The Texas Medical Center Digestive Diseases Center

8TH ANNUAL
Frontiers in Digestive Diseases Symposium

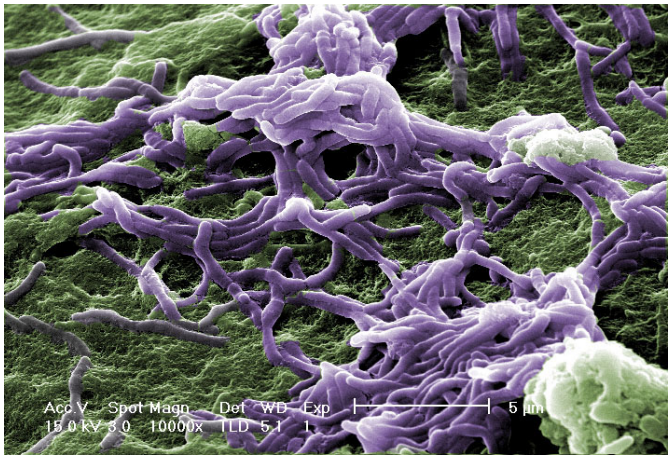
"Emerging Significance of Bile Acids in Digestive and Liver Disease"

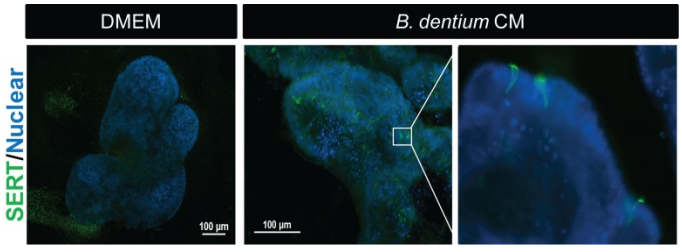
February 11, 2017

Onstead Auditorium
6767 Bertner
Houston, Texas

Texas Medical Center Digestive Diseases Center
8th Annual Frontiers in Digestive Diseases Symposium:
Emerging Significance of Bile Acids in Digestive & Liver Diseases

Saturday, February 11, 2017
Onstead Auditorium, Houston, Texas 77030





Front Cover Image: Scanning Electron Microscopy image of B. dentium (purple) adherence to intestinal mucus (green) in the human goblet cell line LS174T.

Back Cover Image: Mouse ileum enteroids were microinjected with broth control or *Bifidobacterium dentium* conditioned media and stained for the serotonin transporter SERT. Incubation of ileum enteroids with B. dentium conditioned media resulted in significant upregulation of SERT, particularly in enteroendocrine cells.

Image Credit:
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A G E N D A

7:45-8:30 AM	**Breakfast**
8:30 AM – 8:40 AM	**Welcome Remarks** HASHEM EL-SERAG, M.D., M.P.H. Director, Texas Medical Center Digestive Diseases Center
SESSION I	Moderator: BENJAMIN SHNEIDER, M.D.
8:40-9:20 AM	**"Bile Acids in Biology and Medicine-overview"** MICHAEL TRAUNER, M.D., Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna
9:20-10:00 AM	**"Do Bile Acids Mediate Actions of Bariatric Surgery"** RANDY SEELEY, Ph.D., Department of Surgery, University of Michigan.
10:00- 10:20 AM	**"Cholestasis and Bile Acid Signaling in Cardiomyopathy"** MORESHWAR DESAI, M.D., Pediatric Critical Care, Baylor College of Medicine
10:20-10:30 AM	**Coffee break**
SESSION II	Moderator: DOUGLAS BURRIN, Ph.D.
10:30-11:10 AM	**"Interaction of Bile Acids and Microbiome"** JASMOHAN BAJAJ, M.D., Division of Gastroenterology, Hepatology and Nutrition, Virginia Commonwealth University
11:10-11:50 PM	**"Bile Acids and Colonic Function in IBS"** MICHAEL CAMILLERI, M.D., Department of Gastroenterology, Mayo Clinic
11:50-12:30 PM	**"Bile Acids and Norwalk Virus Pathogenesis"** MARY ESTES, Ph.D., Molecular Virology and Microbiology, Baylor College of Medicine
12:30-12:50PM	**"Malnutrition and GI Bile Acid Signaling"** GEOFFREY PREIDIS, M.D., Ph.D., Pediatric GI, Hepatology, Nutrition, Baylor College of Medicine
12:40-2:00 PM	**Lunch / Poster Session**
2:00-2:30 PM	**Poster Awards and Concluding Remarks**

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Texas Children's Hospital®

APPROVED CME ACTIVITY

Directly provided by Texas Children's Hospital
Hosted by the Texas Medical Center Digestive Diseases Center
Saturday, February 11, 2017 | 8:30 am – 2:30pm | Onstead Auditorium

"Bile Acids in Biology and Medicine-Overview"
MICHAEL TRAUNER, M.D., MEDICAL UNIVERSITY OF VIENNA

"Do Bile Acids Mediate Actions of Bariatric Surgery?"
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TARGET AUDIENCE

Internal Audience, Physicians, Specialists Gastroenterology, Research in Digestive Diseases, Medical Students, Residents, Fellows, Any physician or researcher with interest in digestive diseases

EDUCATIONAL OBJECTIVES

At the conclusion of this live activity, participants should be better able to: 1. Define the availability of bile acids in the field of liver and digestive diseases, 2. Apply best clinical practices concerning bile acids for patients with liver and digestive diseases, 3. Identify opportunities to apply bile acids in the treatment of liver and digestive diseases

ACCREDITATION STATEMENT

This live activity has been planned and implemented in accordance with the accreditation requirements and policies of the Texas Medical Association through the joint providership of Texas Children's Hospital and Texas Medical Center Digestive Disease Center. Texas Children's Hospital is accredited by the TMA to provide continuing medical education for physicians.

CREDIT DESIGNATION

Texas Children's Hospital designates this live activity for a maximum of *4.0 AMA PRA Category 1 Credit(s)™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

DISCLOSURE

Drs. Trauner, Seeley, Desai, Bajaj, Camilleri, Estes, and Preidis have reported no relationships with proprietary entities related to the content of this activity. Persons involved in the planning of this activity have reported no relevant financial relationships with any commercial interest.

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LIST OF ABSTRACTS

(Listed in alphabetical order by author.)

Texas Medical Center Digestive Disease Center
CENTER OVERVIEW

The Texas Medical Center Digestive Disease Center (DDC) facilitates on-going digestive diseases research, promotes translational research between basic and clinical areas, develops new projects, nurtures new investigators, and provides GI educational activities.

The DDC is a federally funded center (NIH P30DK056338) designed to serve basic and clinical scientists at institutions within the Texas Medical Center, including Baylor College of Medicine, The University of Texas Health Science Center at Houston and the MD Anderson Cancer Center. It is one of only 17 Digestive Diseases Research Core Centers in the country and the only center in the southeast United States.

The DDC director is Hashem B. El-Serag, M.D., M.P.H., Margaret M and Albert B Alkek Professor and Chair, Department of Medicine at Baylor College of Medicine. The DDC was founded by Mary K. Estes, Ph.D., emeritus director and professor of molecular virology and microbiology at Baylor. The DDC supports three basic science cores: Cellular and Molecular Morphology, Functional Genomics and Microbiome, Integrative Biology; and one clinical core: Study Design and Clinical Research.

For more information, visit https://www.bcm.edu/research/centers/digestive-disease.

Early Childhood Rotavirus Infection Alters Microbial Geography of The Small Intestine

Lori D. Banks[1,2,] Diane S. Hutchinson[1,2,] Bhanu P. Ganesh[2,3,] Jennifer Auchtung[1,2,] Nadim J. Adjami[1,2,] James Versalovic[2,3,] Joseph F. Petrosino[1,2,] Joseph M. Hyser[1,2].

Alkek Center for Metagenomic and Microbiome Research[1], Department of Molecular Virology and Microbiology[2], Baylor College of Medicine, Houston, TX, 77030, United States. Department of Pathology[3], Texas Children's Hospital, Houston, TX, 77030, United States.

Rotavirus (RV) is a major cause of acute gastroenteritis in children, leading to severe dehydration and often death. RV diarrhea involves cross-talk between viral factors, the host cells, and the intestinal microbiome, yet little is known about temporal and compartment-specific changes to the microbiome during acute diarrhea. A better understanding of these communication dynamics can inform the development of novel anti-diarrheal drugs. To investigate the effects of acute infant RV infection on the gut microbiome, we orally gavaged 8 day old BALB/c mouse pups with rhesus rotavirus (RRV), and measured diarrhea severity over 3 days. To first determine acute changes in the small intestinal microbiome upon onset and peak diarrheal disease, we collected the expressed contents or whole tissues from selected compartments of the gastrointestinal (GI) tract at 1 or 3 days post-infection. Samples were then analyzed by 16S DNA sequencing for bacterial composition. One day post infection, we observed a significant decrease in Lactobacillus and Enterococcus species in the contents of the small intestine. Conversely, increases were seen in Enterobacter, Bacteroides, and Akkermansia species in that same compartment. While the specific taxa affected in whole tissue studies were different, significant changes in bacterial community composition were consistently in the terminal ileum and colon, and distinct from fecal communities. Notably, these sites are immediately downstream of the primary rotavirus infection and replication site in the gut. Three days post infection, significant changes in all compartments resolve, as diarrheal disease also wanes. These data suggest that given canonical hallmarks of RV disease, the gut microbiome has a site-specific response to rotavirus diarrhea. We did observe community shifts proximal to the site of infection and in feces, but not other upstream GI compartments. Ongoing experiments seek to determine the immunological context of the acute infection, and the distribution of the post-infection microbiome relative to sites of RV replication.

Therapeutic effect of *Lactobacillus reuteri* DSM 17938 on Treg-deficiency-induced autoimmunity (IPEX syndrome) via the inosine-adenosine 2A receptors

Baokun He[1,2], Yuying Liu[1,2], Thomas K. Hoang[1,2], Ting Wang[1,2], Michael Ferris[3], Christopher M. Taylor[3], Xiangjun Tian[4], Meng Luo[3], Dat Q. Tran[2], Jain Zhou[5], Nina Tatevian[5], Fayong Luo[6], Jose G. Molina[6], Michael R. Blackburn[6], Thomas H. Gomez[7], Stefan Roos[8,9], J. Marc Rhoads[1,2]

[1]Pediatrics Gastroenterology, [2]Pediatric Research Center, [5]Pathology and Laboratory Medicine, [6]Biochemistry and Molecular Biology, [7]Center for Laboratory Animal Medicine and Care, The University of Texas Health Science Center at Houston McGovern Medical School, Houston, TX 77030, United States, [3]Department of Microbiology, Immunology & Parasitology, Louisiana State University School of Medicine, New Orleans, Louisiana, 70118, United States, [4]The Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology, One Baylor Plaza, Baylor College of Medicine, Houston, TX 77030, United States, [8]Department of Microbiology, Uppsala BioCenter, Swedish University of Agricultural Sciences, Uppsala, [9]BioGaia AB, Stockholm, Sweden

BACKGROUND: Regulatory T-cell (Treg) deficiency causes lethal, $CD4^+$T cell-driven autoimmune diseases. Stem cell transplantation is used to treat these diseases, but this procedure is limited by the availability of a suitable donor. The intestinal microbiota drives host immune homeostasis by regulating the development of Treg, Th1 and Th2 cells. It is currently unclear if Treg-deficiency autoimmune disorders can be treated by targeting the enteric microbiota.
OBJECTIVES: To determine the autoimmunity, gut microbiota, and plasma metabolomics affected by probiotic *Lactobacillus reuteri DSM 17938* (LR), and to further identify the mechanism of modulated metabolite(s) in suppressing autoimmunity in Treg-deficient scurfy (SF) mice.
METHODS: Each male mouse pup was given by gavage either MRS media as control or LR in MRS (10^7 CFU/day), water, or inosine (800 mg/kg/day), once daily, starting from 8 days of age (d8) to d22 for analyzing metabolomics, pathology and immunology; to infinite for observing survival rates. Inflammation in the lung and liver was measured by area of lymphocyte infiltration. IFNγ$^+$CD4$^+$T (Th1) and IL-4$^+$CD4$^+$T (Th2) cells were evaluated by flow cytometry. Plasma IFNγ and IL-4 were assessed by ELISA. Spleens from adenosine receptor ($A1^{-/-}$, $A2A^{-/-}$, $A2B^{-/-}$, and $A3^{-/-}$) knockout mice were used to isolate naïve $CD4^+$T cells for testing *in vitro* $CD4^+$T cell differentiation.
RESULTS: We demonstrated that Foxp3$^+$Treg deficiency results in gut microbial dysbiosis and autoimmunity over the lifespan of SF mouse. Remodeling microbiota with LR prolonged survival and reduced multi-organ inflammation in SF mice. LR changed the metabolomics profile disrupted by Treg-deficiency with a major effect of restoring levels of the purine metabolite inosine. Feeding inosine itself prolonged life and inhibited multi-organ inflammation by reducing Th1/Th2 cells and their associated cytokines. Mechanistically, the inhibition of inosine on the differentiation of Th1 and Th2 cells *in vitro* depended on adenosine A2A receptors. Both A2A receptor specific antagonist or genetically knockout A2A to SF mice reversed the anti-inflammatory effects of both inosine and LR *in vivo*.
CONCLUSIONS: A2A receptors mediate a substantial protective effect of inosine and LR. The LR-modulated-microbiota-inosine-A2A axis might represent a potential avenue for combatting autoimmune diseases mediated by Treg dysfunction.

This study was supported NIH/NCCIH R01 AT007083 and by BioGaia AB (Sweden).

Rotavirus infection activates calcium- and cyclic nucleotide-dependent chloride channels in cells

Alexandra L. Chang-Graham[1,2], Nina K. Ramachandran[2], and Joseph M. Hyser[1,2]

[1]Integrative Molecular and Biomedical Sciences Graduate Program, Baylor College of Medicine, [2]Department of Molecular Virology and Microbiology, Baylor College of Medicine,

Rotavirus (RV) causes severe diarrheal disease in children under the age of 5 worldwide. Secretory diarrhea results from overstimulation of chloride (Cl^-) channels that are regulated by cyclic nucleotides (cAMP and cGMP) and calcium (Ca^{2+}) signaling pathways. Although several bacterial toxins are well studied, the Cl^- channels and their molecular mechanisms of activation in RV infection have yet to be identified. RV elevates cytosolic Ca^{2+} and activates store-operated Ca^{2+} entry (SOCE), a mechanism for extracellular Ca^{2+} entry. SOCE can activate Ca^{2+}-activated Cl^- channels (CaCCs) such as anoctamin 1 (Ano1), but whether this contributes to fluid secretion during RV infection is unknown. Furthermore, crosstalk mechanisms between Ca^{2+} and cAMP pathways may contribute to activation of cAMP-dependent Cl^- channels, such as cystic fibrosis transmembrane conductance regulator (CFTR) during RV infection. Therefore, we characterized Cl^- channel expression and performed live cell fluorescent imaging in HEK293 cells and human intestinal enteroids (HIEs) to determine Cl^- channel activation during RV infection.

Using qRT-PCR and western blot, we first determined expression of Cl^- channels in HEK293 cells and HIEs. Next, we created HEK293 cell lines stably expressing halide-sensitive YFP, a fluorescent Cl^- channel biosensor, and either mouse Ano1 or CFTR. We found that RV increased Cl^- channel activation in both mouse Ano1- and CFTR-expressing HEK293 cells and activation was attenuated with channel-specific blockers. Finally, we measured increased fluid secretion and Ca^{2+} flux in RV-infected three-dimensional HIEs stably expressing the fluorescent Ca^{2+} biosensor GCaMP6s. Overall, these results support that RV can activate Ano1 as well as CFTR in infected cells, which may contribute to overall fluid secretion during RV infection. Determining the molecular mechanisms that regulate CaCC and CFTR activation during RV infection is critical for developing life-saving anti-diarrheal drugs and establishing a paradigm to study diarrheal diseases caused by other enteric viruses using the HIE system.

Gfi1⁺ Secretory Cells Revert to Stem Cells Following Crypt Injury

Min-Shan Chen[1], Yuan-Hung Lo[1], Hsin-I Jen[3], Andrew Groves[3], Noah F. Shroyer[1,2]

[1.] Integrative Molecular and Biomedical Sciences Graduate Program, Baylor College of Medicine, Houston, Texas [2.] Section of Gastroenterology & Hepatology, Baylor College of Medicine, Houston, Texas [3.] Program in Developmental Biology, Baylor College of Medicine, Houston, Texas

Background: The intestinal epithelium undergoes rapid turnover which is maintained by small numbers of stem cells located in the base of glandular crypts. Intestinal stem cells play an important role in homeostasis and regeneration after injury. Severe injury caused by cytotoxic drugs or radiation results in depletion of the active stem cell pool, and can result in ulceration and severe barrier dysfunction of the intestinal epithelium. These toxicities can be dose-limiting and life threatening in the context of chemoradiation therapy. Recent reports suggest that secretory progenitor cells can reverse the process of differentiation to function as stem cells and contribute to epithelial regeneration upon injury. Growth Factor-Independent 1 (GFI1) is a zinc finger transcriptional repressor implicated in the differentiation of secretory precursors into goblet and Paneth cells in the intestinal epithelium. Here, we test the hypothesis that Gfi1⁺ secretory cells revert to stem cells to assist epithelial regeneration following crypt damage.

Method: In this study, we generated Gfi1 reporter mice, Gfi1cre; *ROSA26*$^{floxSTOP\text{-}YFP}$. Mice were treated with 20 mg/kg Doxorubicin through intraperitoneal *injection to cause crypt injury.* Intestinal tissues were harvested at different time points and Gfi1⁺ cells were observed by immunohistochemical staining or immunofluorescence staining. For organoid cultures, crypts were isolated from mouse intestinal tissue and cultured in matrigel. Lineage tracing was observed using live confocal image.

Results: Lineage tracing in Gfi1 reporter mice reveals that YFP+, Gfi1-lineal cells were secretory Paneth and Goblet cells, which were not part of the stem cell pool under homeostatic conditions. After treatment with doxorubicin, YFP+, Gfi1-lineal cells became proliferative cells, expanded to fill regenerating crypts and contributed to all cell lineages of the intestinal epithelium*, indicating that Gfi1-lineal cells reverted to stem cells. Reversion of Gfi1-lineal cells was most prominent in the duodenum, where ~60% of crypts showed evidence of reversion, whereas the distal small intestine showed less reversion (~20% in the jejunum and ~10% in the ileum). The extent of reversion was generally correlated with the severity of injury as assessed by dysmorphic crypts.* In addition, pS6 IHC staining revealed that the regenerating intestinal epithelium activates mTOR signaling. To identify potential boosters of intestinal regeneration, we generated three-dimensional organoid cultures from Gfi1 reporter mice which provide a convenient and physiologically relevant model for large-scale screening to identify key pathways regulating stem cell injury and regeneration caused by chemotherapy drugs. Our preliminary results showed that PI3kinase/mTOR activation using PTEN inhibitor improved cell survival, and high R-spondin treatment increased both survival and efficiency of Gfi1+ cell reversion upon injury. R-spondin and PTEN inhibition combined to enhance both survival and reversion upon injury. These data indicated that mTOR and R-spondin may be key regulators of cell survival and stem cell reversion after tissue injury. Our studies in intestinal stem cells may improve our current understanding of regeneration of intestinal epithelium following injury and identify potential therapeutic strategies to mitigate the effects of injury.

The Role of Homeobox Genes in Conferring Intestinal Regional Identity

Zachary K. Criss II, Yuan-Hung Lo, and Noah Shroyer Ph.D.

Homeobox (HOX) genes are known for their role in anterior-posterior patterning of the developing embryo. As in other tissues, *HOX* genes are generally expressed in a spatial collinear fashion along the gastrointestinal tract. Ectopic expression of *hoxd13*, a posterior *HOX* gene, caused partial distalization of the developing avian proximal gut, implicating *HOX* genes in influencing intestinal patterning and regional identity. Our unpublished data, in accord with others, shows that the crypt compartment, which houses stem cells necessary for intestinal epithelial renewal, retains intestinal regional specific identity. The objective of this project is to determine the requirement and sufficiency of *HOX* genes to maintain and establish intestinal regional identity. Here, three-dimensional human intestinal enteroids (HIEs) are used as a model to study the establishment and maintenance of intestinal regional identity. RNA-seq and ChIP-seq revealed an enriched expression of several mid-cluster HOX genes in human ileal crypts compared to jejunal crypts. Additionally, qPCR analysis indicates that mid-cluster HOX9 paralogs (HOXA9, HOXB9, HOXC9 and HOXD9) are markers of ileal and colonic stem cells. I plan to use lentiviral shRNA and inducible cDNA to manipulate *HOX* expression. HOX9 paralogs will be knocked down in HIEs, in order to elucidate the role of *HOX* genes in maintaining and conferring intestinal regional identity in adult stem cells. Furthermore, I will manipulate expression of the HOX9 paralogs in pluripotent stem cell-derived intestinal organoids (HIOs) to test the role of HOX genes in the initial establishment of intestinal regional identity.

The role of the gut microbiota in obstructive sleep apnea induced hypertension

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Obstructive sleep apnea (OSA), characterized by repeated closure of the upper airway during sleep, is a significant clinical problem. OSA is an independent risk factor for systemic hypertension, and the most common underlying cause of resistant hypertension. The importance of a healthy gut microbiota, and detriment of a dysbiotic microbiota, on host physiology is becoming increasingly evident. We have previously observed that gut dysbiosis is associated with the development of hypertension in a rat model of OSA. Furthermore we have shown that transplantation of the dysbiotic microbiota from a hypertensive OSA rat into a normotensive rat is capable of inducing hypertension. These studies demonstrate, for the first time, a causal relationship between gut dysbiosis and hypertension. However, the mechanisms linking gut dysbiosis to hypertension are unknown. We tested the hypothesis that OSA-induced gut dysbiosis leads to impaired gut barrier function, systemic inflammation, and inflammation of the brain, which is linked to hypertension. Using an *in vivo* rodent model of OSA, we exposed high fat fed rats to 2 weeks of sham or OSA (60 apneas/hr). Relative to sham rats, OSA led to a significant increase in TNFα mRNA expression in the cecum wall as well as decreased goblet cells/crypt in the cecum and colon ($n=4$, $p<0.05$). Consistent with impaired gut barrier function and bacterial translocation, we observed bacterial 16S rRNA signatures in the mesenteric lymph node and visceral adipose tissue, as well as elevated adipose IL-6 mRNA expression following OSA ($n=4$-7, $p<0.05$). Flow cytometric analysis revealed a significant decrease in the number of anti-inflammatory T-regulatory cells in the brain of OSA vs. sham rats. This was associated with an increase in the number of activated microglia following OSA ($n=3$, $p<0.05$). Short chain fatty acids play a key role in maintaining gut barrier integrity and regulating immune responses. To further examine the role of gut dysbiosis in the development of OSA-induced hypertension we treated sham and OSA rats with a prebiotic (diet enriched with 20% resistant starch) or probiotic (Clostridium butyricum; 10^9 CFU by gavage every three days) to increase short chain fatty acids and improve gut barrier function. Pre- and probiotics successfully prevented OSA-induced loss of goblet cells and TNFα expression in the cecum and colon. Compared to vehicle treated rats, the probiotic *C. butyricum* prevented the OSA-induced increase in adipose IL-6 expression, increased total T-regulatory cells in the brain, and decreased activated microglia ($n=3$-6, $p<0.05$). Importantly, both the pre- and probiotic successfully prevented the development of hypertension in OSA rats. These data demonstrate a causal role for gut dysbiosis in the development of hypertension, which involves gut barrier disruption, bacterial translocation, and brain inflammation. Our data suggest that manipulation of the gut microbiota, through pre- or probiotics, may serve as a novel therapy in the prevention of hypertension.

Funding Information
This project was funded by NINDS R01NS080531 (RMB), AHA 16SDG29970000 (DJD), and by Public Health Service grant DK56338, which funds the Texas Medical Center Digestive Diseases Center (DJD).

Performance of AFP-Based Hepatocellular Carcinoma Surveillance in Cirrhosis Patients with Cured HCV

Nabihah Tayob[1,3], Pete Richardson[1,2], Fasiha Kanwal[1,2], Donna White[1,2], Xiaoying Yu[1,2], Winnie Zou[1], Diana Castillo[1,2], Jessica Davila[1,2], Hashem El-Serag[1,]
[1]Medicine, Baylor College of Medicine, [2]HSR&D IQUEST Center, Michael E. DeBakey VA Medical Center, and [3]University of Texas - MD Anderson Cancer Center, Houston, TX

Background and Aim: Patients with HCV cirrhosis remain at a high risk for hepatocellular carcinoma (HCC) following sustained virological response (SVR). Performance of HCC surveillance in this subgroup is not well understood. Serum alpha-fetoprotein (AFP) is commonly used (with ultrasonography) for HCC surveillance. Our aim was to evaluate performance of AFP based algorithms for HCC detection in cirrhosis patients with active versus cured HCV.

Methods: We compared two approaches to improving standard AFP surveillance: 1) a laboratory-based algorithm that incorporates current AFP, AFP rate of change in the last year, serum alanine aminotransferase (ALT), platelets and age in a six-month risk prediction model, and 2) a parametric empirical Bayes (PEB) surveillance algorithm that incorporates each patient's available AFP history to identify significant changes in AFP. We used a retrospective cohort ascertained from the VA HCV Clinical Case Registry that included 12,124 HCV-cirrhosis patients (with 902 incident HCC cases) with ≥1 AFP in the VA during 1997-2006. Our validation cohort included 21,707 cirrhosis patients with *active HCV* (with 2,190 incident verified HCC cases) and 3,979 cirrhosis patients *with SVR* (with 168 incident verified HCC cases) who had ≥1 AFP at the VA during 2010-15. We calculated patient-level true positive rate (TPR), the proportion of HCC cases with at least one positive screen in the T months prior to HCC diagnosis, and test-level false positive rate (FPR), the probability of a positive screen in those who did not develop HCC or in HCC cases more than T months prior to diagnosis.

Results: In Table 1, we report the TPR corresponding to 10% test-level FPR for the standard AFP threshold approach (AFP only), the laboratory-based algorithm (AFP+ Lab+ ΔAFP) and the PEB algorithm (PEB with AFP). In patients with active HCV, the laboratory-based algorithm increased the TPR by 2.3-3.0% while the PEB algorithm increased the TPR by 4.1-11.8%, compared to the standard AFP threshold only approach. In those with SVR, TPRs were considerably lower for all three methods; TPR of the standard AFP threshold approach decreased by 13.5-15.9%. However, we observed relative improvements similar to active HCV, for the laboratory-based algorithm (2-2.5%) and the PEB algorithm with AFP (3.9-12.5%) compared to the standard AFP threshold approach.

Conclusions: HCC surveillance with AFP has reduced TPR in treated patients that achieved SVR compared to those with active HCV infection. This may be related to reduced baseline AFP levels. Sensitivity in both groups can be improved using either a laboratory-based screening algorithm or the PEB algorithm with AFP.

Table 1: TPR corresponding to 10% test-level FPR for each surveillance approach.

TPR at 10% FPR	**Time (T) prior to HCC diagnosis**			
	Active HCV			
	6 months	**12 months**	**24 months**	**Ever** (max follow-up time)
AFP only	43.7	43.8	44.1	45.0
AFP + Lab + ΔAFP	46.2	46.1	47.1	47.3
PEB with AFP	47.8	50.2	52.7	56.8
	Treated with SVR			
	6 months	**12 months**	**24 months**	**Ever** (max follow-up time)
AFP only	28.5	27.9	28.8	31.5
AFP + Lab + ΔAFP	30.7	29.9	31.3	33.9
PEB with AFP	27.7	31.8	38.0	44.0

Time (T) is months prior to HCC diagnosis where cancer is assumed to be screen detectable and a positive test is considered to be a true positive screen.

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Evaluation of Screening Approaches for Hepatocellular Carcinoma In Cirrhosis Patients from the Veteran's Affairs Health Care System

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Background and Aim: Serum alpha-fetoprotein (AFP) is commonly used (with ultrasonography) to screen cirrhosis patients for hepatocellular carcinoma (HCC). The reported performance of AFP for HCC detection varies widely. We considered two approaches to improving HCC surveillance with AFP: (1) a laboratory-based algorithm that incorporates current AFP, AFP rate of change in the last year (if available), serum alanine aminotransferase (ALT), platelets and current age in a six-month risk prediction model, and (2) a parametric empirical Bayes (PEB) surveillance algorithm that incorporates each patient's available AFP history to identify significant changes in AFP. Our aim was to evaluate the improvement in screening performance of the two proposed approaches compared to using only the current AFP. ***Methods:*** We have developed the two algorithms using a retrospective cohort derived from the Department of Veterans Affairs (VA) Hepatitis C Virus (HCV) Clinical Case Registry. The cohort included 12,124 HCV-cirrhosis patients (with 902 incident HCC cases) who had at least one AFP at the VA between 1997 and 2006. Our validation cohort consisted of 51,230 patients with *cirrhosis of any etiology* (with 3,319 incident verified HCC cases) who had at least one AFP at the VA between 2010-2015. For each patient, at each AFP test, the laboratory-based algorithm has a positive screen if their six-month HCC risk estimate exceeds a fixed threshold. The PEB algorithm has a positive screen if their AFP exceeds an individual test-specific threshold. We calculated the patient-level true positive rate (TPR): the proportion of HCC cases with at least one positive screen in the T months prior to HCC diagnosis; and the test-level false positive rate (FPR): the probability of a positive screen in cirrhosis patients who did not develop HCC or a positive screen in HCC cases that occurred more than T months prior to HCC diagnosis. ***Results:*** In Table 1, we report the patient-level TPR corresponding to 10% test-level FPR for the standard approach that used a fixed threshold for AFP (AFP only), the laboratory-based algorithm (AFP + Lab + ΔAFP) and the PEB algorithm (PEB with AFP). We considered four scenarios for when HCC is screen detectable: 6 months, 12 months, 24 months, and any time prior to HCC diagnosis. At 10% test-level FPR, the fixed threshold for AFP varied between 19.6ng/mL and 21ng/mL depending on time (T). The laboratory-based algorithm has a TPR that is 1.7-2.6% higher while the PEB algorithm with AFP has a TPR that is 2.9-9.5% greater than the standard AFP only threshold approach. ***Conclusions:*** In a national cohort of VA patients with all possible etiologies of cirrhosis, HCC surveillance with AFP can be improved using either a laboratory-based screening algorithm or the PEB algorithm with AFP. Implementation of these methods at the point of care should be considered.

Table 1: Patient-level true positive rate (TPR) corresponding to 10% test-level false positive rate (FPR) for each surveillance approach.

TPR at 10% FPR	**Time (T) prior to HCC diagnosis**			
	6 months	**12 months**	**24 months**	**Ever (max follow-up time)**
AFP only	49.4	49.5	49.6	50.3
AFP + Lab + ΔAFP	51.1	51.5	52.2	52.8
PEB with AFP	52.3	54.0	56.4	59.8
Time (T) is the months prior to HCC diagnosis where cancer is assumed to be screen detectable and a positive screen is considered to be a true positive screen.				

Phase 3 Biomarker Study for HCC Surveillance Using AFP, AFP L-3 and DCP: A Prospective Collection with Retrospective Blinded Evaluation

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Background: The triple biomarkers (AFP, AFP L-3, DCP) are extensively used and validated for HCC surveillance in Japan. However, there is a paucity of data generated from longitudinal (phase 3) studies in North American populations on the performance of these biomarkers for HCC surveillance.

Methods: We present interim results from a prospective cohort study (8/2014-8/2016) at the Houston VA Medical Center. We enrolled consecutive patients with confirmed cirrhosis irrespective of etiology and no past or present HCC in a 6 monthly surveillance program consisting of liver imaging (mostly ultrasound) combined with AFP. HCC was diagnosed according to AASLD and EASL criteria. Blood samples were prospectively collected every 6 months and underwent retrospective blinded assays for AFP L-3 and DCP. The measurements were undertaken using a microchip capillary electrophoresis and liquid-phase binding assay on uTASWako i30 automated analyzer (Wako Life Sciences, Inc. in Mountain View, CA USA). We calculated sensitivity and false positive rate (FPR) for detecting HCC using cutoffs recommended by the Japan Society of Hepatology (AFP>20 ng/ml. AFP-L3>7%, DCP>0.48 ng/ml, or any of the three). We also calculated the sensitivity and FPR for the GALAD score at a threshold of -1.95 chosen by Berhane et al (2016) to maximize both sensitivity and specificity in a Japanese cohort. Sensitivity was defined as the probability of at least one positive screen within 6, 12 or 24 months before HCC diagnosis. The FPR was defined as the probability of a positive screen in all the screenings conducted in control patients or in HCC cases more than 6, 12 or 24 months prior to HCC diagnosis.

Results: A total of 493 patients contributed to a total of 828 surveillance episodes (52% 1 episode, 32% 2 episodes, 14% 3 episodes and 3% 4 episodes), of whom 14 patients developed HCC. We limited the analysis to 12 HCC cases with complete information on biomarkers before HCC development and 449 controls with consistently negative imaging and complete biomarker information. The mean age of the overall cohort was 63.35 (SD 6.63) and 98% were men. The cirrhosis etiology was HCV in 72%, alcohol 59%, NAFLD in 17% and HBV in 2%. Cases had higher AFP-L3 (6.53 v 3.55, $p=0.048$), lower platelet count (93.5K v 147.1K, $p<0.001$) and lower albumin (3.23 v 3.68, $p=0.045$) at baseline. There were no significant differences in the baseline age or serum levels of AFP, DCP, or ALT. At the time of diagnosis the mean tumor size was 2.01 cm (SD: 0.82), 7 patients had only one HCC nodule (3 had 2 nodules and 2 had 3 nodules), all but two received HCC specific treatment (1 resection, 5 RFA, 4 TACE) and one is listed for liver transplant. The sensitivity of all three biomarkers combined was higher than using any of the markers alone but the FPR was high (Table). Cutoff based on GALAD score further increased FPR. Three out of 12 patients with HCC had elevated AFP and 9 out of 12 patients with HCC had any of the three biomarkers elevated in the presence of a normal surveillance imaging.

Conclusions: In North American patients with cirrhosis, the use of biomarkers with existing cutoffs (AFP, AFP-3, DCP) results in a considerable improvement in sensitivity for HCC detection at an early stage but may also result in an increase in false positive results. Elevated biomarkers occur in the presence of normal surveillance imaging in the study. Further refinement of cutoffs may further increase the accuracy for early HCC detection.

Modulation of intestinal serotonin by *Bifidobacterium dentium*

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Background: The metabolite and neurotransmitter serotonin (5-HT) has a diverse physiological repertoire. 5-HT is primarily synthesized by the enterochromaffin cells (ECCs) in the intestinal epithelium where it regulates intestinal secretion, myenteric neurons and gastrointestinal motility. The activity of 5-HT is tightly modulated by uptake via the serotonin transporter (SERT). Decreased SERT has been implicated in intestinal inflammatory and diarrheal disorders, highlighting the critical role of SERT and 5-HT in intestinal homeostasis. *Bifidobacterium* species have been shown to modulate the gut brain axis and have been proposed to beneficially influence the serotonergic system. We hypothesize that intestinal commensal *Bifidobacterium dentium* stimulates 5-HT production by ECCs and upregulates SERT. **Methods & Results**: The human cell line, HT29, was exposed to *B. dentium* conditioned media (CM) overnight and SERT expression was analyzed by qPCR and immunofluorescence (IF). Addition of *B. dentium* filtered CM to HT29 cells resulted in significant upregulation of SERT mRNA and protein after 24 hrs. *B. dentium* CM stimulated phosphorylation of JNK, a pathway shown in previous studies to be involved in SERT regulation. Moreover, pharmacological inhibition of JNK prevented the *B. dentium* induced upregulation of SERT. To assess 5-HT regulation in a system with multiple epithelial cell types, mouse ileal enteroids and colonoids were exposed to *B. dentium* CM and SERT was examined by qPCR and IF. 5-HT levels were measured in CM and media collected from CM treated enteroids by mass spectrometry (MS/MS). *B. dentium* alone was unable to produce 5-HT. However, incubation of mouse ileal enteroids with *B. dentium* CM resulted in a dose-dependent upregulation of tryptophan hydroxylase (*Tph1*) and release of 5-HT. Mouse colonoids also exhibited a dose-dependent upregulation of SERT mRNA. Staining of mouse enteroids and colonoids revealed significant increases in SERT, which co-localized with 5-HT. Human jejunum enteroids exhibit 1-2% ECC cells, but enteroids over-expressing neurogenin 3 (NGN3) contains ~50% of cells to differentiate into ECCs. Incubation with *B. dentium* CM stimulated enhanced 5-HT release in NGN3 human enteroids. *In vivo*, germ-free mice mono-associated with *B. dentium* exhibited increased ileal and colonic luminal 5-HT, as well as blood serotonin, and increased SERT mRNA. SERT protein was also regulated in *B. dentium* colonized mouse colon, compared with germ-free controls. **Conclusions:** These data demonstrate that the commensal microbe *B. dentium* is capable of regulating key components of the intestinal serotonergic system. As downregulation of SERT has been implicated in the pathophysiology of several functional gut disorders, our data support the consideration of next generation probiotics as potential therapies for serotonin-associated disorders.

Circadian dysfunction in bile acid metabolism promotes NAFLD-induced hepatocarcinogenesis

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Hepatocellular carcinoma (HCC), previously considered a rare cancer, has increased steadily in incidence since the 1980s and is the fastest rising cause of cancer-related death in the U.S (1). Increased HCC incidence is coupled with that of obesity and non-alcoholic fatty liver disease (NAFLD), which is predicted to become the leading cause of HCC in the 21th century, as well as population-wide chronic circadian disruption (2-4). We previously reported that circadian gene mutant mice show cholestasis and increased risk of spontaneous HCC (5, 6). We report here that chronic circadian disruption following a human nightshift schedule increases the risk of obesity and induces spontaneous HCC in wild-type (WT) mice that are normally resistant to spontaneous and carcinogen-induced HCC. This hepatocarcinogenesis is independent of exogenous genotoxic stress and diet manipulation, and occurs after a prolonged period of complex liver metabolic diseases (7, 8). The pathophysiological progression starts with early onset NAFLD, hepatomegaly and cholestasis, with NAFLD progressing to nonalcoholic steatohepatitis (NASH) and fibrosis prior to HCC detection, just as described for obesity-related hepatocarcinogenesis in humans. Genome-wide microarray analyses identified a large number of deregulated hepatic genes in WT mice shortly after the initiation of chronic jet-lag, which overlap significantly with profiles of human HCCs. Strikingly, the top deregulated gene sets include nuclear receptor controlled bile acid and xenobiotic metabolic pathways throughout the lifespan of jet-lagged WT mice. In accord with our previous studies (6), hepatic bile acid levels are substantially increased and the relevance of this bile effect is strongly supported by our observation that ablation of the bile acid receptor FXR (NR1H4) further increases enterohepatic bile acid levels in response to chronic circadian disruption, and dramatically increases HCC incidence. In addition, loss of constitutive androstane receptor CAR (NR1I3), a well-known liver tumor promoter that is activated by elevated bile acid levels, completely prevents circadian dysfunction-induced hepatocarcinogenesis (8). Our findings have important therapeutic implications as the similarity in the pathophysiology and deregulation of FXR and CAR of HCCs found in jet-lagged WT mice and obese humans suggest restoration of bile acid homeostasis and inhibition of CAR as promising complementary strategies for HCC prevention in shift workers or other individuals experiencing chronic circadian disruption.

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Dgk and histamine mediated crosstalk between *Lactobacillus reuteri* and mammalian intestinal epithelium

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Lactobacillus reuteri ATCC PTA 6475, probiotic bacterium, with an intact *hdc* gene cluster is known to synthesize and secrete histamine, and can suppress inflammation in mammalian systems via specific activation of the type 2 histamine receptor (H2R). However, it is unclear if *L. reuteri*-derived histamine also modulates activity of pro-inflammatory H1 receptors in the intestinal epithelium. In this work, we identified a soluble secreted isoform of diacylglycerol kinase (Dgk) from *L. reuteri* 6475. DGKs belong to a distinct, conserved family of intracellular lipid kinases that phosphorylate diacylglycerol (DAG), catalyzing conversion of DAG to phosphatidic acid (PA). This reaction may diminish DAG quantities in the cell membrane possibly modifying host intracellular signaling downstream of DAG. Histamine binding to H1R can cause phosphorylation of PKC via DAG activation. We found that *L. reuteri* 6475 suppressed basal levels of the pro-inflammatory cytokines IL-6, IL-1, Eotaxin (eosinophilic chemoattractant proteins) and G-CSF in the luminal mucosa and in blood plasma. *L. reuteri* lacking Dgk could not suppress the aforementioned pro-inflammatory biomarkers. In addition, we demonstrated that histamine synthesized by *L. reuteri* 6475 activates both H1 and H2 receptors, but Dgk synthesized by the bacterium suppresses H1R downstream signaling. Inhibition of signaling downstream of H1R was supported by diminished PKC phosphorylation in the intestines of wild-type (WT) *L. reuteri* treated but not in *ΔdgkA* mutant treated germ-free (GF) mice. In addition, we also report suppression of $CD11b^{+}Gr1^{+}$ $Ly6G^{hi}$ immature myeloid cells (IMCs) after WT *L. reuteri* treatment. The proportion was consistent with in vivo experiments in our mouse model, PKC phosphorylation was reduced in human epithelial cells after treating the cells with *L. reuteri* derived conditioned media. *L. reuteri* suppressed immune responses by direct effects of the metabolite, histamine, and a secreted bacterial enzyme, diacylglycerol kinase, which converts a membrane lipid signal to an inactive form. These findings provide a deeper mechanistic understanding of intestinal immunomodulatory probiotics and e microbiome: host interaction.

Histamine H2 receptor agonists suppress pro-inflammatory cytokines and colonic inflammation

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Histamine is a biogenic amine that exerts various pathophysiological functions via four receptors. Previous studies showed that bacteria-derived histamine suppresses TNF production in human monocytes and attenuates colonic inflammation in experimental mouse models. These anti-inflammatory effects were diminished by adding histamine H2 receptor (H2R) antagonists, indicating a potential H2R mediated anti-inflammatory signaling pathway.

To confirm whether H2R activation is responsible for anti-inflammatory effects, amthamine and dimaprit, two H2R specific agonists were evaluated in cell culture and preclinical models. In phenylalanine-chloromethyl ketone (PCK) activated THP-1 cells, treatment with amthamine or dimaprit significantly inhibited TNF production in a dose dependent manner, similar to the effect of histamine. In a trinitrobenzene sulfonic acid (TNBS)-induced mouse model of colitis, oral administration of amthamine or dimaprit for 5 days protected Balb/c mice from colitis compared to the control mice that did not receive H2R agonists. Suppression of colitis was indicated by significantly decreased weight loss, macroscopic colonic injury evaluated by a Wallace score system, and reduced serum amyloid A protein concentrations. The plasma concentrations of pro-inflammatory cytokines IL-1β, IL-6 and TNF were significantly decreased in mice treated with amthamine or dimaprit compared to controls by the MAGPIX assay. These combined investigations confirmed that H2R may mediate anti-inflammatory responses in the intestine and may result in opportunities for new therapeutics for inflammatory bowel disease.

Phytosterols have weak direct effect on hepatocyte transporter expression but enhance Kupffer cell activation by endotoxin

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Background: Phytosterols may drive parenteral nutrition associated liver disease pathogenesis through a two-hit mechanism: suppression of hepatic bile clearance through downregulation of the bile salt export pump (BSEP) and increased hepatic inflammation. Inflammation can further suppress BSEP and potentially the ATP-binding cassette transporters ABCG5/8, the hepatic phytosterol clearance transporter, creating a feed-forward loop leading to further hepatic injury. Two nuclear hormone receptors, Farnesoid X Receptor (FXR) and Liver X Receptor (LXR) regulate bile acid metabolism and have been shown to suppress the inflammatory response. The aim of our study was to first, determine if the hepatic transport dysfunction and inflammatory response is hepatocyte independent or requires activation of the resident macrophages, Kupffer cells; second, determine if ligand-mediated activation of FXR and LXR can restore inflammation-induced suppression of transporter function.
Methods: Preterm piglets were administered parenteral nutrition for 10 d containing SO lipid emulsion. On day 10, piglets were given an 8-h infusion of saline or LPS (10 μg*kg-1*h-1). In a separate experiment, primary hepatocytes and Kupffer cells were isolated from neonatal piglets. Cells were treated with media supplemented with 1% SO with or without the addition of phytosterols. After incubation for 24 h, cells were additionally treated with FXR ligand Obeticholic acid (OCA), or LXR ligand GW3694 in the presence or absence of 50 ng/mL LPS or 10 ng/mL IL-1β for 24 h.
Results: In piglets, administration of LPS lead to a significant suppression of BSEP and ABCG5/8 expression compared to saline infused piglets. In cell culture, administration of IL-1β and LPS led to suppression of both BSEP and ABCG5/8. In hepatocytes co-treated with IL-1β and OCA or GW3694, only BSEP expression was rescued. In Kupffer cells, phytosterols alone failed to activate expression of cytokines, however, when treated with both LPS and phytosterols increased cytokine expression significantly more than with LPS alone.
Conclusions: From this study, we conclude inflammation can suppress the transporters for bile acid and phytosterol clearance. Our in vitro data shows that Kupffer cells are susceptible to enhanced activation of cytokine expression by phytosterols when cells are primed with LPS; however, hepatocytes are not. These results suggest that phytosterols may contribute to the progression of liver disease through inflammation.

EriC-family transporters regulate histamine production machinery in *Lactobacillus reuteri*

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Background: Inflammatory bowel diseases (IBDs), including Crohn's Disease and Ulcerative Colitis, affect over 3.5 million people in the U.S. and Europe. Patients with these conditions suffer from chronic intestinal inflammation and epithelial barrier damage, and are at higher risk for certain cancers. Specific strains of human commensal *Lactobacillus reuteri* have been shown to reduce inflammation and tissue damage in mouse models of IBD. The anti-inflammatory capacity of this bacteria stems in part from its ability to produce and secrete histamine, which can act on specific receptors present on mammalian cells. In the bacterial cell, histamine is generated from L-histidine by histidine decarboxylase (HdcA), and released by a histidine/histamine exchanger (HdcP). This process consumes protons, resulting in an increasingly alkaline intracellular pH and an inside-negative membrane potential. These factors can suppress the activities of HdcA and HdcP. Additionally, *hdcA* is not constitutively expressed, and it is unclear which signals may trigger its upregulation. It is currently unknown how *L. reuteri* balances its internal ion environment in order to sustain histamine production. Two proton/chloride antiporters, EriC and EriC2, have been identified in *L. reuteri* as potential regulators of intracellular pH and membrane potential, and thus histamine synthesis. We hypothesize that EriC-family transporters maintain intracellular pH and membrane potential at levels that allow continued histamine production. **Methods & Results:** To investigate how EriC-family ion transport might alter expression and function of the histamine production machinery in *L. reuteri*, single-strand recombineering was used to generate protein knockout, proton transport deficient ('gate-open'), and transport null ('gate-closed') strains in each transporter alone and in combination. These mutations were stable, and did not significantly alter the growth of the resulting strains compared to wild-type (WT) *L. reuteri*. In a histamine production assay, all 9 mutant strains produced approximately half as much histamine as the WT strain. However, in 7 of the 9 mutant strains, *hdcA* expression was ~1.5-fold upregulated relative to WT. Membrane potential was evaluated in each mutant using a fluorescent dye-aggregation assay. The single and double gate-closed mutants carried weaker membrane potentials than the WT strain. Interestingly, this reduction in potential was not observed in any of the transporter knockout strains, suggesting additional compensatory mechanisms in the absence of EriC-family proteins. Differences in intracellular pH were observed across EriC/EriC2 mutants using a pH-sensitive fluorophore assay. **Conclusion:** These data establish a regulatory role for EriC-family proton/chloride antiporters in the expression and function of histamine production machinery. Mechanistically, these transporters can affect membrane potential and may alter the internal pH of the bacteria. In the gut, pH and chloride gradients may alter histamine production, and thus the efficacy of *L. reuteri* as an anti-inflammatory probiotic.

Bile acid signaling as a therapeutic target for liver disease in alpha-1 antitrypsin deficiency

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Background: Alpha-1 antitrypsin deficiency (ATD) can progress to cirrhosis and hepatocellular carcinoma; however, not all patients are susceptible to severe liver disease. In ATD, a toxic gain-of-function mutation generates insoluble ATZ "globules" in hepatocytes, which overwhelm protein clearance mechanisms. The relationship between bile acids (BAs) and hepatocytic autophagy is less clear, but may involve FXR and other nuclear receptor pathways. Based on previous reports that bile duct ligation (BDL) induces autophagy in wild-type mice, we hypothesized that BAs may have hepatoprotective effects in PiZZ transgenic mice, which model ATD liver disease.
Methods: To study endogenous BAs, we performed BDL and partial BDL (pBDL) in PiZZ mice, followed by detailed analysis of liver tissues. In pBDL, the unligated (right) lobe served as an internal control for the ligated (left) lobe. We are also investigating effects of exogenous BAs and nuclear receptor agonists on ATZ globule clearance.

Results: At baseline, several genes involved in bile and lipid metabolism were over-expressed in globule-devoid hepatocytes, compared to globule-containing cells. PiZZ mice subjected to BDL showed up to 50% clearance of ATZ globules, with enhanced proliferation and increased expression of autophagy proteins. Analysis of transcription factors revealed significant changes. Nuclear induction of FXR was an early event. Surprisingly nuclear TFEB, a master regulator of autophagy, remained unchanged. pBDL confirmed that ATZ globule clearance was induced by localized stimuli rather than diet or systemic effects. In separate experiments, significant improvement was also observed with administration of FXR agonists

Conclusions: Retained BAs led to a dramatic reduction of ATZ globules, with enhanced hepatocyte regeneration and autophagy. Treatment with FXR agonists also showed positive effects. These findings support further investigation of BA signaling and receptor agonists as potential therapeutic strategies for ATD liver disease.

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Hepatic FXR/SHP axis modulates systemic glucose and fatty acid homeostasis in aged mice

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The nuclear receptors farnesoid X receptor (FXR; NR1H4) and small heterodimer partner (SHP; NR0B2) play crucial roles in bile acid homeostasis. Global disruption of both FXR and SHP signaling (DKO) causes severe cholestasis and liver injury at early ages. Here, we report an unexpected beneficial impact on glucose and fatty acid metabolism in aged DKO mice, which show suppressed body weight gain and adiposity when maintained on normal chow. This phenotype was not observed in single *Fxr* or *Shp* knockouts. Liver-specific *Fxr/Shp* double knockout (FS^{LKO}) mice fully phenocopied the DKO mice, with lower hepatic triglyceride accumulation, improved glucose/insulin tolerance and accelerated fatty acid utilization. In both DKO and FS^{LKO} livers, these metabolic phenotypes were associated with altered expression of fatty acid metabolism and autophagy-machinery genes. Loss of the hepatic FXR/SHP axis reprogrammed white and brown adipose tissue gene expression to boost fatty acid utilization. In conclusion, combined deletion of the hepatic FXR/SHP axis improves glucose/fatty acid homeostasis in aged mice, reversing the aging phenotype of body weight gain, increased adiposity and glucose/insulin tolerance, suggesting a central role of this axis in whole body energy homeostasis.

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SPDEF shifts the transcriptional targets of activated β-catenin to enforce tumor quiescence

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Background and Aims: Canonical Wnt/β-catenin signaling pathway activation, with resultant high β-catenin transcriptional activity, is frequently implicated in human colorectal cancers (CRCs); however, there are currently no treatments targeting this pathway. We have previously reported that SAM Pointed Domain Ets transcription Factor (SPDEF) is a colonic tumor suppressor. However, the molecular mechanism by which SPDEF mediates colorectal tumor repression is unclear. Here we aim to elucidate the molecular mechanism of SPDEF-mediated colonic tumor suppression and its repression of Wnt/β-catenin signaling in CRCs.

Methods: We analyzed the effects of SPDEF expression on β-catenin-driven intestinal tumors in a new mouse model (*Lgr5*^{CreERT2}; *β-catenin*^{exon3}; *Rosa26*$^{LSL\text{-}rtta\text{-}ires\text{-}EGFP}$; *TRE-Spdef*), human colon cancer xenografts, and patient-derived cancer colonoids. Moreover, wildtype or truncated SPDEF mutants were used for β-catenin activity assay, co-immunoprecipitation, and chromatin immunoprecipitation.

Results: SPDEF is sufficient to inhibit β-catenin-driven intestinal tumorigenesis and restrict established tumor cells' growth, and enforces a quiescent state on β-catenin-driven tumor cells. SPDEF inhibits canonical β-catenin transcriptional activity through protein-protein interaction, independent of its DNA binding capacity. SPDEF disrupts the binding between β-catenin and its DNA binding partners TCF1 and TCF3, selectively displacing β-catenin from the promoter/enhancer regions of cell cycle genes without affecting stem cell signature genes.

Conclusions: These results unveil a novel mechanism by which SPDEF directs tumor cells to switch between active and quiescent states, by shifting the transcriptional targets of activated β-catenin. We define a new paradigm in which the transcriptional program driven by oncogenic β-catenin is modifiable to confer proliferation or quiescence in the absence or presence of SPDEF.

Early colonization with *Bifidobacterium* species increases postnatal CNS expression of genes important to neurodevelopment and affects behavior of adult mice

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Background: *Bifidobacterium* species have been shown in recent studies to alter CNS gene expression, neurotransmitter function, and behavior in adult rodents. In humans, *Bifidobacterium* species are detectable within the first week after birth and are a predominant genus of the infant intestinal microbiota. We have previously shown that colonization of adult germ-free mice with *Bifidobacterium dentium* results in altered CNS expression of genes important to postnatal neurodevelopment. Given that *Bifidobacterium* colonizes the host during a critical period of neural circuit development and organization, *Bifidobacterium*-specific CNS modulation early in life may have pervasive and lasting effects on brain function and behavior. We hypothesized that neonatal colonization with a consortium of "infant-type" *Bifidobacterium* species would alter CNS gene expression throughout neurodevelopment and affect adult behavior. **Methods and Results:** In order to examine the effects of early *Bifidobacterium* colonization, neonatal germ-free mice were treated with a mixture of "infant-type" *Bifidobacterium* species, including *B. bifidum*, *B. longum* ss. *infantis*, *B. breve*, and *B. dentium (*~1x10^9 CFUs/treatment). The treatment began at postnatal day 1 and was repeated every other day. This treatment resulted in immediate and stable colonization of the pups over the course of the experiment. Control (germ-free) mice received sterile saline gavages, and all mice were raised in identical isolator units. Cerebellar tissue was collected from mouse pups at postnatal days 4, 10, and 20. Gene expression was analyzed via qRT-PCR, and our data suggest that relative to germ-free mice, *Bifidobacterium*-colonized mice have increased expression of synaptic plasticity-related genes at P4, and increased expression of neuronal migration/differentiation and neurotransmitter receptor genes at P10 and P20. The GABA receptor A α5 subunit (*Gabra5)*, which plays an important role in long-term potentiation was observed to have between a 2- and 20-fold increase at all three postnatal timepoints. At 5-6 weeks of age, the mice underwent a battery of behavior testing to assess a variety of motor and non-motor behaviors. Our data indicate that early colonization with *Bifidobacterium* restores normal anxiety-like behaviors and improves recognition memory in adult mice. **Conclusion**: These data demonstrate that neonatal colonization with *Bifidobacterium* species results in altered expression of genes important to neurodevelopment and can impact long-term adult behavioral phenotypes.

Lactobacillus reuteri – synthesized folates regulate bacterial histamine production and modulates folate transporters in human enterocytes

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Background: The human gut microbiome consists of complex, site specific, adaptive ecosystems that are uniquely attuned to changing host conditions. This reciprocal relationship is achieved, in part, through the production of diverse bacterial metabolites which are required for human health. Folates are essential vitamins required for human health. *Lactobacillus reuteri* ATCC PTA 6475, a human probiotic gut commensal bacterium, utilizes a series of *fol* genes to synthesize the reduced folate, 5, 10-methenyl-tetrahydrofolate. Site directed mutagenesis of several *L. reuteri* folate metabolism genes revealed that an early stop mutation in the gene encoding bifunctional dihydrofolate synthase/folylpolyglutamate synthase type 2 (*fol*C2), a required gene in the folate synthesis pathway, secretes less histamine. *L. reuteri*-derived histamine has been demonstrated to suppress gut inflammation-markers in human cell lines and mice. Because of histamine's role in host immunomodulation, understanding the bacterial mechanisms regulating histamine synthesis could generate better ways to promote histamine production *in vivo*. **Methods & Results**: To assess the co-regulation mechanism between the metabolites folate and histamine, mutations were made in several folate pathway genes in *L. reuteri*. Mass spectrometric examination of bacterial supernatants revealed a significant reduction in histamine in the *fol*C2STOP mutant. Quantitative real-time PCR and Western blot analysis indicate that reduced histamine production is not due to a reduction in the RNA or protein levels of the *L. reuteri* histidine decarboxylase, HdcA, respectively. Chemiluminescent analysis of *L. reuteri fol*C2Stop supernatants indicated a reduction in folic acid, demonstrating that *fol*C2 is required for further conversion of folic acid. No changes in intracellular pH were found between mutants and WT, indicating that intracellular pH alone was not responsible for alterations in histamine concentration. With regards to host physiology, human epithelial cell line HT-29 and biopsy derived human colonoids exhibit varying levels of folate receptors and transporters. Incubation of cells with *L. reuteri* conditioned media resulted in increased levels of folate receptor β and reduced folate carrier compared to untreated cells. The connection between histamine and folate metabolic processes in *L. reuteri* is being further examined using flow cytometry and isotopic labeling. To assess the effects of *L. reuteri*-secreted factors on non-cancerous cells, human colonoid monolayers were treated with *L. reuteri* conditioned media. This treatment resulted in changes to cell metabolic activity and folate transporter expression. **Conclusion:** Folate synthesis regulates the production of histamine in probiotic *L. reuteri* in a pH- and *hdc*A-independent manner. By delineating the mechanisms regulating the production and secretion of molecules beneficial to the host, such as histamine and folate, we can better understand the effects this probiotic has on the human host.

Examining the role of gut dysbiosis in cerebral small vessel disease

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The importance of a healthy gut microbiota on host physiology is becoming increasingly evident. Recent studies suggest that alterations to the microbiota can have adverse effects beyond the GI tract, and has been linked to hypertension and stroke. Thus we hypothesized that gut dysbiosis could contribute to the development of cerebral small vessel disease (CSVD). Giving merit to this hypothesis, we found that the microbiota of the spontaneously hypertensive stroke prone rat (SHRSP; a model of CSVD) is significantly different than that of WKY controls. Using 16s rRNA sequencing of bacterial DNA we found that SHRSP animals had decreased bacterial richness (p=.005) and diversity (p=.028), indicative of gut dysbiosis. Phenotypically, CSVD includes vessel remodeling, BBB breakdown and neuroinflammation. Gut dysbiosis is often associated with a leaky gut barrier that allows bacteria to enter the systemic circulation. We observed significantly greater permeability of the SHRSP colon barrier when compared to WKY (p=.026). We next sought to determine if impaired colon barrier function in SHRSP could lead to increased bacterial translocation to the periphery and ultimately to the brain. Brains were harvested from 24 week old SHRSP and WKY animals and qRT-PCR of the 16s rRNA gene was performed to detect the presence of bacteria in the brain. We discovered that SHRSP animals displayed a 50% increase in bacterial 16S rRNA load in the brain compared to WKY animals (p=.0063), confirming that bacteria are not only present in brain of CSVD rats, but also in greater abundance than WKYs. Taxonomically we discovered that the *Proteobacteria* and *Firmicutes* phyla made up 60% and 20% of the bacterial DNA found in the brains of SHRSP respectively, compared to 10% P*roteobacteria* and 50% *Firmicutes* in WKY brains (p=.024). Similarly, we found that SHRSP animals displayed a near 2-fold increase of the bacterial endotoxin LPS in the brain, as compared to WKY (p=.01). Finally, to further confirm the presence of bacterial components in the brain we used immunofluorescence imaging to visualize peptidoglycan (PG), a molecule found solely in bacterial cell walls. We discovered that we could visualize the presence of PG in the brains of SHRSP animals, and note that PG was commonly observed inside microglia, which are the resident immune cells of the brain. We conclude from these findings that SHRSP rats exhibit gut dysbiosis, gut barrier breakdown, and bacterial products in the brain. Further studies will examine how this bacterial presence contributes to CSVD phenotypes.

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Clinical features and molecular epidemiology of diarrheagenic *Escherichia coli* pathotypes identified by fecal gastrointestinal multiplex nucleic acid amplification in patients with cancer and diarrhea.

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Background: Diarrheagenic *E. coli* (DEC) pathotypes cause disease in children residing in developing countries, travelers to at-risk regions of the world and, to a lesser extent, in persons residing in the US. We describe the clinical characteristics, outcomes and molecular features of DEC in patients with solid (SM), hematologic malignacies (HM) or stem cell transplant (SCT) recipients at a major cancer center in the US.

Material/methods: We studied SM, HM and SCT patients with suspected infectious diarrhea for whom a fecal GI Biofire multiplex GI PCR (BFM) was positive (n= 250) from November 2015 through October 2016. Ten coliform colonies from stool cultures with BFM DEC were probed for DEC-associated virulence factor genes by PCR. DEC isolates were then studied for antimicrobial susceptibility and HEp-2 adherence.

Results: DEC were identified in 49/250 (20%) of BFM positive patients (Table 1.). Mixed DEC were seen in 7 of 49 (14%) cases. DEC occurred year-round but were more frequent in the summer months. Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC) and enterotoxigenic (ETEC) were more commonly seen in SCT patients than in SM. One of 5 Shiga-toxin producing E. coli (STEC) was O157:H7 and resulted in fatal hemolytic uremic syndrome. Only one Enteroinvasive *E. coli* (EIEC) was identified. In three patients, EPEC and EAEC resulted in chronic infections. Culture confirmed infections in 4/11 (36%) EAEC (three typical *AggR* $^{+}$). Genes coding for dispersin (*aap*) and its transporter (*aatA*) were found in three isolates. One carried *aaiC* alone. All were HEp-2^{+} adherent. EPEC was recovered in 5/22 (23%) samples (all atypical *eaeA*$^{+}$, *bfp*$^{-}$). Two SCT patients carried EAEC strains resistant to multiple antibiotics including fluoroquinolones and expressed extended spectrum beta lactamases. Seven cases had more than one DEC identified.

DEC* (N=49/250 20%)	EPEC (n=36	EAEC (n=11	ETEC (n=5)	STEC (n=5)	EIEC (n=1)
Male 31 (63%)	23 (64%)	7 (64%)	5 (100%)	2 (40%)	0
SM 10 (20%)	6 (17%)	1 (9%)	0	3 (60%)	1 (100%)
HM 14 (29%)	13 (36%)	3 (27%)	0	0	0
SCT 25 (51%)	17 (47%)	7 (63%)	5 (100%)	2 (40%)	0
Fever 15 (31%)	12 (33%)	1 (9%)	1 (20%)	0	1 (100%)
Neutropenia 17 (35%)	15 (42%)	4 (36%)	0	0	0
Lymphopenia 34 (69%)	26 (72%)	8 (73%)	0	2 (40%)	1 (100%)
Chronic Infections 3 (6%)	2 (6%)	1 (9%)	0	0	0
C. difficile coinfection 8 (16%)	3 (8%)	2 (18%)	2 (40%)	3 (60%)	0

Conclusions: In cancer patients with diarrhea, DEC pathotypes can cause mixed infections and are seasonal. EPEC and EAEC were the most frequent pathotypes found in highly immunosuppressed patients. In some instances causing chronic infections with multidrug resistant organisms. The use of highly sensitive BFM testing is identifying new patient populations at risk for DEC in the US children with biliary diseases, particularly PSC and CBD, may lead to earlier detection of CCA, thus providing sufficient time for surgical intervention, potentially leading to better survival outcomes.

Cholangiocarcinoma Among Children And Adolescents: A Retrospective Analysis Of Comorbid Conditions, Treatment, And Survival Outcomes

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Background: Cholangiocarcinoma (CCA) is a bile duct malignancy found primarily in the adult population. The incidence of CCA in children is unknown, and current literature consists primarily of case reports. Risk factors for developing CCA in adults include co-existing diagnoses of biliary disease and other gastrointestinal disease states such as inflammatory bowel disease (IBD), liver fluke infestation and cirrhosis. The few published case studies describing incidences of CCA in children include children with co-existing diseases such as IBD, primary sclerosing cholangitis (PSC), congenital biliary dilation (CBD), biliary atresia, and viral disease states such as HIV infection. Complete surgical resection is the only potential chance of cure in CCA, thus making early diagnosis of the disease essential to survival.

Objective: The objective of this study was to describe the incidence and the clinical characteristics and outcomes of CCA among children and adolescents in the US. Specific factors of interest included patient demographics, tumor location, treatment methods, survival outcomes, and gastrointestinal comorbidities. The understanding of these factors will enable strategizing cost effective surveillance in pediatrics.

Method: This was a retrospective study of pediatric patients diagnosed with biliary tract malignancies using hospital database systems. Cases of pediatric biliary malignancies were identified using the Surveillance, Epidemiology, and End Results Program (SEER 18) and the Healthcare Cost and Utilization Project (HCUP)-Kids' Inpatient Database (KID). The SEER 18 registry includes data from up to 18 US cancer registries collected from 1973-2013, representing 28% of the US population. Data from SEER 18 was used to identify patient demographics, treatment methods and survival outcomes for patients with CCA. The HCUP-KID includes three million pediatric discharges per year from more than 4,100 US community hospitals in 44 states. HCUP-KID from 2003, 2006, 2009, and 2012 were analyzed using SPSS software to identify secondary gastrointestinal diagnoses.

Results: Fifteen pediatric patients with CCA were identified from SEER 18 with an incidence rate of 0.036/1,000,000. Two-thirds of cases were male and the majority Caucasian (n=10). The median age at diagnosis was 17 years (range: 11-19). Nine tumors were intrahepatic, three extrahepatic, and three unspecified. One-third had distal metastases at diagnosis. Nine patients underwent surgical resection including liver transplant in two. One patient was treated with radiation alone, three received no treatment and treatment information was not available in two. Seven of the nine patients who underwent surgical resection were alive at follow-up. All cases without surgical treatment did not survive. The estimated three-year survival (Kaplan-Meier) was 51.85%. Twenty-three cases of CCA were identified from the HCUP-KID with a median age at diagnosis of five years (range: 1-20). Twelve patients were male and the majority Caucasian (n=13). Twelve tumors were intrahepatic and 11 extrahepatic. Nine patients had at least one underlying gastrointestinal comorbidity, with cholangitis most prevalent (n=4).

Conclusions: CCA is a rare malignancy among children and adolescents. Survival without surgical treatment is unlikely. Comorbid gastrointestinal disease states found more frequently in children and adolescents with CCA included PSC, biliary atresia, CBD, and cirrhosis of the liver and bile tract with PSC most prevalent. Early detection of CCA is essential for survival. Surveillance of

Optimizing *E. Coli* Expression Of NSP4 For Structure And Function Analyses

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Rotavirus (RV) remains a leading cause of viral diarrhea in children under 5 years old, and causes more than 200,000 deaths annually, largely due to the poor efficacy of the current RV vaccines. A hallmark of RV infection is the elevation of cytoplasmic calcium ($[Ca^{2+}]$cyto) due to nonstructural protein 4 (NSP4) functioning as a viroporin (virus-encoded ion channel) in the endoplasmic reticulum (ER), which is critical for RV replication and RV-mediated diarrhea. NSP4 is an ER-localized transmembrane protein that carries out multiple functions through distinct functional domains, but there are no NSP4 structures of the viroporin domain (VPD). Our goal is to determine the NSP4 VPD structure; however, NSP4 has multiple properties that make it challenging to do structural studies, including: [1] the VPD makes NSP4 highly cytotoxic to *E. coli*, [2] NSP4 requires detergent extraction for purification, [3] NSP4 has multiple oligomeric states, and [4] two conserved cysteine residues form intra-molecular disulfide bonds. To optimize NSP4 expression, we tested expression levels of full-length NSP4 from simian (SA11), murine (EC) and avian (Ty1) RV, and various truncations of SA11 NSP4. We found that expression of the $NSP4_{(47\text{-}146)}$ truncation had the highest expression and yielded ~30mg/L of purified protein. However, gel filtration studies showed a substantial degree of protein aggregation. To reduce NSP4 aggregation, we engineered mutations to remove the two cysteine residues (C63S and C71S) both individually and in tandem. Using an *E. coli* lysis assay, we observed that normal viroporin function was retained in $NSP4_{(47\text{-}146)}$ C63S/C71S single and double mutants. Next, we tested oligomerization by glutaraldehyde crosslinking and gel filtration and found that the C63S/C71S double mutant formed stable tetramers with only minimal aggregation. We are currently using this NSP4 construct in crystallography studies, and to determine whether putative Ca^{2+} binding sites in the NSP4 VPD bind Ca^{2+}.

Diminished Citrulline-Arginine-Nitric Oxide Production Rates are Associated with Necrotizing Enterocolitis Incidence in Premature Pigs

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Background: Necrotizing enterocolitis (NEC) is a major gastrointestinal disease in premature infants that is associated with formula feeding and intestinal hypoxia. Low arginine availability in these infants has been linked to NEC since arginine is the sole precursor of nitric oxide (NO), a critical mediator of vasodilation, blood flow and tissue oxygenation. Arginine availability depends on its de novo production from gut-derived citrulline and the action of argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL) in the gut and other tissues. However, arginine availability is balanced by arginine catabolism to either NO by NO synthase 2&3 (NOS2&3) or to ornithine by arginase 1&2 (Arg1&2). We hypothesized that citrulline-arginine-NO production rates are limiting in premature pigs, which predisposes them to NEC. **Methods:** Cesarean-derived preterm (90% term) and term (98-100% term) pigs were provided TPN for 24 h, followed by enteral formula for 48 h to induce NEC. Intravenous infusions of [$^{13}C_6$]arginine, [^{15}N-ureido]citrulline and [$^{15}N^{18}O_3$]nitrate were provided throughout feeding to quantify in vivo citrulline-arginine-NO kinetics. **Results:** Intestinal gene expression was assessed after formula feeding, and Arg2 and NOS3 expression were increased in preterm vs term pig and ASS expression was low in fed preterm pigs ($P<0.05$). Clinical scoring showed that NEC incidence was 90% in preterm pigs, whereas term pigs did not get NEC. Importantly, the rates of arginine-citrulline-NO flux showed that preterm pigs produced ~40-50% less citrulline, arginine and NO than term pigs regardless of feeding mode ($P<0.05$). To better assess NO metabolism we also quantified its oxidation products, nitrate and nitrite. Nitrate flux was ~60% lower in preterm pigs during TPN ($P<0.05$), and all pigs exhibited a drastic reduction in nitrate flux after the introduction of enteral formula. Strikingly, there was a marked supraphysiological nitrite surge in plasma prior to and immediately after oral feed initiation in preterm piglets that was absent in term pigs ($P<0.05$) that may be a novel marker of hypoxia. **Conclusions:** Our results suggest that depressed intestinal blood flow and oxygenation that are implicated in NEC may be linked to the diminished capacity to produce citrulline-arginine-NO necessary for vasodilation. These findings provide a rationale for arginine supplementation of preterm infants at risk of NEC to overcome the limited capacity NO-mediated vasodilation and tissue oxygenation.

Rates, risk and predictors of barrett's esophagus recurrence after radiofrequency ablation in a community practice setting: a national veterans cohort study

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Background: Radiofrequency ablation (RFA) therapy is effective for treating Barrett's esophagus (BE). However, even in specialty treatment centers, a fraction of patients experience BE recurrence after complete eradication of intestinal metaplasia (CEIM). Rates and predictors of BE recurrence after CEIM in a community setting have not been well described.

Aim: To determine the incidence and predictors of BE recurrence after CEIM in a national, multi-center, community practice cohort of BE patients treated with RFA therapy.

Methods: This retrospective cohort study from 40 facilities in the Veterans Health Administration included veterans with a diagnosis of BE between 2004 and 2009 and ≥1 RFA treatment who were followed until BE recurrence, death or October 31, 2016. Recurrence of BE and clinical predictors of recurrence were determined on structured EMR review. We calculated the incidence rates of BE recurrence after CEIM (number of cases divided by total number of follow-up years contributed by the study population at risk) and examined associations with time to BE recurrence using a Cox proportional hazards model.

Results: We identified 469 BE patients treated with RFA, of whom 303 achieved CEIM and underwent surveillance endoscopy post-CEIM and constituted our study population. Most were men (98.3%) and White (82.2%), with a mean age of 64.6 years and median follow-up of 2.5 years. Of these patients, 115 (38.0%) had no dysplasia/cancer, 161 (53.1%) dysplasia, and 27 (8.9%) intramucosal cancer (IMC) at baseline. BE recurrence was observed in 98 (32.3%) over 826.8 patient-years total follow-up (incidence rate 11.9% per patient-year). EAC after CEIM developed in 3 patients (incidence rate 0.4% per patient-year). BE recurrence after CEIM was higher among those with baseline dysplasia (n=59, 14.2%/year) or IMC (n=14, 21.1%/year) than those with non-dysplastic BE (n=25, 7.3%/year). Patients undergoing RFA at VA facilities in the lowest quartile of ablations performed (<2 procedures/year during 2005-2014) had higher rates of BE recurrence after CEIM (61.6%/year) than patients at facilities in the highest quartile (>8 procedures/year) (10.6%/year). BE recurrence also increased with increasing age and BE length. On multivariate analysis, baseline dysplastic BE (HR=1.74; 95% CI 1.07-2.84) and facility RFA experience (lowest vs. highest quartile, HR=7.75, 95% CI 2.23-26.92) remained significant predictors of BE recurrence, while BE length and age were not associated with BE recurrence risk.

Conclusions: In this national, VA multi-center cohort study of community practice outcomes after RFA, we found baseline dysplasia and treatment at facilities in the lowest quartile of ablations performed were significant predictors of increased BE recurrence risk after RFA with CEIM. The findings call for performing these procedures in specialized high-volume center.

Accuracy of post-hoc high-resolution microendoscopy for diagnosis of esophageal squamous cell neoplasia

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Background: High-resolution microendoscopy (HRME) is a novel, low-cost, portable optical technology that allows subcellular imaging in patients at risk for esophageal squamous cell neoplasia (ESCN). Dissemination of this and other optical biopsy technologies is limited by the need for expert microendoscopic interpretation. Automated software interpretation would facilitate usage in resource-poor areas without experienced microendoscopists by allowing immediate diagnosis. The objective of this study was to evaluate the accuracy of a fully automated software-based algorithm compared to endoscopists' interpretation of post-hoc HRME images in diagnosing ESCN.
Methods: 87 HRME images were collected from 72 consecutive patients undergoing screening and surveillance for ESCN from 1 US and 2 Chinese tertiary care centers. Eight gastroenterologists (4 expert microendoscopists and 4 novice microendoscopists) underwent training in HRME interpretation using 10 representative images then were tested on the remaining 77 images. The same 77 images underwent automated interpretation using a previously validated image analysis algorithm. Both gastroenterologists and the software algorithm were blinded to the interpretation by the other and to the histopathology. All HRME imaging sites were biopsied and reviewed by two expert pathologists blinded to HRME results who gave a consensus histopathology read of neoplasia (severe dysplasia, cancer) vs. non-neoplasia, which was used as the gold standard. Measures of validity were calculated for each rater and interrater reliability was calculated using kappa statistic. Comparisons between endoscopist and automated interpretation was done using Wilcoxon rank-sum test.
Results: Based on histopathology, 54 imaging sites were neoplastic and 23 were non-neoplastic. Overall, the endoscopists had a sensitivity of 64% [95% CI: 55-72%], specificity of 89% [95% CI: 82-96%], and accuracy of 71% [95% CI: 66-77%]. The automated software analysis had a sensitivity of 80% (p=0.12 when compared to endoscopist), specificity of 78% (p=0.24), and accuracy of 79% (p=0.12). There were no significant differences in sensitivity, specificity, and accuracy between novice and expert endoscopists (p= 0.44, 0.30, and 0.67, respectively). Kappa statistic for all raters was 0.60 (0.55 among novices and 0.60 among experts).
Conclusions: Based on these results, the automated image analysis algorithm is non-inferior to endoscopist post-hoc HRME interpretation without significant differences in sensitivity, specificity, or accuracy in evaluating ESCN. Reliable, automated HRME image interpretation obviates the need for an expert microendoscopist, allowing wider dissemination of HRME to resource-poor areas where this portable, low-cost technology is most needed.

Acid suppression therapy reduces risk of progression from barrett's esophagus to esophageal adenocarcinoma: a nested case-control study in US veterans

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Background: Observational studies suggest that proton pump inhibitors (PPIs) may reduce the risk of esophageal adenocarcinoma (EAC) in patients with Barrett's esophagus (BE). However, the results from these studies are mixed, potentially due to small numbers of EAC cases and inability to adjust for concomitant use of other medications (NSAIDs/aspirin/statins) known to be associated with EAC. It is also unknown whether histamine-2 receptor antagonists (H2RAs) have an independent chemopreventive effect in patients with BE.

Aim: To evaluate the independent effects of PPIs and H2RAs on risk of EAC in patients with BE.

Methods: We conducted a nested case-control study in a cohort of male veterans diagnosed with BE between 2004-2009, identified in the national Veterans Affairs Corporate Data Warehouse. Cases with incident EAC were matched by incidence density sampling to controls with BE who did not develop EAC by the time of EAC diagnosis in the corresponding case. This entailed individual matching on birth year (+/− 1 year) and BE diagnosis date (+/− 3 years). We identified prescriptions for medications (PPIs, H2RAs, NSAIDs/aspirin, statins) that were filled between the BE diagnosis date and 3 months prior to the EAC diagnosis date. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using conditional logistic regression.

Results: In a cohort of 29,536 patients with BE, we examined 311 EAC cases and 856 matched controls. More cases than controls were overweight or obese (86.8% vs. 80.6%, p=0.04) and smoked (19% vs. 13%, p=0.02). Cases were less likely than controls to have filled at least one prescription for PPIs (65% vs. 83%, p<0.01) or H2RAs (8% vs. 14%, p<0.01). In the multivariable model adjusted for age at BE diagnosis, smoking, body mass index and number of endoscopies before EAC diagnosis date, PPI use was associated with 73% lower risk of EAC in patients with BE (OR 0.27, 95% CI 0.18-0.39). The association was independent of duration of use (among PPI users, p-trend 0.31). Likewise, H2RA use was associated with 48% lower risk of EAC (OR 0.52, 95% CI 0.32-0.84). However, unlike PPI use, greater risk reduction associated with H2RA use was seen among those with ≥6 months of H2RA use (vs non-users: <6 months, OR 0.89, 95% CI 0.49-1.61; 6-18 months, OR 0.25, 95% CI 0.09-0.72; >18 months, OR 0.29, 95% CI 0.08-0.98). PPI and H2RA use remained independently associated with lower risk of EAC after mutual adjustment and adjusting for concomitant use of NSAIDs/aspirin and statins (PPIs, OR 0.31, 95% CI 0.21-0.47; H2RAs, OR 0.55, 95% CI 0.34-0.89).

Conclusions: These findings confirm that use of acid suppression therapy, including PPIs and H2RAs, is independently associated with decreased risk of progression from BE to EAC. Further experimental studies are needed to confirm the chemopreventive effect of acid suppression therapy on EAC.

Temporal trends in pre-diagnosis screening and surveillance among patients with newly diagnosed CRC. a healthcare system quality measure?

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Background
Incident colorectal carcinoma (CRC) may be diagnosed in the absence of prior screening, or post-colonoscopy either before or after an appropriately scheduled follow-up colonoscopy. In closed or semi-closed healthcare systems, the distribution of these categories of incident CRC may serve as a useful quality indicator, reveal gaps in screening and surveillance, and offer opportunities for improvement.

Methods
We conducted a retrospective cohort study of all newly diagnosed first CRC in a single large VAMC between October 2007 and October 2016. The entire medical history was reviewed and data were systematically abstracted about age, gender, race, colonoscopy dates, recommended surveillance intervals, and CRC location. We defined interval CRC as those diagnosed after a CRC-negative colonoscopy but before the next recommended follow-up exam. We defined non-interval CRC as those diagnosed in patients who had never had colonoscopy, as well as those diagnosed in patients who had a prior colonoscopy but were diagnosed with CRC after the next recommended follow-up exam. The proportions of interval and non-interval CRC were calculated both overall and yearly. Differences between the interval and non-interval CRC groups were tested using Chi square for categorical variables, while continuous variables were compared using t-tests. The Cochran-Armitage trend test was used for comparing proportions over time. In a multivariate logistic regression, we examined the temporal changes in the interval CRC rate adjusting for age, sex, and race.

Results
A total of 572 CRC cases were identified; 96.9% men, mean age 66.5 years, 28.5% were black and 81.5% white or Hispanic. Overall, interval cancers accounted for 35 of 572 total CRC (6.1%) and non-interval cancers accounted for 93.9% of all CRC (5.6% diagnosed after the next recommended colonoscopy, 88.3% diagnosed at the first colonoscopy ever). Interval CRC were more commonly found in elderly patients and the proximal colon than non-interval CRC. There was no significant change over time in the proportions of interval CRC or CRC diagnosed after recommended follow. In a multivariate logistic regression, the year of diagnosis was not associated with interval CRC rate after adjusting for age, sex and race. Compared with 2007-2010, the OR adjusted for age, race and sex was 1.91 (0.80-4.52) and 1.63 (0.65-4.08) for 2011-2013 and 2014-2016, respectively.

Conclusion:
The proportion of interval CRC has not changed in the last decade. Most CRC cases (88.3%) are diagnosed in patients with no previous screening and surveillance, 5.6% are diagnosed in patients with overdue surveillance and only 6.1% of CRCs are diagnosed after colonoscopy and before the next recommended one. These proportions have remained relatively stable over time. Each category must be addressed using different strategies.

Predictors of Hepatocellular Carcinoma in HIV/HCV Co-infected Patients With Cirrhosis

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Background: Cirrhosis is the most important risk factor for hepatocellular carcinoma (HCC) in patients with HIV/HCV co-infection. However, even among patients with cirrhosis, HCC risk is not uniform. Indeed, most HIV/HCV co-infected patients with cirrhosis do not progress to HCC.

Aim: We sought to determine risk factors for progression from cirrhosis to HCC in HIV/HCV co-infected patients.

Methods: We used the Veterans Affairs HIV and HCV Clinical Case Registries and identified HIV/HCV co-infected patients that were diagnosed with cirrhosis (defined by ICD-9-CM codes [571.5, 571.6, and 571.2] or an aspartate aminotransferase to platelet ratio index >2) from 1999-2010. We excluded female veterans (due to small numbers; <2%), as well as patients lacking HCV RNA, follow-up CD4 count or HIV viral load information, and patients diagnosed with cirrhosis within 90 days of HIV diagnosis. The outcome was incident HCC as indicated by ICD-9-CM (155.0 without 155.1). Patients were censored at death, date of last health care encounter or 12/31/2010. We examined associations with age at HIV diagnosis, race/ethnicity, HCV genotype, and HIV-related (combination anti-retroviral therapy era of diagnosis, CD4 cell count, percent time with undetectable HIV viral load, and use of highly active anti-retroviral therapy [HAART]), clinical (ALT and Deyo without AIDS) and behavioral factors (alcohol use, smoking, hard-drug use). Cox proportional hazards analysis was used to estimate Hazard ratios (HR) and 95% confidence intervals (CI) for associations with HCC.

Results: We included 2689 patients; the majority were aged >40y, African American, most recent CD4 count>200, and HCV genotype 1 or 4. Over a median follow-up of 5.0 (SD, 3.4) years, 88 patients (3.3%) developed HCC. In univariate analysis, HCC incidence varied by age at HIV diagnosis, race/ethnicity, ever HAART, alcohol use, and hard-drug use (all $p<.10$). Older age at HIV diagnosis (>50 vs. <40y; HR=2.19; 95%CI 1.02-4.70), Hispanic ethnicity (vs. non-Hispanic white; HR=2.46; 95%CI 1.05-5.76), and HCV genotype 1/4 (vs. HCV genotype 2/3; HR=1.95; 95%CI 1.06-3.57) were associated with higher risk of HCC in the multivariable model. Percent undetectable HIV viral load and CD4 count <200 (nadir or most recent) were not associated with HCC.

Conclusions: HCC is common in HIV/HCV co-infected patients with cirrhosis (3.3%) and risk varies by age, ethnicity and HCV genotype. If confirmed in other populations, these findings may lead to enhanced HCC prevention and surveillance efforts.

Targeting the nuclear receptor LRH-1 to treat inflammatory bowel disease in mice

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Background: Inflammatory bowel diseases (IBD) affect approximately 1.5 million patients in the US. It is a complex and chronic autoimmune disease group of unknown causes and without a cure. Murine models of colitis are intensely used in IBD research to identify its pathogenesis or novel therapeutic targets. Nuclear receptor Liver receptor homolog-1 (LRH-1) has been proposed to inhibit intestinal and colonic inflammation by inducing epithelial glucocorticoid synthesis in response to inflammatory signals. We have previously identified DLPC (dilauroyl phosphatidylcholine; C12:0-12:0 PC) as an extrinsic agonist ligand for LRH-1. The aim of this study was to test whether DLPC treatment can impact on the inflammatory response in murine models of colitis.

Material/Methods: DSS IBD model: 2.5% Dextran sodium sulfate (DSS) was added to the autoclaved drinking water of intestinal epithelial cell-specific *LRH-1* knockout (*LRH-1*$^{IEC\text{-}KO}$) mice and control littermates for 5 days. Autoimmune IBD model: Wild type splenic naïve CD4+ T cells were intraperitoneally (ip) injected to *LRH-1*$^{IEC\text{-}KO}$*Rag2*$^{-/-}$ mice, human *LRH-1* transgenic (*hLRH-1*$^{IEC\text{-}Tg}$)*Rag2*$^{-/-}$ mice, and control *Rag2*$^{-/-}$ mice to induce a chronic immune modified enterocolitis (T cell transfer or Tct model). DLPC (30mg/kg body weight) was ip injected daily for 5 days in DSS fed mice after stopping DSS and for 7 days for Tct mice at the 8-9th week after T cell transfer.

Results: In the DSS model, DLPC treatment decreased indirect colitis severity scores (diarrhea, hematochezia, weight loss, and sick appearance) and histological severity. DLPC induced steroidogenic enzymes Cyp11A1 and Cyp11B1 and anti-inflammatory cytokines (IL-10), while suppressing pro-inflammatory cytokines (TNF-alpha and IL-1beta). These anti-inflammatory effects of DLPC were decreased in *LRH-1*$^{IEC\text{-}KO}$ mice. In the Tct model, *LRH-1*$^{IEC\text{-}KO}$ mice showed higher disease activities, *hLRH-1*$^{IEC\text{-}Tg}$ mice showed lower disease activities than those of controls respectively. Similar to the DSS model, DLPC treatment suppressed pro-inflammatory cytokines, reduced colonic damage and disease activities in the Tct model. In both models, treatment with a control PC Dipalmitoyl PC (DPPC), or vehicle, did not reveal any significant effects.

Conclusion: LRH-1 activation was beneficial in suppressing colonic inflammation in both chemial and autoimmune IBD models in mice, whereas its suppression may contribute to developing chronic colitis. These findings indicate the protective role of LRH-1 in mammalian colitis by triggering local corticosteroid production in response to injury and autoimmunity. LRH-1 activation might serve as a therapeutic intervention to treat human IBD patients in the future. This work is supported by NIH NIDDK grant, NIH T32 training grant, NIH supported digestive disease center (DDC), NIH supported cytometry & cell sorting core.

Alterations in autophagy signaling pathways in Wilson's disease (*Atp7b*$^{-/-}$ mouse)

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Wilson's disease (WD) is an autosomal recessive disease caused by loss of function mutations in the Cu-transporting P-type ATPase, ATP7b, which results in a variety of symptoms including, hepatic copper accumulation, cholestasis, cirrhosis, liver failure, and neurological dysfunction. Current therapies for WD are limited to chelation and/or zinc therapy or liver transplantation, which has been associated with several side-effects and poor patient compliance. Uncontrolled WD can result in liver failure and requires liver transplantation for patient survival. Despite the severity of WD, insights regarding copper-mediated changes in metabolism are limited. In the WD mouse model (*Atp7b*$^{-/-}$ mouse) and WD patients, we found that binding of the nuclear receptors FXR, HNF4α, and LRH-1 to promoter response elements was decreased, as well as nuclear receptor target gene mRNA expression. In addition to controlling hepatic metabolic signaling pathways, the nuclear receptors FXR and PPARα regulate autophagy in mice. Autophagic response to nutrient signaling is critical to maintaining metabolic homeostasis in the liver, and changes in autophagy have been demonstrated in liver disease, including non-alcoholic steatohepatitis (NASH), primary biliary cirrhosis, and cholestatic animal models (bile duct ligation). Reports linking autophagy to Wilson's disease have been limited to correlative studies demonstrating increased copper accumulation in lysosomes with Wilson's disease progression; however, mouse models of Wilson's disease clearly demonstrate increased lipid droplets and steatosis, damaged nucleoi, and damaged mitochondria. We measured autophagy component genes involved in autophagosome assembly (LC3a, LC3b), autophagy effectors (Bnip3), and autophagy gene regulator (Sesn2). LC3a, LC3b, Bnip3, and Sesn2 mRNA expression were increased in response to fasting in wild-type mice and returned to baseline levels in the re-fed group. In the *Atp7b*$^{-/-}$ group, mRNA expression of LC3a and LC3b was unchanged suggesting a defect in autophagosome assembly. The basal levels of Bnip3 mRNA expression were decreased in the *Atp7b*$^{-/-}$ mice, but the levels did increase in response to fasting, albeit only to the basal level observed in the fed WT mice. In contrast, the basal mRNA expression of Sesn2 was increased in the *Atp7b*$^{-/-}$ mice and showed only a modest response to fasting. Overall, these studies strongly support the prediction that autophagy pathways are dysregulated in *Atp7b*$^{-/-}$ livers.

Role of MicroRNA-200 Family Members in Chronic Pancreatitis

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Introduction: MicroRNA (miR)-200 family consists of five members (a, b, c, 141, 429) and has anti-fibrogenic functions in the lungs and liver, but their effects in the pancreas are not clear. We reported that bone morphogenetic protein (BMP)2 opposes the pro-fibrogenic effects of transforming growth factor (TGF)-β in the pancreas. We hypothesize that miR-200 family mediates the opposing effects of TGF-β and BMP2 on pancreatic fibrosis; thus miR-200 family level may be a defining factor during chronic pancreatitis (CP) progression. To test our hypothesis in this study, we examined miR-200 family level in mouse CP in vivo and the regulation in pancreatic cells in vitro.
Methods: CP was induced in C57BL/6 mice by cerulein (50μg/kg, 5 IP injections/day, 3 days/wk for 4wks). Control mice received normal saline (n=4/group). Pancreatic fibrosis was assessed by Sirius red staining and qPCR for extracellular matrix (ECM) collagen and fibronectin measurement. In vitro, primary human pancreatic stellate cells (hPSCs), the key cells that produce ECM leading to pancreatic fibrosis, were used. The cells were treated with vehicle, TGF-β (1ng/ml), BMP2 (50ng/ml), or TGF-β & BMP2 and harvested 24 hrs after the treatment for the measurement of miR-200 family level by qPCR. The conditioned media were collected 48 hrs after the treatment for the detection of fibronectin secretion by Western blotting. In a separate set of experiments, the cells were transfected with a specific miR-200b inhibitor (30nM), mimic (30nM), or control with transfection reagent alone, and the conditioned media were collected 48 hrs after.
Results: In CP mice, the miR-200 level decreased by 60-70% compared to the control mice and inversely correlated with fibrosis ($p<0.05$). In hPSCs, all miR-200 levels were not altered by TGF-β alone compared to vehicle control. Among miR-200 family members, only miR-200b level was significantly increased by BMP2 alone by 150%, and the BMP2's effect was abolished with the combined treatment. Fibronectin secretion was induced by TGF-β alone by 150%, but unaltered by BMP2, and the TGF-β's effect was abolished with the combined treatment ($p<0.05$). Furthermore, the miR-200b inhibitor induced fibronectin secretion by 135%, while the miR-200b mimic did not alter secreted fibronectin level, compared to control ($p<0.05$).
Conclusions: TGF-β and BMP2 have opposing effects on miR-200b and fibronectin secretion in hPSCs. The inversely correlated miR-200b level with pancreatic fibrosis in CP and the stimulating effect of fibronectin secretion by the miR-200b inhibitor in vitro suggest that miR-200b is anti-fibrogenic in the pancreas. However, whether miR-200b mediates the opposing effects of TGF-β and BMP2 on pancreatic fibrosis warrants further investigation.

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Rotavirus Infection Induces LGR5 Intestinal Stem Cell Population via WNT Pathway Stimulation

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Introduction. The study of stem cell responses to injury has broad implications for understanding divergent roles of stem cell populations. Traditional methods to study the intestinal stem cells (ISCs) under injury use γ-irradiation to target proliferating stem cells. Previous work showed LGR5-labelled ISCs actively proliferate to maintain homeostasis and are highly susceptible to γ-irradiation injury. BMI1-labelled ISCs are normally quiescent but can activate to respond under injury. Here, we use rotavirus (RV) infection *in vivo* and in the Human Intestinal Enteroids (HIEs) as a novel injury model to examine ISC populations and their induction under insult.

Methods. Transgenic mouse models, *Lgr5*$^{CreER\text{-}GFP}$ and *Bmi1*^{CreER}*;R26*^{mTmG}, were used to examine LGR5- and BMI1-labelled ISC populations. HIEs were used to model RV infection in a human, epithelial system. We examined WNT pathway target genes using qRT-PCR and canonical β-catenin activity via TCF/LEF luciferase reporter assay.

Results. In contrast to γ-irradiation, RV infection targets differentiated cells, thus retaining an intact ISC compartment with both LGR5- and BMI1-labelled populations. When compared with mock-infected animals, mice infected with RV show a lengthened and more proliferative ISC compartment. Proliferative marker PCNA is significantly increased at both the mRNA and protein levels after RV infection. Additionally, proliferating cells labeled by EdU migrate faster out of the crypt compartment following infection. To compare ISC involvement, we infected *Lgr5*$^{CreER\text{-}GFP}$ and *Bmi1*^{CreER}*;R26*^{mTmG} mice. Both Lgr5 transcripts and cell number are significantly increased after infection compared to mock animals. Although Bmi1 transcripts are increased following infection, BMI1 cell numbers remain constant. Similar findings are seen in RV-infected HIEs, suggesting that actively-cycling LGR5 cells are the bona fide ISC responder under RV damage. To examine how ISCs are activated following infection, we screened for gene expression involved in the WNT pathway (*Axin2, Ccnd1, Cd44, EphB2, Myc, and Sox9*). We found that all screened genes are upregulated following infection in both mice and HIEs. Furthermore, conditioned media from RV-infected HIEs stimulated canonical β-catenin activity in a TCF/LEF luciferase reporter assay and, when used to culture naïve, un-infected HIEs, can further stimulate the WNT signaling pathway.

Conclusions. In contrast to γ-irradiation, RV infection of the differentiated cell types induces the LGR5 ISCs and *not* the BMI1 cells. This induction depends on canonical WNT signaling pathway activation, which leads to proliferation and repair of the intestinal epithelium. Our data suggest that, when LGR5 population is intact, it remains the primary source of epithelial restitution and does not rely on BMI1 cells, irrespective of insult.

Determinants and Outcomes of Hospice Utilization among Patients with Advanced-Staged Hepatocellular Carcinoma in a Veteran Affairs Population

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Background. Approximately 60% of patients with hepatocellular carcinoma (HCC) are diagnosed with advanced stage disease. Patients who receive treatment for advanced stage HCC typically have poor survival outcomes (< 10% at 3 years for BCLC stage C and < 10% at 1 year for stage D), and many patients do not qualify for HCC treatment. Hospice services provide palliative care to patients with short term survival irrespective of treatment. However, only a few small studies have examined the role of hospice in the management of advanced HCC. The aim of our study was to examine the utilization and determinants of hospice use, as well as the impact of hospice use on survival.

Methods. Using data from the Department of Veteran Affairs, we conducted a retrospective cohort study in a national sample of patients diagnosed with verified HCC between 2004 and 2011. Information on patient demographics, HCC clinical factors, treatments, hospice utilization and death was ascertained from the medical records. We calculated the proportion of patients who utilized VA hospice services within 30 days of HCC diagnosis or post-HCC treatment. Multiple logistic regression models were developed to identify determinants of hospice use. Kaplan Meier and Cox Proportional Hazards models were used to examine survival.

Results. We identified 814 patients with advanced stage HCC (BCLC stage C and D); 397 (49%) received HCC specific treatment. Approximately 73% of patients utilized hospice, of which 332 (40%) occurred at time of HCC diagnosis and 265 (33%) after HCC treatment. Patients who were referred to hospice at diagnosis were more likely to be white than non-white (62.1% vs. 52.9%, $p < 0.01$), resided in the southern United States (39.5% vs. 31.8%, $p < 0.05$), and had an ECOG performance score > 3 (41.9% vs. 31.8%, $p < 0.01$) compared with patients who did not receive hospice or treatment. Among those who received HCC treatment, having insurance in addition to VA benefits was associated with a lower likelihood of VA hospice use (47.2% vs. 60.0%, $p < 0.01$). The most commonly received treatments irrespective of hospice utilization were liver-directed therapy (39%) and sorafenib (29%). A surprisingly large proportion of patients also received curative intent treatments including transplant or resection (23.7% in stage C, and 31.3% in stage D). Hospice use was not associated with significant differences in overall survival following HCC diagnosis in the absence of treatment. On the contrary, among patients who received treatment, hospice use was associated with significantly lower survival. (Stage C median survival: 11.6 vs. 16.1 months, $p < 0.001$; Stage D: 8.7 vs. 15.7 months, $p < 0.002$). These results persisted in Cox Proportional Hazards models adjusting for patient characteristics, stage, and treatment.

Interpretation. Despite very high mortality, 27% of patients diagnosed with advanced HCC did not use hospice at all, and most patients used hospice only after receiving HCC specific treatments. However, there were no differences in survival by hospice use among patients who did not receive treatment. Furthermore, patients who were in hospice following treatment had shorter overall survival than those who did not use hospice post-treatment. Race, geographic region, performance and insurance status were associated with differences in hospice usage.

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