Yale Liver Center
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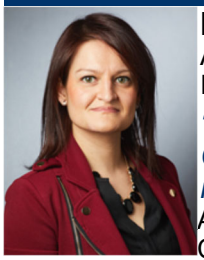
2018-2019 Pilot Project Awards



Mariangela Amenduni, PhD
Associate Research Scientist
Internal Medicine, Digestive Diseases

"Use of hiPSCs to study the function of CFTR mutations in cholangiocyte innate immunity"

About 2000 mutations have been identified in CFTR and multiple studies have been done to establish a correlation between the CFTR genotype and CF disease phenotype, however this relationship is not well understood. Understanding how CFTR mutations translate to altered synthesis or function of CFTR protein in human cholangiocytes and how the genetic defects are connected to the biliary epithelial innate immunity response, will be useful to personalize the therapeutic approach to the patient. In this project we will address these unsolved questions using iPSCs (induced pluripotent stem cells) technology to generate cholangiocytes from patients bearing CFTR mutations belonging to different functional classes.

Venkata Boddupalli, PhD
Associate Research Scientist
Internal Medicine, Digestive Diseases

"Delineating the role tissue resident T cells in mediating immunopathology of PSC-IBD"

We aim to elucidate the underlying role of tissue resident memory T cells (TRM) in immunopathogenesis of PSC and the mechanistic basis for the coexistence of IBD with PSC. We are using Kaede-PSC photoconvertible transgenic mice model to address the role of gut resident TRM cells in causing PSC and liver tissue pathology. We intend to capture and characterize pathogenic T cell clones that have homed from GI tract to liver using Kaede-PSC mouse model. These mice models will improve our understanding of the role played by TRM cells in cholangiopathies. Also, comparing these observations obtained from Kaede-PSC mouse model with human clinical data will further expand our view about PSC biology.

Xinshou Ouyang, PhD
Assistant Professor
Internal Medicine, Digestive Diseases

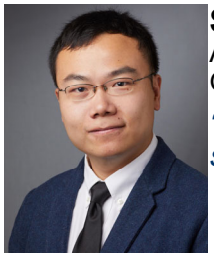
"RNA methylation landscaping of gene regulation in macrophage-mediated inflammation in NASH"

N^6-methyladenosine (m^6A) is the abundant internal modifications in many sites of messenger RNA, modulated by adenosine methyltransferases ('writers'), demethylases ('erasers') and RNA binding proteins ('readers') to shape the cellular 'epitranscriptome'. m^6A thus functionally influences all fundamental aspects of mRNA metabolism, mainly mRNA stability. However, its physiological function in immune cells is yet not fully understood. Mechanisms for non-alcoholic steatohepatitis (NASH) development are under investigation in an era of increased prevalence of obesity and metabolic syndrome. The goal of this proposed research is to delineate the signaling events of m^6A directed macrophages in the existence of redox status and chronic inflammation in NASH.

Gregory Tietjen, PhD
Assistant Professor
Surgery, Transplant

"Personalized diagnostic profiling of donor livers during ex vivo perfusion"

A severe shortage of viable livers and the declining health of the donor population represent the two most significant challenges facing clinical liver transplantation today. These challenges are exacerbated by the lack of robust diagnostic tools capable of providing a clear assessment of donor liver viability. Historically, the decision to transplant a given organ is highly subjective, which can lead to systemic inefficiencies, the potential for discard of usable organs and increased waitlist mortality. Ex vivo normothermic perfusion of deceased donor livers has emerged as a platform that can enable more sophisticated assessment of organ viability and even provide an opportunity to repair marginal organs to make them suitable for transplantation. In this project, we aim to develop quantitative diagnostic imaging tools for use during ex vivo normothermic liver perfusion to provide personalized assessments of each donor liver prior to transplantation. We believe this work has the potential to improve utilization of marginal liver allografts and thereby reduce waitlist mortality.



Steven Wang, PhD
Assistant Professor
Genetics | Cell Biology

"Imaging-based 3D genomics and transcriptomics in aging liver"

Aging poses a major risk for many liver diseases. At the cellular level, the occurrence and accumulation of senescent cells is a main characteristic of aging liver. The molecular mechanism governing cellular senescence and its downstream effects have been studied relatively extensively in cell culture systems, but a detailed picture of the native molecular compositions and molecular/cellular spatial organizations of senescent cells in an aging liver remains elusive. In this study, we aim to profile single-cell transcriptome and to trace chromatin organization in the native liver tissue context through the aging process, with our state-of-the-art imaging-based in-situ transcriptomics and genomics technologies. The study will reveal the in situ molecular mechanism underlying cellular senescence and aging, as well as the interactions between senescent cells and their microenvironment, at different ages.

AVAILABLE CORE SERVICES

ADMINISTRATIVE

PILOT FEASIBILITY PROGRAM
Pilot grants given annually to promote studies of liver disease

ENRICHMENT PROGRAM
Weekly seminar series, annual Klatskin Lectureship, bi-annual Center retreat

MORPHOLOGY

CONFOCAL, SUPER-RESOLUTION, MULTIPHOTON IMAGING
Leica SP5
Zeiss LSM 710 duo
Leica SP8 gated STED 3X
Bruker Luxendo MuVi SPIM
Swept-field (Opterra II, Bruker)
Vutara 252 super-resolution
Zeiss LSM 880 AiryScan Fast

ELECTRON MICROSCOPY
Tecnai 12. biotwinFEI Tecnai TF20 FEG

EPIFLUORESCENCE MICROSCOPY INCLUDING QUANTITATIVE & RATIO IMAGING
Zeiss Axio Observer epifluorescence microscope
Olympus BX51 multi-headed brightfield microscope
Dissecting microscope
Zeiss Discovery 8 SteReo

CELLULAR-MOLECULAR

ISOLATED CELL PREPARATIONS
Hepatocytes, cholangiocytes, endothelial cells, stellate cells, portal fibroblasts and hepatic lymphocytes, primarily from mice and rates. Human hepatocytes when available.

CELL CULTURE FACILITIES
Available for short– and long– term cultures and cell lines

PROTEIN & GENE EXPRESSION
Quantitative real time PCR and infrared imaging detection . Altering gene expression in these cells using siRNA transfection and adenovirus infection technologies

IPSC & ORGANOIDS
Developed from skin fibroblasts, liver tissue, and bile

CLINICAL-TRANSLATIONAL

BIOSTATISTICAL SUPPORT
Two biostatisticians available for expertise in the design, conduct, and analysis of patient-oriented studies, as well as methodological development, education, and training

PATIENT REGISTRY
Patient databases on diagnoses including: chronic hepatitis C, cirrhosis, chronic hepatitis B, PBC, autoimmune hepatitis, PSC, hepatocellular carcinoma, NAFLD, and cholangiocarcinoma

BIOSPECIMEN & LIVER BIOPSY REPOSITORY
Recruitment of patients and collection of blood samples

IPSC/LIVER ORGANOID
On request, PBMCs are transferred to the Yale Stem Cell Center (YSCC) for reprogramming into iPSC. YSCC will generate at least 3 clones of iPSCs for each PBMC sample. iPSCs can be differentiated into liver cells (biliary cells or hepatocytes) and made available. Liver organoids available upon request

Most of these services are available to liver center members at no cost.

For more information please contact Christine.abu-hanna@yale.edu

Congress Announcement: 4th International Conference on Alcohol and Cancer

WHEN
Sunday, April 14, 2019 - Thursday, April 18, 2019
8:00 AM - 6:00 PM

WHERE
Newport Marriot Hotel
25 America's Cup Avenue
Newport, Rhode Island 02840

REGISTRATION
Alcoholandcancerconference.org

SESSIONS
- Epidemiology and alcohol public policies
- Reproducible research practices and transparency
- Big data, deep learning and artificial intelligence
- Systems approaches (metabolomics, epigenetics, genomics and imaging of alcohol-related cancer)
- Molecular mechanisms of alcohol-induced carcinogenesis (including signaling pathways and non-coding RNAs)
- Stem cells and genomic instability
- Alcohol and cancer (colon, liver, pancreatic, breast, and aerodigestive cancers)
- Inflammation, microbiome and nutrition
- Developmental origins of alcohol and cancer

KEYNOTE SPEAKERS
Elisabete Weiderpass
Director of the International Agency for Research on Cancer (IARC)

Richard M. Caprioli
Vanderbilt University

Hidekazu Tsukamoto
University of South California

Charles S. Fuchs
Director of Yale Cancer Center

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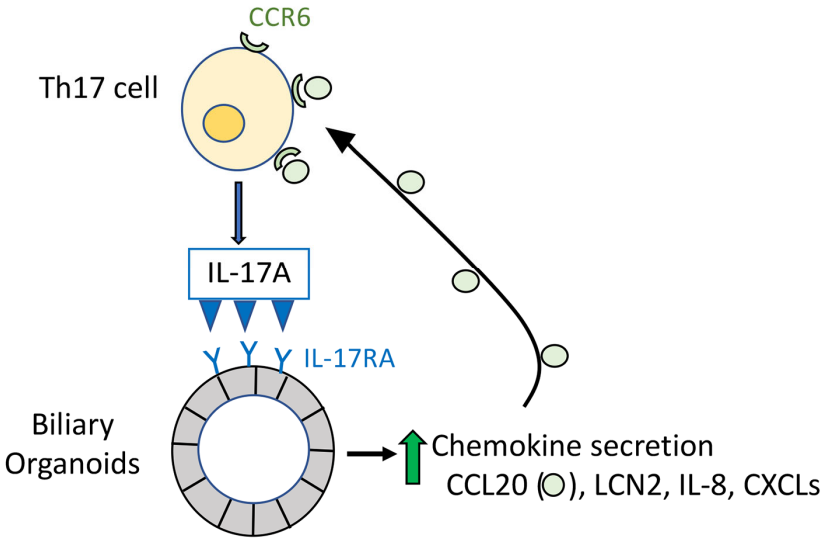
Featured Publications

Bile-derived organoids from patients with primary sclerosing cholangitis recapitulate their inflammatory immune profile

Carol J. Soroka, David N. Assis, Leina S. Alrabadi, Scott Roberts, Laura Cusack, Ariel B. Jaffe, and **James L. Boyer**
Hepatology, 2019

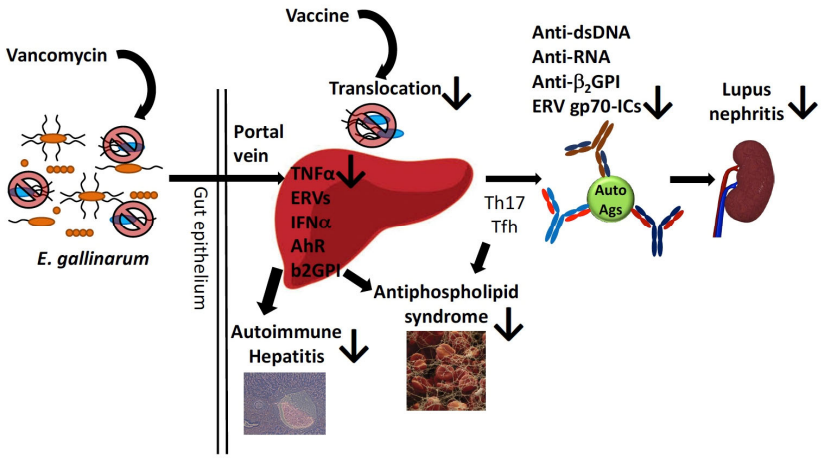
Cholangiopathies such as Primary Sclerosing Cholangitis (PSC) are a heterogenous group of diseases affecting the intra- and extra-hepatic bile ducts of the liver. PSC is characterized by fibrosing strictures of the small and large bile ducts, and is believed to be an immune-mediated disorder in which patients commonly also have associated inflammatory bowel disease. Research on PSC is hampered by difficulties in studying the cholangiocyte which makes up such a small portion of total liver cells, as well as being restricted to obtaining tissue/cells from explants late in the progression of the disease. In this paper, Soroka et al describe a novel method of isolating progenitor cells from bile of PSC patients undergoing diagnostic and therapeutic ERCP for clinical care. These stem cells proliferate well in culture as hepatic organoids, maintain a biliary genotype with previously characterized PSC gene markers, and can be biobanked for future analyses. These organoids can be stimulated to secrete chemo/cytokines which act as pro-inflammatory mediators to attract and activate various immune cells which could further exacerbate liver damage. We are currently exploring how stimulation of the IL17A pathway in bile-derived organoids can lead to secretion of CCL20, LCN2 and CXCL1 which in turn could attract Th17 lymphocytes to damaged bile ducts in PSC patients (see cartoon). For the first time researchers have an in vitro model to maintain biliary cells and to study cell-cell interactions and drug therapies which may further our understanding of this serious disease.



The Enemy Lies Within: Spontaneous Translocation of a Gut Pathobiont Drives Autoimmunity

Manfredo-Vieira S, Hiltensperger M, Kumar V, Zegarra-Ruiz D, Dehner C, Khan N, Costa FRC, Tiniakou E, Greiling T, Ruff W, Barbieri A, Kriegel C, Mehta SS, Knight JR, **Jain D**, Goodman AL, Kriegel MA.
Science. 2018; 359(6380):1156-61.

This study shows that bacteria found in the small intestines of mice and humans can travel to other organs and trigger an autoimmune response. Gut bacteria have been linked to a range of diseases, including autoimmune disorders. This study looked at Enterococcus gallinarum, a bacterium that has been shown to spontaneously "translocate" outside of the gut to lymph nodes, the liver, and spleen. In genetically susceptible mice, the researchers observed that E. gallinarum initiated the production of auto-antibodies and inflammation in tissues outside the gut. They confirmed the same mechanism of inflammation in cultured liver cells of healthy people, and the presence of this bacterium in livers of patients with autoimmune disease. This study further showed that the autoimmunity could be suppressed in mice with an antibiotic or a vaccine aimed at E. gallinarum. With either approach, the researchers were able to suppress growth of the bacterium in the tissues and blunt its effects on the immune system. The study provides further support for microbial pathogenesis for autoimmune disorders and suggests unique way of treating it with a vaccine or antibiotic.



Featured Publications

Effects of Endotoxin on Type 3 Inositol 1,4,5-Trisphosphate Receptor in Human Cholangiocytes

Franca A, Carlos Melo Lima Filho A, **Guerra MT, Weerachayaphorn J**, Loiola Dos Santos M, Njei B, **Robert M**, Xavier Lima C, Vieira Teixeira Vidigal P, Banales JM, Ananthanarayanam M, Leite MF, **Nathanson MH**.
Hepatology. 2019;69(2):817-830.

Clinical conditions that result in endotoxemia, such as sepsis and alcoholic hepatitis (AH), often are accompanied by cholestasis. Although hepatocellular changes in response to lipopolysaccharide (LPS) have been well characterized, less is known about whether and how cholangiocytes contribute to this form of cholestasis. We examined effects of endotoxin on expression and function of the type 3 inositol trisphosphate receptor (ITPR3), because this is the main intracellular Ca2+ release channel in cholangiocytes, and loss of it impairs ductular bicarbonate secretion. Bile duct cells expressed the LPS receptor, Toll-like receptor 4 (TLR4), which links to activation of nuclear factor-κB (NF-κB). Analysis of the human ITPR3 promoter revealed five putative response elements to NF-κB, and promoter activity was inhibited by p65/p50. Nested 0.5- and 1.0-kilobase (kb) deletion fragments of the ITPR3 promoter were inhibited by NF-κB subunits. Chromatin immunoprecipitation (ChIP) assay showed that NF-κB interacts with the ITPR3 promoter, with an associated increase in H3K9 methylation. LPS decreased ITPR3 mRNA and protein expression and also decreased sensitivity of bile duct cells to calcium agonist stimuli. This reduction was reversed by inhibition of TLR4. ITPR3 expression was decreased or absent in cholangiocytes from patients with cholestasis of sepsis and from those with severe AH. Conclusion: Stimulation of TLR4 by LPS activates NF-κB to down-regulate ITPR3 expression in human cholangiocytes. This may contribute to the cholestasis that can be observed in conditions such as sepsis or AH.

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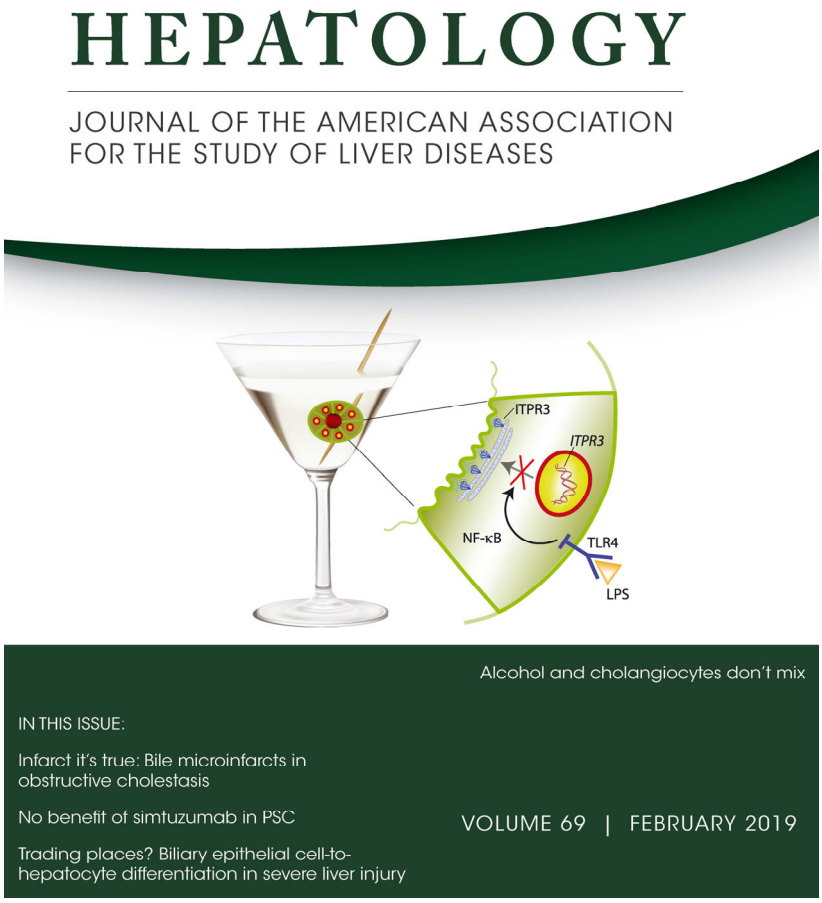

Members' Original Recent Publications

Levels of circulating follicular helper T cells, T helper 1 cells, and the prognostic significance of soluble form of CD40 ligand on survival in patients with alcoholic cirrhosis. Hollister K, Kusumanchi P, Ross RA, Chandler K, Oshodi A, Heathers L, Teagarden S, Wang L, Dent AL, Liangpunsakul S. Liver Res. 2018; 2:52-59. PMID: 30221017

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Mitohormesis in Mice via Sustained Basal Activation of Mitochondrial and Antioxidant Signaling. Cox CS, McKay SE, Holmbeck MA, Christian BE, Scortea AC, Tsay AJ, Newman LE, Shadel GS. Cell Metab. 2018; 28:776-786. PMID: 30122556

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Association Between Aldehyde Dehydrogenase 2 Glu504Lys Polymorphism and Alcoholic Liver Disease. Chang B, Hao S, Zhang L, Gao M, Sun Y, Huang A, Teng G, Li B, Crabb DW, Kusumanchi P, Wang L, Liangpunsakul S, Zou Z. Am J Med Sci. 2018; 356:10-14. PMID: 29779728

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Mif-deficiency favors an atheroprotective autoantibody phenotype in atherosclerosis. Schmitz C, Noels H, El Bounkari O, Straussfeld E, Megens RTA, Sternkopf M, Alampour-Rajabi S, Krammer C, Tilstam PV, Gerdes N, Bürger C, Kapurniotu A, Bucala R, Jankowski J, Weber C, Bernhagen J. FASEB J. 2018; 32:4428-4443. PMID: 29543531

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