomegaly, and gastrointestinal bleeding. To determine the cell-type(s) responsible for fibrosis, we performed inducible postnatal *Dcdc2a* deletion in cholangiocytes, hepatocytes, or hepatic stellate cells. However, none of these compartment-specific manipulations could recapitulate cirrhosis, indicating that loss of *Dcdc2a* in multiple cell types or earlier developmental timepoints were needed for fibrogenesis. Next, we asked if any aspects of cirrhosis could be reversed if *Dcdc2a* expression was rescued using gene therapy. Remarkably, add-back of *Dcdc2a* in hepatocytes and biliary cells using AAV/DJ-CMV-Cre at 4 weeks of age led to near-complete resolution of the gross and histologic findings of advanced fibrosis by 8 weeks of age. To explore the mechanistic basis of the KO phenotypes, we used bulk RNA-seq transcriptomics. *Dcdc2a* KO livers showed upregulation of fibroblast activation and fibrogenesis genes (*Vim*, *Tgfb1*, *Tgfb2*, *Tgfb3*, *Col1a1*, *Col1a2*, *Col3a1*). In addition, *Secreted Phosphoprotein 1* (*Spp1*), also known as osteopontin, was overexpressed specifically in cholangiocytes. To test if *Spp1* could be driving hepatic fibrogenesis in *Dcdc2a* KO mice, we deleted *Spp1* using *Alb-Cre*. *Spp1* heterozygosity reduced liver fibrosis, while homozygosity completely prevented nodular cirrhosis. Thus, *Spp1* hyperactivation plays a central role in

Dcdc2a KO-mediated hepatic fibrogenesis. **Conclusions:** The *Dcdc2a* KO model is one of the strongest mouse models of cirrhosis currently available, and promises to reveal cellular and genetic drivers of end-stage liver disease and its reversibility.

Abstract 034

Irradiated Humanized Liver Mice Develop Early Features of Radiation-induced Liver Disease That Are Absent in Conventional Mice

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Background: Radiation-induced liver disease (RILD) is a major complication of liver irradiation in patients, characterized by endothelial damage, oxidative stress, hypoxia, and fibrosis. Traditional rodent models are highly resistant to such insults, even at extreme single-fraction doses, limiting their translational relevance. We hypothesized that species-specific differences in liver physiology and immune/endothelial composition contribute to the observed discrepancies in radiation sensitivity between human and rodent livers. **Methods:** Eight-week-old C57BL/6 mice underwent image-guided focal irradiation of the right hepatic lobe using the Small Animal Radiation Research Platform (SARRP), with cone beam CT–based treatment planning to ensure precise targeting while sparing surrounding organs. Mice received single-fraction doses of 50 Gy. MISTRG6 mice (Balb/c-129 background) were engrafted with human CD34^{+} cells to generate a humanized liver, harboring human immune, stellate cells and endothelial cells or left non-engrafted. Twelve weeks post-transplantation, MISTRG6 mice received a single image-guided 24 Gy dose to the right hepatic lobe. Hypoxia was assessed using intravenous pimonidazole hydrochloride, followed by staining for human or mouse CD31^{+} endothelial cells. Lipid profiling was performed to assess oxidized lipids and endothelial-related lipid mediators. Liver damage was assessed by plasma bilirubin. **Results:** C57BL/6 mice showed no fibrosis, hypoxia, hepatocyte death, or endothelial damage three months post-irradiation. In contrast, irradiated humanized liver mice, at seven weeks post-irradiation exhibited elevated plasma bilirubin and circulating soluble human VCAM1, indicating liver and endothelial injury. Hypoxia was observed in human CD31^{+} liver sinusoidal endothelial cells (LSECs) and other human non-parenchymal cells (NPCs), but was absent in mouse CD31^{+} cells in irradiated non-engrafted MISTRG6 mice or non-irradiated controls. Lipid analysis revealed increased Prostaglandin F2α-III (oxidized lipid), decreased Docosahexaenoic acid (a protective lipid of the cell membrane), increased 5,6-Dihydroxyeicosatrienoic acid (an Oxylipin metabolite associated with endothelial dysfunction) in irradiated humanized liver mice, compared with irradiated non-engrafted MISTRG6 mice and non-irradiated controls. These results reflecting oxidative damage and endothelial dysfunction, which were absent in conventional mice. **Conclusions:** Humanized liver mice replicate key features of human RILD, including endothelial hypoxia, oxidative lipid changes, and liver damage, which are not observed in conventional rodent models. These mice provide a translationally relevant platform to study human-specific liver responses to irradiation and to test therapies targeting endothelial injury. **Acknowledgements:** We thank the UPenn CARC core for SARRP access and imaging support, the Xenograft Transplantation and the Histology core at Penn.

Abstract 035

Thrombin Induces Rapid Contraction in Hepatic Stellate Cells Driven by PAR1 Signaling

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Background: Clinical and experimental evidence suggests that the coagulation factor thrombin plays a pathologic role in liver fibrosis. One hypothesis linking thrombin to liver fibrosis proposes that thrombin signaling through protease-activated receptor-1 (PAR1) promotes activation of hepatic stellate cells (HSCs), the primary collagen-producing cells in the fibrotic liver. Prior studies report that thrombin promotes HSC contraction *in vitro*. HSC contraction has been linked to fibrosis by increasing intrahepatic vascular resistance and promoting portal hypertension, as well as inducing mechanosensitive transcription factors involved in HSC activation. The aim of this study is to test the hypothesis that thrombin-mediated PAR1 activation induces HSC contraction driven by both calcium-dependent (Gq) and –independent (G12/13) signaling pathways. **Methods:** Immortalized human

HSCs (LX-2) were cultured for 48h then stimulated with human α-thrombin (0.01-1 U/mL) or control. In some experiments, cells were pre-treated with a PAR1 competitive antagonist, vorapaxar (1 μM, VPX), a PAR1-Gq intracellular inhibitor, parmodulin-2 (10 μM, PM2), or a Rho kinase inhibitor, Y-27632 (10 μM) 1h prior to stimulation. Cell contraction was measured using magnetic twisting cytometry. Calcium mobilization and gene expression changes were also assessed. **Results:** Thrombin induced rapid cell contraction (within 60 seconds) in a concentration-dependent manner, which was preceded by intracellular calcium mobilization (within 30 seconds). Thrombin-driven contraction and calcium mobilization were completely inhibited by VPX. Inhibition of Gq signaling with PM2 completely suppressed thrombin-driven calcium mobilization and delayed HSC contraction. On the other hand, inhibition of the Rho-ROCK pathway by Y-27632 did not affect thrombin-driven calcium mobilization, but decreased peak HSC contractility. Combined inhibition of Gq and Rho-ROCK signaling completely inhibited thrombin-driven HSC contraction. Mechanical stress is known to activate mechanosensitive transcription factors such as Yes-associated protein (YAP) in HSCs. Interestingly, thrombin induced expression of two prototypical YAP target genes *ANKRD1* (cardiac ankyrin repeat protein) and *CCN2* (Cellular communication network factor 2) in HSCs after 24 h, which was suppressed by VPX pre-treatment. **Conclusions:** These results show that thrombin activation of PAR1 induces HSC contraction both calcium-dependent (Gq) and –independent (G12/13) signaling pathways and upregulates genes known to be downstream of a mechanosensitive transcriptional regulator. In conclusion, thrombin may contribute to liver disease pathogenesis by driving HSC contraction to increase portal pressure and regulate transcription. **Acknowledgements:** Financial support provided by the NIDDK, R00 DK129710, NHLBI, NIH R01HL164404 and P01HL180318, and Rutgers Center for Environmental Exposures and Disease.

Abstract 036

The Impact of Mitochondrial Haplotype on Inflammation and Fibrosis In Novel OKC-HET$^{B/W}$ Rats

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Background: Non-resolving chronic inflammation is a key contributor to aging and plays a significant role in the development of many age-associated diseases, including chronic liver diseases such as metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, and liver cancer. However, limited information is available on how the host environment such as the mitochondrial haplotype (mt-haplotype) influences inflammation, fibrogenesis, and liver cancer. While obesity and Western diet-associated metabolic stress are central to liver pathology and cancer development, this study uniquely and directly tests the impact of mitochondrial haplotypes on key factors involved in diet-induced liver diseases. **Methods:** We developed a novel rat model (OKC-HET) by crossbreeding four inbred rat strains-Brown Norway (BN), Fischer 344(F344), Lewis (LEW), and Wistar Kyoto (WKY)-in a heterogeneous nuclear background resulting in two mitochondrial haplotypes (OKC-HET^{B} and OKC-HET^{W}), which differ by 94 nucleotides. These rats were fed a Western diet (WD) for 6 months starting at 3 months. Liver samples were collected from chow-diet fed and WD fed male OKC-HET^{B} and OKC-HET^{W} rats. **Results:** We observed elevated inflammation (e.g., TNFα, IL-1β, IL-6), fat accumulation, and fibrosis (Col3α, TGFβ, Colα1 transcript levels and accumulation of collagen fibers as shown by trichrome, PSR staining) were observed in the WD fed male rats of both B- and W-haplotypes compared to their chow-fed counterparts. Importantly, these phenotypes were increased significantly in W-haplotype compared to the B-haplotype. **Conclusions:** As fibrosis is a recognized risk factor for cancer, our data suggest that mitochondrial haplotype modulates western diet-induced liver inflammation and fibrosis, potentially contributing to the development of liver cancer in response to western diet exposure. **Acknowledgements:** U.S. Department of Veterans Affairs,16005238, 1IK6BX005238, Arlan Richardson.

Abstract 037

Perturbed Neutrophil Responses in *Irf3*-deficient Mice Protect From CCl_4-induced Liver Fibrosis in Mice

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Background: Interferon regulator factor 3 (IRF3) executes multiple transcriptional and non-transcriptional functions in the development of metabolic liver diseases. Although *Irf3*-deficient mice are protected from CCl_4 and CDAA-induced models liver fibrosis, with non-transcriptional activity of IRF3 acting as a key driver of hepatic fibrogenesis, the specific cell types mediating its effects remain unclear. Liver fibrosis is characterized by

excessive extracellular matrix accumulation primarily produced by activated myofibroblasts. The recruitment and activation of innate immune cells are pivotal to both the progression and resolution of fibrosis. Neutrophils play a dual role in fibrotic process. While neutrophil-derived cytokines and granule proteins promote fibrosis, neutrophils also promote degradation of matrix. Neutrophil recruitment and NET formation are facilitated by IRF3 in other systems, at least in part by inhibiting NF-κB to promote NETosis. Yet neutrophils regulation by IRF3 is largely unstudied in liver. Therefore, we investigated the role of neutrophils in liver fibrosis and assessed the contribution of IRF3 to neutrophil responses. **Methods:** C57BL/6J (WT), *Irf3*-deficient (*Irf3*$^{-/-}$), and mice expressing only non-transcriptional IRF3 function (*Irf3*$^{S1/S1}$) were exposed to CCl_4-induced liver fibrosis. To determine the functional role of neutrophils, mice were treated with anti-Ly6G antibody 6 hours prior to the last CCl_4 injection to deplete neutrophils. Primary neutrophils were isolated from control WT, *Irf3*$^{-/-}$, and *Irf3*$^{S1/S1}$ mice to characterize their functional activity including NETosis and degranulation. **Results:** NIMPR14 staining revealed that chronic CCl_4 triggered robust infiltration of neutrophils into the liver. Interestingly, neutrophil accumulation was higher in *Irf3*$^{-/-}$ mice compared to both WT and *Irf3*$^{S1/S1}$ mice, but NET formation, assessed by citrullinated histone (CitH3) staining was lower in *Irf3*$^{-/-}$ mice. Neutrophil depletion reduced the protective effect of *Irf3*-deficiency on CCl_4-induced fibrosis. Primary neutrophil degranulation and NETosis were induced by challenge with PMA. Similar to the *in vivo* response, NET formation, evidenced by accumulation of extracellular DNA and CitH3 staining, and release of MPO were reduced in neutrophils isolated from *Irf3*$^{-/-}$ mice compared to neutrophils from WT and *Irf3*$^{S1/S1}$. **Conclusions:** *Irf3*-deficiency enhanced neutrophil recruitment in liver after CCl_4 exposure. However, it impaired NETosis both after CCl_4 treatment *in vivo* and in stimulated primary neutrophils from *Irf3*$^{-/-}$ mice. Importantly, normal neutrophil responses were restored in *Irf3*$^{S1/S1}$ mice, indicating that the non-transcriptional function of IRF3 contributes to critical neutrophil NETosis, likely assisting in the progression of hepatic fibrosis.

Abstract 038

The Fontan Associated Liver Disease (FALD) Atlas: A Spatiotemporal Single-cell Transcriptomics Map of Coordinated Multicellular Remodeling in the Liver.

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Background: Complex single-ventricle congenital heart disease culminates in Fontan circulation, which has a common sequela of hepatic congestion and cirrhosis called Fontan Associated Liver Disease (FALD). Building on our previous single-cell spatial FALD atlas, which revealed zonation disruption and distinct senescence-linked hepatocyte subpopulations with severe disease, we now expand our characterization to the full spectrum of FALD severity. Our analysis reveals a complex remodeling landscape with heterogenous transcriptomic signatures across hepatic cell populations. **Methods:** Single-cell spatial transcriptomics was performed on biopsy and explanted liver tissue from FALD patients across disease severity (congestive hepatic fibrosis scores 2-4; n=6) and histologically normal controls (n=2) using CosMx Spatial Molecular Imager with in-situ hybridization of 6,000 genes. Data were analyzed in R using Seurat, with cell-cell communication and pathway enrichment assessed via CellChat and GSEA, respectively. Spatial neighborhood analysis employed kNN with a permutation-based null model (1000 permutations). **Results:** Unbiased clustering revealed progressive sinusoidal endothelial cell (SEC) capillarization and extracellular matrix deposition, both directed toward inflammatory cell homing. A senescent hepatocyte population (p21^{high}), transcriptionally distinct from the metabolically exhausted hepatocytes in our previous work, showed a pro-fibrotic profile in intermediate FALD (CHF score 2-3) and an inflammatory senescence-associated secretory phenotype (SASP) in advanced FALD (CHF score 4). Enrichment analysis of this population demonstrated downregulation of metabolic pathways and upregulation of epithelial-to-mesenchymal transition (EMT) and cell-cycle arrest (G2M and E2F target signatures). Hepatic stellate cell and myofibroblast proportions expanded with disease severity, with two functionally distinct myofibroblast subtypes: a matrix-depositing pro-fibrotic population and an immune-modulatory C7^{high} subtype. Disease progression was marked by preferential expansion of the inflammatory subtype, which showed significant spatial colocalization with monocyte-derived macrophages. **Conclusions:** We have identified stage-specific transcriptomic signatures across hepatic subpopulations during FALD

progression. Intermediate FALD is characterized by pro-fibrotic extracellular matrix deposition across various cell types including SECs, hepatocytes and myofibroblasts. In late FALD, the senescent hepatocytes upregulate proinflammatory SASP pathways, leading to increased infiltration of monocyte-derived macrophages. The spatial association between inflammatory myofibroblasts and monocyte-derived macrophages suggests a coordinated multicellular remodeling axis driven by upstream senescence signaling. Further characterization of the SASP secretome is needed to establish its role in vascular remodeling and immune activation driving fibrotic progression. **Acknowledgements:** This work was supported by NIH grants R01DK062277 and SVC Endowed Chair in Pathobiology and Therapeutics to SPM. This work was supported in part by T32EB001026 and F30CA284540 to BML. AS was supported through K08 HL161440 and AHA CDA 852875. Support through The Center for Computational Immunogenetics and Drug Repurposing and the Heart Institute was also provided.

Abstract 039

ProtoHep: 878 Hepatocyte Differentiation Protocols Extracted from Biomedical Literature via Agentic LLM Pipeline

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Background: Hepatocyte-like cells derived from induced pluripotent and embryonic stem cells are central to disease modeling, drug screening, and regenerative medicine. Despite two decades of protocol development, no structured, machine-readable resource captures differentiation conditions and outcomes at scale. Existing resources catalog cell lines but omit protocol details, and manually curated databases cover only a fraction of the literature. With over 800 relevant publications, comprehensive manual curation is impractical, motivating automated extraction. **Methods:** We developed ProtoHep using a 16-step automated pipeline. Source papers were identified through three PubMed Central queries, yielding 824 papers. LLM-based triage (DeepSeek v3, T=0) classified papers into six categories; 544 proceeded to extraction. A three-pass agentic architecture was employed: Pass 1 identifies protocol structure, Pass 2 performs detailed extraction using three purpose-built tools (search_corpus for cross-referencing, fetch_reference for resolving citations, flag_incomplete for recording missing data), and Pass 3 enriches from supplements. Papers were processed in citation-graph order for reference resolution. Anti-hallucination defenses included verbatim-only prompts, structured incompleteness recording, and post-extraction grounding against a 99-term MeSH-enriched alias table. Total cost was $25 over 16 hours. **Results:** ProtoHep contains 878 protocol records from 544 publications (2000-2026), capturing cell source, culture conditions, stage-by-stage reagents and concentrations, endpoint markers, and functional assays. Analysis reveals a convergent three-stage architecture (definitive endoderm, hepatic specification, maturation) in over 50% of protocols, with mean duration of 17.6 days. Activin A dominates endoderm induction (60%), while HGF and OSM co-occur in 42-44% of maturation protocols. Core signaling shows stability across 15 years, though CHIR99021 rose from 0% pre-2012 to 29% by 2025-2026. Grounding-based hallucination detection removed 197 fabricated reagent terms across 97 protocols, revealing that LLMs preferentially hallucinate canonical pathway components. For 68 protocols with public RNA-seq data, expression values were integrated for a 47-gene marker panel (390,040 measurements). Median extraction confidence was 0.80, with 86.3% of protocols at or above 0.7. **Conclusions:** ProtoHep is the first comprehensive, machine-readable hepatocyte differentiation protocol database, enabling systematic cross-study comparison and meta-analysis. The pipeline is lineage-agnostic, requiring only four configuration files to adapt to new targets. The finding that LLMs preferentially fabricate canonical pathway components has broad implications for LLM-based scientific extraction. **Acknowledgements:** This work was supported by NIH R01 DK132129and the UCSF Liver Center P30 DK026743.

Abstract 040

Development of Human iPSC-derived Hepatocytes for Drug Discovery, Translational Research, and Toxicity Testing

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Background: Hepatocytes comprise over 80% of the liver mass and are responsible for most of its functions, including lipid and glucose metabolism, storage of macronutrients, secretion of plasma proteins, detoxification and xenobiotic metabolism. With liver diseases accounting for 4% of global mortality annually, there is an urgent need for reliable hepatic models. Addressing this burden requires robust cellular models for clinical applications and predictive screening of Drug-Induced Liver Injury (DILI). While primary human hepatocytes (PHHs) are traditionally used for in vitro drug testing, their utility is restricted by batch-to-batch variability and poor long-term survival in culture. Furthermore, alternative systems like animal models exhibit species-specific differences, whereas immortalized cell lines possess physiological discrepancies when compared to PHHs. Here, we used opti-ox™, a deterministic cell programming technology, to generate consistent, scalable, human pluripotent stem cell (hiPSC)-derived hepatocytes, termed ioHepatocytes, with functional similarity to PHHs, and with demonstrated suitability for toxicology screening, research and disease modelling. **Methods:** ioHepatocytes were generated through forward programming of hiPSCs using proprietary opti-ox™ technology and were characterised by immunofluorescence, transcriptomics, and assays for hepatic function (e.g., albumin secretion) and metabolic competency (CYP450 activity). DILI prediction was validated by assessing viability (CellTiter-Glo) after challenge with known hepatotoxins. **Results**: ioHepatocytes display a classic cobblestone morphology with distinctive nuclei and well-defined borders. Cells express key pan-hepatocyte markers including ALB, HNF4A, ASGR1 and SERPINA1, and present a transcriptomic signature like PHHs. Additionally, ioHepatocytes derived from hiPSCs with opti-ox demonstrate high batch-to-batch consistency. Functionally, ioHepatocytes perform critical hepatocyte functions including albumin secretion, glycogen storage, ammonia clearance and accumulation of lipids. Most importantly, ioHepatocytes show expression of genes involved in phase I, II and III of drug metabolism and have functional cytochrome P450 enzymes (CYP3A, CYP2B6 and CYP1A2). When challenged with compounds known to cause toxicity, ioHepatocytes show a clear dose-dependent decrease in cell viability that strongly correlates with the known DILI severity of the compounds. Importantly, the toxic response very closely mimicked that of PHHs, validating the predictive capability of ioHepatocytes. **Conclusions:** ioHepatocytes offer a highly consistent, functional and scalable human hepatocyte model, overcoming the limitations of existing technologies. Their demonstrated ability to respond to toxic insults similarly to PHHs suggests their use as a reliable, predictive tool for improving DILI risk assessment in drug development.

Abstract 041

Hepatocyte Growth Factor and Epidermal Growth Factor Delivered Via mRNA In Lipid Nanoparticles Improve Engraftment of Human Primary and iPSC-Derived Hepatocytes In Mice

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Background: Thousands of patients die each year awaiting liver transplantation due to end-stage liver failure. To address the scarcity of donors, transplantation of donor primary human hepatocyte (PHH) or patient-specific induced pluripotent stem cell (iPSC)-derived hepatocyte-like cells (iHeps) is explored as alternative therapies. To promote cell engraftment, we propose to deliver key mitogens, hepatocyte growth factor (HGF) and epidermal growth factor (EGF), using safe and non-integrative nucleoside modified mRNA encapsulated in lipid nanoparticles (mRNA-LNP). **Methods:** Our mouse model is the NSG-PiZ mouse which recapitulates human alpha-1 antitrypsin deficiency (AATD)-associated liver disease. We precondition NSG-PiZ mice to express the cell-cycle inhibitor p21 under a hepatocyte specific promoter to recapitulate p21-induced hepatocyte senescence observed in virtually all AATD patients. HGF and EGF mRNA-LNP are delivered twice weekly via retro-orbital injections. One million PHHs or iHeps are transplanted intra-splenically. **Results:** We demonstrate that biweekly injections of HGF+EGF mRNA-LNP lead to 30% repopulation of livers 5 weeks after PHH transplantation and significantly reduces AATD liver disease burden. To test this enhanced engraftment strategy with iHep, we genetically engineered iPSC lines to express physiological levels of HGF Receptor (HGFR) comparable to those in PHH. Importantly, HGFR overexpression does not affect iPSC pluripotency or hepatic differentiation potential into iHeps. We optimized a differentiation protocol yielding nearly 100% FOXA2+ HNF4α+ AFP+ cells by day

10, indicative of hepatic commitment. Transplantation of day 10 enhanced HGFR iHeps is ongoing. **Conclusions:** HGF and EGF mRNA-LNP augment PHH engraftment in vivo in the p21/NSG-PiZ model by improving survival and proliferation of transplanted cells. We also establish a foundation for improving iHep engraftment through enhanced growth factor signaling. Taken together, our research provides insights toward developing patient-specific cell therapies for chronic liver diseases such as AATD. **Acknowledgements:** R01-DK124361 and Alpha-1 Foundation research awards to Valerie Gouon-Evans, and F31-DK135378 to Anna Smith.

Abstract 042

Upregulation of IRF9 Increases Interferon Signaling During Induced Pluripotent Stem Cell Differentiation to Hepatocytes

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Background: Interferons (IFNs) are pivotal components of the innate immune response, orchestrating a multifaceted defense against viral infections, including Hepatitis C virus (HCV). HCV infection induces antiviral responses in hepatocytes through upregulation of interferon-stimulated genes (ISGs), including ISG15, a critical effector that undergoes ISGylation to enhance cellular defense. During iPSC differentiation toward hepatocyte-like cells (HLCs), cells progress through Definitive Endoderm (DE) and Hepatoblast (HB) stages, progressively acquiring interferon responsiveness. This study focuses on the developmental regulation of Interferon Regulatory Factor 9 (IRF9) and its role in establishing antiviral competence during hepatic differentiation. **Methods:** IRF9 expression and function were evaluated across iPSCs, HLCs, hepatic progenitor cells, HepaRG cells, Huh7.5.1 cells, and primary human hepatocytes. IRF9 was overexpressed in iPSCs, and knockdown experiments were performed in HepaRG cells. Protein and mRNA expression levels of IRF9, STAT1, phosphorylated STAT1, and ISG15 were assessed using Western blot and qPCR. Immunofluorescence colocalization analysis was used to evaluate nuclear localization of IRF9 and STAT1. In silico analysis of published whole-cell RNA-seq was also performed. **Results:** IRF9 was absent in iPSCs, correlating with low baseline STAT1 expression and undetectable ISG15 expression and ISGylation, indicating a dormant interferon response. As cells differentiated toward hepatocyte-like cells, IRF9 expression increased, aligning with enhanced interferon sensitivity. Overexpression of IRF9 in iPSCs partially restored STAT1 expression, STAT1 phosphorylation, ISG15 induction, and nuclear colocalization of IRF9 with STAT1 following IFNα treatment. IRF9 and STAT1 were sufficient to drive nuclear colocalization. In HepaRG cells, IRF9 knockdown significantly reduced STAT1 phosphorylation, ISG15 expression, and ISGylation, impairing antiviral responses. IRF9 overexpression in HepaRG and Huh7.5.1 cells, combined with IFNα treatment, enhanced STAT1 activation and ISG15 expression. **Conclusion:** IRF9 functions as a developmental regulator of interferon signaling, controlling the transition from an interferon-unresponsive pluripotent state to an antiviral competent hepatocyte phenotype. Several novel findings emerged when comparing iPSCs to HLCs and hepatic progenitor cells, highlighting IRF9's critical role in regulating type I interferon sensitivity during differentiation.

Abstract 043

MASH Patient-derived iPSC Liver Organoids Show Increased Susceptibility to Disease and Acetaminophen-induced Toxicity

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Background: Organoids generated from patient-derived induced pluripotent stem cells (iPSCs) have revolutionized human disease modeling by providing a physiologically relevant platform that preserves the patient-specific genetic background. However, current liver organoid models often lack the cellular diversity needed to fully recapitulate native tissue, and most rely on xenogeneic matrices such as matrigel, limiting their suitability for translational applications. These limitations underscore the need for approaches capable of generating multilineage, vascularized liver organoids in a matrigel-free environment. **Methods:** Using an air-liquid interface approach, highly vascularized, multicellular liver organoids were generated from de-identified control and metabolic dysfunction–associated steatohepatitis (MASH) donor-derived iPSCs obtained from the CDI-CIRM stem cell repository, eliminating the need for matrigel embedding. Phenotypic and functional analysis were conducted using specific markers and various functional assays. To model steatohepatitis, organoids were

exposed to free-fatty acids and evaluated using transcriptomics, ELISA and lipidomics. Drug-induced hepatotoxicity was assessed following acetaminophen (APAP) treatment via apoptotic, transcriptional, and mitochondrial dysfunction analysis. **Results:** Control and MASH donor-derived liver organoids exhibited major hepatic cell types, including hepatocytes, cholangiocytes, stellate cells, sinusoidal endothelial cells, and Kupffer-like cells, confirmed by phenotypic analysis and functional assays. Upon free-fatty acid exposure, MASH-donor derived liver organoids exhibited increased susceptibility to steatosis, inflammation and fibrosis. Lipidomic profiling showed that MASH phenotype in organoids induced global lipidomic shifts that closely resembled those observed in MASH liver biopsies. APAP treatment induced a dose-dependent increase in apoptosis, inflammation and mitochondrial oxidative stress, accompanied by reduced ATP levels and downregulation of antioxidant pathways, with MASH-derived organoids exhibiting increased sensitivity to APAP toxicity. **Conclusion:** This study presents a robust, matrigel-free approach for generating vascularized, multicellular liver organoids and demonstrates their utility for personalized disease modeling, and drug-toxicity assessment. **Acknowledgement:** This study was supported by NIH R01DK132873 grant awarded to Jason A. Wertheim.

Abstract 044

Differential Expression of SOCS1 Increases Interferon Signaling Throughout Stem Cell Differentiation to Hepatocytes

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Background: Hepatocytes infected with hepatitis C virus (HCV), mount an antiviral response restricting viral replication and recruiting immune cells for viral elimination. Viral clearance failure results in chronic inflammatory conditions that can drive hepatic malignant transformation. During chronic HCV, expression of interferon-stimulated genes (ISGs) is altered, but the canonical expression and regulation of individual ISGs in hepatocytes requires further investigation. **Methods:** We profiled ISG expression in patient samples and in vitro models. ISG induction was evaluated post-stimulation of innate antiviral pathways via measuring mRNA and protein levels. Regulatory mechanisms were examined by sodium bisulfite sequencing (SBS) and chromatin immunoprecipitation qPCR (ChIP-qPCR). **Results:** We show the expression and regulation of ISGs critical for interferon signaling, such as suppressor of cytokine signaling 1 (SOCS1), are controlled to modulate interferon-responsiveness throughout development. Throughout maturation, SOCS1 expression progressively decreases culminating in robust interferon responsiveness in HLCs. SBS revealed increased DNA methylation at the SOCS1 promoter in hepatocytes, correlating with reduced basal SOCS1 expression. ChIP-qPCR further demonstrated decreased enrichment of activating histone markers at the SOCS1 locus. **Conclusion:** Taken together, these findings identify multiple epigenetic mechanisms that fine-tune ISG expression and interferon responsiveness and advances our understanding of how inflammatory gene regulation shapes the antiviral response.

Poster Session – Liver Cancer

Abstract 045

Liver and Biliary Tract Cancer Incidence and Mortality in the United States, 1999–2023: A 25-Year Epidemiologic Analysis

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Background: Liver and intrahepatic bile duct (LIBD) cancer is among the fastest-rising malignancies in the United States (U.S.). We analyzed national incidence (1999–2022) and mortality (1999–2023) from the Centers for Disease Control and Prevention (CDC) United States Cancer Statistics (USCS) program, stratified by year, sex, age, race, and state, excluding Puerto Rico. **Methods:** This is a population-based surveillance study using publicly available CDC USCS data (*USCS CDC, International Classification of Diseases, 10th Revision (ICD-10) code C22, incidence 1999–2022 / mortality 1999–2023. Excludes Puerto Rico*). Age-adjusted rates (AAR, per 100,000, 2000 U.S. standard) for new cases and deaths were examined descriptively across calendar year, sex, race, age bands, and all 50 states plus D.C. Absolute counts were also reported. **Results:** *Incidence:* New

LIBD cases rose from 13,098 (AAR 4.8) in 1999 to 38,345 (AAR 9.0) in 2019 — an 87.5% rise. A COVID-19-related drop to AAR 8.3 in 2020 partially recovered to 8.7 (2021) and 8.3 in 2022 (36,666 cases). *Mortality:* Annual deaths rose from 12,382 (AAR 4.5) in 1999 to 29,911 (AAR 6.6) in 2023 (+141.6%). The mortality AAR peaked at 6.7 (2016–2018) and stabilized thereafter. The mortality-to-incidence ratio narrowed from 0.94 (1999) to 0.79 (2022), suggesting incremental survival gains, yet prognosis remains poor. *Sex:* Males consistently showed ~2× higher rates throughout the study period. By 2023, male mortality AAR was 9.3 vs. 4.4 for females (64.9% of all deaths), with female rates rising proportionally faster (+51.7% vs. +43.1%). *Race:* Asian/Pacific Islander (API) individuals had the highest mortality AAR in 1999 (10.2), declining to 8.1 by 2021 (−20.6%). Black/African American (B/AA) rates rose from 5.9 to 8.5 (2016), easing to 7.4 (2021; net +25.4%). White Americans: 4.1 → 6.4 (+56.1%). American Indian/Alaska Native (AI/AN) rates fluctuated (5.3–8.1), reaching 7.3 in 2021. *Age:* Mortality was heavily concentrated in older adults. In 2023, crude rates were 51.8/100k (≥85 yrs), 45.9 (80–84), 39.6 (75–79), and 35.7 (70–74). Rates fell sharply below age 55 (<10/100k) and were rare under 45 (<1.1/100k). *Geography:* In 2023, highest mortality AARs were in Louisiana (9.5), Texas (8.7), Arkansas (7.9), and Oklahoma (7.6), with Southern/Gulf Coast states consistently overrepresented. Lowest rates were in North Dakota (4.3), Idaho (4.5), and New Hampshire (4.6). **Conclusions:** This surveillance analysis documents a sustained rise in LIBD cancer incidence and mortality across the U.S. over 25 years, affecting all demographic groups yet falling disproportionately on males, B/AA individuals, and Southern state residents. The narrowing mortality-to-incidence ratio signals modest survival improvement, though the overall burden continues to grow. These findings underscore the need for heightened attention toward HBV, hepatitis C (HCV), MASLD, alcohol use, and greater equity in access to early detection and cancer care.

Abstract 046
Genetic Prediction of Hepatocellular Carcinoma in a Prospective Cohort of Patients With Cirrhosis

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Background: The effectiveness of hepatocellular carcinoma (HCC) surveillance is limited in patients with liver cirrhosis, with the potential for novel biomarkers to improve risk stratification. A previous GWAS study identified five single nucleotide polymorphisms (SNPs) associated with HCC risk in patients without viral hepatitis: *PNPLA3* rs738409, *TM6SF2* rs58542926, *MAU2* rs58489806, *MOBP* rs9842969, and *TERT* rs2242652. We evaluated the utility of these SNPs in predicting HCC risk in a prospective cohort of patients with cirrhosis under surveillance for HCC. **Methods:** The performance of the five SNPs in predicting HCC was evaluated in a multicenter prospective cohort of patients with cirrhosis undergoing surveillance with contrast-enhanced MRI (HCC-SC, n=748). Association with liver fibrosis was also assessed for these SNPs, in the Cameron County Hispanic Cohort (CCHC), a population with high prevalence of liver steatosis and cirrhosis (n=797). For each SNP, association with HCC or liver fibrosis was evaluated by logistic regression, adjusting for gender. In HCC-SC patients with metabolic dysfunction-associated steatotic liver disease (MASLD), a Polygenic Risk Score (M-PRS) was developed. Its performance in predicting HCC risk, alone and combined with selected circulating inflammation-associated proteins, was evaluated. Finally, impact of selected SNPs on serum cell-free RNA (cf-RNA) profiles, was determined. **Results:** Significant associations with HCC risk were found in HCC-SC patients with MASLD, for *MAU2* rs58489806, *TM6SF2* rs58542926, and in females only, for *PNPLA3* rs738409. No association with HCC risk was found for *MOBP* rs9842969 or TERT rs2242652. In the CCHC, *PNPLA3* rs738409, *TM6SF2* rs58542926 and *MOBP* rs9842969 were significantly associated with liver fibrosis. M-PRS was developed based on *PNPLA3* rs738409, *TM6SF2* rs58542926, and *MAU2* rs58489806. The performance of M-PRS was limited in predicting HCC in HCC-SC patients with MASLD (AUC=0.659 [95% CI=0.574-0.745]). In addition, inflammation-associated-proteins (n=247) were measured in serum from 190 HCC-SC patients with MASLD. While AFP outperformed all inflammatory proteins in predicting HCC in males (AUC=0.748 [95% CI=0.604-0.891], TGFB1 had the greatest discriminatory power in females (AUC=0.815[95% CI=0.691-0.938]). Combining TGFB1 and AFP with M-PRS improved the performance of M-PRS (AUC=0.808 [95% CI=0.706-0.910] vs AUC=0.713 [0.603-0.824]), reaching 73% sensitivity and 83% specificity. Finally, these three SNPs had a significant effect on cf-RNA profiles in patients with MASLD, consistent with a dysregulation of HNF4A and RYR1. **Conclusions:** In patients with MASLD-associated cirrhosis, a three SNP-based M-PRS combined with AFP and TGFB1 could have utility in predicting HCC in a surveillance setting. **Acknowledgements**: Other Contributors: Megha B. Bhongade[1], Ahmed El Sabagh[1], Darrel W. Cleere[1], David M Baskin[2], Nakul Gupta[3], Stefanie Weinstein[2,4], Prasun K. Jalal[1], David W. Victor[5] Susan P. Fisher-Hoch[6], Joseph B. McCormick[6]

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Abstract 047

The Post-Weaning Involutional Microenvironment Restricts β-Catenin-Driven Hepatocellular Carcinoma

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Background: The liver undergoes significant growth during pregnancy and lactation to meet increased metabolic demands, followed by tightly regulated growth regression during involution after weaning. The molecular mechanisms governing this regression remain poorly understood. However, we hypothesized that this physiological window may harbor endogenous tumor-suppressive signals that was directly investigated in the current study. Gain-of-function mutations in *CTNNB1* are evident in 26% of all HCCs, and loss-of-function alterations in *AXIN1* and *APC* are seen in another 10% of cases, collectively activating Wnt/β-catenin signaling in around 35-40% of all hepatocellular carcinomas (HCCs). This subset of HCCs respond poorly to the immune checkpoint blockade combinations, the current standard of care. Thus, identifying mechanisms that could restrict active-β-catenin in HCC would be highly significant. Here, we investigate if post-weaning involutional microenvironment selectively suppresses β-catenin-dependent hepatocarcinogenesis. **Methods and Results:** Using the Sleeping Beauty transposon/transposase hydrodynamic tail vein injection (SB-HDTVi) system, we delivered hMet+S45Y-β-catenin oncogene constructs into postpartum day 5 and age-matched nulliparous female mice and sacrificed after 2 and 7 weeks. Our preliminary data demonstrate that while comparable plasmid uptake in both groups is evident at 2 weeks, the postpartum mice at 7 weeks exhibited a striking reduction in glutamine synthetase-positive tumor area compared to nulliparous controls (3.25% vs. 37.92%, p=0.0001), alongside reduced Ki-67 staining. **Conclusions:** These studies suggest physiological liver involution generates an endogenous β-catenin-suppressive niche, with implications for understanding sex-based differences in HCC incidence and identifying novel targets for personalized liver cancer therapy.

Abstract 048

Hepatocyte-specific Loss of a Nuclear Envelope Protein LAP1 Promotes Hepatocellular Carcinoma Through Epigenetic Dysregulation

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Background: Hepatocellular carcinoma (HCC) often arises in chronic liver diseases, including steatohepatitis. Lamina-Associated Polypeptide 1 (LAP1) is a nuclear envelope protein and activator of torsinA ATPase. We previously reported that loss of LAP1 or torsinA in hepatocytes causes steatosis and steatohepatitis due to impaired lipid secretion. More recently, we found that the hepatocyte-specific LAP1 knockout (L-CKO) mice develop liver tumors with chow-diet feeding. This study investigates the molecular mechanisms linking LAP1 deficiency to hepatocarcinogenesis. **Methods:** Histopathological analysis of L-CKO liver sections was conducted to assess tumor formation and HCC. Tumor susceptibility was evaluated using low-dose diethylnitrosamine (DEN)-induced and oncogene-driven models (hydrodynamic injection of AKT/nRAS with Sleeping Beauty). Transcriptomic profiling of isolated hepatocytes was conducted to identify mechanisms of LAP1 loss-induced tumorigenesis. Epigenetic changes were assessed by ATACseq and Nanopore-based DNA methylation

profiling. **Results:** We have observed visible liver tumor nodules in L-CKO mice after 1 year of age, with 65% showing histopathological features of HCC. LAP1 deficiency significantly accelerated tumor development in both DEN-induced and oncogene-driven models, indicating increased susceptibility to hepatocarcinogenesis. RNAseq analysis at a pre-tumor stage revealed induction of hepatocyte progenitor-like gene signature, including abnormal activation of a genetic loci regulated by DNA methylation. Genome-wide profiling revealed widespread DNA hypomethylation, and ATAC-seq identified altered chromatin accessibility in LAP1-deficient hepatocytes, indicating early epigenomic changes preceding tumor formation. **Conclusions:** LAP1 loss in hepatocytes induces early epigenetic dysregulation, including DNA hypomethylation and altered chromatin accessibility, prior to tumor development. These changes are associated with progenitor-like transcriptional reprogramming and increased tumor susceptibility. Our collective data show that LAP1 is identified as a key regulator of epigenetic integrity in hepatocytes, and nuclear envelope defects are implicated as a novel driver of HCC. Further integrative analysis is ongoing to define the underlying mechanisms. **Acknowledgements:** This work was supported by the National Institutes of Health 1R01CA283566 (to JYS) and a Pinnacle Research Award from the American Association for the Study of Liver Disease (JYS).

Abstract 049

Using Omics Technologies to Understand Hepatoblastoma Heterogeneity and Identify Tumor Cell Types and Their Microenvironment for Precision Medicine

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Background: Hepatoblastoma (HB) is the most common primary pediatric liver cancer. Among childhood tumors, it has one of the highest mortality rates, with 20% high-risk HB cases resulting in death or liver transplantation. Identifying high-risk tumors has been challenged by tumor heterogeneity. Current clinical high-risk stratification relies on imaging and histology, with serum alpha-fetoprotein (AFP) as the only molecular biomarker. Therefore, it is necessary to improve molecular characterization to enable more accurate patient risk stratification. We previously used single-cell RNA sequencing (scRNA-seq) and identified 5 tumor cell populations shared across HB tumors. We are expanding this analysis by including additional freshly resected and frozen banked tumors, using single-nuclei RNA sequencing (snRNA-seq). In parallel, we used spatial transcriptomics on HB tissue microarrays (TMA) to decipher the microenvironment associated with each tumor cell population. **Methods:** Samples from 28 patients were included among three platforms. scRNA-seq was performed using microwell capture on freshly dissociated tumors and snRNA-seq using droplet capture on banked frozen tumors. Libraries were sequenced on an Illumina NovaSeqX and data was analyzed using Scanpy. TMAs were generated from 23 banked FFPE-HB samples for 10X Genomics Xenium spatial transcriptomics, with a 480-gene custom human liver gene panel, and analyzed using Scanpy and Squidpy. **Results:** We confirmed the reproducibility of tumor cell populations across techniques using matched scRNA- and snRNA-seq patient samples. These findings highlight the utility of snRNA-seq for characterizing banked HB samples. Our expanded sc/snRNA-seq datasets validated the previously identified 5 tumor cell populations, and identified a new population, enriched in *AFP* and potentially associated with high-risk tumors. Spatial transcriptomics revealed that distinct tumor cell populations occupied discrete spatial regions within the tumor and had distinct microenvironments. Hepatoblast signatures were enriched for vascular endothelial cells and tumor-associated fibroblasts at the tumor-stroma interface, with ligand-receptor analysis identifying *HGF-MET* and EGF-family signaling. In contrast, neuroendocrine signatures exhibited somatostatin autocrine signaling and *SLIT*-mediated fibroblast crosstalk. **Conclusion:** By combining these complementary omics techniques, we established a molecular atlas of HB and identified clinically relevant shared tumor signatures across patients and within tumors, each with distinct microenvironments. These results could improve risk stratification and identify signature-specific microenvironmental interactions that represent therapeutic target candidates.
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Abstract 050

Single-Cell Atlas of Chronic Woodchuck Hepatitis-induced HCC Highlights Conserved Tumor-Driven Immune Exhaustion Shared with Human HCC

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Background: Despite the use of immune checkpoint inhibitors (ICI) targeting well-characterized immune exhaustion pathways, the ICI response rate in chronic hepatitis B (CHB)-induced HCC remains below 40%. This may be attributed to a limited cellular-level understanding of the HCC niche. Thus, a systematic characterization of the HCC ecosystem across paired, spatially distinct HCC-related regions is crucial for identifying major contributors to immune dysfunction. **Methods:** Single-cell RNA sequencing was performed on peripheral blood mononuclear cells (PBMCs) and liver biopsies from healthy (n=8) and woodchuck hepatitis virus (WHV)-induced HCC woodchucks (n=9). Spatial sampling from 9 HCC animals included matched non-malignant liver tissue (n=8), HCC margins (n=20), and multiple intratumoral regions (n=23). The transcriptomic analysis was coupled with histology to visualize changes in liver morphology. **Results:** An integrated single-cell map of 275,220 cells was generated from a total of 74 samples, which captured 5 major immune cell lineages shared with human HCC. T cell profiling revealed an increase in the ratio of exhausted CD8+ T to effector CD8+ T cells in HCC animals compared to healthy controls (****p<0.0001). Within HCC animals, a significant enrichment of exhausted CD8+ T cells was observed in HCC margin and tumor compared to non-malignant tumor-distal tissues (**p<0.01 and *p<0.05), which was characterized by an increase in the expression of exhaustion-related markers (e.g., TOX, CTLA4, PDCD1) and a decrease in the expression of cytotoxicity-related markers (e.g., GZMB, IFNG, GZMK). Pseudotime trajectory analysis using Monocle3 suggested a diverged developmental path in HCC CD8+ T cells from healthy CD8+ T cells. Coinciding with a higher CD8+ T cell exhaustion score, WHV-induced HCC margin and tumor were enriched in immunoregulatory myeloid lineages with high expression of angiogenic and immunomodulatory mediators (e.g., VEGF, GPNMB, MIF). Analysis of cell-cell interaction using CellChat revealed multiple regulatory signaling pathways between tumor-enriched myeloid cells and CD8+ T cells that might promote T cell dysfunction. **Conclusion:** Our single-cell characterization of WHV-induced woodchuck HCC demonstrated a transcriptionally and morphologically proliferative tumor subtype, and revealed an immune-exhausted tumor landscape that mirrors human HCC. Future in vitro validations on woodchuck myeloid-T cell interaction will aim to dissect the mechanistic role of myeloid-derived factors in modulating T cell exhaustion, which will reveal therapeutic targets that can be directly tested using WHV-induced woodchuck HCC. Moreover, longitudinal studies using woodchucks could track the emergence of the CD8+ T cell population that gives rise to the exhausted CD8+ T cells with hepatitis progression, as they might represent a more therapeutically targetable group to impair the development of HCC.

Abstract 051

Robust Inference of Liver Zonation Reveals a Targetable PPARα Dependence in Liver Cancer

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Background: Liver zonation constitutes heterogeneous gene expression and ensuing compartmentalized functions, including in hepatocytes, based on location relative to the portal triad (zone 1) or the central vein (zone 3). Zone 3 identity is regulated by the Wnt-β-catenin pathway, in which endothelial cell-derived Wnt ligands establish a hepatocyte-β-catenin activation gradient driving zone 3 functions. Although central to homeostasis and regeneration, zonation remains inconsistently characterized, limiting generalizability. Likewise, despite numerous multi-omic studies in hepatocellular carcinoma (HCC), zonation in this disease and its implications for therapeutic response remains ambiguous. **Methods:** We developed *RIZLiver* (Robust Inference of Zonation in Liver), a tool that infers hepatocyte zonation from gene expression via a regularized expectation-maximization algorithm. After benchmarking on simulated and annotated human spatial data, Xenium ST of six HCCs were characterized by RIZLiver, which derived HCC-specific zonation signatures applied to The Cancer Genome Atlas (TCGA) bulk RNA-seq to identify subtypes. Overall survival (OS), pathway enrichment, and top driver mutations were assessed across subtypes. A candidate target nominated by zone-3 enrichment was tested in a *CTNNB1-NFE2L2* preclinical HCC model for functional and therapeutic validation. **Results:** *RIZLiver* achieved robust prediction accuracy in simulated and annotated spatial transcriptomic benchmarking analysis. Xenium profiling revealed intra- and inter-tumoral zonation heterogeneity, with zone-3 enrichment in *CTNNB1*-mutated tumors. HCC-specific zonation signatures stratified TCGA patients by OS (p=0.024). Zone-3 HCC was enriched for *CTNNB1* (p<0.001) and *TERT* promoter (p=0.003) mutations and for fatty acid omega-oxidation and *NFE2L2* activation signatures while negatively associating with *TP53* mutations (p<0.001). Pharmacologic inhibition of PPARα, a regulator of hepatic β-oxidation, reduced tumor burden in the *CTNNB1-NFE2L2* HCC model (p<0.05). **Conclusions:** *RIZLiver* enables systematic, robust inference of liver zonation across diverse transcriptomic studies. By applying this tool to HCC ST data and integrating these results with bulk RNA sequencing in a large cohort, we identify enrichment of a zone-3 transcriptional program in a *CTNNB1*-driven, PPARα-dependent HCC subtype – implicating zonation as a transcriptional axis central not only to homeostasis but also to disease pathogenesis. **Acknowledgements:** This work was supported by NIH grants to YCC (R00CA248944, R35GM154967, R03CA305794), SPM (R01DK62277, R01DK103775, R01CA251155, R01CA250227), and TMY (F30CA298277), a UPMC Hillman Cancer Center Team Shark Tank Award to TMY (P30CA047904), and a University of Pittsburgh CTSI HITS Pilot Award to TMY, BML, YCC, and SPM (UL1TR001857).

Abstract 052

Alcohol Alters AhR/β-catenin Signaling and Reshapes Liver Zonal Plasticity Leading to Immune-Metabolic Reprogramming in Metabolic-Syndrome Associated Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) arises in metabolic dysfunction-associated steatohepatitis (MASH), alcohol-related liver disease (ALD), and their overlap (MetALD), yet how metabolic zonation shapes tumor lineage and immunity remains unclear. **Methods:** MASH-associated HCC, which harbors frequent Ctnnb1 mutations in the Stelic Animal model (STAM), was induced by the combined administration of the diabetogenic agent streptozotocin (STZ) and a high-fat diet (HFD)18. Alcohol-associated HCC was generated using a 5% ethanol Lieber-DeCarli diet. MetALD-associated HCC was modeled by adding 10% ethanol in the STAM models. Zonal plasticity, immunometabolic reprogramming, and differential responses to aPD1 therapy across these

disease contexts were analyzed using bulk RNA sequencing, digital spatial profiling (DSP), lineage tracing, whole-exome sequencing (WES), single-cell RNA (ScRNA) sequencing, and liquid chromatography-mass spectrometry (LC-MS) metabolomics. **Results:** We established three complementary mouse models that recapitulate MASH-, ALD-, and MetALD-associated HCC in a diabetic context. Lineage tracing and RNA sequencing revealed that STAM tumors frequently harbored missense mutations resembling human *CTNNB1*-mutant HCC. Metabolomic analysis showed that these MASH-HCCs, characterized by diffuse glutamine synthetase (Gs) staining, originated from periportal/midlobular hepatocytes, lost periportal identity, and acquired β-catenin-driven perivenous programs that activated the IDO1-kynurenine-AhR (Aryl hydrocarbon receptor) axis, promoting resistance to anti-PD-1 therapy. In contrast, ethanol in a diabetic context induces Gs^- ALD-HCC with suppressed β-catenin/AhR/constitutive androstane receptor (CAR) signaling and reduced perivenous/xenobiotic programs. Superimposed ethanol further drove steatosis, ductular reaction, and senescence, generating heterogeneous tumors, including Gs^- MetALD-HCC from zone 3 and Gs^+ HCC of HPC/biliary origin. These Gs^- MetALD-HCC showed reduced kynurenine, increased IL-7, enhanced $CD4^+/CD8^+$ T cell infiltration, and decreased myeloid-derived suppressor cells. Consistently, pharmacologic inhibition of AhR or hepatocyte-specific deletion of β-catenin reduced MASH-HCC burden and restored sensitivity to anti-PD-1 therapy. **Conclusions:** These results identify AhR as a key mediator of β-catenin-driven tumor immunosuppression and a potential therapeutic target in *CTNNB1*-mutant HCC, highlighting context-dependent immune escape in alcohol-associated liver cancer. **Acknowledgements:** This study is supported by the National Institutes of Health NIAAA RO1AA028035 grant and the Lavin Bernick Grant Funding awarded to LP, and the DOD grant HT9425-23-1-0737 awarded to CL and LP.

Abstract 053

Characterizing Autoimmune Hepatitis (AIH) Using Complementary Single-nuclei and Spatial Transcriptomics Techniques

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Background: Autoimmune hepatitis (AIH) is a chronic liver disease characterized by immune cells attacking hepatocytes, leading to chronic inflammation, cirrhosis, and death if untreated. AIH incidence appears to be on the rise, disproportionately affecting women and people of color. Diagnosis often requires an invasive liver biopsy, as serum biomarkers, including autoantibodies, lack sufficient sensitivity and specificity. Treatment can require lifelong immunosuppression with corticosteroids, and up to 25% of patients do not respond to treatment. Much remains unknown about the biological basis of AIH, including the autoantigen that leads to hepatocyte damage, the immune cell profile, and which molecular signaling pathways are active in the disease. **Methods**: Here, we use the Prospective Observational Study to Understand Liver Disease (POSULD), a well-characterized, diverse patient cohort with integrated clinical and molecular data to investigate disease mechanisms in AIH using a combination of single-nuclei RNA sequencing and spatial transcriptomics. To define the cellular and molecular mechanisms driving AIH, we performed single-nuclei RNA sequencing on liver tissues from 7 patients with AIH. We also included publicly available snRNA-seq data from 3 healthy controls. Next, we applied high-resolution spatial transcriptomics using the 10x Genomics Xenium platform to characterize disease-related changes in situ. To do so, we created a custom 480-gene human liver panel informed by our single-cell and bulk sequencing data for AIH. We then generated liver tissue microarrays using liver biopsy samples from 54 patients with AIH, control patients with metabolic-associated steatotic liver disease (MASLD), and healthy human controls. This approach enabled high-resolution in situ mapping of liver cell types and spatially informed identification of lobular regions (periportal, pericentral, and portal) using connectivity-based analysis. **Results**: Single-nuclei analysis revealed a massive increase in T lymphocytes in AIH and upregulation of the potential autoantigen AFF3 in periportal hepatocytes, implicating the periportal region in disease pathogenesis. We validated these findings in the spatial transcriptomics data. Unsupervised clustering based on spatial cell-type composition and regional gene expression profiles identified a subset of AIH patients characterized by shrunken periportal regions and expanded portal regions enriched for myeloid and lymphocyte populations. This subgroup also demonstrated elevated AIH panel scores, increased TGF-β signaling, and reduced STAT5B and heme biosynthesis pathway activity, highlighting spatially organized immune-hepatocyte interactions associated with

severe disease. The spatial analysis showed that T cell expansion in AIH is localized to the portal region, where they form clusters with B cells. Cell-to-cell signaling analysis of our single-nuclei dataset identified CD40-CD40LG signaling between B and T cells as specifically upregulated in AIH livers, which we validated in the spatial transcriptomics data. **Conclusions**: These findings provide insight into the spatially organized immune-hepatocyte interactions that characterize AIH and highlight candidate pathways for targeted therapeutic intervention, advancing efforts toward precision medicine in this disproportionately affected population. It also demonstrates how a complementary approach combining single-cell and spatial transcriptomics can reveal novel molecular and cellular insights into liver disease pathophysiology. **Acknowledgements**: This project was funded by UCSF ImmunoX pilot study, UCSF Department of Medicine Cohort grant, UCSF Liver Center (P30DK026743), and Chan Zuckerberg Initiative.

Abstract 054

Developing a Human Precision-cut Liver Slice Platform to Investigate the Immune Microenvironment in Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality worldwide (PMID 35000616). Frontline treatment options include immunotherapy, though response rates remain limited (~36%) (PMID 32402160). This is partly due to immunosuppressive features of the tumor microenvironment (TME), including macrophage differentiation (PMID 39709141). We hypothesize that macrophage-mediated immunosuppression and spatial disorganization contribute to dysfunction of the surrounding immune cells and immunotherapy resistance. Macrophages are highly plastic *in vitro*, highlighting the need for physiologically relevant models that preserve tissue architecture to study their function in the context of HCC (PMID 40482642). We therefore seek to develop a three-dimensional TME platform using precision-cut liver slices (PCLS) from human HCC. **Methods:** 3mm punch biopsies from human HCC tumor and matched uninvolved liver tissue were embedded in 5% low-melt agarose. Using a vibratome, 250 µm slices were generated and cultured in organotypic transwell inserts for up to 7 days. Slices were formalin-fixed and paraffin-embedded for histology, fixed in Cytofix/Cytoperm for whole-mount immunofluorescence (IF) or enzymatically and mechanically dissociated for flow cytometry. Supernatants were collected daily for analysis of secreted factors by cytometric bead array. To assess the impact of macrophages in the TME on the function of immune cells such as T cells, we depleted macrophages from HCC-PCLS with clodronate liposomes. $CD14^+$ monocytes were added directly to slices or below transwells to assess macrophage differentiation in the TME. **Results:** HCC-PCLS remained viable for 5-7 days, as assessed by production of intracellular ATP. HCC-PCLS maintain disease features during culture as assessed by histological analyses including hematoxylin and eosin and trichrome for collagen, as well as supernatant profiling by cytometric bead array. $CD68^+$ macrophages and $CD3^+$ T cells were detected within PCLS by immunohistochemistry. Co-cultured $CD14^+$ monocytes infiltrated the tumor slices and differentiated into $CD68^+$ macrophages, gaining characteristics of tumor macrophages during 5 days in culture, as demonstrated by flow cytometry and IF. **Conclusions:** We established a human HCC-PCLS platform that preserves TME architecture while enabling manipulation of immune cell composition. This system enables investigation of how macrophages influence immune dysfunction and may inform immune profiling strategies to optimize immunotherapy in HCC. **Acknowledgements:** We thank the Toronto General Hospital Departments of Surgery and Pathology for tissue collection, the University Health Network (UHN) Pathology Research Program for histology support, the UHN Advanced Optical Microscopy Facility for imaging, as well as all patients and families for their contributions. We acknowledge the National Sciences and Engineering Research Council Discovery Grant Program.

Abstract 055

Wnt Receptor Fzd10 Marks CAR-induced Tumor-associated Hepatocyte Population with Cancer Stem Cell-like Features

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Background: Constitutive androstane receptor (CAR, *Nr1i3*) is a hepatocyte-expressed nuclear receptor that mediates widespread transcriptional responses to xenobiotics, promotes steatosis, and with prolonged exposure, induces mouse hepatic tumorigenesis. Mice were treated with TCPOBOP, a selective CAR agonist xenobiotic, to identify early CAR-induced gene responses that drive liver tumor formation, with the goal of interdicting liver disease progression at an early stage. **Methods:** snRNA-seq, ChIP-seq and ATAC-seq datasets were analyzed for male CD1 mouse livers exposed to TCPOBOP persistently, with liver tumors first detected at 14 wk and becoming extensive by 20-22 wk. smFISH validated localization and co-expression. Human HCC data were analyzed using UALCAN. DAVID and Ingenuity Pathway Analysis (IPA) identified enriched pathways and upstream regulators. **Results:** *Fzd10* was rapidly (within 3 hr) and strongly (>90-fold) induced by TCPOBOP in mouse liver. ATAC-seq sites proximal to *Fzd10* responded to TCPOBOP by chromatin opening, were linked to *Fzd10* expression by multiomic analysis, and acquired enhancer histone marks (H3K27ac, H3K4me1). A subset of these *Fzd10*-proximal enhancers showed strong CAR binding, indicating that *Fzd10* is a direct CAR target gene. Fzd10 and Sox4 RNAs co-localize within an snRNA-seq pericentral hepatocyte subcluster where CAR is enriched, in both high and low Fzd10- and Sox4-expressing subpopulations. The Sox4 locus was increasingly accessible (snATAC-seq) in Fzd10+ cells, suggesting that Fzd10 and Sox4 define a common cancer stem cell-like population. smFISH validated the expression and co-localization of both gene transcripts. DAVID analysis indicated cancer-associated pathway enrichment, and IPA identified Tbx3, a Wnt/β-catenin target that maintains cancer stemness, as an activated upstream regulator in Fzd10+ and Sox4+ hepatocytes. Hepatocyte re-clustering using 433 tumor-specific gene features, including 32 well-defined liver cancer markers representing distinct tumorigenic programs, revealed high- and low-expression patterns aligned with Fzd10-high and Fzd10-low subclusters, suggesting shared tumor-related transcriptional programs. Human HCC data showed FZD10 overexpression and association with aggressive tumor features. **Conclusions:** CAR induces both Fzd10-high and Fzd10-low pericentral hepatocytes, which may represent distinct tumor-associated reprogramming events. Fzd10+ hepatocytes are proposed to acquire cancer stem cell-like and tumor-associated features through increased Wnt/β-catenin signaling. These findings support Fzd10 as an early marker and a potential therapeutic target for CAR-driven HCC. **Acknowledgements:** NIH grant ES024421 (to DJW) & GSI 2025 Pilot Grant

Abstract 056

β-Catenin Inhibition Delays Tumor Progression in an hMET–NRF2 Hepatocellular Carcinoma Model via Non-Canonical Signaling

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Background: Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer-related mortality worldwide. We generated an hMET–NRF2 driven HCC model that produces glutamine synthetase (GS)-negative tumors, suggesting tumor development occurs independent of the canonical β-catenin activation. Based on this assumption, siRNA-mediated β-catenin knockdown was initially used as a specificity control for β-catenin–dependent tumor models. Unexpectedly, β-catenin inhibition significantly delayed tumor progression as was reported earlier. **Methods and Results:** To investigate the molecular basis of this effect, bulk RNA sequencing was performed using liver samples from wild-type mice (n=4), tumor mice treated with si-control (n=4), and tumor mice treated with si-β-catenin (n=4). Differential gene expression analysis was conducted using DESeq2 (fold change >1.5, FDR <0.05). Pathway enrichment analysis was performed using Ingenuity Pathway Analysis (IPA) and integrated with β-catenin activity signatures and non-canonical β-catenin gene sets. Compared with wild-type liver, the hMET–NRF2 tumor model exhibited 3059 upregulated and 2356 downregulated genes. Following si-β-catenin treatment, 1066 genes were upregulated and 1641 genes were downregulated relative to untreated tumors. Pathway analysis revealed enrichment of Rho/cytoskeleton signaling, extracellular matrix remodeling, inflammatory and stromal signaling, and secreted-factor pathways. Analysis of 669 genes showing opposite regulation between tumor development and β-catenin inhibition identified strong enrichment of SRC–PAK–Rho GTPase signaling networks. Key genes, including *SRC, PAK1, PAK3, DIAPH3, IQGAP3,* and *CDH1*, emerged as hub regulators linking cytoskeletal remodeling, adhesion, and signaling pathways. **Conclusions:** These findings suggest that β-catenin inhibition can delay tumor progression even in GS-negative HCC through non-canonical signaling pathways centered on SRC–PAK–Rho cytoskeletal networks. This work highlights a

previously underappreciated role of non-canonical β-catenin signaling in HCC and suggests that targeting β-catenin may have therapeutic potential beyond canonical β-catenin-activated tumors.

Abstract 057

Orthotopic Xenografts of Human Hepatoblastoma Exhibit Delayed Growth in Neonatal Compared to Adult Livers of Immunodeficient Mice

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Background: Hepatoblastoma, the most common primary liver malignancy in children, arises most frequently in young infants and rarely after age 4. How the microenvironment of the developing liver modulates tumorigenesis remains poorly understood. While orthotopic xenografts of human and mouse hepatoblastoma cell lines in neonatal immunodeficient mice develop lung metastases more often than in adult mice (Fan L et al., Hepatology 2022; Klein P et al., Oncology 2025), the growth rates of primary tumors in neonatal vs. adult liver have not been compared. Here, we report on growth dynamics of luciferase-expressing xenografts of the HepG2 cell line and a primary human hepatoblastoma tumoroid line in neonatal vs. adult livers of NRG mice. **Methods:** Patient-derived hepatoblastoma tumoroids were obtained and cultured as previously described (Wu PV et al., Nat Commun 2024). HepG2 was purchased from ATCC. Cells were lentivirally transduced with a red-shifted Firefly luciferase under blastidicin selection (Biohippo). NRG mice were used to generate orthotopic xenografts. For neonatal xenografts, 10^6 cells in 10μl Matrigel were injected percutaneously into the liver of awake animals at P2-4. For postnatal week 4 and adult (>8-12 week) xenografts, animals were placed under anesthesia with isoflurane, a transverse upper abdominal incision was made to expose the liver, and 10^6 cells in 30μl Matrigel were injected into the left lobe. Luciferase-expressing cells were detected by IVIS at 15-25 minutes after IP luciferin injection at 2-week intervals starting at week 4 after tumor cell injection. **Results:** Luciferase-expressing HepG2 tumors were detectable by week 4 after injection of adult mice (n=3) but not detectable until week 6 after injection of neonatal mice (n=5). At 6 weeks, tumor size measured by average total flux was 4-fold greater in adult vs. neonatal xenografts ($p<0.01$). Of three different patient-derived hepatoblastoma tumoroids tested, only HB1 (derived from a lung metastasis) successfully engrafted in the liver. Whereas luciferase-expressing HB1 tumors were detectable by week 4 after injection of postnatal week 4 (n=3) and adult mice (n=4), tumors were not detectable until week 8 after injection of neonatal (P2-4) mice (n=2). **Conclusions:** Growth of tumors from both HepG2 and patient-derived hepatoblastoma tumoroids exhibited a longer latency period after injection into neonatal compared to adult mouse livers. Studies are ongoing to identify signals that suppress or promote growth of human hepatoblastoma in the developing mouse liver. **Acknowledgements:** This research was supported by a Hyundai Hope on Wheels Bridge2K Award (PVW), the Burroughs Wellcome Fund Career Award for Medical Scientists (PVW), and NIH grant P30 DK078392 (*Integrated Morphology Core* of the Digestive Disease Research Core Center in Cincinnati). Mouse work was approved by the IACUC at Cincinnati Children's Hospital Medical Center.

Poster Session – Liver Gene Expression

Abstract 058

Zinc Finger Transcription Factor FANIN is the Upstream Regulator of Pioneer Factors Foxa1 and Foxa2 in the Liver

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Background: Transcription factors regulate gene expression that orchestrates many processes, including development and physiology. Winged-helix (f*orkhead*) factor paralogs Foxa1 and Foxa2, originally named HNF3α and HNF3β, are called pioneer factors for their ability to independently bind closed chromatin and change chromatin accessibility allowing other factors to bind. Foxa1 and Foxa2 are essential to liver function, both in development and differentiated tissue. How these pioneer factors are regulated was unknown. Based on computational analysis, we have identified the upstream regulator of Foxa1 and Foxa2 we named FANIN.

Methods: We performed ChIP-Seq to identify FANIN binding, and ATAC-Seq & RNA-Seq in FANIN liver-specific mutants to assess chromatin accessibility and differential gene expression. Functional analysis of genomic data was completed to identify the role of FANIN in the liver. **Results:** Deletion of FANIN leads to loss of Foxa1 and Foxa2 expression in the liver. Since Foxa1 and Foxa2 are pioneer factors, we assessed whether deletion of FANIN affects chromatin accessibility. Indeed, ATAC-Seq signal is reduced in both male and female FANIN mutants, in contrast to Foxa2 mutants in control condition. FANIN also regulates sexually dimorphic gene expression in the liver in multiple ways, including sex-specific binding and effect on chromatin accessibility, as well as differential regulation of Foxa1 and Foxa2 paralogs. **Conclusions:** We conclusively show that Foxa1 and Foxa2 are regulated differently whether deleted in development or postnatally and confirm distinct non-redundant roles in both settings. Our work provides unparalleled novel insight into control of the genetic circuit involving two pioneer factor paralogs that control fundamental mechanisms of chromatin dynamics and gene regulation. **Acknowledgements:** We thank X. Wei and Y. Hao for technical assistance. I.M.B. is supported by National Institute of Diabetes and Digestive and Kidney Diseases R01 DK121059.

Abstract 059

Sustained Loss of ESRP2 Rewires Hepatocyte Splicing to Drive MetALD and Block Recovery

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Background: How post-transcriptional programs govern the balance between liver injury and regeneration remains poorly defined. RNA splicing is increasingly recognized as a driver of hepatocyte identity and plasticity. We previously showed that ESRP2 (Epithelial Splicing Regulatory Protein 2) controls adult hepatocyte-specific splicing networks. Yet low human transcriptomic resolution and inadequate models of human liver pathology have hindered mechanistic insight. Redefinition of alcohol-related liver disease as metabolic dysfunction and alcohol-associated liver disease (MetALD) in 2023 further highlights the need to define how diet–alcohol interactions reshape core regulatory networks in human liver. **Methods:** We generated ultra-deep RNA sequencing datasets (>100M reads/sample) from human liver across alcohol-related hepatitis (AH), cirrhosis (AC), and non-alcoholic steatohepatitis (NASH). While standard analytical pipelines confirmed known global changes, we applied novel computational approaches to resolve hepatocyte-specific transcriptional and splicing programs from our bulk data. These features were used to train a machine learning framework to classify >200 independent, shallow human liver transcriptomes into clinically-defined disease states. To establish causality, we developed a 12-week MetALD mouse model to mimic human disease. Combining ESRP2 loss- and gain-of-function mouse models with alcohol withdrawal-based recovery paradigms we probed the functional role of ESRP2 in MetALD progression and resolution using molecular and histological analyses. **Results:** Unprecedentedly deep RNA-seq revealed a hidden layer of hepatocyte-intrinsic regulation, resolving disease-specific splicing patterns missed by conventional datasets. These signatures distinguished inflammatory AH from fibrotic AC, partially overlapped with NASH, and identified ESRP2 as a consistently depleted splicing regulator. Hepatocyte-specific patterns derived from deep sequencing also classified 200+ shallow human datasets, supporting translational scalability. The MetALD mouse model mirrored key human liver pathobiology, including ESRP2 downregulation, and delineated diet and alcohol-driven changes. Functionally, ESRP2 deficiency in mice exacerbated MetALD-linked liver injury that failed to resolve upon alcohol withdrawal and instead exhibited progressive damage. Sustained hepatocyte-specific ESRP2 expression, however, ameliorated disease severity and aided recovery. **Conclusions:** We identified post-transcriptional reprogramming as a defining and predictive feature of human MetALD. Disorders in ESRP2-driven hepatocyte splicing program linked metabolic and alcohol-induced liver injury as maladaptive ESRP2 downregulation trapped hepatocytes in neither functionally competent nor regeneration-permissive pseudo-fetal states, that worsened disease pathology and blocked recovery. Restoring ESRP2 rescued these phenotypes, implicating ESRP2 as a central regulator of hepatocyte plasticity and a tractable therapeutic target in MetALD. **Acknowledgements:** We acknowledge support from the Histology and Microscopy core facilities at Veterinary Medicine and Carl R. Woese Institute for Genomic Biology (IGB), University of Illinois, Urbana-Champaign, respectively. This work was supported by the NIH grants R01-AA010154 and R21-HD104039, the Chan-Zuckerberg Biohub Chicago Award, Muscular Dystrophy Association Research Grant MDA1072487, and the Phillip A. Sharp Endowment (to AK), the Charles F. Kade Fellowship in

Biochemistry (to DD), the Ruppel Spudich Scholarship in Molecular and Cellular Biology (to AR), and the IGB Undergraduate Research Scholarship (to AB).

Abstract 060

Pioneer Factor FOXA-mediated DNA Demethylation in Human Hepatic Fate Programming

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Background: The pioneer transcription factor (TF) FOXA is essential for initiating hepatogenesis in the foregut endoderm and for liver development and homeostasis. By opening chromatin, FOXA facilitates recruitment of hepatic TFs to activate lineage-specific transcriptional networks. This property has been leveraged to directly reprogram fibroblasts into induced hepatocyte-like cells (iHeps); however, current approaches remain inefficient and yield off-target cells. One of the major barriers is the incomplete erasure of DNA methylation, a repressive epigenetic mark that restricts lineage plasticity. FOXA has been proposed to cooperate with TET DNA demethylases to prime hepatic enhancers, yet the mechanisms governing this interaction during hepatogenesis remain unclear. Moreover, low TET expression in fibroblasts may limit efficient DNA demethylation during iHep reprogramming. Here, we aim to **(1)** define if and how FOXA selectively and dynamically recruits TET to robustly establish and stabilize the hepatic fate program, and **(2)** enhance FOXA-TET cooperation to improve iHep reprogramming efficiency and fidelity. **Methods: (1)** Using a human pluripotent stem cell differentiation system that generates homogeneous foregut and hepatoblast populations, we established a doxycycline-inducible CRISPRi model to simultaneously knock down *FOXA1/2/3* (*FOXA*-TKD), followed by genome-wide TET ChIP-seq profiling. **(2)** Human fibroblast-to-iHep reprogramming was performed using FOXA3, HNF1A, and HNF4A. To test the role of DNA demethylation, TET3 was ectopically expressed, or 5-AzaC (DNA methyltransferase inhibitor) was applied at defined time points, followed by RNA-seq at intermediate and endpoint iHep stages. **Results: (1)** *FOXA*-TKD resulted in genome-wide redistribution of TET binding in foregut and hepatoblasts. FOXA-dependent TET binding sites were uniquely enriched for FOXA motifs and endoderm/hepatic TF motifs, and were associated with foregut and hepatic GO terms. **(2)** In iHep reprogramming, exogenous TET expression enhanced activation of some key hepatocyte genes, whereas global DNA demethylation by 5-AzaC preferentially increased epithelial gene expression without improving hepatocyte gene expression. **Conclusions:** These findings provide direct evidence that FOXA not only opens chromatin but also recruits TET DNA demethylases to hepatic enhancers during human hepatogenesis, potentially in cooperation with hepatic partner TFs. Furthermore, our data suggest that FOXA-TET-mediated, locus-specific DNA demethylation, rather than global demethylation, is critical for efficient and faithful iHep reprogramming. Ongoing studies will identify partner TFs and optimize TET induction, coupled with single-nucleus multiome profiling to benchmark iHeps against human liver datasets. This work establishes a mechanistic framework for improving iHep generation and suggests that precise epigenetic editing strategies may overcome current barriers in regenerative medicine.
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Poster Session – Liver Genetic Disease and Gene Therapy

Abstract 061

Impacts of Fluid Shear Stress on NOTCH Signaling in a *JAG1* KO Model of Alagille Syndrome Using Cholangiocytes

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Background: Liver-on-a-chip (LOC) systems are microscale platforms that have been used to examine liver metabolism, repair, and injury under dynamic, 3D conditions *in vitro*. One advantage of LOCs is their ability to incorporate physiologic stimuli absent in standard 2D cell culture, such as fluid shear stress using pressure-driven perfusion, that can encourage mechanobiological interactions between cells. The integration of human-derived cell sources in LOC platforms allows for a unique opportunity to examine the manifestation of rare genetic liver diseases affecting the liver, such as Alagille syndrome (ALGS), to model variations in phenotype between

patients. ALGS is characterized by a paucity of interlobular bile ducts arising from *JAG1* or *NOTCH2* haploinsufficiency, which disrupts NOTCH signaling during embryogenesis and impairs hepatobiliary development (*Nature Genet*. 1997, 16:243-251; *Nature Genet*. 1997, 16:235-242; *Am J Hum Genet*. 2006, 79:169-173 ; *J Pediatr*. 1987, 110:195-200; *J Med Genet*. 1997, 34:152-157). Despite informative knockout experiments using murine models, species-specific cell and molecular differences underscore the need for patient-derived, human-relevant systems to capture genotype–phenotype diversity and therapeutic responses (Kamath BM, Loomes KM, eds. *Alagille Syndrome: Pathogenesis and Clinical Management*. Springer International Publishing; 2018:167-193; *Drug Discov Today*. 2019, 24:2139-2151). The role of JAG1 in endothelial cells under fluid shear stress have previously been examined by Rodriguez et al. using the Ibidi microfluidic chip platform (FEBS J. https://doi.org/10.1111/febs.70466). This study similarly aims to examine the unique relationship between fluid shear stress but in the context of ALGS by perfusing *JAG1* knockdown cholangiocytes spheroids in-chip and observing how flow perturbs downstream receptor-ligand interactions via the NOTCH pathway. **Methods:** To engineer a perfusable vascularized human liver on a chip, we utilized engineered endothelial cells which we re-expressed a vasculogenic transcription factor ER71. We have been previously shown that upon re-expression of ER71, the engineered ECs intermingled with primary human hepatocytes in a microfluidic device. The device is fabricated with two large biomimetic vessels to represent an arteriole and a venule. The engineered ECs formed an inter-connected network of capillary vessels that span the interstitial space between the arteriole and venule vessels. These capillary vessels also connected the arteriole and venule vessels. After the device was connected to peristaltic pump to simulate blood circulation from arteriole to capillary to venule, we used this system to investigate long term function of hepatocytes. Primary human biliary organoids, pluripotent stem cell derived cholangiocytes or the cholangiocyte cell line MMNK-1 was used to create an integrated biliary tree in a similar fashion to the perfusable vascularized platform previously generated with human hepatocytes. To determine the impact of shear stress on NOTCH signaling, an shRNA was used to create a *JAG1* knockdown in the respective cell systems and verified using qPCR and western blotting. Wild-type cells were transduced with a fluorescent NOTCH reporter lentivirus reporter. NICD transduced cells were used as a positive control. Cells seeded in the Ibidi µ-Slide VI system and were perfused at varying flow rates using a peristaltic pump (and hence at varying shear stress exposures). The effects of step-wise shear stress on NOTCH signaling were then qualified using fluorescence microscopy and proximity ligation assays. **Results and Conclusion**: LOC fluid shear stress studies specifically examining ligand-receptor interactions in the context of ALGS remains an underdeveloped area of research (*Adv Healthc Mater*. 2025, 14:2501776). Future work could benefit from additional complexity in-chip through dynamic co-culture with other hepatobiliary cell populations to more accurately represent the heterogeneity of the liver. Overall, this study aims to explore the effects of fluid flow rate in microfluidic chips on binding and signaling in the context of *JAG1* in activating downstream NOTCH signaling to better understand how biliary development manifests in ALGS patients. **Acknowledgements:** This work was supported by the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK; R01DK146438 and R01DK138677).

Abstract 062

Discovering Nanoparticle Corona Ligands for Liver Macrophage Capture

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Background: Researchers design therapeutic nanoparticles to deliver drugs to disease sites. The liver captures and removes nanoparticles from circulation, preventing them from targeting extrahepatic diseased tissues and cells. Kupffer cells take up most circulating nanoparticles. Nanoparticles accumulate a layer of serum proteins on their surfaces in the bloodstream. The adsorbed serum proteins and their cognate cell receptors used to remove nanoparticles from the bloodstream have not been linked. Here, we identify the adsorbed serum proteins that bind to specific liver macrophage receptors. **Methods:** We used a multi-omics approach to identify these interactions. We used a genome-wide CRISPR knockout screen to identify macrophage receptors used to bind nanoparticles. Then we used proteomic mass spectrometry to identify the absorbed nanoparticle serum proteins. We matched receptors to ligands on the nanoparticle with STRING. After further experimental validations, we identified these interactions. **Results:** We discovered six absorbed serum proteins that bind to two liver macrophage receptors. Nanoparticle physicochemical properties can affect the degree of the six serum proteins adsorbing to the surface, the probability of binding to cell receptors, and whether the liver removes the nanoparticle from circulation. Identifying the six adsorbed proteins allowed us to engineer decoy nanoparticles that prime the liver to take up fewer therapeutic nanoparticles, enabling more nanoparticles for targeting extrahepatic tissues. **Conclusions:** Elucidating the molecular interactions governing the nanoparticle journey in vivo will enable us to control nanoparticle delivery to diseased tissues. **Acknowledgements:** This work was supported by the Canadian Institute of Health Research, the NanoMedicines Innovation Network, and the Canadian Research Chairs Program.

Abstract 063

5’ UTRs Contain Disease-relevant, Targetable Motifs to Treat Genetic Disease

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Background: 5’ UTRs serve as loading points within mRNA for translational machinery components to ensure the regulated production of the gene products of protein-coding genes. Over evolutionary time, the lengths of 5’UTRs and distribution of RNA-regulatory elements (RREs) within them, have fine-tuned translational output to appropriate levels. Naturally occurring human genetic variation within these untranslated regions can be statistically associated with disease phenotypes to nominate motifs of potential clinical importance. **Methods**: We have used computational predictions, dual luciferase assays, Western blots, site-directed mutagenesis, and antisense oligonucleotides (in cultured cell lines, primary patient cell lines, and *in vivo* in mouse) to nominate, test, and validate functional RREs in clinically actionable target pathways, including the BMPR2 and Notch signaling pathways. We have developed a dual-fluorescent protein screening platform to deeply probe 5’UTR regulation in individual transcripts and broadly probe perturbation of hundreds of transcripts, generating datasets by FACS-Seq for machine learning rule prediction. **Results**: Our data provide novel, well-validated targets for the functional bidirectional manipulation of translational output from disease relevant genes, in particular of the BMPR2 receptor: a haploinsufficient risk factor for pulmonary arterial hypertension that also regulates fibrosis in liver and lung. **Conclusions**: 5’ UTR RREs represent an underappreciated axis for potential therapeutic intervention that has implications for a broad range of diseases, including haploinsufficient conditions like Alagille syndrome. **Acknowledgments**: The authors thank the following funding sources: the UPenn Digestive and Liver Center (P30DK050306), Pilot Grant (NH), The University of Pennsylvania, Dean’s Innovation Fund (YB), NIH/NIGMS R01GM147739 (MPI: NH, YB).

Abstract 064

Dissecting Hepatocyte Heterogeneity in Liver Growth to Improve *in vivo* Gene Therapy

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Background: The liver plays a central role in many physiological processes, and mutations in hepatocyte-expressed genes cause monogenic disorders that are potential targets for liver-directed gene therapy. Despite being the platform of choice for *in vivo* gene therapy, the episomal nature of adeno-associated viral (AAV) vectors limits transgene durability during postnatal liver growth, making integrating lentiviral vectors (LV) and genome editing preferable for long-lasting correction in young patients. Our recent work showed that most of the adult liver arises from a small subset (15-20%) of newborn hepatocytes, which are the most proliferative and least differentiated. Spatial transcriptomics revealed that these clonogenic hepatocytes co-localize with hematopoietic progenitors in a niche enriched for pro-proliferative signals. The initial distribution of genetic modification across proliferating vs. quiescent hepatocytes shapes long-term outcomes: homology-mediated editing in newborns is enriched in clonogenic hepatocytes and expands over time, whereas neonatal LV delivery drives uniform integration and stable maintenance of the modified fraction (Milani, Starinieri et al., J Hep 2025). **Methods and Results:** To further dissect hepatocyte heterogeneity during postnatal maturation, we are combining high-resolution VISIUM HD spatial transcriptomics with CARLIN lineage tracing (Bowling et al. *Cell* 2020). Preliminary experiments assessed CARLIN barcode generation, via embryonic labeling with doxycycline at gestation day 17 or postnatal induction with viral-like particles or lipid-nanoparticle (LNP) delivery of Cas9 plus CARLIN guide RNAs in 2-day-old CARLIN mice. These experiments revealed high variability in liver barcoding across methods and litters, with top samples achieving ~25% editing. Unsupervised clustering of VISIUM HD datasets from 2-day, 2-week and adult livers confirmed expected transitions: abundant hematopoietic and proliferating hepatocytes in neonates, reduced hematopoiesis and emergence of zonation at 2 weeks, and full periportal-pericentral organization in adults. Current analyses focus on proliferating hepatocytes and their interaction with the hematopoietic niche. To further assess the impact of gene engineering in wild-type (WT) mice, we developed a homology-based genome-editing strategy using the second intron of the murine albumin locus as a safe harbor for the integration of a therapeutic transgene. This strategy employs co-administration of AAV8- donor DNA and LNP-Cas9/mRNA. We will initially evaluate the editing efficiency *in vivo* using a reporter transgene. Then, we will assess how stable transgene integration—achieved through LV or genome editing—affects the transcriptional landscape of distinct hepatocyte subsets during postnatal liver growth. **Conclusion:** Overall, this work lays the groundwork for an in-depth investigation of the molecular and cellular dynamics during liver growth and maturation, and of how in vivo liver-directed gene engineering affects these processes.

Abstract 065

Vascular Endothelial Growth Factor (VEGFA) mRNA in Lipid Nanoparticles as a Bridge Therapy for AATD Liver Disease

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Background: Alpha-1 antitrypsin deficiency (AATD) is caused by a single base-pair mutation in the *SERPINA1* gene, leading to the production of the aberrant Z-AAT protein, which misfolds and polymerizes within hepatocytes, resulting in hepatotoxicity. Liver transplantation remains the only treatment for AATD-associated chronic liver disease. However, due to the scarcity of donors, many patients die while awaiting transplantation, highlighting the urgent need for alternative therapies. Hepatocyte proliferation is the primary mechanism of liver regeneration; however, this process is impaired in chronic liver diseases especially in AATD with the accumulation of Z-AAT globules within hepatocytes. In such contexts, biliary epithelial cells (BECs) can compensate by proliferating and differentiating into functional hepatocytes. The goal of this study is to investigate BEC-driven liver repair in alleviating AATD-associated liver disease and serve as a bridge therapy for patients awaiting transplantation. **Methods:** We generated a BEC lineage-tracing mouse model using tamoxifen-inducible KRT19-CreERT tdTomato reporter mice crossed into the PiZ background recapitulating AATD-associated liver disease. Mice were pretreated with a single injection of AAV8-TBG-p21 to induce hepatocyte senescence, a feature observed in AATD patients. VEGFA mRNAs in lipid nanoparticles (LNPs) were administered four times to promote BEC-to-hepatocyte conversion, as we previously demonstrated in a diet-induced chronic liver injury model (Rizvi et al., 2023). Cellular conversion was quantified by enumerating tdTomato^{+} hepatocytes. Additionally, single-nucleus RNA sequencing data from six homozygous ZZ patient livers and two normal MM livers were analyzed to assess evidence of BEC-to-hepatocyte conversion in human

AATD. **Results:** Mice treated with VEGFA mRNA-LNPs exhibited a significant increase in tdTomato+ hepatocytes that appear healthy as they lacked Z-AAT globules compared to control mRNA-LNP-treated groups where tdTomato+ hepatocytes were absent. However, the efficiency of conversion was lower than previously observed in the diet-induced injury model. This reduced conversion correlated with the accumulation of CD45+ immune cells surrounding BECs, potentially inhibiting VEGFA-mediated conversion. Consistent with these findings, human data revealed a transitional cell population bridging BECs and hepatocytes, characterized by a pronounced inflammatory transcriptomic profile. **Conclusions:** Our findings demonstrate that VEGFA mRNA-LNPs significantly promote BEC-to-hepatocyte conversion in an AATD mouse model. However, this process is less efficient than expected, likely due to the heightened inflammation associated with this disease. Future studies will focus on mitigating inflammation to enhance VEGFA-driven liver repair and evaluating the function of newly generated hepatocytes. **Acknowledgements:** NIHR01DK133404 and alpha 1 Foundation ID:1255268 award to VGE.

Abstract 066

A Forward Genetic Approach to Identify Modifiers of Liver Disease Risk in Alpha-1 Antitrypsin Deficiency (ATD)

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Background: Alpha-1 antitrypsin deficiency (AATD) is a monogenic disorder most commonly associated with the Z mutation (E342K) in *SERPINA1* that causes misfolding, polymerization, and accumulation of mutant alpha-1 antitrypsin (ZAAT) protein within the endoplasmic reticulum of hepatocytes. Intracellular ZAAT polymerization induces proteotoxic stress and contributes to liver injury, while systemic deficiency of functional AAT predisposes patients to pulmonary emphysema. Although all individuals with the homozygous “PiZZ” genotype produce misfolded ZAAT, only a subset develop progressive liver disease, suggesting that, in addition to environmental risk exposures, genetic factors also contribute to disease susceptibility and severity. We hypothesize that genes regulating ZAAT intracellular trafficking, processing, degradation, and secretion modulate ZAAT accumulation associated with hepatocellular injury and clinical disease. **Methods:** To identify the effects of targeted gene perturbations on intracellular ZAAT accumulation, we engineered fluorescent ZAAT reporter cell lines and optimized a fluorescence-activated cell sorting (FACS)-based CRISPR interference (CRISPRi) screening platform. We additionally utilized induced pluripotent stem cell lines derived from well-characterized PiZZ patients to generate hepatocytes (iHeps) to model liver-specific disease biology. Candidate modifier genes identified from the screen will be further validated in iHeps to characterize their mechanistic contribution to protein processing and global hepatocellular transcriptomic regulation. **Results:** We established stable expression of mEmerald-ZAAT and ZIM3-dCas9 CRISPRi machinery in the hepatic HepG2 cell line via lentiviral transduction. Using these engineered cells, we performed a genome-scale FACS-based CRISPRi screen targeting protein-coding genes to identify perturbations capable of either reducing or increasing intracellular ZAAT levels. In parallel, we established fluorescent ZAAT reporter knock-ins at the endogenous *SERPINA1* locus in multiple AATD patient-derived iPSC lines. These edited patient lines have been successfully characterized and demonstrate reporter expression patterns comparable to their unedited controls. **Conclusions:** This platform provides a scalable and disease-relevant approach to identify genetic modifiers that influence ZAAT-induced proteotoxic injury in AATD. Identification and mechanistic characterization of modifier genes may help explain phenotypic heterogeneity among PiZZ individuals and reveal novel therapeutic targets for AATD-associated liver disease.
Acknowledgements: This project was supported by a postdoctoral research grant from the Alpha-1 Foundation.

Poster Session – Liver Homeostasis and Regeneration

Abstract 067

Temporally Controlled Expression of a Splicing Factor in Single Cells Coordinates the Metabolic and Proliferative Activities of Regenerating Livers

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Background: The exact mechanics of liver regeneration, such as how quiescent hepatocytes transition into a proliferative state and how regenerating livers sustain normal metabolic activities while the tissue recovers from

injury, are largely unknown. The role of alternative splicing and RNA binding proteins has remained completely uninvestigated. Epithelial splicing regulator protein 2 (ESRP2) is an RNA splicing factor that acts as the developmental switch for splicing targets during liver maturation, including in the Hippo signaling pathway. ESRP2 promotes the production of adult Hippo pathway splice variants thereby limiting hepatocyte proliferation in a quiescent mature liver. We previously reported ESRP2 and its splicing targets are transiently reprogrammed to the fetal stage in hepatocytes during active phases of regeneration. We further hypothesized that ESRP2 is temporally controlled within regenerating hepatocytes to promote a proliferative or metabolic state via its splicing targets. **Methods:** We used the ⅔ partial hepatectomy procedure on 8wk old mice to study the role of ESRP2 during liver regeneration. Comparisons on KO and OE mice against appropriate controls were used to determine the relative changes in expression of known splicing targets involved in metabolism, proliferation, and the Hippo signaling pathway. We used single-cell RNA sequencing to determine altered cell states in ESRP2KO cells compared to WT during the initiation and termination stages of liver regeneration. Finally, we used ASOs to switch alternative splicing of key Hippo signaling pathway targets to validate the functional impact of ESRP2 during liver regeneration in mice. **Results:** Forced expression of ESRP2 in the mouse liver inhibits the proliferation of hepatocytes, whereas ESRP2 deletion in hepatocytes increases their proliferative index during liver regeneration. The temporal regulation of ESRP2 during liver regeneration is also accompanied by splice isoform switching of Hippo signaling genes. Using a mix of ASOs targeting the Hippo signaling pathway, we could partially rescue the proliferative defect of ESRP2OE mice. Here we also show that hepatocytes bifurcate into proliferative or metabolic cell states, such that ESRP2 is highly expressed in the metabolically active hepatocytes and is absent in the proliferating hepatocytes. **Conclusions:** Taken together, these data imply that tightly controlled expression of ESRP2 coordinates the metabolic and proliferative activities of hepatocytes during liver regeneration, primarily through the splicing of key Hippo signaling pathway genes. **Acknowledgements:** CZI; CBI; NIH.

Abstract 068
Human-length Telomeres Delay Regeneration of the Liver Parenchyma in Mice
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Background: Telomeres are critical for maintaining genomic integrity and naturally shorten during DNA replication due to the “end replication problem” unless extended by telomerase. Telomeres shortened below a critical length become uncapped, activating the DNA damage response pathway leading to replicative senescence. The commonly used C57BL/6 mouse strain has telomeres about 5 times longer than those present in humans. We recently engineered the C57BL/6 “Telomouse” to enable the study of human length telomeres, which we used here to examine the effects of shortened telomeres on liver regeneration. **Methods:** Telomere length was measured at base resolution using *NanoTelSeq*. We performed partial hepatectomy (PHx) experiments with Telomice using wild type C57BL/6 mice as controls. Staggered injections of the thymidine analogs CldU and IdU were used to assess proliferation. Transplantation of isolated hepatocytes into Fah (fumarylacetoacetate hydrolase) null mice was performed to determine hepatocyte repopulation capability. **Results:** Human-length telomeres limited the proliferative capacity of cholangiocytes and hepatocytes in short-term liver regeneration. While control mice exhibited significant cholangiocyte proliferation at 36 hours post-PHx, which remained stable at 46 hours post-PHx, Telomice exhibited decreased cholangiocyte proliferation at both time-points. Both control and Telomice exhibited increased hepatocyte proliferation at 46 hours compared to 36 hours post-PHx. However, Telomice exhibited less proliferation than controls at both time points and increased DNA damage response after partial hepatectomy. Over time, proliferation caught up such that the total number of hepatocytes that have undergone S-phase in Telomice is comparable to that of the wild type mice. In the experiments using the Fah null mouse model of conditional hepatocyte ablation, Telomice hepatocytes also exhibited reduced efficacy in repopulation. **Conclusions:** Short, human-length, telomeres induce DNA damage in the regenerating liver, hampering its ability to accelerate cell proliferation and regenerate the liver. These findings may provide a potential explanation for the delayed regenerative capacity of human livers. The significant effect of telomere length on the liver regeneration potential described here highlights the importance of telomere testing in donors of liver transplants to ensure higher transplantation success. **Acknowledgements:** We thank Monica Neustadter-Blackman (Tzfati lab) for advice on *NanoTelSeq* and the Molecular Pathology and

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Abstract 069
Endothelial ARNT is Required for Wnt-dependent Metabolic Zonation and Liver Regeneration
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Background: The liver performs essential functions required for survival. For maximal efficiency, the liver is spatially organized into functional zones along the portal-central axis, a phenomenon known as liver zonation (LZ). Zone 1 (periportal) is closest to the portal vein; zone 2 is an intermediate zone; finally, zone 3 (pericentral) is a less oxygenated region closest to the central vein. Previous studies have established that Wnt-β-catenin signaling is a critical regulator of LZ and liver regeneration (LR) after partial hepatectomy (PH) via *Wnt2* and *Wnt9b* production from zone 3 endothelial cells (ECs). What remains unknown is the upstream regulators of Wnt-β-catenin signaling. We hypothesize that relative hypoxia in Zone 3 drives Hypoxia-inducible factor (HIF) signaling in endothelial cells to regulate *Wnt2* and *Wnt9b* expressions, thereby controlling LZ and LR after PH. **Methods**: For our study, we generated *Lyve1-Cre; Arnt$^{f/f}$* (*Lyve1$^{\Delta Arnt}$*) mice to delete *Arnt* (HIF-1β) from ECs. PH was performed on 10-12-week-old mice to assess proliferation at 40 hours post-PH. **Results**: At baseline, endothelial *Arnt* deletion resulted in significant portal liver necrosis and injury, portal fibrosis, inflammation, and compensatory enhanced pericentral hepatocyte proliferation. This observation was associated with capillarization of zone-1 sinusoidal endothelial cells. In addition, there was a notable expansion of zone 1 and constriction of zone 3, indicated by immunohistochemistry and spatial transcriptomics. *Wnt2* and not *Wnt9b* expression and WNT target genes were significantly downregulated in whole liver qPCR. Additionally, bulk RNA-seq revealed global changes in metabolic functions in KO livers typically reminiscent of zone 3 functions such as lipogenesis, xenobiotic metabolism, and bile acid synthesis. After PH, mice hepatocytes reach peak mitosis at 40h through the panzonal expansion of WNT/β-catenin signaling, as also indicated by cyclin-D1 expression. When *Arnt* was deleted, there was a significant decrease in Ki-67$^+$ and cyclin-D1$^+$ hepatocytes at 40h post-PH. **Conclusions**: Our data suggest that endothelial ARNT is required for maintenance of sinusoidal endothelial cell phenotype, especially in zone-1. Additionally, ARNT in endothelial cells controls zone-3 hepatocyte β-catenin activity and zonation by regulating *Wnt2* expression. Likewise, endothelial cell ARNT is essential for inducing panzonal endothelial *Wnt2* expression and hepatocyte β-catenin activation during regeneration post-PH.

Abstract 070
Macrophage Colony-stimulating Factor Prolongs Kupffer Cell Survival, Phenotype, and Phagocytic Potential Function *in vitro*
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Background: Macrophage depletion models following liver injury (PMID:33958644) or partial hepatectomy (PMID:17322066) impairs liver resolution and regeneration, highlighting the importance of hepatic myeloid cells in maintaining tissue homeostasis. These cells promote immune tolerance while surveying the sinusoidal lumen (PMID:29328785) and coordinate innate and adaptive immune responses. Their phenotypic and functional plasticity makes them a promising therapeutic target (PMID:33041338). Macrophage colony-stimulating factor (M-CSF) is widely used to support myeloid cell survival and differentiation *in vitro* (PMID:3604662). However, its influence on the phenotypic identity of primary Kupffer cells (KCs) remains poorly defined. This study aims to investigate the effects of M-CSF supplementation on primary murine KCs cultures. **Methods:** Murine livers were harvested using an in-situ two-step collagenase perfusion and digestion, followed by non-parenchymal cells isolation and F4/80+ enrichment. Cells were cultured with 0-100 ng/mL of M-CSF for up to 7 days. IncuCyte live-cell imaging was used to measure confluency and early apoptosis using an Annexin V Red assay. Phenotypic characterization across M-CSF concentrations and timepoints was performed using flow cytometry. Phagocytic

capacity was assessed by measuring E. coli pHrodo uptake over 24 hours under the same culture conditions using IncuCyte imaging. Additionally, cultures in the presence and absence of 20 ng/mL M-CSF were analyzed across defined timepoints using bulk RNA-sequencing to profile transcriptomic changes. **Results:** Cultures without M-CSF failed to recover to baseline confluency by day 7, unlike supplemented cultures (n=4). Optimal survival of cultured cells occurred at 20–60 ng/mL M-CSF, as determined by Annexin V assay (n=3). CD206 expression increased and MHC-II expression decreased over time (n=3), with higher CD206 expression at day 7 in 10–40 ng/mL M-CSF conditions. Phagocytosis was greatest at 10–40 ng/mL M-CSF (n=4), while cultures treated with 60 ng/mL M-CSF was comparable to inhibitor-treated controls (n=4). The transcriptomic analysis revealed culturing duration had a greater effect than M-CSF (n=3–4), though M-CSF better preserved hallmark pathways associated with cell cycle, survival and metabolism. **Conclusion:** M-CSF (20–40 ng/mL) supports survival and phagocytic function of primary hepatic myeloid cell cultures. While phenotypic changes and the transcriptomic landscape are largely time-dependent, M-CSF enhances CD206 expression and preserves key pathways. Collectively, these findings establish a platform for maintaining viable primary KCs and support therapeutic development. **Acknowledgements:** Bulk RNA sequencing performed by the Princess Margaret Genomics Centre.

Abstract 071

Spatial Resolution of Gene Functions in Lipid Metabolism and Liver Regeneration

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Background: Metabolic dysfunction–associated steatotic liver disease (MASLD) impairs hepatic regeneration, yet the mechanisms linking accumulation of lipid species to hepatocyte fitness remain unclear. Conventional approaches lack resolution to connect gene perturbations with local metabolic phenotypes. We aimed to integrate CRISPR interference (CRISPRi) with spatial lipidomics and transcriptomics to identify how altering gene expression can influence lipid metabolism and affect liver regeneration. **Methods:** We performed pooled in vivo CRISPRi targeting 16 lipid-handling genes (e.g., APOE, CIDEB, CPT1A, CPT1B, CPT1C, FOXO1, GCKR, GPAM,HSD17B13, INSR, MBOAT7, MTTP, PLIN2, PNPLA2, PNPLA3, TMC4) under chow and choline-deficient high-fat diet (CDHFD) conditions in the *Fah-/-; dCas9* mouse model of liver repopulation. Guide RNA enrichment/depletion during clonal repopulation was quantified by sequencing. Spatial lipidomics (MALDI-MSI) and 10x Visium transcriptomics will be applied to regenerating livers to map lipid species and gene-expression programs linked to each perturbation. We further evaluated the regenerative role of CIDEB through CRISPRi knockdown in the *Fah-/-; dCas9* mouse model. **Results:** CRISPRi screening revealed distinct regulators of regenerative fitness. MTTP and INSR knockdown impaired repopulation (gRNA depletion), whereas CIDEB and HSD17B13 repression enhanced regeneration (gRNA enrichment), consistent across diets. PNPLA2 knockdown conferred selective advantage under CDHFD, suggesting diet-specific modulation of triglyceride hydrolysis. Integration of MALDI-MSI and Visium datasets is underway to spatially resolve lipid remodeling and transcriptional programs associated with these perturbations. **Conclusions:** Our findings identify CIDEB and HSD17B13 as promising targets for enhancing regeneration in steatotic livers and highlight PNPLA2 as a diet-sensitive regulator. This work establishes a spatial functional-genomics framework combining CRISPRi, lipidomics, and transcriptomics to dissect localized metabolic networks driving liver repair. These insights may inform therapeutic strategies for MASLD and related chronic liver diseases. **Acknowledgements:** Mayo Clinic.

Abstract 072

Aging Disrupts Hepatic Zonation and Architecture Through Circadian Regulation of Liver Homeostasis

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Background: Aging-associated changes in the liver are not inherently pathological but increase susceptibility to chronic liver disease. However, the mechanisms underlying aging-induced hepatic dyshomeostasis, particularly alterations in liver architecture, intercellular communication, and hepatocyte zonation, remain poorly defined. **Methods:** We performed histological and immunostaining analyses on young (2-month-old) and aged (>24-month-old) wild-type mouse livers, combined with single-nucleus RNA sequencing to assess cell-type–specific transcriptional and intercellular changes. Zonation markers (ASS1, CYP2E1, GS) were used to evaluate spatial organization. Human liver biopsies from young (≤25 years) and aged (>60 years) donors were analyzed for translational validation. Transcriptional network analysis identified candidate regulators, and hepatocyte-specific *Bmal1* floxed mice were generated to assess circadian regulation of liver zonation. **Results:** Aged mouse livers showed preserved architecture without fibrosis but exhibited hepatocyte enlargement. Single-nucleus RNA sequencing revealed altered cell–cell communication and zone-specific transcriptional reprogramming, indicating disrupted hepatocyte zonation. Immunostaining confirmed expansion of ASS1$^+$, CYP2E1$^+$, and GS$^+$ zones and the emergence of aberrant ASS1$^+$/GS$^+$ bi-zonal hepatocytes. These changes were associated with downregulation of key zonation regulators (Ctnnb1, Foxo1, Tcf7l2) and compensatory alterations in Wnt and Rspo3 signaling from non-parenchymal cells. Similar zonal disruptions were observed in aged human liver samples. Regulatory analysis identified CLOCK as a master transcription factor governing zonation pathways. Hepatocyte-specific deletion of BMAL1 caused early disruption of zonation (by 2 months), including reduced GS expression, patchy CYP2E1 distribution, and hepatocyte hypertrophy, which worsened with age (10 months). **Conclusions:** Aging disrupts hepatic zonation and intercellular communication through coordinated transcriptional and architectural remodeling. The emergence of bi-zonal hepatocytes and zone expansion are key hallmarks of liver aging. Circadian regulation via the CLOCK–BMAL1 axis is critical for maintaining zonal integrity, highlighting a potential therapeutic target for age-associated liver dysfunction.

Poster Session – MASLD and MASH

Abstract 073

Roles of TorsinA in Regulating Lipid Metabolism in Human Hepatocytes

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Background: Abnormalities of liver lipid metabolism cause metabolic dysfunction-associated steatotic liver disease (MASLD), a growing public health epidemic estimated to affect approximately 25% of the U.S. population. Our previous research established torsinA, an AAA+ ATPase in the endoplasmic reticulum/nuclear envelope as a key regulator of hepatic lipid metabolism. Conditional deletion of *Tor1a* in mouse hepatocytes caused marked steatosis, characterized by lipid droplet accumulation resulting from impaired apolipoprotein B (ApoB)–dependent very-low-density lipoprotein (VLDL) secretion. While these findings demonstrated a significant function of torsinA in hepatic lipid metabolism in mice, its role in VLDL secretion in human hepatocytes remains unknown. Here, we investigate the role of torsinA in regulating lipid metabolism in human induced pluripotent stem cell (hiPSC)-derived hepatocytes (iHeps). **Methods:** Multiple *TOR1A* knockout (*TOR1A*-KO) lines were generated from a commercially available hiPSC line using CRISPR-Cas9 genome editing. Mutant and isogenic control iPSC lines were then differentiated to hepatocytes following an established protocol. Hepatic lineage progression was validated by RT-qPCR of stage-specific markers. To assess steatosis, iHeps were stained with BODIPY to visualize the neutral lipid droplets using confocal microscopy. ApoB secretion into the culture medium was measured using an ELISA kit. **Results:** *TOR1A*-KO iPSCs successfully differentiated into iHeps (Day 21 onwards), progressing through Definitive Endoderm (DE) and Hepatic Endoderm (HE) stages with phenotypes comparable to the isogenic control. Stage-specific expression of DE, HE and iHep markers confirmed normal developmental progression, indicating that loss of *TOR1A* does not disrupt the overall hepatic differentiation trajectory. We confirmed that torsinA expression was absent in the *TOR1A*-KO line throughout differentiation and in the iHeps. Steatosis analysis in Day 25+ iHeps revealed pronounced lipid accumulation in *TOR1A*-KO cells compared to controls, recapitulating the steatotic phenotype previously observed in the mouse model. Consistent with the increased steatosis phenotypes, ApoB secretion was significantly reduced in the *TOR1A*-KO iHeps relative to controls, indicating that torsinA depletion causes impaired VLDL secretion in human

hepatocytes. **Conclusions:** Our findings demonstrate that torsinA has a crucial role in ApoB-mediated VLDL secretion and is required for maintaining lipid homeostasis in human hepatocytes. These results suggest torsinA as a potential therapeutic target for addressing dysregulated lipid metabolism in MASLD. **Acknowledgements:** This work was supported by the National Institutes of Health 1R01CA283566 (to JYS), Pinnacle Research Award from American Association for the Study of Liver Disease (JYS) and NIHR Imperial Biomedical Research Centre (BRC) (TR).

Abstract 074
Genome Editing Highlights SUGP1 as a Key Contributor to MASLD and Lipid Accumulation
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Background: Metabolic dysfunction-associated steatohepatitis (MASH) is a leading cause of chronic liver disease, yet effective therapies remain limited. Genome-wide association studies have identified genetic risk variants at the TM6SF2 locus, including SUGP1 (rs10401969, C/T allele) and TM6SF2 (rs58542926, C/T allele)**,** which increase MASH susceptibility. These two variants are in high linkage disequilibrium, and the genetic association at this locus is typically attributed to the TM6SF2 coding variant. Recent evidence indicates that the SUGP1 “C” allele is a functional variant contributing to MASLD development. SUGP1 knockdown in cellular and animal models (see Teker et al abstract) has been shown to increase cellular steatosis and accelerate MASLD/MASH progression. The protective T alleles at these loci are associated with reduced disease risk, but their functional mechanisms remain poorly understood. We hypothesized that CRISPR-mediated correction of the SUGP1 “C” risk variant would reverse disease-associated lipid accumulation phenotypes and reveal convergent protective mechanisms contributing to MASLD susceptibility at the TM6SF2 locus. **Methods:** Using CRISPR/Cas9, we edited iPSCs from a MASH patient carrying heterozygous risk alleles (C/T) at SUGP1 rs10401969 and TM6SF2 rs58542926 loci. Three lines were generated: SUGP1-corrected, TM6SF2-corrected, and double-corrected. All lines, along with parental MASH-carrier and healthy non-carrier iPSCs, were differentiated to hepatocyte-like cells (iHeps). Intracellular lipid accumulation was quantified after oleate treatment, and RNA-sequencing was performed. **Results:** Parental MASH-carrier iHeps (C/T) exhibited robust (5.5 ± 0.5 fold-change, FC) lipid accumulation compared to BSA control. Correction of the SUGP1 risk variant reduced lipid content by 52% (FC = 2.6 ± 0.4, $p < 0.01$), while TM6SF2 correction reduced accumulation by 55% (FC = 2.5 ± 0.3, $p < 0.001$). Correction of both alleles led to the greatest (64%) reduction (FC = 2.0 ± 0.2, $p < 0.0001$). RNA-seq analysis revealed transcriptional reprogramming following CRISPR correction of SUGP1 and TM6SF2 risk alleles. Lipid metabolism pathways were remodeled (60–66% of 983 lipid metabolism genes altered), and ECM pathway activity was reduced compared to MASH lines, suggesting attenuation of fibrosis-related processes. Key lipid genes exhibited distinct regulation: FASN was downregulated across all corrected backgrounds, while SCD was specifically upregulated in TM6SF2-corrected cells. These findings align with previous studies showing SUGP1’s role in MASLD progression (see Teker et al abstract), supporting its involvement in lipid metabolism and ECM remodeling. **Conclusions:** Correction of SUGP1 and TM6SF2 independently reduces hepatocellular lipid accumulation, with additive effects when both variants are corrected. Transcriptomic profiling reveals shared and distinct lipid metabolic pathways, validating SUGP1 and TM6SF2 as causal MASH risk genes and therapeutic targets. **Acknowledgements:** This work was supported by NIH R01 DK130391.

Abstract 075
The Role of Hepatic Mitochondrial Acyl-CoA Metabolism in Steatotic Hepatocytes
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Background: Metabolic-associated steatotic liver disease (MASLD) is characterized by dysregulated lipid homeostasis and persistent metabolic stress. Mitochondrial acyl-CoA thioesterases (ACOTs) modulate the balance between acyl-CoAs and free fatty acids, yet their transcriptional contributions to MASLD are not well defined. In this study, we examined the effects of two mitochondrial ACOTS, Acot2 and Acot9, knockdown (KD) on lipid metabolism and hepatocyte stress related gene expression. **Methods:** AML12 mouse hepatocytes were

subjected to shRNA-mediated knockdown of Acot2 and Acot9, followed by induction of steatosis using a physiologically relevant palmitic/oleic acid mixture (1:2 ratio). Gene expression levels were assessed by qPCR, while degree of steatosis was determined by staining of lipid droplets with Oil Red O. **Results:** Steatosis markedly induced the expression of genes involved in lipogenesis and lipid processing (Fas, Acc, Srebp1, Dgat1/2, Gpat1, Acsl1/3/5, Cpt1/2, Plin2) as well as markers of cellular reticulum stress (Grp78, Atf4/6, Xbp1, Ddit3, Sod1, Ero1a, Traf2) in liver cells. Knockdown of either Acot2 or Acot9 substantially blunted this transcriptional response, returning most gene expression levels toward physiological baseline, which resulted in significant decrease in lipid droplet accumulation. Notably, Acot9 knockdown elicited a pronounced compensatory upregulation of Cpt2, consistent with an adaptive shift toward enhanced mitochondrial fatty acid oxidation. In contrast, Acot2 knockdown failed to suppress Plin2 and further increased Fabp5 expression, suggesting augmented lipid droplet stabilization and fatty acid trafficking as protective mechanisms against lipotoxicity. **Conclusions:** Mitochondrial ACOT2 and ACOT9 play a central role in driving the maladaptive transcriptional response to lipid excess. Silencing either enzyme confers a protective effect by reestablishing normal expression patterns of lipogenic and cellular stress-associated genes. Together, these findings indicate that targeting ACOT activity may rebalance cellular metabolic homeostasis, positioning these enzymes as promising therapeutic candidates for MASLD.

Abstract 076

Senescence Mediated Hepatocyte Injury Drives MASLD Progression

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Background: Senescence of hepatocytes is a central driver of MASLD progression, but the mechanisms that initiate and sustain this senescent state remain unclear. Senescence-associated DNA damage, mitochondrial dysfunction, and metabolic reprogramming may contribute to persistent liver injury. In this study, we investigated whether loss of Ubc13, a K63-linked ubiquitin-conjugating enzyme involved in DNA repair and mitochondrial homeostasis, promotes senescent hepatocyte accumulation and accelerates MASLD progression. **Methods:** Senescence was studied in hepatocyte-specific Ubc13 knockout mice ($Ubc13^{\Delta hep}$) and littermate controls ($Ubc13^{fl/fl}$) fed normal diet or high-fat diet (HFD). Senescent cells were targeted using dasatinib plus quercetin (D+Q). Liver injury, steatosis, senescence, and regeneration were assessed by ALT/AST, histology, γH2AX, p21, p16, PCNA, transcriptomics, and proteomics. Senescence-associated glutaminolysis was evaluated using CB-839, a glutaminase-1 inhibitor, in human hepatocyte organoids and mice. **Results:** In aged/MASLD human and mice liver samples we found an increased expression of senescence markers p21 and p16, while Ubc13 expression was reduced ($p<0.05$). Senescent hepatocytes were significantly increased in HFD-fed $Ubc13^{\Delta hep}$ mice, with elevated γH2AX and p21, steatosis, and higher ALT/AST compared with controls ($p<0.01$–0.05). Senolytic treatment with D+Q reduced steatosis, ALT/AST, and p21-positive hepatocytes ($p<0.05$–0.01), supporting senescence as a functional mediator of disease severity. Liver transcriptomic analyses revealed a pro-senescent shift with increased anti-apoptotic genes (Bcl2, Mcl1) and reduced proliferation and ubiquitination pathways ($p<0.05$), along with increased SASP expression. Pathway analysis showed activation of p53 signaling and cell cycle arrest, with suppression of DNA replication and metabolic pathways. Senescence-associated mitochondrial dysfunction was confirmed by proteomics, with reduced MFN2, OPA1, TOMM20, and TOMM70 under HFD (FD>1.5, FDR<0.05); these changes were improved by D+Q and further worsened in $Ubc13^{\Delta hep}$ mice ($p<0.05$–0.01). Senescence-associated glutaminolysis inhibition by CB-839 reduced lipid accumulation ($p<0.05$) and p21-positive senescent cell populations ($p<0.05$) in human hepatocyte organoids and mice. **Conclusion:** Senescence is a major driver of MASLD progression and is further amplified by reduced hepatocyte Ubc13. This promotes a self-sustaining senescent state characterized by DNA damage, mitochondrial dysfunction, and glutaminolytic reprogramming, leading to steatotic liver injury. Senolytic therapy and GLS1 inhibition attenuate these disease features, identifying a senescence-centered Ubc13–mitochondria–glutaminolysis axis as a therapeutically actionable pathway in MASLD.

Abstract 077

Stung by a Broken Clock: Circadian Disruption Drives cGAS-STING Activation, Pyroptosis, and Fibrogenic Remodeling in MASH

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Background: Circadian misalignment is increasingly linked to metabolic disease; however, the mechanisms by which hepatocyte clock disruption promotes metabolic dysfunction associated steatohepatitis (MASH) remain poorly defined. Here, we define hepatocyte-intrinsic circadian disruption as a proximal trigger of oxidative stress driven innate immune activation that initiates fibrogenic remodeling through intercellular crosstalk. **Methods:** Systemic circadian disruption was modeled in C57BL/6 mice subjected to alternating 12-hour light-dark phase shifts with high-fat feeding (60% kcal fat). Metabolic phenotyping, histology, immunostaining, and molecular analyses (qRT-PCR, western blotting) were performed. BMAL1-deficient human iPSC-derived hepatocytes (iHEPs) were used to model hepatocyte clock disruption. Oxidative stress and antioxidant response was assessed using MitoSOX and qRT-PCR following H_2O_2 exposure. Hepatocyte-macrophage crosstalk was evaluated using conditioned media (CM) from palmitate + IL-1β treated iHEPs applied to THP-1 macrophages. Macrophage-stellate cell interactions were assessed using CM from LPS + dsDNA stimulated macrophages. Inflammasome modulation was tested using the clock enhancer, Nobiletin in BMAL1-deficient macrophages in a liver-on-a-chip platform using SEAP-based IL-1β reporter readout. **Results:** Circadian disruption combined with high-fat feeding induced steatosis, glucose intolerance, macrophage accumulation, and fibrogenic remodeling. These changes were accompanied by activation of cGAS-STING signaling, NLRP3 inflammasome, and pyroptotic responses. BMAL1-deficient iHEPs exhibited heightened oxidative stress (MitoSOX) with induction of antioxidant and innate immune gene programs. CM from BMAL1-deficient iHEPs activated STING signaling in THP-1 macrophages, demonstrating that hepatocyte-derived stress signals initiate innate immune activation. CM from clock-dysregulated macrophages further promoted stellate cell activation, establishing a feed-forward hepatocyte–macrophage–stellate cell axis. Notably, Nobiletin attenuated IL-1β (inflammasome activation) in BMAL1-deficient macrophages in the liver-on-a-chip system, indicating pharmacologic reversibility of this pathway. **Conclusions:** Circadian disruption promotes MASH progression through hepatocyte oxidative stress driven activation of cGAS-STING and inflammasome pathways, leading to pyroptosis-associated paracrine signaling that propagates macrophage activation and stellate cell mediated fibrogenesis. These findings identify hepatocyte circadian clock disruption as a mechanistic driver of immuno-fibrogenic crosstalk and demonstrate that circadian-inflammasome signaling is pharmacologically targetable in MASH.
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Abstract 078

Tropomyosin Receptor Kinase Signaling Induces mTOR Thereby Leading to Steatosis and Metabolic Reprogramming of Hepatocytes

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Background: Metabolic dysfunction-associated steatohepatitis (MASH) represents a public health burden that worsens in elderly and often concurs with hyperinsulinemia. Hepatic stellate cells (HSCs) drive fibrosis; however, their contribution to liver metabolism remains poorly defined. We identified a neurotrophin-3 (NTF3)-tropomyosin receptor kinase B (TRKB) signaling axis in which HSCs promote hepatocyte proliferation and survival through paracrine signaling. As we observed steatosis in NTF3-treated hepatocytes during our studies, we hypothesized that TRK-signaling plays a critical role in hepatic lipid metabolism. **Methods:** We assessed healthy livers from young and aged WT mice, from human and murine models of MASH by IF, WB, snRNA-seq and/or bulk RNA-seq. Co-localization of HSC markers and neurotrophins was assessed by IF and by WB of HSC cell lines. NTF3-dependent steatosis was assessed by Oil red O (ORO) staining in primary mouse hepatocytes, and in young

and aged primary hepatocytes that were incubated with oleic acid. Human ductal organoids (HDOs) were incubated with oleic and palmitic acid, and neurotrophin-dependent steatosis determined by co-incubation with TRK inhibitors. In vivo models included NTF3-treated HSC-depleted mice and murine models of MASH (GAN diet) with AAV.GFAP.Cre-induced NTF3- overexpression (LSL-NTF3), and TRKB knock out (Alb-CreER; TRKB$^{flox/flox}$). mTOR signaling was assessed by WB of cell lysates from mouse passaged human hepatocytes, and HDOs that had been incubated with NTF3 and/or TRK-inhibitors, insulin or torin. Autophagy was assessed by WB in HepG2 and in LC3-GFP-LC3mt-RFP transfected HepG2. **Results:** NTF3 and brain-derived neurotrophic factor (BDNF, high-affinity ligand of TRKB), were detected in mouse and human HSCs, while TRKB expression was observed in hepatocytes. TRKB, BDNF and NTF3 were increased in murine models of MASH and in human MASH samples by WB and bulk RNA-seq. NTF3 increased steatosis in primary mouse hepatocytes. BDNF increased lipid accumulation in HDOs following fatty acid treatment, which was significantly reduced by TRK inhibition. Consistently, mice with elevated NTF3 levels seemed to increase steatosis after GAN-diet feeding, while hepatocyte-specific TRKB deletion seemed to reduce macrosteatosis. Mechanistically, neurotrophin stimulation enhanced mTOR signaling, and suppressed autophagy, effects that were further amplified in the presence of insulin suggesting synergistic effects. Increased mTOR is known to drive MASH and decreased autophagy is a hallmark of aging and associated with steatosis. We therefore assessed if this pathway might be enhanced during aging: Aged-mouse livers exhibited increased *Ntrk2* (which encodes for TrkB) mRNA and protein expression compared to young controls, and increased *Ntrk2*, *Ntf3* and *Bdnf* expression in zone 1 hepatocytes by snRNA-seq. This was accompanied by a trend towards increased *NTRK2* expression in portal hepatocytes in human MASH by snRNA-seq. Primary hepatocytes from aged mouse livers exhibited increased lipid uptake under lipid-rich culturing conditions. Further analyses are ongoing. **Conclusions:** These findings identify a neurotrophin-TRK-mTOR signaling axis in hepatocyte lipid metabolism which is enhanced by aging and metabolic cues. This reveales a potential mechanism for aging-dependent metabolic reprogramming that worsens MASH in the elderly, thereby underscoring TRK signaling as a potential therapeutic target.

Abstract 079

Clearance of Cholesterol-containing Lipid Crystals Reverses Liver Stiffening and Fibrosis in a Dietary Rat Model of MASLD

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by liver steatosis, but how lipid accumulation contributes to disease progression is not well understood. We showed in a rat model that while lipid droplets (LDs) are associated with liver softening, increased cholesterol storage in LDs causes lipid crystal formation in the liver akin to the crystals observed in some human MASLD livers, causing stiffening that precedes fibrosis. However, the mechanism by which crystals stiffen the liver is unclear, and the impact of removing crystals on fibrosis regression is unknown. We examined the mechanics of matrices with embedded simple LDs or lipid crystals to determine how lipid crystals stiffen tissue. We also investigated whether removing crystals from the liver affects liver mechanics and fibrosis regression in rat dietary models of MASLD. **Methods:** Rats were fed control, high fat (27.5% palm oil, HF), and high-fat/high-cholesterol (27.5% palm oil, 2.5% cholesterol, 2% cholate; HFHC) diets for 9 weeks. Artificial LDs (aLDs) and crystals (with composition based on lipidomics analysis of control, HF, and HFHC livers) were physically characterized by scanning ion conductance microscopy. HFHC lipid crystals were incubated with HF LDs or aLDs to test solubility. For regression studies, rats fed HFHC diets for 9 weeks were then fed either a control diet for 9 to 15 more weeks, or control diet with 0.4% added ursodeoxycholate, control diet with 2 mg/mL aspirin in the drinking water, or HF diet for 9 more weeks. Rheometry was used on liver tissue and fibrin tissue mimics with or without either aLDs/crystals or polystyrene (PS) beads with different aspect ratios to measure stiffness. **Results:** Lipid crystals were stiffer than LDs and crystals, unlike LDs, stiffened tissue mimics. Similarly, anisotropic but not spherical PS

beads (of the same stiffness and volume) caused stiffening. In vitro, lipids from HF-fed rat livers solubilized lipid crystals extracted from a HFHC liver. In vivo, changing from an HFHC diet to an HF diet led to crystal solubilization and significantly reduced lipid crystals, liver stiffness, and fibrosis compared with changing to a control diet, which had minimal reduction. Ursodeoxycholate, but not aspirin, also led to reduced liver stiffness. **Conclusions:** The structure and shape of lipid inclusions regulate liver mechanics, such that cholesterol-containing lipid crystals result in a stiff liver. Removing lipid crystals from the steatotic liver leads to significant liver softening and fibrosis regression in a rat model. This suggests that ursodeoxycholic acid, a diet low in cholesterol but high in vegetable fats, and other therapies that promote solubilization of lipid crystals could improve liver fibrosis in MASLD. **Acknowledgements:** NIDDK P30DK050306, NIBIB R01EB017753 (to PAJ. and RGW.), ALF Postdoctoral Research Fellowship and NIDDK T32DK007066 (to DL), NSF CMMI1548571.

Abstract 080

Epigenetic Modifications Suppress HNF4α Expression in MASLD iPSC-derived Hepatocytes: Effect of Vitamin C

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Background: Hepatocyte nuclear factor 4 alpha (HNF4α) is a master regulator of hepatocyte differentiation whose expression is reduced in chronic liver diseases. Our group recently reported that HNF4α expression in iPSC-derived hepatocytes (iPSC-Heps) from MASLD patients is 50% lower than that in iPSC-Heps from healthy subjects. Lower HNF4α expression in MASLD iPSC-Heps is associated with increased methylation of the HNF4α P1 promoter. The goal of this study was to investigate the mechanism underlying the excess methylation of the HNF4α promoter in MASLD iPSC-Heps and to determine whether pharmacologic agents that promote DNA demethylation restore HNF4α expression. **Methods:** iPSCs from healthy controls and MASLD patients were differentiated into iPSC-Heps over 21 days. Gene expression was assessed by qPCR. On day 10 of differentiation, hydroxymethylated DNA and chromatin accessibility were analyzed using hydroxymethylated DNA immunoprecipitation (hMeDIP) and ATAC-seq, respectively. MASLD iPSC-Heps were treated with vitamin C (100ug/ml) as a demethylating agent. Following vitamin C treatment, HNF4α promoter methylation and gene expression were assessed by methylation-specific qPCR and qPCR, respectively. **Results:** *HNF4A* expression was reduced in MASLD vs. control iPSC-Heps from day 9 to day 13 of differentiation, corresponding to the endoderm-to-hepatoblast transition. This was accompanied by a similar reduction in the expression of genes encoding ten-eleven translocation (TETs), which initiate demethylation of DNA by converting 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). On day 10 of differentiation, 5hmC in the region of the HNF4α P1 promoter was significantly decreased in MASLD vs. control iPSC-Heps by hMeDIP ($P < 0.05$). Furthermore, ATAC-seq on day 10 demonstrated a decrease in chromatin accessibility at the HNF4α P1 promoter in MASLD vs. control iPSC-Heps. In an effort to reduce the excess DNA methylation in MASLD iPSC-Heps we treated them with vitamin C, a pharmacologic inducer of DNA demethylation that stimulates TET activity. Preliminary data showed that vitamin C treatment from day 9 to 11 of differentiation lowered methylation of the HNF4α P1 promoter, as measured by methylation-specific qPCR. Moreover, treatment of MASLD iPSC-Heps with vitamin C vs. vehicle from day 9 to 11 increased *HNF4A* expression 3.5-fold on day 21 ($P < 0.05$). **Conclusions:** *HNF4A* expression is reduced in MASLD vs. control iPSC-Heps in the early stages of hepatocyte differentiation, coincident with lower *TET* expression and decreased hydroxymethylation of DNA at the HNF4α P1 promoter. Pharmacologic demethylation of DNA with vitamin C increased *HNF4A* in MASLD iPSCs 3.5-fold at the end of differentiation. These findings suggest an epigenetic mechanism for the dysregulation of HNF4α in MASLD.
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Abstract 081

A SNP, a miRNA, and a Mask: Precision Rescue of TM6SF2 in Genetic Steatosis

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Background: The TM6SF2 rs58542926 (E167K) variant is a major genetic risk factor for metabolic dysfunction–associated steatotic liver disease (MASLD) and steatohepatitis (MASH), yet the mechanisms underlying TM6SF2 insufficiency remain incompletely defined. Beyond its coding effect, rs58542926 creates a de novo exonic binding site for miR-432, suggesting a post-transcriptional regulatory mechanism. We investigated

whether rs58542926 establishes a genotype-specific miR-432–TM6SF2 axis driving steatosis, and whether this interaction can be selectively neutralized using competitive microRNA masking. **Methods:** Human iPSC-derived hepatocytes (iHeps) from rs58542926 carriers and wild-type controls were analyzed. miR-432 and pri-miR-432 expression were quantified by qRT-PCR. CRISPR-corrected isogenic revertant cells (D2) and lentiviral overexpression of WT or rs58542926 TM6SF2 were used for genetic interrogation. RISC dependence was assessed by siRNA knockdown of AGO2 and TNRC6A. Chromatin accessibility and transcription factor enrichment were evaluated by ATAC-seq with HOMER analysis. Two chemically distinct oligonucleotide masks (miR-Mask 01 and miR-Mask 02) targeting the rs585-created miR-432 site were tested. TM6SF2 mRNA/protein levels and lipid accumulation under oleate challenge were quantified. **Results:** rs58542926 created a functional miR-432 binding site that rendered TM6SF2 transcripts susceptible to canonical RISC-mediated repression. In the rs58542926 MASH line 7017, pri-miR-432 and mature miR-432 were massively elevated (up to ~1400-fold and ~1000-fold, respectively), while CRISPR correction suppressed miR-432, restored TM6SF2 expression, and reduced lipid accumulation. AGO2 or TNRC6A knockdown rescued TM6SF2 mRNA without altering miR-432 levels, confirming effector-dependent repression. ATAC-seq revealed enrichment of GATA3, AP-1 and TEAD motifs at the miR-432 regulatory landscape. Therapeutically, miRNA masking produced allele-selective TM6SF2 rescue: miR-Mask 02 restored TM6SF2 mRNA up to ~6-fold and reduced lipid accumulation by ~40%, while miR-Mask 01 achieved partial molecular rescue with ~25–30% lipid reduction. Neither mask altered endogenous miR-432 levels. **Conclusions:** These data define a genotype-specific regulatory circuit in which TM6SF2 rs58542926 both induces miR-432 and creates susceptibility to its repression via canonical RISC machinery. Competitive microRNA masking selectively disrupts this pathogenic interaction, restoring TM6SF2 function and improving hepatocyte lipid handling without global miRNA depletion. Together, this work establishes a mechanistic basis for TM6SF2-associated steatosis and demonstrates allele-selective RNA masking as a precision therapeutic strategy for genetically defined MASLD/MASH. **Acknowledgments:** This study was supported in part by National Institutes of Health (NIH) Grant K08DK098270 and R01DK130391.

Abstract 082

A Spatially Resolved Metabolic and Transcriptomic Atlas of Human Metabolic Dysfunction-associated Steatotic Liver Disease

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a global epidemic characterized by spatial heterogeneity in steatosis, inflammation, and fibrosis. Growing evidence indicates that bioactive metabolites are involved in MASLD progression. While spatial transcriptomics has begun to map transcriptional signatures in human MASLD, the application of spatial metabolomics remains limited. We aimed to generate a comprehensive high-resolution atlas of human MASLD livers by integrating spatial transcriptomics and matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI)-based spatial metabolomics. **Methods:** Human liver biopsies spanning normal, MASL, and MASH stages were analyzed by spatial transcriptomics (Stereo-seq) and MALDI-MSI–based spatial metabolomics on serial sections. Spatial registration enabled alignment and paired multi-omic integration. Mouse high-fat diet (HFD) models, human hepatocyte organoids, primary hepatocytes, hepatic stellate cells (HSCs), and THP1-derived macrophages were used for functional validation. LC-MS/MS, RNA-seq, and histological staining were performed. **Results:** We established a comprehensive high-resolution atlas of human MASLD livers by integrating spatial transcriptomics

and spatial metabolomics. This multimodal profiling captures the dysregulated genes and metabolic networks underlying lipid-accumulating region and fibrotic niche during disease progression. We identify widespread lipid accumulation and amino acid depletion as core features of MASLD, which impairs hepatic antioxidant capacity and metabolic homeostasis. Notably, we identify acetylglycine (ACG), a metabolite mainly produced in hepatocytes and excreted in kidney and urine, whose progressive reduction is associated with hepatic lipid accumulation. Functional validation demonstrates that ACG supplementation robustly alleviates hepatic steatosis in mouse models, indicating that ACG acts as a protective molecule. Furthermore, we delineate the metabolic landscape of the fibrotic niche, which is uniquely characterized by hyperactive sphingolipid metabolism that drives inflammatory crosstalk between immune cells and fibroblasts to promote fibrogenesis. **Conclusions:** Our high-resolution spatial atlas reveals MASLD as a regionally compartmentalized disease characterized by lipotoxicity, amino acid deficiency, and niche-specific sphingolipid signaling. ACG is a hepatocyte-derived protective metabolite whose restoration represents a safe therapeutic strategy, while sphingolipid metabolism is a targetable anti-fibrotic node. This study provides a high-resolution spatial blueprint for understanding MASLD pathogenesis and identifies actionable metabolic targets for therapy. **Acknowledgements:** We acknowledge support from all grants and thank all collaborators for their contributions.

Abstract 083

Elucidating the Mechanistic Relationship Between a Genetic Variant of Interest and Increased MASLD Predisposition in Humans

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) affects approximately 38% of the global population; it is associated with increased risk of hepatocellular carcinoma and costs billions in direct medical costs and societal costs (*Fed Pract Health Care Prof VA DoD PHS,* 2019, **36**:14-19; *J Hepatol.* 2023, **79**:842-852). While it is known that various gene variants can increase MASLD risk, there is no consensus on the mechanism of how one of the most well-known of these variants (the E167K variant of the TM6SF2 gene) increases MASLD risk (*Spec Issue Non-Alcohol Fat Liver Dis.,* 2021, **50**:101111); a complicating factor is the poor phenocopying of the E167K TM6SF2 phenotype in mice. This project aims to dissect the mechanism by which E167K TM6SF2 increases MASLD risk using human-relevant *in vitro* models for the goal of superior prevention and treatment of this disease. **Methods and Results:** The primary sequence of TM6SF2 was manually analyzed, and regions were identified where 1) a Cholesterol-Recognition Amino acid Consensus sequence (CRAC) motif or its reverse sequence (CARC) is present and 2) the orientation of the motif(s) is consistent with the 3D predicted structure of TM6SF2 from AlphaFold. This analysis revealed four such regions of possible cholesterol recognition. When further analyzing the TM6SF2 structure in its predicted dimer form, only one of these regions appears to be fully accessible to potential interaction partners in the protein's native ER membrane; if so, that would suggest that TM6SF2 has 2 highly likely CARC/CRAC domains in its WT form, and 4 in its E167K form. This increase in potential cholesterol-binding domains in TM6SF2 may increase its affinity for cholesterol in its native ER membrane, thereby increasing its colocalization near or within cholesterol-rich ER lipid-rafts and, in turn, with components of the ERAD pathway that are known to interact with TM6SF2. To ameliorate the issue of mice poorly phenocopying the E167K TM6SF2 phenotype, multiple human-relevant *in vitro* models of simple steatosis/MASLD were established. These include a 3D spheroid model composed of primary human hepatocytes and stromal cells as well as an iPSC-derived hepatocyte (iHep)-based model, both treated with a lipid solution to induce hepatic steatosis. These models' human cellular nature and demonstrated ability to successfully develop a hepatic steatosis phenotype without worsening hepatic function make them suitable models for mechanistic study. **Conclusion:** Through their use, multiple possible mechanisms are being explored to deduce the relationship between E167K TM6SF2 and increased MASLD predisposition in humans. Defining this mechanism has the potential to support superior prevention and treatment efforts for MASLD patients. **Acknowledgements:** This work was supported by the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK; R01DK146438 and R01DK138677).

Abstract 084

LNP-mediated Targeting of a Conserved Non-canonical MST1/2-FOXO3 Survival Axis in Liver Myofibroblasts Reverses MASH Fibrosis

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Background: Metabolic dysfunction-associated steatohepatitis (MASH) drives progressive liver fibrosis, cirrhosis, and liver failure, yet effective antifibrotic therapies remain lacking. **Methods:** To address this, we engineered a fully human 3D MASH fibrosis model by co-culturing MASH patient liver-derived organoids with primary human myofibroblasts (MFBs) and peripheral blood monocytes, generating scar-like structures containing senescent parenchymal cells, proliferative collagen-producing MFBs, and TREM2^{+} scar-associated macrophages. Single-cell transcriptomics demonstrated strong concordance with human MASH fibrotic scars. **Results:** Using this platform, we identified a non-canonical MST1/2-FOXO3 survival axis in liver MFBs as a therapeutic vulnerability. This disease-promoting pathway, previously only described in pulmonary arterial adventitial fibroblasts, was highly active in MASH-associated liver myofibroblasts and is likely relevant across multiple etiologies of liver fibrosis. PDGFRβ-targeted MST1/2 siRNA lipid nanoparticles (LNPs) selectively halted MFB proliferation and induced MFB death while sparing parenchymal cells in vitro. In a mouse model of MASH, the corresponding targeted mouse siRNA LNP formulation reversed established fibrosis by ~68% within 10 days. **Conclusions:** In contrast to iPSC-derived liver organoid systems our human liver derived 3D fibrosis platform preserves the MASH epigenetic memory and reproduces key aspects of human fibrotic scar tissue not previously modeled. Using this system, we identify a cross-organ conserved non-canonical Hippo signaling survival axis (MST1/2-FOXO3) as a selective therapeutic vulnerability in pathogenic myofibroblasts. These findings are important for researchers, translational scientists, and clinicians because they demonstrate rapid reversal of established fibrosis using myofibroblast specific MST1/2 siRNA LNPs, a therapeutic modality promising broad clinical translation. More generally, our study provides a practical framework for developing and evaluating cell-selective antifibrotic RNA nanomedicines towards improved treatments and outcomes for patients with MASH and potentially other chronic liver diseases. **Acknowledgements:** This work was supported by National Institutes of Health grant 1R21AI183111-01(TR), the Arno A. Roscher Foundation (TR), and the American Society of Gene and Cell Therapy/Cystic Fibrosis Foundation Career Development Award (JN). We thank Kim Olthoff and Abraham Shaked from the Penn Transplant Institute of the University of Pennsylvania for the human liver specimens. We are grateful to the Single Cell Technology Core and the High Throughput Screening Core of the Children's Hospital of Philadelphia Research Institute for assistance in acquiring our single cell data. We thank the Henderson lab for providing the RAW data of their human MASH liver single cell analysis. We thank the Penn Immunology Core for preparation of human peripheral monocytes. We thank the members of the Muzykantov, Weissman, and Rader labs for helpful discussions.

Abstract 085

Reduced ZMPSTE24 Expression Leads to Prelamin Accumulation and Development of Steatosis in MASLD Patients

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Background: Metabolic-associated steatotic liver disease (MASLD) is highly prevalent in type 2 diabetes mellitus (T2D) and its incidence has increased with the obesity epidemic. Mutations of nuclear lamina-associated genes including *LMNA* have been associated with fatty liver. We previously described a mechanism relating changes at the nuclear lamina that lead to opening of previously repressed chromatin and upregulation of lipid synthesis and storage pathways to development of steatosis in MASLD. Genetic variants in nuclear lamina-related genes have been associated with MASLD and changes in nuclear morphology have been observed in

MASLD patients. Here we extend our findings to identify an upstream regulator of the nuclear lamina changes driving steatosis in MASLD. **Methods:** Snap-frozen liver biopsies from male and female MASLD patients (ages 31–47) with moderate to severe steatosis were obtained and compared to healthy controls. RNA-seq and ChIP-seq were performed to assess differential gene expression and FOXA2 chromatin occupancy genome-wide. Prelamin-A accumulation and nuclear morphology were evaluated by immunofluorescence and immunoblotting. *Zmpste24* mutant mice were used as a complementary in vivo model to validate findings from human patient data. Ingenuity Pathway Analysis (IPA) was employed to identify upstream regulators of differentially expressed genes and FOXA2 target genes. **Results:** We report that changes at the nuclear envelope in MASLD patients are caused by downregulation of zinc metalloproteinase Ste24 (ZMPSTE24), an enzyme that processes prelamin to mature lamin A, leading to accumulation of prelamin in hepatocytes of both male and female patients. *Zmpste24* mutant mice develop hepatic steatosis and exhibit upregulation of p53 target genes. Functional analysis determined p53 as a regulator of genes differentially expressed and bound by FOXA2 in MASLD patients, corresponding to observations in *Zmpste24* knockout animals. In male MASLD patients, ZMPSTE24 expression is repressed by mir-141-3p. In female patients, pathway analysis revealed activation of nuclear receptors FXR, CAR, LXR, and PXR, as well as cholesterol biosynthesis and fatty acid metabolism pathways, demonstrating sexual dimorphism in downstream transcriptional responses. Furthermore, expression of glucose and insulin-regulated genes is reduced in MASLD patients, suggesting altered glucose metabolism and insulin resistance consistent with hallmarks of T2D. **Conclusions:** Downregulation of ZMPSTE24 leads to the nuclear lamina changes responsible for development of MASLD we had previously reported, placing ZMPSTE24 upstream of our described FOXA2-dependent mechanism driving steatosis. Hence, MASLD should be considered as a type of laminopathy, and approaches to restore ZMPSTE24 expression and nuclear lamina function should be tested for treatment of the disease. These findings underscore the need for intervention at early stages of steatosis, prior to progression to metabolic-associated steatohepatitis (MASH).

Abstract 086

TorsinA ATPase Activity is Essential for ApoB-mediated VLDL Secretion in Hepatocytes

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Background: Abnormalities in hepatic lipid metabolism lead to Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), which is a growing public health concern. Our previous research has demonstrated that torsinA plays a key role in apolipoprotein (apoB)-mediated very low-density lipoprotein (VLDL) secretion in hepatocytes. Hepatocyte-specific torsinA-deficient mice (A-CKO) exhibit a profound hepatic steatosis due to impaired VLDL secretion. TorsinA is a member of the AAA+ ATPase family located in the endoplasmic reticulum (ER). This study aimed to determine whether ATPase activity of torsinA is necessary for VLDL secretion and how its dysfunction affects hepatic lipid metabolism. **Methods**: We used adenoviral and adeno-associated viral vector-mediated gene delivery to re-express wild-type (WT) or ATPase hydrolysis-defective (EQ mutant) torsinA in A-CKO mice, and assessed *in vivo* functional rescue of steatosis and VLDL secretion. Co-immunoprecipitation assays were used to determine the interaction of apoB with WT or EQ torsinA. Confocal microscopy and flow cytometry were employed to investigate cellular lipid dynamics. Genome-wide transcriptomics and spatial lipidomics were used to examine metabolic pathways and lipid species distributions. **Results:** Re-expression of WT torsinA in A-CKO mice successfully normalized VLDL secretion and resolved the severe steatosis. In contrast, the EQ torsinA failed to rescue either phenotype, demonstrating that torsinA's ATPase activity is indispensable for this process. These findings were confirmed in isolated hepatocytes. Mechanistically, the interaction between apoB and the EQ torsinA was significantly reduced compared to WT torsinA. Transcriptomic analysis demonstrated specific dysregulation of the sterol biosynthesis pathway in A-CKO mice or upon re-expression of EQ torsinA. Furthermore, spatial lipidomics identified a fatty acyl-dependent dysregulation of phospholipid species, alongside major changes in neutral lipids, indicating altered phospholipid remodeling. **Conclusions:** Our data establish that the ATPase activity of torsinA is essential for the VLDL secretion from the ER and required for proper interaction with apoB, potentially affecting the initial assembly of VLDL particles. The profound steatosis observed in the absence of functional torsinA ATPase activity is a direct result of defective lipid secretion, not increased lipogenesis. These findings highlight torsinA's enzymatic function as a critical node

in hepatic lipid metabolism and suggest that modulating torsinA ATPase activity could represent a novel therapeutic strategy for MASLD. **Acknowledgements**: This work was supported by the National Institutes of Health [grant number 1R01CA283566 (to JYS)] and a Pinnacle Research Award from the American Association for the Study of Liver Disease (JYS).

Abstract 087

Reprogramming Chronic Liver Disease Through Physiological Regeneration: Transient ZNRF3/RNF43-β-Catenin Activation Reverses MASH

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Background: A fundamental barrier in metabolic dysfunction-associated steatohepatitis (MASH) therapy is whether regenerative signaling can be reactivated to reverse chronic disease without promoting tumorigenesis. In acute liver injury, transient activation of regenerative pathways restores tissue function in a tightly controlled manner. We hypothesized that applying this physiological paradigm to chronic liver disease—through temporally restricted activation of WNT/β-catenin signaling—could enable disease reversal. ZNRF3 and RNF43 are key negative regulators of this pathway and provide a tunable entry point for its modulation. **Methods:** We used hepatocyte-specific deletion of ZNRF3/RNF43 to activate β-catenin signaling in diet- and toxin-induced MASH mouse models. Disease regression was assessed by histological and transcriptomic analyses. In parallel, we explored translational feasibility using siRNA-mediated approaches to achieve transient pathway activation. **Results:** Activation of β-catenin through ZNRF3/RNF43 deletion induced rapid and coordinated regression of steatosis, inflammation, and fibrosis, representing a multidimensional reversal of MASH. This response was spatially organized, with periportal hepatocytes contributing prominently to metabolic recovery. Mechanistically, pathway activation drove metabolic reprogramming, including activation of the alternative bile acid synthesis pathway and enhanced hepatic cholesterol clearance. Rather than targeting a single axis, β-catenin activation engaged an integrated regenerative program that simultaneously improved metabolic and structural features of the diseased liver. Notably, preliminary siRNA-based modulation recapitulated key aspects of this response, supporting the feasibility of transient pathway activation. These findings suggest that temporally controlled activation can capture regenerative benefits while avoiding risks associated with sustained signaling. **Conclusions:** These results demonstrate that principles of physiological regeneration can be leveraged to reverse chronic liver disease. ZNRF3/RNF43-mediated control of β-catenin signaling enables a tunable therapeutic strategy in which transient activation drives multidimensional MASH regression while maintaining safety. This work establishes a conceptual framework for regenerative therapy in metabolic liver disease. **Acknowledgements:** We thank Dr. Jan Tchorz for providing the ZNRF3/RNF43 floxed mice used in this study.

Abstract 088

SUGP1 Knockdown Promotes MASH in Hepatic TM6SF2 Knockout Mice

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Background: Transmembrane 6 superfamily 2 *(TM6SF2)* rs58542926, a missense (E167K) variant, is robustly associated with metabolic dysfunction-associated steatotic liver disease (MASLD) and progression to steatohepatitis (MASH), such that it is widely thought to be causal of disease. However, it is in near-perfect linkage disequilibrium (>0.9) with SURP and G-Patch Domain Containing 1 (*SUGP1)* rs10401969, an intronic variant that affects splicing, reduces SUGP1 protein levels, and impacts cholesterol metabolism. In iPSC-derived hepatocytes (iPSC-Heps), we found i) *SUGP1* knockdown increased lipid accumulation, ii) iPSC-Heps from donors with both *TM6SF2* and *SUGP1* risk alleles had greater cellular steatosis than those with both non-risk alleles, and iii) genomic editing to revert the *SUGP1* risk allele to the non-risk allele mitigated this effect (see abstract by Keshavarz Aziziraftar *et al*). Thus, we hypothesized that loss of *SUGP1* contributes to the association

between *TM6SF2* rs58542926 and MASLD. To further evaluate this, we tested how loss of SUGP1 impacts MASLD in a hepatic *Tm6sf2* knockout mouse. **Methods:** Hepatocyte-specific *Tm6sf2* knockout was generated by injecting *Tm6sf2* homozygous floxed mice with AAV-TBG-iCre at 7 weeks of age. Thereafter, mice received weekly IP injections of liver-specific GalNAc-conjugated antisense oligonucleotides (ASOs) targeting *Sugp1* or a non-targeting control (NTC), n=8/sex/ASO. Mice were kept on a chow diet. After 8 weeks, tissues were collected for analysis. **Results**: Hepatic *Tm6sf2* and *Sugp1* transcript levels were reduced >80%, and there were no differences in body weight by ASO. In male mice, *Sugp1* knockdown caused statistically significant increases in all measures of MASLD, including hepatomegaly, steatosis, lobular and portal inflammation, and hepatocyte ballooning—resulting in an increased composite MASLD activity score (6 ±1.06 fold, $p<0.0001$). Males also showed increased numbers of mitotic figures, acidophil bodies, and microgranulomas. Similar results were observed in females. In both sexes, hepatic RNAseq analysis found that *Sugp1* knockdown significantly upregulated genes involved in cell division and collagen/extracellular matrix organization, and downregulated genes involved in lipid and retinol metabolism, and cholesterol transport. Similar pathways were identified in a parallel analysis of *SUGP1* knockdown in iPSC-Heps (see abstract by Keshavarz Aziziraftar et al). **Conclusions:** Our findings indicate that hepatic *Sugp1* loss in the context of *Tm6sf2* knockout is sufficient to drive MASLD progression to MASH, supporting a causal role of SUGP1 in disease pathogenesis and implicating *SUGP1* rs10401969 in the genetic association observed at the *TM6SF2* locus. **Acknowledgements:** This work was supported by NIH R01 DK130391, the UCSF Liver Center P30 DK026743, and UC Davis West Coast Metabolomics Center. *Tm6sf2* floxed mice were provided by Dr. Nicholas O. Davidson, and ASOs by Ionis Pharmaceuticals.

Abstract 089

Genotype-Specific MASLD Progression in Human Liver Microphysiology Systems: Implications for Precision Medicine

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Background: Metabolic dysfunction–associated steatotic liver disease (MASLD) is a highly heterogeneous disorder shaped by genetic risk and protective variants that influence disease progression and therapeutic response. Among these, PNPLA3 I148M variant is strongly associated with increased hepatic steatosis, fibrosis, and adverse clinical outcomes. Despite this, mechanistic insight into how such variants modulate molecular pathways and drug responses remains limited, largely due to the lack of physiologically relevant human models. To address this gap, we integrated patient digital twins (PDTs)—computational representations built from clinical and multi-omics data—with patient biomimetic twins (PBTs) derived from patient primary cells or iPSCs, establishing a precision medicine platform. **Methods:** We developed human liver acinus microphysiology systems (LAMPS) using genotyped primary hepatocytes and key non-parenchymal cells to model MASLD progression under defined metabolic stress. LAMPS incorporated wild-type, high-risk (PNPLA3, TM6SF2, MBOAT7, GCKR), and protective (HSD17B13, MTARC1) variants and were maintained under fasting, early, and late metabolic syndrome conditions. Disease phenotypes and drug responses were evaluated using imaging and biochemical assays, and integrated with PNPLA3-stratified human liver transcriptomic analyses to identify genotype-dependent pathways and candidate biomarkers. **Results:** LAMPS constructed with high-risk MASLD variants exhibited increased hepatic steatosis compared to wild-type systems, while protective variants demonstrated reduced lipid accumulation, consistent with clinical observations. Drug testing revealed genotype-dependent responses to resmetirom, with greater efficacy observed in wild-type compared to PNPLA3 GG systems. Computational analyses identified PNPLA3 GG–specific amplification of pathogenic pathways, including inflammation and mitochondrial dysfunction, alongside suppression of protective signaling. Notably, fibroblast growth factor 21 (FGF21) emerged as a key genotype-dependent regulator, with reduced expression and signaling in PNPLA3 GG carriers, accompanied by downregulation of AMPK-related pathways. These findings suggest that impaired FGF21 signaling contributes to increased disease susceptibility and altered therapeutic responsiveness in high-risk genotypes. **Conclusion:** Our integrated experimental and computational approach demonstrates that MASLD genetic variants drive distinct molecular and functional phenotypes in human liver microphysiology systems. In PNPLA3 high-risk variants, suppression of the FGF21 axis may mechanistically link genetic susceptibility to heterogeneous drug responses. These findings support genotype-

stratified LAMPS as a precision platform for MASLD biomarker discovery and therapeutic evaluation.
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Abstract 090

A PNPLA3-deficient iPSC-derived Hepatocyte Screen Identifies Pathways to Potentially Reduce Steatosis in Metabolic Dysfunction-associated Fatty Liver Disease

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Background: The incidence of metabolic dysfunction-associated fatty liver disease (MAFLD) is dramatically increasing in adults and children, while effective pharmacological treatments remain unavailable. MAFLD can progress from lipid accumulation in the liver to more severe inflammation, cirrhosis, and cancer. It is the most common cause of chronic liver disease and is projected to be the leading cause of end-stage liver disease in the next decade. Many etiologies contribute to MAFLD. A polymorphism in the Patatin-like Phospholipase Domain Containing Protein (PNPLA3 I148M) has the most significant association with the disease and all stages of its progression. A roadblock to identifying potential treatments for PNPLA3-induced MAFLD is the scarcity of a cellular platform that recapitulates PNPLA3 I148M-mediated onset of steatosis in human hepatocytes. We used hepatocytes generated from our PNPLA3 I148M induced Pluripotent Stem Cells (iPSCs) to model the effect of the polymorphism on lipid content and lipid droplet accumulation, providing a platform to identify pathways with potential therapeutic value using an established high-throughput screening platform. **Methods:** We generated *PNPLA3* I148M and PNPLA3 -/- iPSCs using CRISPR-Cas9. Using established protocols, we differentiated the resulting cells into hepatocytes and measured the efficiency of differentiation. Using BODIPY 493/503 staining, we measured lipid accumulation in our iPSC-derived hepatocytes. We then used a small molecule screen to identify lead compounds that reduce lipid accumulation. **Results:** Both our PNPLA3 I148M and PNPLA3 -/- iPSC-induced hepatocytes showed hepatic marker expression as well as significant increase in lipid content compared to wildtype iPSC-induced hepatocytes. Our small molecule screen resulted in lead compounds that give insight to pathways involved in PNPLA3-mediated lipid accumulation. **Conclusion:** We conclude that human iPSC-derived hepatocytes can be used to effectively model the onset of MAFLD in the presence of the PNPLA3 I148M variant, which we show acts through a loss of function. Our model also provides a platform to identify small molecules with potential therapeutic value. Lead compounds were used to find common pathways that could play a role in PNPLA3-mediated lipid accumulation.

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