Rho-kinase inhibitor coupled with peptide-modified albumin carrier reduces fibrogenesis and portal pressure in cirrhotic rats without systemic effects

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Published in:
Hepatology

1

Risk of Hepatitis C transmission from antibody positive-nucleic acid negative liver organs to antibody negative recipients

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Background: Occult Hepatitis C Infection (OCI) is defined as detectable Hepatitis C Virus (HCV) RNA in liver tissue of patients with spontaneous or treatment-induced HCV RNA clearance from serum (anti-HCV positive/serum nucleic acid test (NAT) negative). While described in literature, clinically significant infections have rarely been reported. Given ongoing shortage of liver grafts, transplantation of such organs, hereby referred to as HCV increased risk donor (IRD) to anti-HCV negative recipients could potentially expand the donor pool. The purpose of this project was to evaluate the risk of HCV transmission from HCV-IRD to anti-HCV negative candidates at our center. **Methods:** A prospective cohort study of all anti-HCV negative liver transplant (LTx) recipients transplanted with an HCV-IRD organ between March 2016 and February 2017. HCV-IRD is defined as (1) donor that meets the Public Health Services (PHS) criteria for increased risk of infection transmission AND (2) anti-HCV positive and HCV NAT negative. All HCV-IRD organ recipients underwent HCV-NAT testing 3 months post-LTx or earlier if clinically indicated by sudden rise in liver enzymes to determine HCV transmission. **Results:** Twenty-five anti-HCV negative candidates received HCV-IRD organs; 58% male, age 58 years (36-69) with an average model for end-stage liver disease score 22. One recipient was excluded from the analysis due to death from primary graft non-function. HCV disease transmission occurred in 4 recipients (16%) by 3 months post-LTx, predominantly in females (3/4), one with a prior history of HCV/HIV co-infection who had undergone successful HCV treatment 2 years prior to LTx. Three of the 4 patients were treated with direct acting antiviral (DAA) therapy. One patient completed 12 weeks of treatment and achieved end of treatment response. The remaining two patients are currently undergoing HCV treatment and are aviremic at weeks 2 and 4 of treatment. The final patient had a post-operative course that was complicated by pulmonary hypertension, renal failure, and recurrent infections that precluded treatment and died 10 months post-LTx. Although all the donors in our cohort were PHS high risk and likely still within the eclipse period after a re-infection; 16% rate of HCV transmission is much higher than expected for "eclipse-period", thus suggesting OCI in donors as the most likely mode of HCV transmission. **Conclusion:** Our experience suggests that risk of early HCV transmission from this very high risk donor pool of anti-HCV+ve/NAT-ve is 16%. Due to availability of safe and effective HCV DAA therapies, use of such organs should be considered to increase the donor organ pool.

Disclosures:

Madison Cuffy - Speaking and Teaching: Ethicon

The following people have nothing to disclose: Khurram Bari, Keith Luckett, Tiffany E. Kaiser, Tayyab Diwan, Michael R. Schoech, Nadeem Anwar, Kamran Safdar, Flavio Paterno, Senu Apewokin, Shimul Shah

2

Optimizing Allocation of Grafts From Hepatitis C Virus Antibody-Positive Donors Through Urgent Serum HCV RNA Determination

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Background & aims. With the advent of direct-acting antivirals (DAA) therapy (Tx), HCV antibody-positive donors (HCVAb+D) are likely to become more frequently HCVRNA negative and their use is increasing in liver transplant (LT) setting. Although infection transmission has not been detected in recipients of livers from HCVAb+ HCVRNA negative donors, viral load is often unavailable prior to allocation due to technical issues. We evaluated the role of urgent serum HCVRNA determination in HCVAb+D to optimize liver allocation. *Patients & methods*. From 08/2014 to 03/2016, we transplanted 11 livers from brain-dead HCVAb+D, accepting a graft fibrosis up to 3/6 according to Ishak score (IS). All donors underwent liver biopsy and serum HCVRNA determination by the time of harvesting. Patient survival, post-LT/Tx sustained virological response (SVR) at week 48 and 1-year liver stiffness (LS, kPa) were evaluated. *Results.* Median donor age was 51 years, median cold ischemia time 432 minutes. Seven donors were HCVRNA positive (median 6.1 Log IU/mL) and were allocated to untreated viremic recipients (median HCVRNA 6.1 Log IU/mL), who underwent DAA Tx immediately after LT. They became HCVRNA <15 IU/mL within week 4 and achieved SVR48. Four donors tested HCVRNA negative at LT and were allocated to on-DAA HCVRNA negative (median time 70 days) recipients; Tx was stopped at LT: 3 achieved SVR48; 1 genotype 4 recipient, being negative before LT for 78 days with sofosbuvir+ribavirin, relapsed at week 4 post-LT a genotype 4 virus; sequencing confirmed the recipient origin of the virus (Pt 4 on the table), which was subsequently successfully eradicated with another DAA Tx course. All 11 recipients are alive after a median follow-up of 662 days. Median LS at 1-year after LT is 7.3 kPa. *Conclusions.* Urgent serum HCVRNA determination in our HCVAb+D allowed a paired donor/recipient virological allocation of the livers, thus optimizing cost-effectiveness of antiviral Tx. Furthermore, these data suggest the potential safety of the allocation of non viremic HCVAb+D organs also to non-HCV recipients.

Patient N.	Recipient HCV genotype	Donor HCV genotype	Donor age (years)	Liver biopsy (grading and staging according to Ishak score)	Post-transplant HCV genotype	One-year post-transplant liver stiffness (kPa)
1	4	3	51	4/18; 1/6	3	7.8
2	3	No viremia	41	0/18; 1/6	No viremia	6.7
3	1b	No viremia	54	0/18; 1/6	No viremia	5.8
4	4	No viremia	52	2/18; 0/6	4	6.8
5	3	No viremia	68	1/18; 0/6	No viremia	Not available
6	1b	1b	47	2/18; 0/6	1b	5.6
7	3	1b	43	2/18; 2/6	1b	8.3
8	1	4	34	3/18; 2/6	1	8.6
9	3	2	79	3/18; 2/6	Not available	9.9
10	1b	3	46	4/18; 3/6	3	7.8
11	1b	2	73	3/18; 1/6	2	5.2

Disclosures:

Giorgio Maria Saracco - Advisory Committees or Review Panels: GILEAD, ABBVIE, MSD; Grant/Research Support: BMS

The following people have nothing to disclose: Silvia Martini, Francesco Tandoi, Fabrizia Pittaluga, Ezio David, Silvia Strona, Donatella Cocchis, Antonio Amoroso, Rossana Cavallo, Mauro Salizzoni, Renato Romagnoli

3
Transplanting HCV-Positive Livers into HCV-Negative Patients with Preemptive DAA Therapy: Outcomes of a Modeling Study

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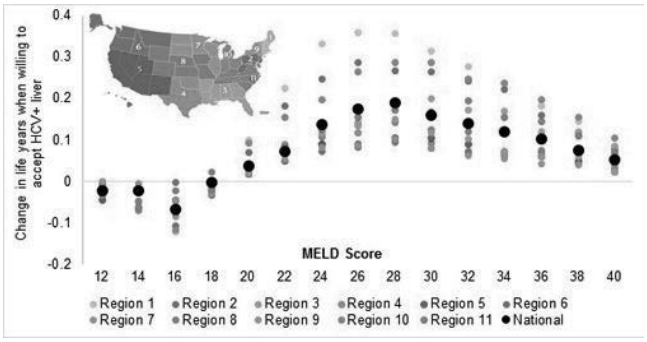
Background: Under current guidelines hepatitis C virus (HCV) positive (+) livers are not transplanted into HCV negative (–) recipients because of adverse post transplant outcomes associated with allograft HCV infection. However, with the advent of direct-acting antivirals (DAAs), HCV can now be cured post liver transplant (LT) with >90% success. Given the limited number of transplant viable organs, and high waitlist mortality, HCV- patients on the LT waiting list may benefit from accepting HCV+ organs. Our objective was to evaluate in which HCV- patients (by MELD score) the potential benefit of accepting an HCV+ organ outweighed the risks associated with HCV allograft infection. **Methods**: We developed a Markov-based mathematical model that simulated a virtual trial of HCV- patients on the LT waiting list to compare long-term outcomes in patients willing to accept HCV+/- livers versus those willing to accept only HCV- livers. Patients receiving HCV+ livers were treated preemptively with 12 weeks of DAA therapy and had a higher risk of graft failure than those receiving HCV- livers. We integrated data from published studies and the United Network for Organ Sharing (UNOS), and subsequently tracked patients' MELD scores over time. **Results**: The Figure shows the difference in life years by individual MELD score when patients are listed for both HCV+ and HCV- livers versus listed for only HCV- organs. Accepting HCV+/- livers versus HCV- livers resulted in an increase in patients' life expectancy when MELD was ≥ 20 (range 18–20, depending on UNOS region). The magnitude of clinical benefit was greatest in UNOS regions with the highest HCV+ donor rates. Sensitivity analysis on a wide range of parameters demonstrated that model outcomes remained robust. **Conclusions**: Transplanting HCV+ livers into HCV- patients receiving preemptive DAA therapy could be a viable option for improving patient survival on the LT waiting list. Clinical trials are needed to confirm the benefits of preemptive DAA therapy in HCV- recipients who receive HCV+ livers.



Disclosures:

Jagpreet Chhatwal - Consulting: Merck & Co., Inc., Gilead, Complete HEOR Solutions; Grant/Research Support: NIH/National Center for Advancing Translational Sciences, American Cancer Society

Fasiha Kanwal - Grant/Research Support: Gilead, Merck

Raymond T. Chung - Advisory Committees or Review Panels: Alnylam; Grant/Research Support: Gilead, Abbvie, Merck, BMS, Boehringer Ingelheim, Janssen

The following people have nothing to disclose: Sumeyye Samur, Emily D. Bethea, Mark S. Roberts

4
Improved Short-Term Survival in HCV Patients following Liver Transplantation in the Era of Direct Acting Antiviral Agents

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Background: Survival in liver transplant (LT) recipients with hepatitis C virus (HCV) following the approval of direct acting antiviral (DAA) agents remains unknown. We studied the impact of DAA era on the short-term survival rate following liver transplantation. **Methods:** Using the United Network for Organ Sharing database from 2010 to 2016, we analyzed short-term (1-year) post-LT survival in HCV patients without hepatocellular carcinoma (HCC). Kaplan-Meir survival analysis was performed to compare post-LT survival before (pre-DAA era: before 2014) and after (DAA era: after 2014) the approval of DAA agents in United States (October-November 2013). LT recipients with 1-year follow-up survival period that overlapped into the DAA era were censored. LT recipients in pre-DAA and DAA eras were matched to adjust for recipient and donor demographics (age, gender, and ethnicity), cold ischemia time (CIT) and Model for End-Stage Liver Disease (MELD) score at LT. **Results:** From 2010-2016, 11,223 LT recipients with non-HCC HCV and at least 1-year follow-up demonstrated a 1-year post-LT survival rate (1YR-PLT-SR) of 90.1%

(95% CI: 90.0%-90.2%). Overall 1YR-PLT-SR increased 5.3% to 92.5% (95% CI: 91.8%-93.3%) in 2016 and was significantly higher among HCV LT recipients during the DAA era (pre-DAA: 89.0%, 95% CI: 88.4%- 89.5% *vs*. DAA: 92.1%, 95% CI: 92.0%-92.2%, p <0.001). After adjusting for recipient and donor characteristics, CIT and MELD score at LT, the DAA era was still associated with significantly improved 1YR-PLT-SR (p <0.001) (Figure). **Conclusion:** We report improvement in one-year short-term survival rate in LT recipients with HCV during the DAA era. Future studies with longer follow-up are needed to confirm these encouraging data.

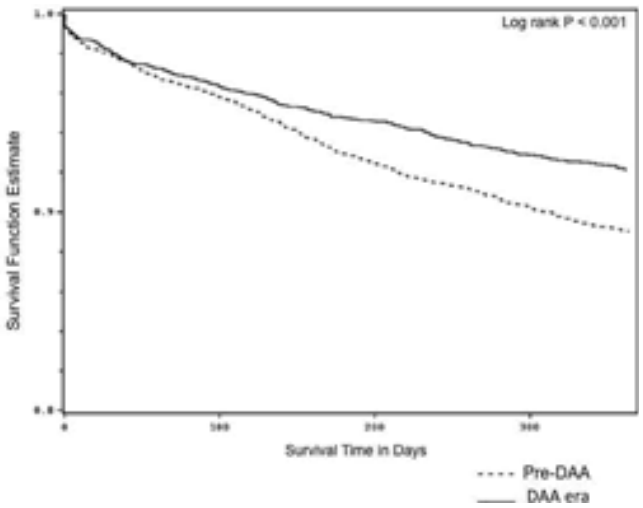

Figure. Kaplan-Meir survival analysis demonstrating improved 1-year post-LT survival in the DAA era in matched pre-DAA and DAA era cohorts (pre-DAA: 89.5% *vs.* DAA: 92.7%, p < 0.001).

Disclosures:

Stevan A. Gonzalez - Speaking and Teaching: Gilead, Salix, AbbVie, Merck

Aijaz Ahmed - Consulting: Bristol-Myers Squibb, Gilead Sciences Inc., Roche, AbbVie, Shire, Janssen pharmaceuticals, Intercept; Grant/Research Support: Gilead Sciences Inc., Intercept

The following people have nothing to disclose: George Cholankeril, Andrew A. Li, Eric R. Yoo, Zobair M. Younossi

5
The Efficacy of Pharmacological Intervention for Reducing Macrovesicular Lipid Accumulation in Primary Human Hepatocytes In Vitro: A Precursor Study to Improvement of Macrovesicular Steatosis in Donor Livers with Normothermic Machine Perfusion of the Liver

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Introduction: Macrovesicular steatosis of donor livers is associated with a high risk of graft dysfunction post-operatively. Increasingly ex vivo machine perfusion is being seen as a potential reconditioning strategy with the aim of amelioration of steatosis thereby making them transplantable. In vitro models offer the opportunity to test the effect of different drugs considering this end. **Methods:** Steatosis was induced in static cultures of isolated primary human hepatocytes (PHH) or the human hepatoma cell line(HepG2) by exposure to high levels of unsaturated and saturated free fatty acids (palmitic, oleic and linoleic acid). This was followed by defatting using control medium or medium supplemented with a cocktail of defatting agents for up to 48 hours. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used to assess viability. Intracellular triglyceride quantification and oil red O staining were used to assess lipids content. Ketone bodies (3-hydroxybutyric acid+acetoacetic acid-mM) were measured in the supernatant at time points up to 48 hours. A flow based cell culture system which assessed lipid content under conditions which mimicked hepatic sinusoidal flow was also developed. **Results:** The initial experiments with lipid laden HepG2 cells showed an increase in the cell viability in the group supplemented with the cocktail. Moreover, it promoted a significant reduction in the level of intracellular fat droplet content after 48 hours of treatment which coincided with a median decrease of 33% (13-45%) in intracellular triglyceride levels. Interestingly, under flow conditions designed to replicate a typical shorter period of normothermic machine perfusion of the liver (NMP-L) still achieved an approximate 25% median (25-27%) reduction within 6 hours. For PHH the cocktail resulted in a median decrease of 28% (6-43%) and 46% (33-67%)in the area of lipid droplets and the intracellular triglyceride dropped by 18% (17-19%) and 32% (28-34%) during 24 and 48 hours of treatment respectively. In addition the total ketone bodies measured in the supernatant (a marker of fatty acid oxidation) increased 1.22 and 1.40 fold by 24 and 48 hours in the treated group compared with controls. **Conclusion:** Using primary human hepatocytes, these data demonstrate for the first time that pharmacological defatting strategies can be used effectively decreasing intracellular lipid content, deviating fatty acids towards β-oxidation and with minimum hepatotoxicity in a in vitro primary cell culture model. The present study suggests that such strategies may be well tolerated for reduction of steatosis in donor livers undergoing NMP-L prior to transplantation.

Disclosures:

The following people have nothing to disclose: Yuri L. Boteon, Wallace L. Lorraine, Ricky H. Bhogal, Richard W. Laing, Amanda P. Boteon, Darius Mirza, Simon C. Afford

6
Blood and Graft Biomarkers Predictive of Operational Tolerance in Liver Transplant Recipients on Sirolimus

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Background: Conversion from CNI to sirolimus (SRL), an mTOR-inhibitor, changes cellular/genomic regulatory signatures in liver transplant recipients (LTR). Whether these signatures can also predict successful mTOR-I withdrawal (tolerance) is not known. AIM: To determine if regulatory cells and gene expression signatures can predict successful SRL withdrawal in LTR. METHODS: A prospective trial of SRL monotherapy withdrawal was performed in non-immune, non-viremic LTR > 3 years post-LT.

Once baseline biopsy was deemed acceptable, SRL was weaned over ~6 months and biopsies performed 12 months post-weaning or at concern for AR. Samples were collected for immunophenotyping (blood) and gene expression (blood/biopsy RNA) at baseline and at study end or AR. RESULTS: 21 LT recipients were consented. 6 were excluded due to subclinical AR on baseline biopsy or other reasons. 15 with acceptable baseline biopsies underwent SRL weaning: age 61.3±8.8 yrs; LT to SRL weaning 6.7±3 years. Eight (53%) achieved operational tolerance (TOL). Of the 7 non-TOL, 3 had mild AR near end of weaning, 3 mild AR on study end biopsy, and 1 removed due to cancer recurrence. There was no difference in age, time on SRL monotherapy, or time from LT to weaning between TOL vs. non-TOL. At baseline, there were statistically higher tolerogenic dendritic cell and regulatory B cell % in the TOL vs. non-TOL groups. For gene expression, there were 153 probesets in blood and 93 completely different probesets in biopsy distinguishing TOL vs. non-TOL at baseline. A previously identified biopsy gene signature (Bohne et al, JCI 2012) accurately predicted TOL in 12/13 LTR, serving as additional signature validation in a novel mTOR-I treated LTR cohort. Additionally, the 153 blood gene signature was able to predict TOL in the end of study samples with 82% sensitivity and 100% specificity (AUC - 0.88). Conclusion: In this pilot study, withdrawal from mTOR-I therapy was achievable in >50% of our LTR. High regulatory B cell and dendritic cell percentages in blood and gene expression signatures in both blood and biopsy might predict successful withdrawal prior to consideration of weaning. Larger studies of mTOR-I conversion and weaning, in conjunction with predictive blood/biopsy assays, are needed to validate our results.

Disclosures:

Josh Levitsky - Consulting: Transplant Genomics Incorporated; Grant/Research Support: Novartis; Speaking and Teaching: Gilead, Salix, Novartis

Sunil M. Kurian - Stock Shareholder: Transplant Genomics Inc

The following people have nothing to disclose: Alberto Sanchez-Fueyo, James M. Mathew, Jessica Heinrichs, Bryna E. Burrell

13
Minocycline and anti-epileptics are the leading causes of DILI in Children

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Background: The etiologies and outcomes of idiosyncratic drug induced liver injury (DILI) in children are not well established. The aim of this study was to provide an overview of the presenting features, etiologies, and outcomes of children with suspected DILI enrolled in the ongoing DILIN prospective and retrospective studies. **Methods:** The DILIN Prospective Study follows subjects for up to 24 months after DILI onset while the Retrospective Study collects more limited data. This analysis was based upon all cases adjudicated via consensus expert opinion as definite, highly likely, or probable that involved children < 18 years of age enrolled in DILIN between 9/2004 and 1/2017. **Results:** Among 69 enrolled children with suspected DILI, 57 were adjudicated as definite (14), highly likely (30), or probable (13) and constituted the study group. Median age was 14.3 years (range: 1.7-17.9), 67% female, and 82.5% Caucasian. 35% reported a history of drug allergy but none of liver disease. Median duration of therapy was 140 days (5-569). At onset, median ALT was 411 U/L (range: 33-4185), alkaline phosphatase 203 U/L (62- 1177), and total bilirubin 3.3 mg/dL (0.2- 33.9). Based upon R ratios at onset, 82% had hepatocellular injury. Immunoallergic features included fever (37%), rash (25%) and peripheral eosinophilia (15%). Antimicrobials were the most frequently implicated agents (51%) followed by antiepileptics (21%). The top 5 implicated agents were minocycline (n=11), valproate (n=6), azithromycin (n=4), and isoniazid (n=4). 53% of children were jaundiced, 63% hospitalized and 3 underwent liver transplant because of acute liver failure 2 to 3 weeks after DILI onset (1 due to valproic acid, 1 isoniazid and 1 erythromycin-sulfisoxazole). Among 49 children followed for at least 6 months, 8 (16%) had persistently abnormal liver tests, 4 in children with leukemia (3) or lymphoma (1) receiving chemotherapy and 4 in children receiving minocycline (2) or azithromycin (2). Blinded central review of 16 liver biopsies demonstrated moderate/severe inflammation in the lobules (n=8) or interface (n=6) and less frequently in the portal tracts (n=3). 6 biopsies had early fibrosis (stage 1-2), and 3 had bridging fibrosis (stage 3-4). Steatosis was seen in 5 biopsies but was a prominent feature in only 2 children with due to atomoxetine without obesity. **Conclusions**: Antimicrobials and antiepileptic drugs are the most frequently implicated agents in pediatric DILI. While the majority of cases of DILI in children are self-limited and benign, cases of acute liver failure requiring transplantation account for 5% and chronic injury for another 16% of cases.

Disclosures:

Naga P. Chalasani - Consulting: Abbvie, NuSirt, Lilly, Afimmune, Allergan/Tobira, Axovant, Madrigal, Shire, Ardelyx; Grant/Research Support: Intercept, Cumberland, Lilly, Gilead, Galectin

Robert J. Fontana - Consulting: Abbvie, CLDF, Alnylam; Grant/Research Support: BMS, Gilead

The following people have nothing to disclose: Frank DiPaola, Huiman X. Barnhart, David E. Kleiner, Jay H. Hoofnagle

14
Calmangafodipir is a new treatment for late stage liver toxicity after acetaminophen overdose

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Background Acetaminophen (APAP - paracetamol) is the leading cause of acute liver failure in the USA. The current treatment for APAP poisoning is acetylcysteine (NAC). NAC protects hepatocytes by restoring intracellular glutathione, which increases the detoxification of the APAP metabolite NAPQI. However, NAC has little or no beneficial effect once NAPQI has bound to intracellular proteins and caused oxidative stress. There is an unmet need for new therapeutic agents that are mechanistically active on downstream pathways and so have a wider therapeutic window than NAC. Calmangafodipir ($Ca_4Mn(DPDP)_5$) is a unique chemical species that has superoxide dismutase activity which protects cells from oxidative stress. The objective of this murine study was to determine whether calmangafodipir prevents APAP-induced liver injury at a late time when NAC is no longer active. This is with a direct line-of-sight on translating calmangafodipir into a phase 1 human clinical trial. Methods In these studies male B6C3F1 mice, 5-6 weeks of age, were used. Acute liver injury was

induced by intraperitoneal injection of APAP (300mg/kg). At different time-points after APAP treatment, either NAC (300mg/kg) or calmangafodipir (10mg/kg) or both was administered intravenously. Twelve hours after APAP treatment blood and tissue samples were collected for analysis. Results APAP induced an elevation in serum alanine transaminase activity (ALT - median (IQR): 15,332 U/L (5,216-18,712), N=13) that was significantly attenuated when NAC was administered 1 hour after APAP (ALT 225U/L (162-671), N=5, P=0.004 by Mann-Whitney Test compared to APAP alone). However, there was no significant effect when NAC treatment was delayed to 2.5 hours after APAP. By contrast, calmangafodipir prevented APAP-induced acute liver injury even when administered 6 hours after APAP (ALT 113U/L (60-177) N=5, P=0.001 by Mann-Whitney Test compared to APAP alone). Of clinical relevance, calmangafodipir retained efficacy with regard to preventing liver injury when combined with NAC. This effect on ALT activity was mirrored by prevention of APAP-induced liver injury on histological analysis. Discussion These results suggest that calmangafodipir could be used as an effective treatment for APAP poisoning in patients who present late to hospital when NAC has lost efficacy. Based on these data we have commenced a phase 1 clinical trial exploring safety and tolerability of calmangafodipir in patients attending hospital following APAP overdose.

Disclosures:

Jacques Näsström - Management Position: PledPharma AB; Patent Held/Filed: PledPharma AB; Stock Shareholder: PledPharma AB

The following people have nothing to disclose: James W. Dear, Emma Morrison, Dennis Henriksen

15
Acute liver injury due to immunotherapy for metastatic cancer: a new challenge

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BACKGROUND Antibodies blocking immune checkpoint programmed cell death (PD1)/ programmed cell death ligand 1 (PD-L1) and cytotoxic T-lymphocyte antigen 4 (CTLA4) are now widely used for metastatic cancer but are associated with a spectrum of toxicity as hepatotoxicity. The mechanism of immunotherapy-induced liver injury is unclear, and our aim was to characterize acute hepatitis associated with these new drugs. **METHODS** Among 1425 patients (pts) treated with immunotherapy, 12/2013-05/2017, in a qualified cancer institute, 19 (1.3%) were referred to the liver unit for grade 3-4 hepatitis. Three pts were excluded (acute HEV hepatitis, tumor liver infiltration, absence of liver biopsy). Clinical and biological data were collected. Two blinded pathologists performed histological review. **RESULTS** Sixteen pts were evaluated, 9 (56%) female, median age 63 [33-84] years. Nine (56%) pts received anti-PD1/PD-L1 and 7 (44%) pts anti-CTLA4 therapy (3 in monotherapy and 4 in combination with anti-PD1). Time between therapy initiation and hepatitis onset was 5 [1-49] weeks, median number of injections was 2 [1-36]. Biology at peak was: AST 399 [117-2289] IU/L, ALT 416 [266-3137] IU/L, total bilirubin 18 [6-324] mmol/L, GGT 317 [39-1252] IU/L. No patient developed hepatic failure. Antinuclear and anti-smooth muscle auto-antibodies ≥1:80 were found in 8 (50%) and 3 (19%) pts, respectively. Median IgG was 9 [6-18] g/L. According to RUCAM scale for causal relationship: in 14 (87.5%) pts the association was highly probable and in 2 (12.5%) was probable. Histology related to anti-CTLA4 demonstrated poorly delimitated granulomas, including fibrin ring granulomas, severe lobular necrotico-inflammatory activity and central vein endothelitis. Histology related to anti-PD1/PD-L1 was more heterogeneous with lobular hepatitis and mild to moderate portal activity. In both cases, bile duct injuries with lymphocytic cholangitis were found. Immunostaining found a prevalent CD8+ lymphocyte infiltrate. The management was tailored according to the severity of liver injury: 8 (50%) pts improved either spontaneously (n=6) or with maintenance of 0.2 mg/kg/day of steroids (n=2), 8 (50%) received steroids therapy (0.5-1 mg/kg/day (n=7) or 2 mg/kg/day with the addition of a second immunosuppressive drug (n=1)). In 3 (16%) pts the immunotherapy was safely reintroduced. **CONCLUSIONS** Acute hepatitis due to immunotherapy for metastatic cancer is rare (1.3%) and mostly not severe. Histological assessment can distinguish between anti-PD1/PD-L1 and anti-CTLA4 toxicity. Management should be tailored according to patient's severity and that 50% of patients did not require high dose of steroids.

Disclosures:

Caroline Robert - Consulting: BMS, NOVARTIS, MERCK, AMGEN, GSK, ROCHE

The following people have nothing to disclose: Eleonora De Martin, Stephane Champiat, Salim LAGHOUATI, Aurelien Marabelle, Didier SAMUEL

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Testing for Hepatitis C Virus RNA is Recommended in Patients with Suspected Drug Induced Liver Injury (DILI)

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Background: The diagnosis of DILI relies on compatible history, liver injury tests and exclusion of other causes. The aim of the current study was to determine the need for comprehensive hepatitis C (HCV) testing (anti-HCV antibody and HCV RNA) to exclude acute HCV infection in suspected DILI. Methods: The United States DILI Network (DILIN) prospective study enrolls patients with suspected DILI attributed to any drug or herbal and dietary supplement (HDS) within 6 months of liver injury onset. Patients undergo testing for competing causes of liver injury and complete a 6-month follow-up visit. Patients with known

chronic HCV infection can be included. Causality is adjudicated by consensus expert opinion using a 5-point scale: 1-definite, 2-highly likely, 3-probable, 4-possible, 5-unlikely. Results: From 9/04-10/16, 1734 patients were enrolled of whom 1518 had undergone 6 months of follow up and causality adjudication. Initial anti-HCV results were available in 1457 (96%) and HCV RNA results in only 795 (52%). Stored serum was available in 814 patients and analyzed for HCV RNA. In all, HCV testing results were available on 1518 patients (1457 for anti-HCV and 1482 for HCV RNA) of whom 104 had evidence of HCV infection; 10 HCV RNA alone, 16 anti-HCV alone and 78 both. All 104 cases were reviewed in depth by 4 hepatologists and scored by consensus as having acute HCV (n=23), chronic HCV (n=56), resolved HCV (n=13), false positive (n=2) or inconclusive (n=10). The 23 acute HCV cases included 9 women and 14 men, age 20-83 (mean 47) years. All presented with acute hepatocellular injury with median ALT 1448 U/L (range 458-3501), ALP 232 U/L (range 92-551), and total bilirubin 10.8 mg/dL (range 1.1-23.1). While all cases were suspected DILI at enrollment, at adjudication 6 months later, 19 were were judged as due to HCV and not DILI (10 unlikely and 9 possible); while 4 were still considered DILI (1 highly likely and 3 probable). Seven cases were anti-HCV negative at presentation and were later found to have detectable HCV RNA, in 4 others HCV RNA testing had not been done. The clinical course led to hospitalization in 18, was severe in 4 and 1 patient died (unrelated to HCV). Among 11 patients with 6 month follow up, 6 cleared HCV RNA, 5 had persistence of HCV RNA and 2 had undergone successful antiviral treatment. Conclusions: 23 of 1518 (1.5%) cases of suspected DILI were actually due to acute HCV infection. All patients presented with hepatocellular injury but were misdiagnosed usually due to lack of HCV RNA testing at the time of liver injury onset. These findings indicate that exclusion of acute HCV requires HCV RNA testing in patients with suspected DILI.

Disclosures:

K. Rajender Reddy - Advisory Committees or Review Panels: Merck, Janssen, BMS, Abbvie, Gilead; Grant/Research Support: Merck, BMS, Gilead, Janssen, Abbvie

Hans L. Tillmann - Consulting: Novartis, Novo Nordisk; Employment: AbbVie; Stock Shareholder: AbbVie, Abbott, Gilead

Naga P. Chalasani - Consulting: Abbvie, NuSirt, Lilly, Afimmune, Allergan/Tobira, Axovant, Madrigal, Shire, Ardelyx; Grant/Research Support: Intercept, Cumberland, Lilly, Gilead, Galectin

Robert J. Fontana - Consulting: Abbvie, CLDF, Alnylam; Grant/Research Support: BMS, Gilead

Gavin A. Cloherty - Employment: Abbott Laboratories; Stock Shareholder: Abbott Laboratories

The following people have nothing to disclose: Jawad Ahmad, Paul H. Hayashi, Victor J. Navarro, Andrew Stolz, Huiman X. Barnhart, Jay H. Hoofnagle

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Gender Enigma: Increased Occurrence of Anti-tuberculosis Drug-induced Liver Injury and Acute Liver Failure in Women Despite Tuberculosis Being More Common in Men

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Background: Infectious diseases in general occur more commonly in males. (PLOS one 2013e2390). According to World Health Organization, globally men are significantly more at risk of contracting and dying from tuberculosis (TB) than women (www.who.int). On the contrary gender difference in some liver diseases has been described, but there are conflicting reports with regard to drug-induced liver injury (DILI) in females. Anti-tuberculosis drugs are the most common cause of DILI and drug-induced acute liver failure (DIALF) is middle and low income countries. **Aim:**Therefore the aim of this study was to assess the effect of gender on antituberculosis hepatotoxicity including liver failure and outcome in a large single center DILI registry. **Methods:** We identified all cases of anti-tuberculosis DILI (group 1) and anti-tuberculosis DIALF (group2) from a large DILI registry (1997-April 2016). DILI and DIALF were defined by standard criteria (Clin Pharmacol Ther 2011;89:806). We estimated the proportion of males and females in the anti-tuberculosis DILI and DIALF group and compared that in a cohort of patients with TB reporting to the state TB control program (Group 3). Basic statistics was employed to examine the influence of gender in the development of anti-tuberculosis DILI and DIALF. **Results:** Of the 121461 TB patients reporting to a state TB program in the year 2015 and 2016, 81767 (67.3%) were males and 39677 (32.6%) were females **(Ratio 2:1)**. 17 were transgender. From the DILI registry we identified 421 patients with anti-tuberculosis DILI of whom 230 (54.7%) were males and 191 (45.3%) were females **(Ratio 1.2:1)**. Of 93 patients with antituberculosis DIALF, 43 were males (42.7%) and 50 were females (53.7%) **(Ratio 0.86:1)** of whom 24 (59%) and 39 (81.3%) died respectively. Therefore although more males developed tuberculosis in the population (67.3%) (group 3), a greater proportion of females were represented in the anti-TB DILI group (45.3%) (group 1) (p=0.001). In the anti-TB DIALF group (group 2) more women (53.7%) than men developed DIALF compared to the TB population (group 3) (p=0.001). Further, mortality from anti-tuberculosis-DIALF was more in females (81.3%) than in males (59%) (p=02). **Conclusions:** Although two thirds of TB occur in men, women are more likely to present with antituberculosis-DILI including antituberculosis DIALF. Moreover more women die from DIALF than men. This suggests increased hepatotoxicity in women than men despite TB being less common in women. Sex differences in absorption, distribution, metabolism and excretion (ADME) should be investigated to understand the increased predisposition of anti-tuberculosis hepatotoxicity in women.

Disclosures:

The following people have nothing to disclose: Harshad Devarbhavi

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Clinical Outcomes and Histopathological Patterns in Ayurveda and Herbal Induced Liver Injury: A Single Centre Experience in 27 patients.

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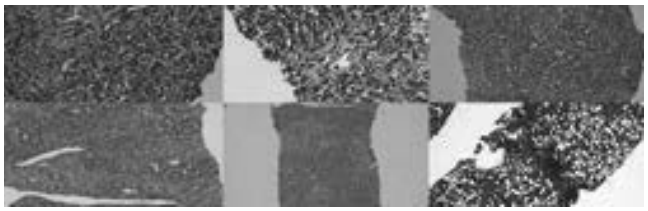
Introduction-Ayurvedic and herbal products (AHP) are known to cause varying degrees of liver injury. Histopathological patterns of liver injury due to AHP is not well studied. **Patients-**Out of 1440 patients(Sep'16-Mar'17), 94 patients (6.52%)diagnosed with AHP liver-injury, by RUCAM.27 patients (28.7%) underwent biopsy after informed consent. **Results-** ***Males***(77.8%, n=27), age 46.8y±15.7;duration of drug intake 74d(range 10-84); onset of liver injury 98d(14-112). 13 (48%) had single and 14.8% had multiple comorbidities;significant alcohol

25.9%; ***commonest use for drug-dyspepsia/abdominal bloating*** (29.6%, n=8) f/b **appetite enhancement**(22.2%, n=6). **Fatigue, anorexia, jaundice** seen in (96.3%, n=26) Abdominal pain in 7.4%(n=2), pruritus 44.4%(n=12), fever 29.6%(n=8) and joint pains 22.2%(n=6). **Hepatic encephalopathy**(HE) at admission in 29.6%(n=8), **ascites** in 37%(n=10) and skin bleeds in 3.7%. **Autoantibodies positive in 37%**(n=10)-ANA(25.9%, n=7); LKM1(7.4%, n=2); AMA(3.7%, n=1); total bili 8.2(2.8-53.5), AST 358(40-1894), ALT 266(23-1447), AP 123(34-431), GGT 118(20-688), INR 1.4(1.05-5.91), albumin 3.4±0.6, creatinine 0.7(0.4-2.6), IgG 1013(628-3428). **Hepatocellular, cholestatic and mixed patterns** seen in 59.3%, 7.4%, 33.3%. Possible, probable and definite liver injury in 59.3%,40.7%,0% respectively. Median f/u 110d(6-255). Six patients **died (22.2%)** including 1 post-LDLT (at 168d).**Chronic active hepatitis in 63%**, lobular/portal inflammation in (74.1%, mild-37%)/ (85.2%, moderate-66.7%), interface-hepatitis (59.3%, severe-11.1%), mixed cellular(55.6%),**necrosis(51.9%, bridging-type 25.9%, zone one 22.2%)**; fibrosis in 66.7 (bridging 33.3%, F2 in 18.5%, cirrhosis in 22.2%); **cholestasis in 63%(mixed type 51.9%)**; ballooning 18.5%,+Mallory Denk in 14.8%, steatosis 37%(mixed 26%, severe 7.4%)& bile duct injury in 33.3%. **Higher age, INR and lower albumin at baseline** predicted mortality in absence of cirrhosis($p<0.05$), also HE ($p=0.001$). Presence of **necrosis and steatosis on biopsy** associated with higher mortality($p=0.03$); 100% mortality-**panacinar submassive and massive necrosis**($p=0.005$).**Conclusion-**AHP-liver injury has high mortality with higher age, coagulopathy, HE, submassive/massive necrosis at baseline. Early liver biopsy, clinical identification of at risk patients expedites definitive treatment with liver transplant.



Significant-patterns.

Disclosures:

The following people have nothing to disclose: Cyriac A. Philips, Rajaguru Paramaguru

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Hepatitis C Care in the Department of Veterans Affairs: A System-wide Process Improvement Project

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As of October 2014, the Department of Veteran Affairs (VA) provided care to >168,000 Veterans with chronic hepatitis C virus (HCV) infection. The availability of direct acting antiviral medications (DAAs) presented an opportunity for VA to identify, evaluate, and effectively treat HCV-infected patients across the system. **Aim:** To describe key processes the VA used to change HCV care. **Methods:** Starting in October 2014, VA Central Office (VACO) funded and provided program support for a collaborative of 18 interdisciplinary Hepatitis Innovation Teams (HIT) charged with improving HCV care within their geographical region. The HIT developed Lean process improvement projects (PIP) based on locally determined goals for HCV care. HITs participated in virtual inter-and intra-team working groups as well as monthly conference calls to share and implement strong practices. Annually, HIT submitted updated PIPs with iterative projects/goals. National targets were established for screening, treatment, and SVR testing, and updated annually. VA measured and reported these outcomes nationally and for each VA facility. In 2016, HITs completed an implementation questionnaire to assess their impact on HCV care. **Results**: Teams developed PIP that were reviewed annually. Attendance on monthly calls was 96% and satisfaction with the process was high. Teams met 83% (49/60) of their individual performance improvement goals. Strong practices disseminated across the HITs included population health management informatics tools, strategies for telemedicine/telehealth, incorporating new technology into clinical care, integrated care management, expansion of scope of practice to include evaluation and treatment by advance practice clinicians, and integration of HCV treatment into substance use treatment programs. From January 2014 through March 2017 birth cohort screening increased from ~65% to 78.7% and >84,000 Veterans received DAAs. 86% have been tested for SVR with ~95% achieving SVR. Respondents attributed ~50% of their implementation strategies to participation in the HIT program. Support from leadership was critical to success of the HIT. **Conclusions**: With financial and project support, and availability of real-time data on HCV care, HIT developed and implemented PIP in their region, and shared strong practices across regions. HITs utilized methods described by the IOM in the Learning Healthcare Project, including current state mapping, development of iterative PIP, and use of real-time data derived from clinical care. This framework could serve as a model to transform clinical practice in large healthcare systems.

Disclosures:

Timothy R. Morgan - Grant/Research Support: Merck, Abbvie, Genentech, Gilead, Bristol Myers Squibb

The following people have nothing to disclose: Rachel Gonzalez, Angela M. Park, William Lukesh, Vera Yakovchenko, Pamela S. Belperio, Maggie Chartier

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Population screening using a point-of-care test reveals unexpectedly low prevalence of active HCV infection in Spain

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Background and Aims: In the USA, the CDC recommends HCV population screening for individuals born between 1945 and 1965, whereas in Spain and other EU countries there is no recommendation because of lack of prevalence data in the general population. We aimed to (1) assess whether this oral fluid test is a suitable non inva-

sive-method for population screening; (2) estimate the true prevalence of undetected HCV infection in our area to identify groups at risk of chronic HCV infection. **Methodology:** The study was divided in two phases: First, a risk factor questionnaire was collected from 317 consecutive patients (anti-HCV positive n=208; anti-HCV negative with other liver diseases n=109) before performing the OraQuick HCV rapid test in oral mucosal transudate (OMT) (n=317) and fingerstick blood (n=252). We calculated the sensitivity and specificity of the test by using anti-HCV serostatus as the gold-standard. Second, the proposal for free HCV infection screening using OraQuick HCV rapid test in OMT was offered by regular mail to 9,000 individuals older than 18 years old, randomly selected from the hospital assigned population. **Results:** Aim 1: In OMT, the clinical sensitivity and specificity of the OraQuick HCV rapid test was 89.9% and 100%, respectively. In fingerstick blood, the sensitivity improved to 98.8%. Among anti-HCV positive patients, the sensitivity was higher both in OMT (97.2%) and fingerstick blood (97.6%) in those who were viremic. In contrast, the sensitivity in OMT in non-viremic patients (treatment-induced clearance) was only 82.2%. There were no differences in sensitivity between viremic and non-viremic individuals when testing fingerstick blood. Finally, extension of the incubation time to 40 minutes enhanced the sensitivity, especially in OMT (up to 94.7%) and in the subgroup of non-viremic, anti-HCV+ patients (up to 90.1%). Aim 2: To date, among 835 persons included in the second phase, only 12 (1.4%) were reactive according to the OraQuick HCV test in OMT. These persons were already aware of their infection. **Conclusions:** The OraQuick HCV test in OMT has a high sensitivity and specificity that decreases substantially in anti-HCV positive HCV-RNA negative patients with treatment-induced viral clearance. This problem can be avoided by increasing the incubation times or by the use of fingerstick blood. In our department, the true prevalence of anti-HCV+ was only 1.4%, and there were no new diagnoses made. These preliminary data suggest a different epidemiological profile in our area compared to other regions (such as the USA).

Disclosures:

F. Xavier Lopez-Labrador - Grant/Research Support: Sanofi-Pasteur

Marina Berenguer - Advisory Committees or Review Panels: Novartis, Astellas, Gilead, BMS, MSD; Grant/Research Support: Gilead

The following people have nothing to disclose: Ângela Carvalho-Gomes, Carmina Pallarés, Vanessa Hontangas, Almudena G. Cubbells, Isabel Conde, Tommaso Di Maira, Victoria Aguilera

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Utilization of Patient-Aligned Care Teams (PACT) clinical pharmacists in the primary care setting to treat and monitor non-cirrhotic patients receiving direct-acting antiviral (DAA) therapy.

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BACKGROUND: For the past 15 years, the Veterans Administration Long Beach Healthcare System (VALBHS) has utilized a liver clinical pharmacist specialist (CPS) to initiate and monitor hepatitis C (HCV) therapy for patients who have been evaluated and approved for treatment by hepatologists. In order to improve access, PACT clinical pharmacists in primary care were trained to treat patients with uncomplicated HCV. **OBJECTIVE:** To compare sustained virologic response (SVR) and adherence to scheduled appointments in uncomplicated HCV-infected patients on DAAs treated by either a PACT CPS in primary care or by a liver CPS in liver clinic. **METHODS:** The liver CPS trained all 13 VALBHS PACT CPS through a series of didactic and practical training sessions. Patients within VALBHS identified with chronic HCV infection were referred to the liver CPS for a chart review and triaged to either the hepatologist or the liver CPS based on severity of liver disease. Patients with possible cirrhosis and/or a complicated medical history were referred to the hepatologist for further evaluation and then referred back to the liver CPS when cleared for treatment. Patients without cirrhosis were referred to the liver CPS for a comprehensive evaluation and to select and prescribe a DAA. Genotypes 2, 3 and 4 and more complicated patients remained with and were treated by the liver CPS in liver clinic. Genotype 1 patients without advanced liver disease and prescribed a DAA without ribavirin were referred to a PACT CPS for treatment in primary care. The liver CPS and hepatologist were available in real-time to answer any questions from PACT CPS. Routine visits were at weeks 2, 4, and 8. End of treatment (EOT) and SVR follow-up were by telephone. **RESULTS:** Medical records of patients treated by PACT pharmacists and liver pharmacists between October 1, 2015 and September 30, 2016 were reviewed. (See table) **CONCLUSIONS**: SVR rates were similar for patients treated by PACT CPS in primary care and liver CPS in liver clinic. Adherence with lab and follow-up appointments were similarly high for both groups. Healthcare systems can effectively treat greater numbers of hepatitis C patients with existing resources by utilizing trained clinical pharmacists in the primary care setting with appropriate support from specialists.

RESULTS

	Primary Care Clinic (n=64)	Liver Clinic (n=74)
Adherence week 4/EOT/SVR follow-up	86%/100%/90%	91%/98%/89%
Lost to follow-up	4 (6.2%)	2 (2.7%)
Self-discontinued	2 (4.7%)	3 (4%)
SVR achieved	52 (89.7%)	62 (89.9%)
SVR not achieved	1 (1.7%)	0
SVR lab not available (at time of data collection)	5 (8.6%)	7 (10.1%)

Disclosures:

Timothy R. Morgan - Grant/Research Support: Merck, Abbvie, Genentech, Gilead, Bristol Myers Squibb

The following people have nothing to disclose: Macy Ho, Jennifer Fong, Thuong PH Tran, Rachel Gonzalez, Suna Chung

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Treatment of Hepatitis C Virus Leads to Economic Gain Related to Reduction in Cases of Hepatocellular Carcinoma (HCC) in Japan

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Background: HCC is a serious and expensive complication of HCV infection. Achieving sustained virologic response is associated with a reduction in cirrhosis, HCC and mortality. These benefits of HCV cure can also be realized at the societal level by preventing cases of HCV-related complications and their costs. Japanese HCV patients have been infected for a longer period and have higher prevalence of HCC with associated mortality and economic burden. Treatment of HCV with highly effective Oral Direct Acting Antiviral (DAA) regimens can lead to high SVR rates potentially reducing HCV complications and their costs. **Aim:** To use a decision analytic Markov model to estimate the economic benefit of HCV cure by reducing HCC and decompensated cirrhosis (DCC) in Japan. **Methods:** A hypothetical cohort of 10,000 HCV GT1b Japanese patients with a mean age of 60 was modeled with a hybrid decision tree and Markov state-transition model capturing the natural history of HCV infection over a lifetime horizon. It was assumed that 15% of the cohort had cirrhosis and 20% were treatment-experienced. Treatment options were assumed to be approved all-oral DAAs vs. no treatment (NT). Treatment efficacy was based on randomized controlled trials of DAA regimens. Transition rates and costs were obtained from Japan-specific data. The number of cases of DCC, HCC and quality-adjusted life years (QALYs) were projected for patients treated with an all-oral DAA vs. NT. QALYs were monetized using a willingness to pay (WTP) threshold which varied from ¥4 to ¥6 million. The incremental savings associated with treatment were calculated by adding the projected cost of complications avoided to the monetized gains in QALYs. **Results:** The model showed that DAA treatment can avoid 2078 cases of HCC and 1495 cases of DCC, saving ¥857,946.9 and ¥341,645.7 per treated patient; respectively. If we combine both DCC and HCC as serious complications of HCV-cirrhosis in Japan, treatment leads to avoidance of 3573 cases of complications and the associated savings of ¥1,199,592.6 per treated patient. Additionally, DAA treatment can lead to an additional 2.67 QALYs gained per patient treated. The indirect economic gains associated with treatment-related QALY improvements was estimated to be ¥10,680,000, ¥13,350,000 and ¥16,020,000 per patient at WTP thresholds of ¥4 million, ¥5 million and ¥6 million. Total economic savings of HCV GT1 treatment with DAAs (vs. NT) was ¥11,879,592.6, ¥14,549,592.6 and ¥17,219,592.6, at these different WTP thresholds. **Conclusion:** Treatment of HCV GT1 with all Oral DAAs in Japan can lead to significant direct and indirect savings related to avoidance of HCC and DCC.

Disclosures:

Rachel Beckerman - Consulting: Gilead Sciences

Masashi Mizokami - Consulting: Gilead Sciences, Sysmex; Speaking and Teaching: Gilead Sciences, Sysmex Company

The following people have nothing to disclose: Zobair M. Younossi, Atsushi Tanaka, Yuichiro Eguchi, Linda Henry

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Baby Boomers Are More Likely To Be Linked to HCV Care Than Non-Baby Boomers

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Background: Screening for hepatitis C (HCV) in the 1945-1965 birth cohort (BC) is recommended by CDC in addition to risk based screening. There are mixed data about whether linkage to care (LTC) between BC and non-BC populations differ. We aimed to examine differences in LTC to a specialist in a large urban population where HCV screening has been scaled up in both outpatient and inpatient settings. **Methods:** We included all patients who had HCV RNA+ in our system in 2016 and quarter 1 (Q1) 2017 in the study population. We compared LTC rates between those in the BC and those outside the BC. LTC was defined as at least one appointment with an HCV specialist. Covariates examined included race, gender, known prior HCV positivity before index test, site of HCV diagnosis (inpatient, outpatient, emergency department, jail), HIV, type of insurance, HCV risk factor (with active and remote intravenous drug use [IDU] coded separately), psychiatric diagnosis and member of BC. **Results**: During the study period, 801 patients had a +HCV antibody. Of those, 570 (71.0%) had HCV RNA+, 242 (42.6%) in the BC and 326 (57.3%) outside the BC. 126 of 242 (52%) of BC patients were linked to care compared to 120/326 (36.8%) of non-BC patients (p<0.0003). Comparing patients who were linked vs not linked, univariate analysis identified HCV risk factor, site of HCV diagnosis, being in the BC, and psychiatric diagnosis as possibly being associated with linkage, with p<0.05. On multivariable analysis, being diagnosed as an inpatient, aOR 0.48 (95% CI 0.27-0.83, p=0.008), having active IDU, aOR 0.40 (95% CI 0.23-0.67, p=0.23-0.67, p=0.005), having risk factor not assessed, aOR 0.012 (95% CI 0.004-0.04), p<0.001) were all associated with not being LTC. Remote IDU was not significant. Being in the BC was positively associated with LTC, aOR 2.27 (95% CI 1.40-3.67, p=0.008). **Conclusions**: HCV patients outside the BC have lower rates of LTC that are not fully accounted for by active IDU or psychiatric diagnoses. Ongoing studies are needed to explore the reasons for this and develop strategies to more fully engage this vulnerable population.

	aOR for LTC (95% CI)	*P*
Site of Diagnosis		
Outpatient	1.0	Ref
Emergency Dept	0.69 (0.33-1.43)	0.32
Inpatient	0.48 (0.28-0.83)	0.008
Jail	0.40 (0.04-3.86)	0.43
Risk Factor		
Other	1.0	
Active IDU	0.40 (0.24-0.67)	0.005
Remote IDU	0.98 (0.57-1.69)	0.93
Not assessed	0.01 (0.004-0.04)	<0.0001
Psychiatric diagnosis	0.75 (0.50-1.1)	0.18
Birth Cohort	2.27 (1.40-3.67)	0.0008

Disclosures:

Melissa K. Osborn - Advisory Committees or Review Panels: Gilead Sciences; Grant/Research Support: Gilead Sciences

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Cost-effectiveness of Treating Acute versus Chronic Hepatitis C Infection Using Direct-Acting Antivirals

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Background: Currently clinical guidelines do not routinely recommend initiating treatment in patients with acute hepatitis C virus (HCV) infection primarily because of (1) the desire to avoid treating patients who would have otherwise spontaneously cleared the infection on their own, (2) the limited data on the efficacy of direct-acting antivirals (DAAs) in the acute phase of HCV infection, and (3) the lack of studies evaluating the cost effectiveness of early treatment strategies. Given recent pilot data supporting the efficacy of DAAs in acute HCV, we aim to assess the trade-offs of treating acute versus chronic HCV infection. **Methods**: We developed a simulation model to compare two HCV management strategies: (1) treat acute HCV immediately with 6 weeks of ledipasvir/sofosbuvir (LDV/SOF), and (2) wait for six months and treat only those who develop chronic HCV infection ('treat chronic HCV' arm) with 8 weeks of LDV/SOF. We incorporated recent clinical data on the efficacy of DAAs in treating acute as well as chronic HCV. Patients who failed treatment progressed through different stages of the natural history of HCV. For each strategy, we estimated total discounted quality-adjusted life years (QALYs), lifetime costs, and the incremental cost-effectiveness ratio (ICER). **Results:** Treating HCV in the acute phase versus deferring treatment until the chronic phase of infection increased QALYs by 0.02 and increased costs by $1030. Treating acute versus chronic HCV resulted in an ICER of $42,700/QALY. When we accounted for the potential reduction in HCV transmission resulting from acute HCV treatment, we found treating acute HCV *increased* QALYs by 0.03 and *decreased* costs by $7,140; in this scenario treating acute versus chronic HCV became cost-saving. Treating acute HCV remained cost-effective/saving irrespective of the duration of DAA therapy in acute phase (4-6 weeks) and chronic phase (8-12 weeks). Probabilistic sensitivity analysis showed that treating acute HCV would be cost-effective/saving with the probability of 99%. **Conclusion**: Immediate treatment of acute HCV infection can improve clinical outcomes and be highly cost-effective or cost-saving compared with deferring treatment until the chronic phase of HCV infection. Given the need to halt the rising HCV incidence in the United States, current treatment guidelines should be revisited to incorporate recommendations that account for the clinical and economic benefits of treatment of acute HCV in the era of DAAs.

Disclosures:

Raymond T. Chung - Advisory Committees or Review Panels: Alnylam; Grant/Research Support: Gilead, Abbvie, Merck, BMS, Boehringer Ingelheim, Janssen

Jagpreet Chhatwal - Consulting: Merck & Co., Inc., Gilead, Complete HEOR Solutions; Grant/Research Support: NIH/National Center for Advancing Translational Sciences, American Cancer Society

The following people have nothing to disclose: Emily D. Bethea, Qiushi Chen, Chin Hur

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Efficacy of tenofovir to prevent progression of chronic hepatitis B in patients with mild elevation of alanine aminotransferase: preliminary results of a triple-blind randomized placebo-controlled trial

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Background: Substantial elevation of alanine transaminase (ALT) is usually required to initiate antiviral therapy for chronic hepatitis B (CHB). Definitive evidence that attests therapeutic efficacy in patients with mildly elevated liver enzyme has been lacking. Objectives: to study whether tenofovir disoproxil fumarate (TDF) could prevent disease progression in CHB patients with serum ALT <2 folds the upper limit of normal (ULN). Methods: In this multicenter triple-blind placebo-controlled trial, we enrolled 161 CHB patients whose serum ALT ranged 1-2 folds ULN despite viral DNA >2,000 IU/mL in a preceding year. They were randomized to receive either TDF (n=81) or placebo (n=80) for 3 years. The primary endpoints were the histological progression of liver fibrosis and necroinflammation as evaluated by the Knodell and Ishak systems. Results: The TDF and placebo groups were similar in the baseline including viral DNA (median, 5.17 vs. 5.34 log IU/mL) and ALT levels (median, 53 vs. 52 U/L). As of May 2017, 53 TDF and 52 placebo receivers have completed the 3-year trial and undergone liver biopsy. We herein reported the preliminary results of these 105 patients while awaiting the last one to finish in 2018. Liver fibrosis progressed (≥1 Ishak stage increase) in 12 (22.6%) and 23 (44.2%) patients in the TDF (n=53) and placebo (n=52) groups, respectively (P=0.02). At the end of the trial, 2 (3.8%) TDF and 8 (15.4%) placebo receivers (P=0.05) developed cirrhosis (Ishak stage 5 or 6). Necroinflammation (Knodell necroinflammatory score increase ≥2 points) worsened in 5 (9.4%) and 10 (19.2%) participants in the TDF and placebo arms, respectively (P=0.17). Eight of the 52 placebo completers took entecavir as a rescue therapy for clinical flare, which did not occur in any patient assigned to TDF. Conclusions: Preliminary results of this trial suggest the efficacy of TDF to prevent liver fibrosis progression in CHB patients with mild ALT elevation (ClinicalTrials.gov identifier: NCT01522625).

Study outcomes

	TDF (n=53)	Placebo (n=52)	P value
Ishak fibrosis ≥1 stage increase, n (%)	12 (22.6%)	23 (44.2%)	0.02
Cirrhosis (Ishak stage 5 or 6), n (%)	2 (3.8%)	8 (15.4%)	0.05
Knodell necroinflammatory score ≥2 points increase, n (%)	5 (9.4%)	10 (19.2%)	0.17
HBV DNA, IU/mL	<20	15,379 (741~245,662)	<0.001
Undetectable viral DNA, n (%)	40/48 (83.3%)	5/50 (10%)	<0.001
ALT, U/L	28.5 (24~39)	43 (31-57)	<0.001
ALT normalization (<40 U/L), n (%)	40 (75.5%)	23 (44.2%)	0.001
qHBsAg, IU/mL	878.7 (320.1~1512.7)	1144.3 (181~3553.6)	0.25
HBsAg loss, n (%)	0	1 (1.9%)	0.5

Continuous variables (median and IQR) were compared using the Wilcoxon rank sum test; categorical variables (number and the percentage) were examined by Fisher's exact test

Disclosures:

Yao-Chun Hsu - Speaking and Teaching: AbbVie, Gilead Sciences, Bristol-Myers Squibb Company

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No Resistance to Tenofovir Alafenamide Detected Through 96 Weeks of Treatment in Patients with Chronic Hepatitis B

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Aim: Presented herein are the post Week 48 through Week 96 resistance analyses for 2 Phase 3 studies (GS-US-320-0108 and GS-US-320-0110) evaluating tenofovir alafenamide (TAF) versus tenofovir disoproxil fumarate (TDF) for the treatment of chronic hepatitis B (CHB) in HBeAg+ and HBeAg- treatment-naïve or treatment-experienced adults. **Methods**: Patients were randomized 2:1 and stratified by HBV DNA and treatment status to receive TAF or TDF. HBV pol/RT population or deep sequencing was conducted for patients with ≥24 weeks of treatment with viremia (HBV DNA ≥69 IU/mL) at Week 96 or at early discontinuation post Week 48. Deep sequencing was conducted for patients with HBV DNA >159 IU/mL and sequence changes at the consensus sequence level (15%) are reported. Virologic breakthrough (VB) was defined as HBV DNA ≥69 IU/mL after achieving <69 IU/mL or a ≥1.0-$\log_{10}$ increase from nadir. Phenotypic analysis using recombinant HBV in HepG2 cells was performed for VB patients who were adherent to study drug (plasma drug levels), patients with conserved site substitutions, or for polymorphic substitutions emergent in >1 patient. **Results:** 1298 patients were randomized and treated (TAF: n=866; TDF: n=432). A similar percentage of patients in the TAF or TDF arms qualified for sequence analysis post Week 48 through Week 96 of treatment (TAF: 10.5%; TDF: 10.9%). In the TAF arm, 87 patients qualified at Week 96: 31 had no sequence change from BL, 15 were unable to sequence (UTS), 32 had polymorphic site substitutions, and 9 had conserved site substitutions. In the TDF arm, 45 patients qualified at Week 96: 26 had no sequence change, 6 were UTS, 11 had polymorphic site substitutions, and 2 had conserved site substitutions. With the exception of the ADV-resistance substitution rtA181T, each detected conserved site substitution was observed in a single patient. Detection of the rtA181T substitution in 2 patients, 1 from each arm, was not associated with increasing plasma HBV DNA levels. At Week 96, a small percentage of patients experienced VB (TAF: 2.4%, TDF: 3.0%), and VB was often associated with documented study drug nonadherence (TAF: 22%, TDF: 46%). 27 patients qualified for phenotypic analysis post Week 48 through Week 96 (TAF: n=19, TDF: n=8) and no patient isolates tested showed a reduction in susceptibility to TAF or tenofovir, respectively. **Conclusion:** Overall, the proportion of patients analyzed and the HBV sequence changes observed were similar between patients in the TAF and TDF arms of these Phase 3 studies. Most substitutions occurred at polymorphic positions and no substitutions associated with resistance to TAF were detected through 96 weeks of treatment.

Disclosures:

Henry Lik-Yuen Chan - Advisory Committees or Review Panels: Gilead, Janssen, Bristol-Myers Squibb, Roche, Abbvie; Speaking and Teaching: Novartis Pharmaceutical, Echosens

Patrick Marcellin - Consulting: GILEAD, MSD, ABBVIE; Grant/Research Support: GILEAD, BMS, janssen, MSD, ABBVIE; Independent Contractor: GILEAD, BMS, JANSSEN, MSD, ABBVIE; Speaking and Teaching: GILEAD, BMS, MSD

Calvin Q. Pan - Advisory Committees or Review Panels: Gilead; Consulting: BMS, Gilead; Grant/Research Support: BMS, Gilead, Merck; Speaking and Teaching: synergy, Gilead, Salix, Allergan, Abbvie

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The following people have nothing to disclose: . Shalimar, Namiki Izumi

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Tenofovir-based combination therapy or monotherapy for multi-drug resistant chronic hepatitis B; Real world data from multicenter cohort study

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Background/Aims: Treatment of multidrug resistant (MDR) chronic hepatitis B (CHB) is still a challenging issue. Although current guidelines recommend tenofovir (TDF) or TDF plus entecavir (ETV) for MDR CHB, real-life data comparing various combinations based TDF with TDF plus ETV are lacking. Herein, we report a multicenter cohort study for the evaluation of TDF-based therapy for MDR CHB. Methods: The inclusion criteria were CHB patients with resistance to more than 2 nucleos(t)ide analogues and hepatitis B virus (HBV) DNA level over 200 IU/mL (or 1000 copies/mL). Patients with decompensated cirrhosis or hepatocellular carcinoma were excluded. Primary end point was virologic response defined by undetectable HBV DNA at month 12. Secondary endpoints were VR at month 24 and 36. Results: A total of 243 patients were enrolled. Mean age of patients were 49 years and 77.4 % were male. Mean baseline HBV DNA level was 4.2±1.5 log IU/mL. Genotypic resistance to L-nucleoside analogues (L-NA)+adefovir (ADV) (59 patients), L-NA+ETV (109 patients), ADV+ETV (4 patients), L-NA+ADV+ETV (40 patients) were confirmed at enrollment. Initial treatments for MDR CHB were TDF+ETV 1 mg (170, 69.9 %), TDF+ETV 0.5 mg (10, 4.1 %), TDF+lamivudine (16, 6.6 %), TDF+telbivudine (1, 0.4 %), and TDF monotherapy (46, 18.9 %). Virologic response rates of the whole cohort at year 1, year 2, and year 3 were 96.7%, 88.9 %, and 73.7 %, respectively. At year 1, virologic response rate was not significantly different between the TDF+ETV 1 mg group and other TDF based therapy groups (75.9 % vs. 75.4 %, respectively, P = 0.930). When we compared the virologic response rate in-between TDF+ETV 1 mg group, other combination group, and TDF monotherapy group, there was no difference in-between the groups (75.9 %, 72.0 %, and 77.3 %, respectively, P = 0.883). TDF monotherapy was not inferior to any combination therapies (77.3% vs. 75.4%, P = 0.793). Result of data analysis at year 2 (86.1 %, 80.0 %, and 86.8 %, respectively, P = 0.746) and year 3 (88.9 %, 87.5 %, and 85.7 %, respectively, P = 0.889) showed the same trends. Among 194 HBeAg positive patients, 6.7%, 6.7%, and 10.8% lost HBeAg at year 1, year 2, and year 3. HBeAg loss rates were not different in-between TDF+ETV 1mg group, other combination group, and TDF monotherapy group (11.5%, 14.3%, and 5.9%, P = 0.552) at year 3. Only one patient lost HBsAg in TDF monotherapy group. Conclusion: TDF based therapy was effective for the treatment of MDR CHB. The efficacy of TDF monotherapy did not differ from the TDF based combination therapies. However, serologic response rates were low despite prolonged therapy.

Disclosures:

The following people have nothing to disclose: Hyung Joon Yim, Sang Jun Suh, Young Kul Jung, Hana Park, Yeon Seok Seo, Soon Ho Um, Young Seok Kim, In Hee Kim, Hyoung Su Kim, Ji Hoon Kim, Young-Sun Lee, Eileen L. Yoon, Myeong Jun Song, Jun Yong Park

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Prospective, Randomized Assessment of HBV-Associated and Other Clinical Outcome Events During Long-Term Therapy With Entecavir or Other HBV Nucleos(t)ide Analogues in Patients With Chronic HBV Infection

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Background: Entecavir (ETV) is a nucleoside analogue widely approved for treatment of chronic hepatitis B virus infection (CHB). REALM was a prospective, randomized, open-label, observational study, conducted to assess rates of long-term clinical outcomes (benefits vs risks) in patients with CHB who were treated with ETV or other standard-of-care HBV nucleos(t)ide analogues (nuc). **Methods:** Treatment-naive or -experienced patients with hepatitis B e-antigen (HBeAg) +ve or -ve CHB were randomly assigned (1:1) to monotherapy with ETV (0.5 mg or 1.0 mg once daily per product label) or a non-ETV nuc (specific agent investigator-selected) and followed for ≤10 years. Primary endpoints were rates of clinical outcome events (COEs) including 1) malignant neoplasms: all, non-hepatocellular carcinoma [HCC], HCC; 2) liver-related HBV disease progression (composite of non-HCC events of HBV disease progression, HCC, and liver-related death); and 3) all-cause death. Secondary endpoints were COE rates of non-HCC malignant neoplasms, HCC, and liver-related death. Investigator-reported COEs were adjudicated by an independent committee. Treatment-related serious adverse events (SAEs) were also assessed. **Results:** Randomized and treated patients included 6216 on ETV and 6162 on non-ETV. Overall, at baseline 54% were HBeAg+ and 20% had cirrhosis; 84% were Asian and 13% white. Patients remained on initial therapy for a mean 86 months (ETV) or 78 months (non-ETV). Six percent of ETV recipients switched to non-ETV regimens after a mean 77 months; 12% of non-ETV recipients switched to ETV or ETV combinations after a mean 48 months. There were no statistically significant differences in COE rates between treatment

groups (Table). Results were consistent when adjusted by baseline covariates. Drug-related SAEs that were not protocol-defined COEs were reported in 12 (0.2%) ETV and 50 (0.8%) non-ETV recipients, with spectra as expected; the most common were ALT increase (n=2) in ETV recipients and primarily muscular (n=24) or neuropathic events (n=5) in non-ETV recipients. **Conclusions:** This large, long-term study found no significant differences in COE rates between patients randomized to ETV vs non-ETV nuc monotherapy. Findings were not impacted by patient attrition or treatment switches. SAEs were uncommon although numerically higher in the non-ETV group.

Table. Analysis of Adjudicated Events

COEs, n (%)	ETV N=6216	Non-ETV N=6162	HR (CI)*	P-Value
Primary Endpoints: Overall malig. neoplasms	331 (5.3)	337 (5.5)	0.93 (0.800, 1.084)	0.36
Deaths	238 (3.8)	264 (4.3)	0.85 (0.713, 1.012)	0.07
Liver-related HBV disease progression	350 (5.6)	375 (6.1)	0.89 (0.769, 1.030)	0.12
Secondary Endpoints: Non-HCC malig. neoplasms	95 (1.5)	81 (1.3)	1.10 (0.817, 1.478)	
HCC	240 (3.9)	263 (4.3)	0.87 (0.727, 1.032)	
Liver-related deaths	46 (0.7)	48 (0.8)	0.91 (0.608, 1.365)	
*Hazard ratio (ETV:non-ETV) and CI: 95.03% CI for 1° and 95% CI for 2° endpoints.				

Disclosures:

Jinlin Hou - Consulting: Gilead, Roche, Novartis, GSK, Abbovir; Grant/Research Support: Novartis, GSK, BMS, J&J

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Tawesak Tanwandee - Grant/Research Support: Contravir, Merck, SillaJen, Gilead, Inovio

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The following people have nothing to disclose: Wei Zhao, Changhyeong Lee, Viacheslav Morozov, Christoph P. Berg, Jose D. Sollano, Adrian Streinu Cercel

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Combination/sequential therapy with ETV, Peg-IFN alpha-2b and GMCSF enhanced HBsAg loss and appearance of HBsAb in NA suppressed CHB patients (the Anchor A study): an interim analysis

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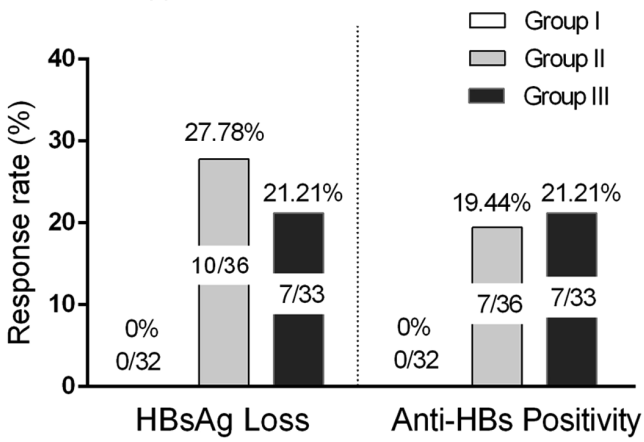
Background: HBsAg loss is considered the optimal endpoint for Chronic Hepatitis B. This multicenter, randomized controlled trial (NCT02327416) was to evaluate whether combination/ sequential therapy with NA, Peginterferon alfa-2b (Peg-IFN α-2b) and granulocyte-macrophage colony stimulating factor (GMCSF) could induce HBsAg loss in CHB patients treated with long-term NA. Methods: 204 CHB patients who had received NA more than one year, with HBsAg <3000 IU/ml and HBV DNA ≤1000 copies/ml, were randomized 1:1:1 to receive ETV (0.5mg/day, oral) for 96 weeks (Group I) or ETV for 48 weeks and Peg-IFN α-2b (180 μg/week, subcutaneous) for 96 weeks (Group II) or ETV and intermittent GMCSF (75 μg/day, first 5 days each month, subcutaneous) for 48 weeks in combination with Peg-IFNα-2b (180 μg/ week, subcutaneous) for 96 weeks (GroupIII). Interim data on 101 patients (32 in Group I, 36 in Group II and 33 in Group III) who have completed 72 weeks of treatment were analyzed.. Results: Baseline characteristics were comparable among three treatment groups. At week 72, patients who received combination/ sequential therapy with NA, Peg-IFN α-2b and GMCSF (Group III) and patients who received combination / sequential therapy with NA and Peg-IFN α-2b (Group II) achieved higher rates of HBsAg loss when compared with those continuing NA treatment (Group I), respectively (Group III vs. Group I, 21.21% vs. 0, p =0.006; Group II vs. Group I, 27.78% vs. 0, p = 0.001). There was no significant difference in HBsAg loss rate between Group III and Group II (21.21% vs. 27.78%, p =0.527). Appearance of HBsAb was only observed in Group III (21.21%) and Group II (19.44%). Combination / sequential therapy with NA, Peg-IFNα-2b and GMCSF were well-tolerated in all patients. Conclusion: For patients who achieved virological suppression with NA, combination / sequential therapy with NA, Peg-IFN α-2b and GMCSF significantly increases rates of HBsAg loss and HBsAb appearance.



Disclosures:

Qin Ning - Advisory Committees or Review Panels: ROCHE, NOVARTIS, BMS, MSD, GSK; Consulting: ROCHE, NOVARTIS, BMS, MSD, GSK; Grant/Research Support: ROCHE, NOVARTIS, BMS; Speaking and Teaching: ROCHE, NOVARTIS, BMS, MSD, GSK

The following people have nothing to disclose: Meifang Han, Di Wu, Deming Tan, Yongping Chen, Xinyue Chen, Xiaoguang Dou, Ke Ma, Li Sun

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HbsAg decline and HbsAg seroclearance in chronic hepatitis B patients on nucleos(t)ide analogues or pegylated interferon therapy – a systematic review and model-based meta-analysis

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Background In chronic hepatitis B (CHB), surface antigen (HbsAg) seroclearance is associated with improved clinical outcome and allows safe discontinuation of treatment. Current treatment with nucleos(t)ide analogues (NA) or Pegylated Interferon (PegIFN) result in low rates of HbsAg seroclearance. New treatment regimens are in development with the aim to improve HbsAg seroclearance rates. The goal of the present study is to quantify, from historical standard of care studies, early HbsAg changes from baseline, HbsAg seroclearance rates and the association between both. **Methods** A systematic review was performed searching PubMed, ClinicalTrials.gov, and conference proceedings for studies of NAs (Tenofovir, Entecavir) or PegIFN in subjects with CHB. This meta-analysis studied HbsAg decline and seroclearance at various timepoints (random-effects method). Adjustments for treatment type, HbeAg status and genotype etc. were explored as potential heterogeneity sources. A model-based meta-analysis was conducted to predict the rate of HbsAg seroclearance on/after treatment based on the on-treatment mean HbsAg decline. **Results** Over 90 studies (randomized controlled, prospective cohorts and retrospective analyses) with HbsAg data were retained. The meta-analysis results are shown in the table. HbsAg decline and seroclearance rate under NA treatment was consistently minimal for HbeAg negative patients. For HbeAg positive patients NA treatment had a larger, albeit limited impact on HbsAg with significant heterogeneity across studies. Overall, the cumulative HbsAg seroclearance rate increased over time but remained low. Under PegIFN there was no notable difference in HbsAg kinetics between HbeAg negative and positive patients and the pooled results are reported. The model-based meta-analysis showed that mean HbsAg decline at week 24 was found to be predictive of the HbsAg loss irrespective of treatment regimen and HbeAg status. **Conclusions** This study established, in a model-based meta-analysis across studies, how early read-outs can be predictive of long term outcome under the current standard of care regimens. For novel agents, the meta-analysis results can be used to quantify the performance of the control arm in study designs with historical borrowing. For example, future studies considering a NA control arm in HbeAg positive patients can anticipate a 24-week mean decline in HbsAg levels ranging from 0.09 to 0.59 log10 IU/mL and a 1-year HbsAg seroclearance rate of 0% to 3%.

Treatment	24-week HbsAg change from baseline (log10 IU/mL)		48-week HbsAg loss (%)	
	N (study arms/cohorts)	Meta-analytic predictive distribution Mean (95% CI)	N (study arms/cohorts)	Meta-analytic predictive distribution Mean (95% CI)
PegIFN (HbeAg-/+)	19	-0.55 (-0.80;-0.31)	34	3% (1%;8%)
NA (HbeAg-)	6	-0.02 (-0.04;0.00)	19	0% (0%;2%)
NA (HbeAg+)	10	-0.34 (-0.59;-0.09)	24	1% (0%;3%)

Disclosures:

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Pre-Transplant Performance Status and Outcome of Liver Transplantation.

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Background Frailty and compromised functional status are independent determinants of mortality in patients with chronic liver disease (CLD), and in the setting of liver transplantation (LT) are closely linked to waitlist mortality. Associations with post-transplant outcomes are less well characterized. In a large national cohort of patients with CLD we examined the association of pre-LT performance status on post–LT patient survival. **Patients** 7053 adult recipients with CLD of defined etiology undergoing first elective LT in the United Kingdom between 1994 and 2016 were studied. Patients with malignancy or requiring critical care organ support at time of LT were excluded. Pre-LT functional status was prospectively assessed using a modification of the Eastern Cooperative Oncology Group (ECOG) performance status, rating ability to perform personal care or work-related activities on a 5-point scale, where 1=no restriction and 5=completely reliant on nursing / medical care. **Results** 1021 (14%) recipients had ECOG status of 4 (only capable of limited self care, mostly confined to bed or chair) and 232 (3%) were status 5 (fully dependent). As compared to those with 5800 recipients with status 1-3, status 4-5 recipients had higher pre-LT MELD scores (19 (IQR 15-25) vs. 16 (13-19)), lower sodium (135 (131-139) vs. 137 (134-140)), more had ascites (80% vs. 58%) and more were hospitalized at time of LT (61% vs. 6%), (all $p<0.001$). Post-LT survival worsened incrementally with increasingly impaired performance status, with 90 day and 1 year patient survival falling from 96% and 93% in status 1 patients to 83% and 77% in status 5 ($p<0.001$ log-rank). When Eras of 1994-2005 and 2006-2016 were compared, survival improved for patients of all status but those of status 4 and 5 remained inferior. On multivariate Cox-regression analysis adjusting for factors including MELD, measures of graft quality and era of transplantation, performance status remained strongly and independently predictive of post-transplant mortality. **Conclusion.** Recipient performance status assessed using a simple measure is independently predictive of post transplant patient survival, with strongest association seen in those with advanced debility. Our findings reinforce the importance of debility as a potentially modifiable determinant of post-LT outcome. We thank UK NHS Blood and Transplant for the provision of data.

Disclosures:

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Reduced Incidence of Kidney Waitlisting After Liver Transplantation in the Post-Share 35 Era

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Background: The Share 35 policy was implemented in June 2013 to improve access to liver transplantation (LT) for patients with end-stage liver disease at the highest risk of mortality (MELD ≥ 35). With a hypothesis that the policy, by shortening of waiting time in patients with hepatorenal syndrome, has reduced the incidence of irreversible renal damage, we assessed the demand for kidney transplantation (KT) after LT. **Methods:** Data for all adults undergoing LT from 1/1/2010 to 12/31/2015 were extracted from the Organ Procurement and Transplantation Network database. Registration on the KT waitlist within 1 year after LT alone (LTA) was determined, with the end of follow-up on 12/31/2016. Competing risks methods were used to compare the 1-year incidence of KT listing after LTA before and after Share 35, with adjustment for Model for End Stage Liver Disease score and dialysis status at LT. In a sensitivity analysis, we extended the analysis to incorporate simultaneous liver-kidney transplantation (SLK). **Results:** There were 35,936 eligible deceased donor LT performed during the study time period. Of these, 302 patients were listed for KT within 1 year of an LT, comprising 0.85% of the study sample. There were 2,879 recipients of SLK, of whom 35 (1.2%) were listed again for KT. The proportion of patients on dialysis at the time of LT increased from 14% to 17% in the post-Share 35 era, while the cumulative incidence of KT listing within 1 year of LTA decreased from 0.9% to 0.7% (p=0.04). In competing risks analysis, the post-Share 35 era was associated with a 29% reduction in risk of 1-year KT listing among LTA recipients (sHR 0.71, 95% CI 0.55-0.91). Among patients with serum creatinine ≥ 1.5 mg/dL or on dialysis at LT, the median duration of renal dysfunction decreased from 10 days (interquartile range [IQR]: 3-35) to 9 days (IQR: 3-33) post-policy. In sensitivity analysis, SLK increased from 7.4% to 9.0% of all LT after Share 35. The proportion of patients with renal failure undergoing either SLK or listing for KT within 1 year of LTA was 24% post-Share 35, compared to 22% pre-Share 35. **Conclusion**: The incidence of listing for KT within 1 year of LTA decreased, as the duration of renal dysfunction shortened, after the implementation of Share 35. With the implementation of the SLK "safety net" policy, which grants priority on the KT waitlist to LT recipients with persistent renal dysfunction after LT, these metrics will need to be closely monitored.

Disclosures:

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The following people have nothing to disclose: Allison J. Kwong, Sumeet K. Asrani, Scott W. Biggins, Julie Heimbach, Peter L. Abt

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Differences in survival after early kidney after liver transplant compared with simultaneous liver-kidney transplant: Evaluating the potential of the "safety net"

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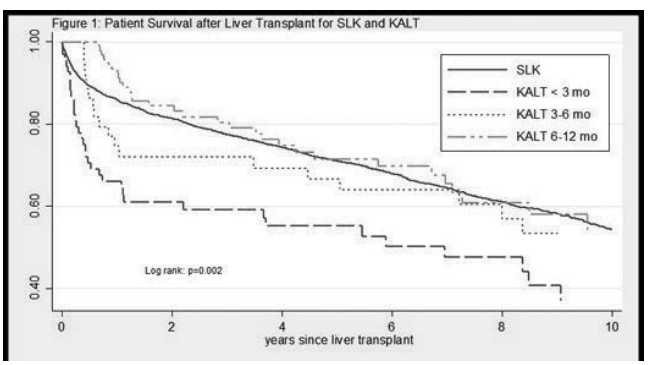
BACKGROUND: Decisions regarding who requires a simultaneous liver-kidney (SLK) transplant remain imperfect with some patients failing to recover their native renal function after liver transplant alone. New UNOS SLK guidelines establish a "safety net" to provide prioritization on the kidney waitlist for patients with early and persistent renal failure after liver transplant. However, there has been little evidence comparing survival for early kidney after liver transplant (KALT) compared with SLK. **METHODS:** Using SRTR data, patient survival from time of liver transplant in adult SLK and KALT transplant recipients who underwent liver transplant from 2002 to 2015 was compared according to unadjusted Kaplan Meier methods with log-rank tests and adjusted Cox regression models. **RESULTS:** During the study period, there were 5,360 SLK transplants, 68 KALT < 3 months, 44 KALT 3-6 months, and 94 KALT 6-12 months. Patients who underwent KALT <3 months had lower survival compared with SLK (HR 1.84, 95% CI=1.32-2.57) after adjustment for recipient age, race/ethnicity, gender, MELD, home/hospital/ICU status, and HCV status. Patients who received a KALT 3-6 months had lower survival early on compared with SLK, but at >6 years there was no significant difference in survival (Figure 1). These differences in survival remained when adjusting for KDPI in deceased donors. In the KALT <3 months group, there was no significant difference in survival in patients who received KALT within < 7 days, 8-30 days, or 31-90 days, with the majority of deaths occurring in the first year. **DISCUSSION:** New SLK guidelines have included a "safety net" to allow for prioritization on the kidney waiting list for those patients who undergo LTA and have persistent renal failure. However, our analysis suggests that early KALT transplant does not have equivalent survival to SLK suggesting continued importance of determining who truly "needs" a SLK. Moreover, given the significant early mortality associated with KALT <3 months, potential futility in these patients receiving a kidney transplant should be considered.



Disclosures:

Glenn A. Halff - Board Membership: texas organ sharing alliance; Stock Shareholder: xenex

The following people have nothing to disclose: Colleen L. Jay, W. Kenneth Washburn, Greg Abrahamian

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A novel equation, GRAIL (GfR Assessment In Liver disease) on the waitlist predicts need for kidney after liver transplantation

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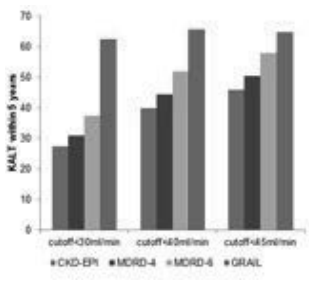
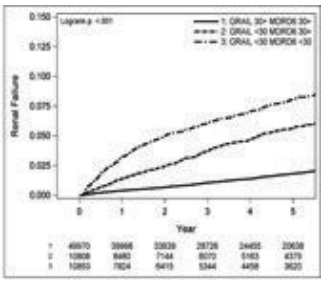
Estimating glomerular filtration rate (CKD-EPI, MDRD-4, MDRD-6) is poor in liver patients with renal dysfunction (GFR<30ml/min) but is routinely used for decisions regarding LT vs. SLKT on the wait list (WL). We developed a model for **G**F**R** **A**ssessment **I**n **L**iver disease (GRAIL) to predict kidney after LT (KALT). **Methods:** GRAIL was derived using objective variables (creatinine, BUN, age, gender, race, albumin) to estimate GFR (eGFR) based on **timing** of measurement relative to LT and **degree** of renal dysfunction. Measured GFR (iothalamate, mGFR) in 12,122 samples (30 yrs) at **protocol** time points before/after LT served as reference. An eGFR<30ml/min by GRAIL on the WL was examined as a predictor of renal outcomes (mGFR<20ml/min, chronic dialysis, KALT) within 5 years after LT in center data. GRAIL on the WL was externally validated using the SRTR (n=68,217, 2001-2015) to assess ability to predict need for KALT. **Results:** GRAIL eGFR<30ml/min on the WL predicted a higher percentage of patients developing renal outcomes within 5 years after LT (38% vs. 12% CKD-EPI, 12% MDRD-4, and 21% MDRD-6). In external validation (SRTR), GRAIL eGFR<30ml/min on the WL predicted highest percentage needing KALT at 1 year (71 % vs. 37% CKD-EPI vs. 41% MDRD-4 vs. 49% MDRD-6) and 5 years (62% vs. 26% CKD-EPI vs. 29% MDRD-4 vs. 35% MDRD-6). (Figure 1) In other words, a majority of cases of KALT at 1 year (51-63%) would not have been predicted by current equations.(Figure). GRAIL eGFR<30ml/min identified 28% (1y) and 36% (5y) more cases of KALT that were not identified by MDRD-6 on the WL. (Figure 2) **Conclusion:** GRAIL eGFR<30ml/min on the WL predicts KALT better than current equations. GRAIL may serve as alternate equation developed in patients with liver disease to enable critical therapy decisions regarding SLKT versus LTA.





Disclosures:

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The following people have nothing to disclose: Sumeet K. Asrani, James F. Trotter, Goran Klintmalm

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Malnutrition in liver transplant recipients is associated with poor outcomes after orthotopic liver transplantation: A potential target for pre transplant intervention

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Background and Aims: Malnutrition affects between 40-80% of patients awaiting orthotopic liver transplantation (OLT), and is associated with increased morbidity and mortality after major surgery. This study investigates the impact of malnutrition on clinical outcome after OLT. **Methods:** This is a retrospective review of prospectively maintained data on 390 adult patients undergoing OLT between January 2009 and June 2016. Patients with fulminant liver failure or those requiring re-transplantation were excluded. Nutritional status was assessed by subjective global assessment (SGA) and functional muscle assessment by handgrip strength (HS) and 6-minute walk test (6MWT). All assessments were made at the time of wait listing, with SGA and HS repeated at OLT. Study outcomes evaluated included length of stay (LOS) in ICU, overall hospital LOS, episodes of infection until hospital discharge, episodes of rejection and biliary strictures within 90 days, readmission within 90 days as well as graft and patient survival. **Results:** The final analysis included 321 patients (69% male, mean age 52.2±11.3years). Malnutrition (SGA B+C) was identified in 67% of patients at time of being placed on the wait list for OLT. There was a progressive decline in nutritional status following a median waiting time of 4.4 months with 77% of patients being malnourished at the time of OLT. Of these 18% (n=58) were severely malnourished (SGA C). Malnutrition at wait listing was significantly associated with severity of liver disease (higher MELD and Child-Pugh scores), reduced HS and 6MWT results (all $p<0.05$). Severe malnutrition at OLT was associated with longer ICU LOS (147hrs vs 89hrs, $p=0.008$), increased hospital LOS (40d vs 16d, $p<0.01$) and increased incidence of infection (55.2% vs 33.8%, $p=0.014$) when compared to well-nourished patients. Occurrence of rejection ($p=0.536$), biliary anastomotic strictures ($p=0.126$) and readmission ($p=0.766$) were not associated with presence of malnutrition at OLT. Overall patient survival at 5 years was 90%, and not associated with nutritional status ($p=0.271$). **Conclusion:** This study validates the high prevalence of malnutrition in patients undergoing OLT and is the largest study to date demonstrating the effect of nutritional status and muscle function on early post-OLT morbidity and mortality. Aggressive strategies to combat malnutrition and deconditioning in the pre-transplant period may lead to improved patient outcomes and economic benefits after OLT.

Disclosures:

Peter W. Angus - Advisory Committees or Review Panels: Gilead Sciences, BMS, Bayer; Grant/Research Support: Gilead sciences

Adam Testro - Grant/Research Support: Qiagen

The following people have nothing to disclose: Brooke Chapman, Su Kah Goh, Sarah Romero, Jacqueline Luke, robert m. jones, Vijayaragavan Muralidharan

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Post-Transplant Outcomes from the Multi-Center American Consortium of Early Liver Transplantation for Alcoholic Hepatitis (ACCELERATE-AH) Study

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INTRODUCTION A European protocol showed that early liver transplant (LT) in severe alcoholic hepatitis (AH) could improve survival with low incidence of alcohol use post-LT. While controversial, more U.S. centers are undertaking LT for this indication. ACCELERATE-AH includes 12 centers from 8 UNOS regions studying outcomes in early LT for AH. **METHODS** Consecutive patients with a new diagnosis of severe AH who underwent LT prior to 6 months of abstinence between 2006 to 2017 were included. All were evaluated by an addiction specialist during LT evaluation. AH was defined clinically (jaundice, prolonged INR, chronic and recent alcohol use, no prior diagnosis of liver disease or episodes of AH). Survival and alcohol use post-LT, defined as a "slip" (any use, followed by sobriety) or sustained alcohol use, were assessed at 1 and 3 years post-LT. **RESULTS** Among 147 LT recipients (71% male, 83% Caucasian, 66% privately insured) with median abstinence pre-LT of 55 days (IQR 36-91), 54% received steroids for AH pre-LT. Median Day 7 Lille score was 0.82 (IQR 0.56-0.97) and LT MELD was 39 (IQR 35-40). Median post-LT follow-up was 1.6 years (IQR 0.8-3.0). Among 141 discharged home, 101 (72%) had alcohol abstinence, 25 (18%) had slips only, and 15 (11%) had sustained use. Cumulative probability of alcohol use was 25% and 34% at 1 and 3 years post-LT. Median time to first drink was 160 days (IQR 79-346) post-LT. In univariate analysis, lack of self-admission at hospitalization (vs. involuntary), lack of complete acceptance of diagnosis, history of alcohol-related legal issues, and younger age were associated with alcohol use, but only lack of self-admission at hospitalization (OR 4.1, 95% CI 1.9-9.0, p<0.001) was predictive in adjusted models. Cumulative post-LT survival was 94% and 84% at 1 and 3 years. Predictors of death were: >10 drinks per day prior to presentation, any alcohol use post-LT, and sustained alcohol use post-LT. In multivariate analysis, only any alcohol use post-LT was associated with death (HR 3.9, 95% CI 1.2-13.0, p=0.03). Of 18 deaths, 9 occurred within 3 months of LT: 8 of these 9 had received steroid therapy (p=0.04 vs. no steroids), of which 5 died of sepsis. The other 9 deaths occurred more than 1-year post-LT; 7 were alcohol-related. **CONCLUSIONS** In this multi-center retrospective cohort of patients with severe AH, early LT achieved excellent short-term survival. However, alcohol use was present in 25% within 1-year post-LT, and the majority of deaths beyond the 1st year were alcohol-related. These results highlight the need to improve pre-LT prognostic assessment for alcohol use, and to develop strategies to prevent and treat alcohol use post-LT.

Disclosures:

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Michael R. Lucey - Grant/Research Support: AbbVie, Gilead, Hologic

Mary E. Rinella - Advisory Committees or Review Panels: Intercept, Abbvie; Consulting: Intercept, Novartis, NGM, Shire, Fibrogen

Norah Terrault - Advisory Committees or Review Panels: Dynavax, Gilead; Consulting: Conatus, BMS, Novartis, Merck, Intercept; Grant/Research Support: Biotest, Vertex, Gilead, AbbVie, Merck, BMS, Eisai

The following people have nothing to disclose: Brian P. Lee, Neil Mehta, Laura Platt, John P. Rice, Gene Y. Im, Hyosun Han, David W. Victor, Oren K. Fix, Lisanne Dinges, Christine C. Hsu, Michael D. Voigt, Haripriya Maddur, Sheila Eswaran, Jessica Hause, David P. Foley, Jennifer L. Dodge, Zhiping Li

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To study efficacy of CRRT on ammonia clearance and mortality in the Acute liver Failure patients- A single centre observational study

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Background and Aim: Hyperammonaemia leads to cerebral edema (CE) and has significant clinical consequences in ALF.There is scarce data on efficacy of CRRT(Continuous Renal Replacement Therapy)in ammonia reduction & outcome. This study aimed to assess efficacy of CRRT on ammonia clearance at 24 hours & outcome. **Methods:** Consecutive adult ALF with arterial ammonia >150lmol/L were prospectively enrolled & given Standard medical therapy(SMT) & CRRT[Group 1] or only SMT [Group 2]. Arterial ammonia measured at baseline and 24 hours after starting of CRRT. The primary endpoint was survival at 28 days. **Results:**Fifty-three patients with ALF were enrolled. Fourty-five patients underwent CRRT+SMT(aged31.6±8 years, 25(55.55%males) and8 patients only SMT(aged 27.7±9.2,3(37.5%) males). Viral hepatitis(44%)was the most common etiology. Hyperacute ALF was the most common presentation(67.92%). Baseline disease severity scores(MELD, SOFA, APACHE)&hemodynamics were comparable between groups. Pre and Post CRRT(at 24 hrs) ammonia (lMol/L) in Group 1 were 249±149 and 145±58; p<0.001:In group 2 Pre & Post CRRT(at 24 hrs) ammonia(lMol/L)167±14.5 &124±31, p=0.02. Similarly Pre and post CRRT lactate in Group 1Vs group 2(3.3±0.92 & 2.7±0.68,; p=0.72. Vs 6.3±4.5 &6.1±4.4, viz; p=0.18. 28-day survival(30/45% vs 7/8 %, log rank p=0.22) were not different between the two groups. **Conclusion:** CRRT is efficient at ammonia clearance in patients with ALF, but this is not translated into better outcomes. The results of the present study would need to be confirmed in larger cohort of patients with acute liver failure.

Disclosures:

The following people have nothing to disclose: Chetan Kalal

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Increasing Acceptance of Severe Acute Alcoholic Hepatitis as an Indication for Liver Transplantation with Outcomes comparable to Fulminant Hepatic Failure

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Background: Although severe acute alcoholic hepatitis (AAH) is a controversial indication for proceeding with liver transplant (LT) surgery, AAH may have similar outcomes to fulminant hepatic failure qualifying for Status 1A. We aim to evaluate and compare liver transplant outcomes in patients waitlisted for AAH versus those waitlisted with Status 1A. **Methods:** Using the United Network for Organ Sharing (UNOS) database between 2011 to 2016, we analyzed all adult LT candidates and recipients listed for AAH or Status 1A including drug-induced liver injury due to acetaminophen (DILI-APAP) and non-DILI. Demographics, clinical characteristics and regional differences were also compared among the cohorts. Competing risks analyses were performed to determine 90-day rates for waitlist mortality and LT. In addition, Kaplan-Meir survival analysis was performed to evaluate post-LT survival. Statistical significance was reached with a $p < 0.05$. **Results:** From 2011-2016, 193 LT candidates were listed for AAH, of which 145 (75.1%) underwent liver transplantation. There was no significant difference in 90-day waitlist mortality in AAH candidates compared to those with Status 1A (Table). Since 2013, an increasing trend in the number of AAH listings and transplants was observed, with 2016 accounting for over 30% of total number of AAH listings and transplants at 28 LT centers. Nearly 40% of AAH LT occurred in UNOS Regions 2 and 7. Compared to Status 1A LT recipients, AAH recipients had a significantly higher prevalence of males (69.0%) and Hispanics (16.6%). In addition, 90-day LT rate was statistically higher in AAH than DILI-APAP (p <0.001). AAH 1-year and 3-year post-LT survival was comparable to DILI-APAP (p =0.10) but significantly higher than non-DILI ($p < 0.001$). In a sub-analysis comparing LT outcomes in AAH to chronic alcoholic liver disease (ALD), AAH was associated with superior 1- and 3-year post-LT survival (p <0.001) **Conclusion:** The recent rise in the number of LT surgeries performed in patients with AAH is suggestive of an increased willingness from LT centers to list AAH candidates. Our results demonstrate that patients with AAH have comparable early post-LT outcomes to those transplanted for Status 1A.

Comparison of Liver Transplant Outcomes in AAH versus Status 1A in the US from 2011-2016.

	AAH	DILI-APAP	Non-DILI	P
Waitlist Registrants	193	314	1405	—
LT Recipients	145	142	1,016	—
90-Day Waitlist Mortality	15.0%	19.7	20.1	0.21
90-Day Transplant Rate	61.1%	36.0%	63.8%	< 0.001
1-Year Post-LT Survival	93.3%	87.7%	85.4%	< 0.001
3-Year Post-LT Survival	93.3%	80.8%	81.4%	< 0.001

Disclosures:

Aijaz Ahmed - Consulting: Bristol-Myers Squibb, Gilead Sciences Inc., Roche, AbbVie, Shire, Janssen pharmaceuticals, Intercept; Grant/Research Support: Gilead Sciences Inc., Intercept

The following people have nothing to disclose: George Cholankeril, Andy Liu, Jeevin Sandhu, Eric R. Yoo, Andrew A. Li, Puneet Puri, Shiv K. Sarin

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Over-expression of c-Met in bone marrow mesenchymal stem cells improves their effectiveness in homing and repair of acute liver failure

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Background: Transplantation of bone marrow mesenchymal stem cells (BMSCs) has emerged as a novel therapy for acute liver failure (ALF). However, the homing efficiency of BMSCs to the injured liver sites appears to be poor. In this study, we aimed to determine if over-expression of c-Met in BMSCs could promote the homing ability of BMSCs to the livers of rats with ALF, thereby improving their capability in repairing ALF. **Methods:** Over-expression of c-Met in BMSCs (c-Met-BMSCs) was attained by transfection of naive BMSCs with the lenti-c-Met-GFP. The impact of transplanted c-Met-BMSCs on both homing and repair of ALF was evaluated, compared with lenti-empty vector transfected BMSCs (control BMSCs). **Results:** After cells were transfected with the lenti-c-Met-GFP vector, the BMSCs displayed high expression of c-Met protein as demonstrated by Western blot. In addition, *in vitro* transwell migration assays showed that the migration ability of c-Met-BMSCs was significantly increased in comparison with that of control BMSCs (P<0.05), and was dependent on HGF concentration. Furthermore, The ALF rats transplanted with c-Met-BMSCs had significantly higher survival rates than ALF rats treated with control BMSCs (83.3% vs 50%, P<0.01) ; this was accompanied by elevated homing ability to the injured liver (as shown in Fig 1) and liver function in the ALF rats transplated with c-Met-BMSCs. Parallel pathological examination further confirmed that transplantation of c-Met-BMSCs ameliorated liver injury with hepatic activity index (HAI) scores reduced, and that the effects of c-Met-BMSCs were more profound than those of control BMSCs. **Conclusions:** Over-expression of c-Met promotes the homing of BMSCs to injured hepatic sites in a rat model of ALF, thereby improving the efficacy of BMSCs therapy for ALF repair.

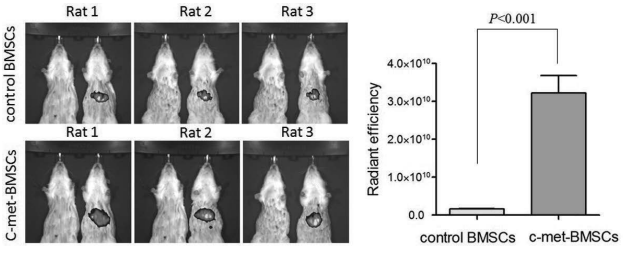

Fig 1: Enhanced ability of homing in c-Met-BMSCs to the liver of rats with ALF

Disclosures:

The following people have nothing to disclose: Chuanlong Zhu, Kun Wang, Yuwen Li, Yongting Zhang, Wenting Li, Wenyu Lin, Jun Li

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Early Predictors of Mortality in Children with Acute Liver Failure with Jaundice to Encephalopathy Interval Less than 7 days

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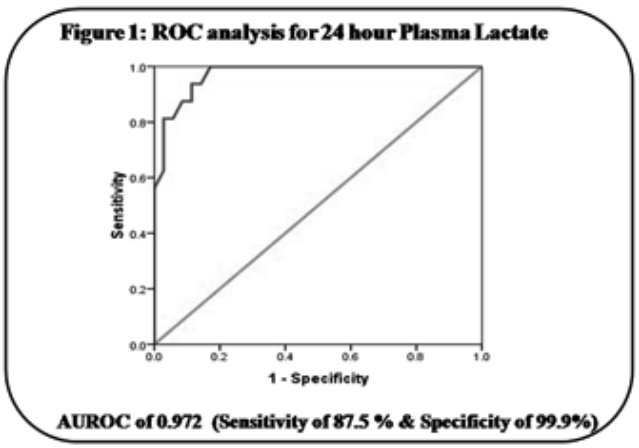
Aim: To identify the predictive risk factors for poor outcome (death/liver transplant) in children with pediatric acute liver failure (PALF) with jaundice to encephalopathy interval (JEI) of less than seven days. There is very limited data regarding this. **Methods:**Conducted between January 2011 to March 2017. Clinical, baseline laboratory parameters and their evolution at 12,24 and72 hrs was recorded. Liver injury Unit score (LIU), King's College Criteria (KCC), Pediatric End Stage Liver disease Score (PELD) was calculated at baseline and 72 hrs. Children were divided into two groups-Group 1:Survival with native liver and Group 2:Death or Liver transplantation. Outcome was assessed at 4 weeks. **Results:**Out of 140 patients of PALF, there were 51 children were with JEI less than 7 days, 55% boys, mean age 9.6yrs ±5.1 yr(0.5-18 yrs). Mean JEI was 2.5±1.5days(0-6 days). Hepatitis A was commonest etiology in 57%. Sixty eight percent (35/51) survived with native liver, 27.4% (14/51) children died and 2 were transplanted. Thirty-five children(68%) presented with grade 3 or 4 hepatic encephalopathy (HE) with features of raised intracranial pressure (ICP), out of which 57% (20/35) survived with native liver. Predictors of mortality on univariate analysis included non viral etiology(p=0.005), INR at 24 hrs(p<0.001), plasma lactate at 24 hours(p<0.001) and LIU score(p<0.001). On multivariate analysis, plasma lactate at 24 hour above 2.9 mmol/L was the most significant predictor of poor outcome with AUROC of 0.972 (sensitivity of 87.5% and specificity of 99.9%), (Adjusted OR 4.91, 95% CI 3.42 - 6.40, p<0.0001) **Conclusion:** Sixty eight percent of children with PALF with JEI less than 7 days survive with native liver. More than half (57%) the children presenting with raised ICP along with HE grade 3 /4 survived with native liver. High plasma lactate at 24 hrs after admission is the best predictor of poor outcome in this group



Disclosures:

The following people have nothing to disclose: Arti Pawaria, Seema Alam, Vikrant Sood, Rajeev Khanna

35
Molecular Adsorbent Recirculating System (MARS) Can Reduce Short-term Mortality among Patients with Acute-on-chronic Liver Failure

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Background: Acute-on-chronic liver failure (ACLF) is associated with numerous consecutive organ failures and a high short-term mortality rate. Molecular adsorbent recirculating system (MARS) therapy has demonstrated beneficial effects on the distinct symptoms, but the associated mortality data remain controversial. We intended to generate new hypotheses that would form the basis for better management strategies and interventions to reduce the high mortality rate associated with ACLF. **Methods:** This analysis was conducted in two parts: First, 101 patients with ACLF grade 1-3 and CLIF-C-OF liver subscore=3 but stable pulmonary function were identified and received either standard medical treatment (SMT, n=54) or SMT and MARS (n=47) at the University Hospital Muenster. Second, the results of this retrospective analysis were tested against the data from the prospective randomized RELIEF trial by applying the recently introduced CLIF-criteria. **Results:** Additionally to improved laboratory parameters (bilirubin and creatinine), the short-term mortality (up to day 14) of the MARS group was significantly reduced compared with SMT. A reduced 14-day mortality rate was observed in the MARS group (9.5% *vs.* 50.0% with SMT, p=0.004), especially in patients with multi-organ failure (ACLF grade 2-3). Concerning the affected organ system, this effect of MARS on mortality was particularly evident among patients with increased kidney, brain or coagulation CLIF-C-OF subscores. Subsequent reanalysis of the RELIEF dataset with adoption of the CLIF-classification resulted in similar findings. **Conclusion:** MARS treatment was associated with an improved short-term survival of patients with ACLF and multi-organ failure. Among these high-risk patients, MARS treatment might bridge to liver recovery or liver transplantation.

Disclosures:

Vicente Arroyo - Advisory Committees or Review Panels: Baxter TheraMARS; Speaking and Teaching: GRIFOLS

Rafael Banares - Advisory Committees or Review Panels: abbvie, gilead, baxter, janssen; Grant/Research Support: gambro; Speaking and Teaching: gilead, bms, janssen

The following people have nothing to disclose: Hans U. Gerth, Michele Pohlen, Gerold Thoelking, Iyad Kabar, Hartmut H. Schmidt

36

Role Of Bedside Ultrasound Guided Measurement Of Optic Nerve Sheath Diameter In Children With Acute Liver Failure – A Prospective Observational Pilot Study.

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Background: Early detection of intracranial hypertension (ICH) by non-invasive methods can improve the outcome in acute liver failure (ALF). Our study evaluated (i) feasibility and role of ultrasound (USG) guided measurement of optic nerve sheath diameter (ONSD) in children with ALF and healthy controls (ii) correlation between ONSD and hepatic encephalopathy (HE), international normalized ratio (INR) and blood ammonia (BA). ***Methods***: Twenty four children with ALF [21 boys, 14 (5–18 years)] and 15 healthy children [8 boys, 13(5.5–18 years)] were enrolled. Demographic, clinical and laboratory parameters were recorded. INR, BA and ONSD were measured at admission, at time of change in clinical grades of HE and at recovery. ALF patients were grouped into 4 groups- (A) no HE, INR>2, (B) HE I-II, (C) HE III-IV and (D) recovery (no HE, INR <2). Healthy controls underwent single ONSD measurement. ***Results***: Acute viral hepatitis was the commonest etiology in 75% of children. Forty six recordings of ONSD were done in 24 ALF cases [median 2 (1–3)]. ONSD was measured in all without adverse effects. Median ONSD was 3.9 (2.8–4.4) mm in healthy controls, 3.3 (2.25–4.35) mm in group A, 5.38 (4.2– 7.35) mm in group B, 5.52 (4.1–8.24) mm in group C and 4.22 (2.2–6.05) mm in group D (Figure 1). ALF cases with HE (group B and C) had significantly higher ONSD than controls, ALF without HE (group A) and ALF at recovery (group D). There was no significant difference in ONSD in ALF with HE I-II versus HE III-IV [5.38 (4.2–7.35) versus 5.52 (4.1–8.24) mm; p= 0.4]. ONSD showed significant correlation with BA (r=0.38, p=0.008) and INR (r=0.34, p=0.02). Intraclass correlation co-efficient for ONSD readings was 0.97. ***Conclusions:*** Bedside measurement of ONSD is feasible, safe and has excellent inter observer reliability in children with ALF. ONSD is significantly higher in ALF with HE than ALF without HE, healthy controls and ALF at recovery. ONSD shows positive correlation with INR and blood ammonia levels.

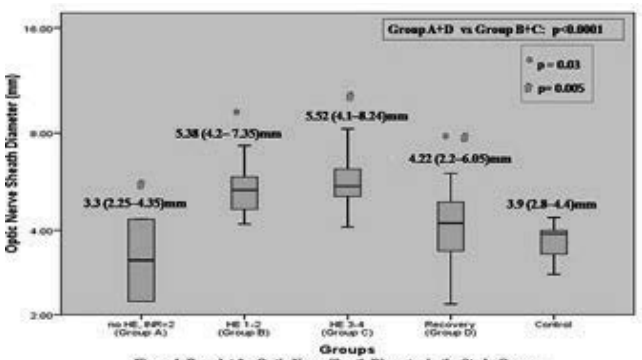

Figure 1: Box plot for Optic Nerve Sheath Diameter in the Study Groups

Optic nerve sheath diameter in children with acute liver failure and healthy control

Disclosures:

The following people have nothing to disclose: Mridul C. Das, Anshu Srivastava, Rajanikant Yadav, Surender K. Yachha, Ujjal Poddar

37

Interim results of a multicenter, open-label phase 2b clinical trial to assess safety and efficacy of Myrcludex B in combination with Tenofovir in patients with chronic HBV/HDV co-infection

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Introduction: Currently there are no specific treatment regimens for Hepatitis D Virus (HDV) infected patients. Myrcludex B (MyrB) is a first-in-class entry inhibitor exerting its antiviral function by blocking the jointly used HBV/HDV receptor sodium taurocholate co-transporter NTCP. In a preceding Phase 2a trial we showed that monotherapy with 2mg MyrB induced HDV RNA decline of >1log in 6/7 patients at w24 and demonstrated synergism with peg IFNα. Here we present interim results of a Phase 2b clinical trial on 120 chronically HBV/HDV co-infected individuals receiving 2, 5, and 10mg MyrB daily in combination with TDF or TDF alone. Methods: 120 HBeAg-negative patients with chronic Hepatitis D were randomized in four treatment arms. Treatment with TDF 245mg/day started not less than 12w prior to MyrB. MyrB was administered s.c. once daily at 2 (A), 5 (B) or 10mg (C) for 24w followed by a 24w period continuing TDF. Patients in arm D received TDF alone. The primary endpoint was HDV RNA negativation or decrease by ≥2log10; secondary endpoints include ALT normalization, AEs, blood bile acids levels and MyrB-antibodies. Results: Safety: MyrB was generally well tolerated with 118 drug related AEs (mild n=90, moderate n=25, severe n=3), mainly temporary injection site reactions and lab abnormalities. From six reported SAEs 5 were unrelated to the study drug and 1 was ALT elevation during follow up. Two SAEs led to study medication withdrawal. Efficacy: At w12, HDV RNA levels declined in all groups treated with MyrB at any dose with mean reductions from BL by 1.24log10 IU/ml in A (n=16), 1.40log10 IU/ml in B (n=18) and of 1.74log10 IU/ml in C (n=17). TDF treated patients (D) showed no serum HDV RNA reduction (0.09log10 IU/ml, n=17). While all patients with elevated ALT levels in the MyrB arms showed a pronounced decline

of ALT-levels (with complete normalization in 7/21 (A), 5/21 (B), and 9/22 (C)) no such tendency was observed for the TDF arm. At w24 HDV serum RNA levels further declined in B (1.63log10 IU/ml; n=7) and C (2.42log10 IU/ml; n=10) regardless of previously normalized ALT. No further decline of HDV RNA was observed in D (0.015log10 IU/ml (n=9)). HBsAg showed no significant changes in all arms. MyrB induced elevations of conjugated bile salts. Conclusion: Administration of MyrB was safe and well tolerated even at doses saturating NTCP (5mg and 10mg). The dose-dependent HDV RNA decline was accompanied by a pronounced tendency of ALT normalization after 12weeks. As expected for an entry inhibitor HDV RNA declined by an elimination kinetic in most patients. Accordingly, HDV negativation may be expected upon prolonged treatment durations.

Disclosures:

Heiner Wedemeyer - Advisory Committees or Review Panels: Falk, Abbvie, Novartis, Roche Diagnostics, Eiger, Janssen, GSK, Transgene, MSD, Roche, Gilead, Abbott, BMS; Consulting: MyrGmbH; Grant/Research Support: Roche Diagnostics, Novartis, Gilead, Roche, Abbott, MSD; Speaking and Teaching: Abbvie, Gilead, BMS, MSD, Novartis, ITF

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Antje Blank - Grant/Research Support: Myr GmbH, Hepatera; Independent Contractor: Myr GmbH

Anita Pathil - Advisory Committees or Review Panels: AbbVie, Alexion; Speaking and Teaching: AbbVie, BMS, Gilead

Mathias Haag - Grant/Research Support: Robert Bosch Foundation, German Center for Infectious Research (DZIF)

Matthias Schwab - Consulting: Astra Zeneca; Grant/Research Support: Robert Bosch Foundation, Deutsches Zentrum für Infektionsforschung, Germany, Lilly Pharma, Robert Bosch GmbH

Walter E. Haefeli - Board Membership: Aqua Institute Göttingen; Consulting: PIQUR Basel; Grant/Research Support: Sumaya Biotec GmbH & Co. KG, Klaus Tschira Stiftung, Vaximm GmbH, German Federal Ministry of Education and Research, BMBF, grant no. 01GK0702, Actelion GmbH, GSK GER/UK, CHIESI GmbH, Smooth ClinicalTrials Ltd., BMBF (DZIF, ESTHER), BayerPharma AG; Speaking and Teaching: Aspen Europe GmbH, GSK GER/UK/Slovakia/France/, Daiichi Sankyo GmbH, Bristol-Myers Squibb GmbH, MSD Sharp & Dohme GmbH, BayerPharma AG, Pfizer GmbH, Berlin-Chemie AG; Stock Shareholder: Dosing GmbH, Heidelberg

Stephan Urban - Advisory Committees or Review Panels: Gilead; Consulting: Gilead, Humabs; Patent Held/Filed: Myr-GmbH, University Hospital Heidelberg, INSERM; Stock Shareholder: StraTarHep-UG, Gilead

The following people have nothing to disclose: Pavel Bogomolov, Birgit Bremer, Natalia Voronkova, Katrin Schöneweis, Patrick Lehmann, Jürgen Burhenne

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Modeling hepatitis delta virus dynamics during ritonavir boosted lonafarnib treatment–the LOWR HDV-3 study

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Background & Aim. Interferon alpha therapy for chronic hepatitis delta (HDV) infection is unsatisfactory. The prenylation inhibitor lonafarnib (LNF) has proven anti-HDV activity in early phase clinical trials. A phase 2 LOWR HDV-3 study with an all-oral combination of once-daily ritonavir (RTV) boosted LNF was reported safe and tolerable in patients for up to 6 months of therapy. Here we sought to provide insights into HDV-host dynamics and LNF+RTV efficacy using mathematical modeling on LOWR HDV-3 study samples. **Methods:** HDV patients were randomized in LOWR HDV-3 into one of six groups: LNF 50/75/100mg + RTV 100mg once daily for 24 weeks (n=12) or 12 weeks of placebo followed by LNF 50/75/100mg +RTV 100mg once daily for 12 weeks (n=9). Since frequent viral kinetics only were available in the 24 week arm of LNF 50/75/100mg + RTV 100mg, the 12 patients in this arm were included in the modeling analysis. All patients were treated with hepatitis B nucleotide analogues. A mathematical model that includes hepatocyte proliferation was used to estimate HDV kinetic parameters and LNF+RTV effectiveness in blocking viral production. **Results:** Four different viral kinetic patterns were identified in each dosing group: (i) a triphasic decline consisting of a first phase with rapid virus load decline, followed by a "shoulder phase" in which virus load decays slowly or remains constant, and a third phase of renewed viral decay, (ii) a flat partial response (FPR), consisting of a first phase with rapid virus load decline followed by a lower set point of viral load), (iii) a rebound, in which FPR or triphasic kinetic patterns were observed followed by a rebound in viral load (due to varying effectiveness of drug) and (iv) non-response (patients were excluded from modeling). Modeling results indicate a delay [median t_0 = 8.50 (IQR: 16) days], in which viral load remained at pre-treatment levels, and that LNF+RTV had a 95% (IQR: 21%) efficacy in blocking HDV production, regardless of LNF dose. Median HDV and infected cells' half-lives ($t_{1/2}$) were estimated as 1.7 (IQR: 0.03) days and 1.2 (IQR: 0.94) days, respectively. Viral rebound is explained by a decline in LNF+RTV efficacy from ~95% to ~50% after 28-137 days post initiation of therapy. Modeling suggests cure (defined as less than one virus in the patients' extracellular body fluid) might have been achieved by increasing duration of therapy to 51 wk, 54 wk, and 94 wk for the 3 triphasic responders. **Conclusions:** Modeling results show high antiviral efficacy (95%) with LNF + RTV and suggest that response guided therapy of longer duration may result in continued anti-HDV activity and viral clearance.

Disclosures:

Ohad Etzion - Advisory Committees or Review Panels: HepQuant LLC

Cihan Yurdaydin - Advisory Committees or Review Panels: Janssen, Roche, Gilead, AbbVie, Merck; Speaking and Teaching: BMS

Harel Dahari - Grant/Research Support: Eiger BioPharmaceuticals

The following people have nothing to disclose: Preeti Dubey, Christopher Koh, Pallavi Surana, Susan L. Uprichard, Ma Ai Thanda Han, Nancy Fryzek, Devika Kapuria, Varun K. Takyar, Scott Cotler, Theo Heller

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SB 9200 an oral selective immunomodulator is safe and efficacious in treatment-naïve, non-cirrhotic HBV patients: Results from Cohort 1 of the ACHIEVE trial.

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SB 9200 is a novel oral selective immunomodulator which targets the host pattern recognition receptors Retinoic Acid Inducible Gene (RIG-I) and Nucleotide Oligomerization Domain protein 2 (NOD2) to activate innate and adaptive immunity. SB 9200 at 400mg daily for 7 days in HCV genotype 1 patients reduced HCV RNA by > 0.9log10 in 30% of patients and was considered equivalent to PEG-IFN. SB 9200 is currently being evaluated in the ACHIEVE trial, a double-blind, placebo-controlled phase 2 study in HBV treatment-naïve patients to identify the optimal dose of SB 9200 as both monotherapy and in combination with tenofovir 300mg daily. PATIENTS: 20 treatment-naïve non-cirrhotic HBV patients were randomized 4:1 to SB 9200 25mg daily or placebo for 12 weeks. Primary end points were safety and antiviral response defined by reduction in HBV DNA at week 12. There were M 12: F 8, mean age 40.5 years with 18 Asians and 2 Caucasians. 11 were HBeAg-positive and 9 HBeAg-negative, genotype A 2, B 9, C 7 and D 2. Baseline viral burden was higher in HBeAg+ (mean HBV DNA 7.1 log10; mean quantitative HBsAg 4.38 log10) compared to the HBeAg- patients (mean HBV DNA 5.5 log10; mean quantitative HBsAg 3.18 log10). RESULTS: There were no clinical, hematological or biochemical SAEs and no interferon-like side effects. 11 patients had treatment-emergent AEs and there was no difference in the number or type of AEs between SB 9200 or placebo patients with the most common (20%) being non-specific GI complaints of constipation, abdominal pain, nausea and diarrhea. All AEs were graded mild to moderate. Three patients had ALT flares > 200 IU. Two placebo patients had viral flares including an HBeAg-neg reversion to HBeAg-pos; the other flare was immune-related at week 4 in a patient on SB 9200 who had an associated 2.32 log10 reduction in HBV DNA and a 1.01 log10 reduction in HBsAg. All 3 patients were dose-reduced to every other day. At week 12, mean change in HBV DNA was -0.58 log 10 in SB 9200 compared to +0.37 log10 in placebo patients ($p = 0.014$). HBV DNA reduction was greater in SB 9200-treated HBeAg-neg patients (mean -0.86 log10) compared to HBeAg-pos patients (mean -0.37 log10). Overall 5 of 16 patients (31%) had a maximal >0.5log10 reduction in HBsAg (range 0.52 – 1.01 log10). CONCLUSION: Low-dose SB 9200 (25mg daily) demonstrated safety and anti-viral activity in treatment-naïve patients consistent with activation of innate immunity and potential suppression of cccDNA. The greater response in HBeAg-neg patients versus HBeAg-pos patients is possibly related to the effect of viral burden at such a low dose of SB 9200.

Disclosures:

Carla S. Coffin - Advisory Committees or Review Panels: Janssen, GSK; Grant/Research Support: BMS, Gilead Sciences, Roche

Magdy Elkhashab - Advisory Committees or Review Panels: Abbvie Inc, Gilead Sciences, Merck; Grant/Research Support: Gilead Sciences, Abbvie Inc, Intercept, EISAI, Roche, Celgene, Genfit, Shire, Spring Bank, Janssen

Alnoor Ramji - Advisory Committees or Review Panels: BMS, Merck, Gilead, Janssen, Lupin, Intercept; Grant/Research Support: BMS, Abbvie, Merck, Gilead, Intercept, Novartis; Speaking and Teaching: Celgene, Merck, Gilead, Abbvie

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Radhakrishnan P. Iyer - Employment: Spring Bank Pharmaceuticals, Inc

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The following people have nothing to disclose: Man Fung Yuen, Susan Greenbloom, Won Kim

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HBcrAg, HBV-RNA declines in A Phase 2a Study Evaluating the Multi-dose Activity of ARB-1467 in HBeAg-Positive and Negative Virally Suppressed Subjects with Hepatitis B

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Background: ARB-1467 is a novel RNA interference product consisting of three synthetic double-stranded, small interfering RNAs directed against hepatitis B virus (HBV) messenger RNAs, targeting three distinct sites in the HBV genome. ARB-1467 is designed to inhibit viral replication, reduce all HBV transcripts, and lower all viral antigens. Hepatitis B core-related antigen (HBcrAg) and HBV-RNA have been suggested as additional markers of HBV infection and used as surrogate for ccc-DNA. **Methods:** Serum was analyzed for levels of HBV DNA (qPCR; LOD 20 IU/mL), polyadenylated full-length HBV RNA (qRT-PCR; LLOQ 10IU/mL), quantitative HBsAg (Architect, LLOQ 0.05 IU/mL), and HBcrAg (Lumipulse; dynamic range 3–7 log10 IU/mL), from Baseline (BL) through Day85. 24 subjects on stable nucleoside therapy enrolled in 3 cohorts (Table below). Subjects were randomized 3:1 (active vs. placebo) and received ARB-1467 as three monthly IV doses. **Results:** BL characteristics were similar for the 3 cohorts; 19 (79%) male, mean age 45.5 yrs and mean BL HBsAg (log_{10} IU/mL): 3.46; 3.38; 3.62 and 3.43 in Cohorts 1, 2, 3 and placebo, respectively. HBcrAg and HBsAg reductions were greater with successive doses (Table below), and 6/11 subjects achieved HBsAg reductions >1 log. HBcrAg (cohort 3) and HBV-RNA testing

are ongoing. Treatment was well tolerated, one SAE not related to ARB-1467. **Conclusions:** On treatment HBcrAg and HBsAg reductions were observed following multiple doses; declines after three monthly 0.4 mg/kg doses were slightly greater than at 0.2 mg/kg for both markers. Further studies are needed to determinate the utility of HBcrAg for monitoring the response to novel CHB treatment.

Cohort	ARB-1467 (mg/kg)	HBeAg		Multiple Dose HBcrAg Reduction (log10 IU/mL)			Multiple Dose HBsAg Reduction (log10 IU/mL)		
			N	Mean(a)	Mean Max(b)	Max(c)	Mean(a)	Mean Max(b)	Max(c)
1	0.2	Neg	6	-0.2	-0.2	-0.6	-0.6	-0.7	-1.3
2	0.4	Neg	5(d)	-0.3	-0.3	-1.2	-0.8	-0.9	-1.1

(a)The mean serum HBsAg/HBcrAg reduction is the nadir value of the arithmetic mean of all values observed at each time point (b)The mean max HBsAg/HBcrAg reduction is the mean of each patient's max reduction in serum HBsAg/HBcrAg (c)Max HBsAg/HBcrAg reduction is the best single reduction among all patients in a cohort (d)4 subjects with HBcrAg data

Disclosures:

Kosh Agarwal - Advisory Committees or Review Panels: Merck, AbbVie, Gilead; Grant/Research Support: BMS, Gilead; Speaking and Teaching: Gilead, Merck, Abbvie

Edward J. Gane - Advisory Committees or Review Panels: Alios, Merck, Janssen, Gilead Sciences; Board Membership: AbbVie; Speaking and Teaching: Gilead Sciences, Merck, Alnylam, AbbVie

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Stuart K. Roberts - Board Membership: AbbVie, Gilead, Bayer, MSD

Yoon Jun Kim - Grant/Research Support: Bristol-Myers Squibb, Roche, JW Creagene, Bukwang Pharmaceuticals, Handok Pharmaceuticals, Hanmi Pharmaceuticals, Yuhan Pharmaceuticals; Speaking and Teaching: Bayer HealthCare Pharmaceuticals, Gilead Science, MSD Korea, Yuhan Pharmaceuticals, Samil Pharmaceuticals, CJ Pharmaceuticals, Bukwang Pharmaceuticals, Handok Pharmaceuticals

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William Symonds - Employment: Arbutus

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The following people have nothing to disclose: Wendy Cheng, Sang Hoon Ahn

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Immunological assessment of HBeAg-negative chronic hepatitis B patient responses following anti-PD-1 treatment.

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Background: Checkpoint blockade inhibitors have shown efficacy in relieving *in situ* T cell dysfunction in established tumour settings. Chronic Hepatitis B infection is associated with T cell dysfunction mediated in part by the PD-1:PD-L1 axis. This study investigated: the *in vivo* durability of PD-1 occupancy by nivolumab; HBV-specific T cell frequency and functionality; and peripheral blood subset composition in patients treated with nivolumab alone or in combination with a therapeutic vaccine. **Methods:** In this Phase I study (GS-US-330-1938) patients received a single dose of nivolumab at 0.1 mg/kg (n = 2) or 0.3 mg/kg (n = 12) or received vaccine GS-4774 at baseline and GS-4774 and 0.3mg/kg nivolumab at Week 4 (n = 10). Through weeks 0-24 of this study: a 19-parameter flow cytometric panel was used to assess changes in the frequency and activation status of immune cell subsets in patient blood; pan-T cell and subset-specific PD-1 receptor occupancy (RO) by nivolumab was determined using a 9-parameter flow cytometric assay; and HBsAg- and HBcAg-specific T cell responses were determined by *ex vivo* IFNγ/TNFα FluoroSpot. **Results:** In direct *ex vivo* FluoroSpot assays 18/24 patients exhibited T cell responses to either HBsAg or HBcAg peptide libraries, with 15/24 patients exhibiting responses to both. One patient treated with 0.3mg/kg nivolumab-only exhibited a decline in HBsAg from 1173IU/ml (baseline) to undetectable (week 20), concomitant with a significant increase in peripheral HBsAg-specific T cells (week 24; ANOVA; Bonferroni post-test *p=<0.001)* and marked increases in circulating $CD8^+$ $CCR7^-$ $CD45RA^-$ T_{EM} (15.4% at baseline, 30% at week 24); $CD8^+$ $CCR7^-$ $CD45RA^+$ T_{EMRA} (5.34%, 26.4%); $CD8^+$ $CD57^+$ (17.6%, 48.9%) and $CD4^+$ $CCR7^-$ $CD45RA^-$ T_{EM} (11.3%, 41%). PD-1 RO was retained at ≥6 weeks post-infusion in all patients assessed, with a subset of patients showing prolonged occupancy to ≥12 weeks post-infusion. Mean total T cell RO across 0.1 and 0.3 mg/kg cohorts weeks 1-6 post-infusion was 72.75% (95% CI 73.64, 76.85). No significant differences in maximal RO (*p=0.839)* or in RO duration were observed between 0.1 and 0.3 mg/kg cohorts. RO was subset-dependent and was most pronounced on T_{EM} and T_{EMRA} populations. **Conclusions:** Single-dose nivolumab treatment at up to 0.3mg/kg resulted in sustained PD-1 occupancy for a minimum period of 6 weeks post-infusion, and in complete HBsAg clearance in one patient. HBsAg- and HBcAg-specific T cell responses could be detected in a majority of patients tested. Anti-PD-1 treatment may be associated with temporal changes in peripheral T cell subset composition.

Disclosures:

Anuj Gaggar - Employment: Gilead Sciences, Inc.

Jacky Woo - Employment: Gilead Science

Mani Subramanian - Employment: Gilead Sciences; Stock Shareholder: Gilead Sciences

Christian Schwabe - Grant/Research Support: Assembly Bioscience

Edward J. Gane - Advisory Committees or Review Panels: Alios, Merck, Janssen, Gilead Sciences; Board Membership: AbbVie; Speaking and Teaching: Gilead Sciences, Merck, Alnylam, AbbVie

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Pharmacokinetics and exploratory exposure-response of siRNAs administered monthly as ARB-001467 (ARB-1467) in a Phase 2a study in HBeAg positive and negative virally suppressed subjects with chronic hepatitis B

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Background: ARB-1467 is a novel lipid nanoparticle small interfering RNA (LNP siRNA) product directed against hepatitis B virus (HBV) messenger RNAs (mRNAs), targeting

three distinct sites in the HBV genome. ARB-1467 is designed to inhibit viral replication, reduce all HBV transcripts, and lower all viral antigens. As previously reported, monthly dosing in subjects with chronic hepatitis B was generally well tolerated and HBsAg declines >1 $\log_{10}$ were observed. Methods: ARB-001467-002 is single-blind, placebo-controlled, multi-dose study in non-cirrhotic, virally suppressed subjects to evaluate safety and efficacy of ARB-1467 over 12 weeks. Subjects (n=24) on stable nucleoside therapy were enrolled in 3 cohorts, randomized 3:1 (active:placebo): Cohort 1, HBeAg- at 0.2mg/kg; Cohort 2, HBeAg- at 0.4mg/kg; Cohort 3, HBeAg+ at 0.4mg/kg. Subjects received ARB-1467 as 3 monthly IV infusions and were monitored for safety, pharmacokinetics (PK) and HBV markers. Serial blood samples for PK analysis of each siRNA were collected up to 168h post-infusion after the 1st dose, and either the 2nd or 3rd. Plasma concentrations were obtained by validated ELISAs. Noncompartmental PK were derived and summary statistics prepared by cohort and dose number. Relationships between maximum concentration (Cmax) and area under the concentration-time curve (AUC(0-t)) vs. HBsAg decline were explored graphically. Results: The PK of each of the siRNAs were characterized by Cmax at the end of infusion with a marked drop in concentration over 6-8h post-dose; a secondary peak 24-36h post-dose was common. Each of the siRNAs showed comparable plasma concentrations, consistent with equimolar siRNA loading within the LNP. Representative PK for one (D6-11) of the siRNAs are shown for Dose 1 (Table). Accumulation of siRNA in plasma was negligible. Cmax and AUC(0-t) values were modestly greater than dose proportional. There were no clear differences in PK between HBeAg+ and HBeAg- subjects. Mean exposures and mean HBsAg decline in HBeAg- subjects were greater with 0.4mg/kg vs. 0.2mg/kg, but no meaningful trends between individual exposure and response were evident. Conclusions: siRNA PK following dosing with ARB-1467 was modestly greater than dose proportional from 0.2 to 0.4mg/kg. Differences in individual patient response were not well explained by plasma PK. Patient- and disease-specific factors affecting response require further evaluation.

Panel Dose # (dose) [N]	Cohort 1 Dose 1 (0.2 mg/kg) [6]	Cohort 2 Dose 1 (0.4 mg/kg) [6]	Cohort 3 Dose 1 (0.4 mg/kg) [6]
Cmax [ng/mL] Geometric Mean (CV%)	420 (30)	1303 (31)	973 (32)
AUC(0-t) [ng*h/mL] Geometric Mean (CV%)	14292 (101)	38183 (80)	31926 (54)

CV% = coefficient of variation

Disclosures:

Timothy Eley - Employment: Arbutus Biopharma

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Edward J. Gane - Advisory Committees or Review Panels: Alios, Merck, Janssen, Gilead Sciences; Board Membership: AbbVie; Speaking and Teaching: Gilead Sciences, Merck, Alnylam, AbbVie

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Yoon Jun Kim - Grant/Research Support: Bristol-Myers Squibb, Roche, JW Creagene, Bukwang Pharmaceuticals, Handok Pharmaceuticals, Hanmi Pharmaceuticals, Yuhan Pharmaceuticals; Speaking and Teaching: Bayer HealthCare Pharmaceuticals, Gilead Science, MSD Korea, Yuhan Pharmaceuticals, Samil Pharmaceuticals, CJ Pharmaceuticals, Bukwang Pharmaceuticals, Handok Pharmaceuticals

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Candidate Liver Safety Biomarkers Provide Prognostic and Mechanistic Insights in Patients with Drug-Induced Liver Injury

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Background: Traditional biomarkers of drug-induced liver injury (DILI) are not mechanistically informative and demonstrate suboptimal performance as predictors of outcome highlighting a need for newer, candidate biomarkers. **Methods:** Candidate biomarkers were quantified in serum samples collected by the DILI Network (DILIN) within two weeks of DILI onset from 145 patients adjudicated as at least probably having DILI (>50% likelihood). Outcome was considered "adverse" if patients required a liver transplant/died within 6 months as a result of their DILI episode (n=17). The following were examined as predictors of adverse outcome: total keratin 18 (K18), caspase cleaved K18 (ccK18), alpha fetoprotein (AFP), osteopontin (OPN), glutathione S-transferase alpha, fatty acid binding protein 1 (FABP1), cadherin 5, arginase 1, and macrophage colony stimulating factor receptor (MCSFR) determined by immunoassay. A subset of patients in this cohort (n=27) had liver biopsies obtained within two weeks of DILI onset and serum collection, and that were blindly interpreted by the DILIN pathologist (DK). Histopathological findings reported from these biopsies were correlated with the biomarker measurements. **Results:** Logistic regression demonstrated that elevated levels of K18, ccK18, AFP, OPN, FABP1, and MCSFR were each significantly predictive of adverse DILI outcome (p<0.05). The serum "apoptotic index", the ratio of ccK18 to K18, was also inversely correlated with adverse outcome (p=0.003). While the international normalized ratio (INR) was the best single predictor of adverse outcome (ROC AUC=0.922), OPN was the second best predictor (AUC=0.871). Multiple forward regression resulted in a predictive model with predictors of INR, total bilirubin, aspartate aminotransferase, OPN and K18 (AUC=0.97). This model was more specific (specificity=0.98 based on predicted probability cutoff=0.19) than "Hy's Law" and Model for End-Stage Liver Disease (MELD) > 20 (specificities=0.64 and =0.73, respectively). Some biomarkers were also correlated with histopathological findings reported from the biopsies. The degree of inflammation was significantly correlated with MCSFR (p<0.05) while the extent of coagulative/confluent necrosis was significantly correlated with AFP and OPN (p<0.01 and 0.001, respectively). The semi-quantitative score of necrosis was inversely correlated with apoptotic index, a ratio of ccK18

to K18 (p<0.05). **Conclusion:** Incorporation of candidate biomarkers with traditional measurements improved six month DILI outcome prediction. In addition, some of the new biomarkers may provide a "liquid biopsy" to assess degree of inflammation and mode of hepatocyte death.

Disclosures:

Gerd A. Kullak-Ublick - Independent Contractor: Novartis Pharma

Herbert L. Bonkovsky - Advisory Committees or Review Panels: Recordati Rare Chemicals, Clinuvel, Inc.; Consulting: Alnylam, Inc, Clinuvel, Inc., Mitsubishi-Tanabe Pharma; Grant/Research Support: Gilead Sciences

Naga P. Chalasani - Consulting: Abbvie, NuSirt, Lilly, Afimmune, Allergan/Tobira, Axovant, Madrigal, Shire, Ardelyx; Grant/Research Support: Intercept, Cumberland, Lilly, Gilead, Galectin

Robert J. Fontana - Consulting: Abbvie, CLDF, Alnylam; Grant/Research Support: BMS, Gilead

Michael Merz - Employment: Novartis Institutes for Biomedical Research

Paul B. Watkins - Consulting: Abbott, Actelion, Boerringer-Ingelheim, Cempra, Alecra, Roche, GSK, Merck, Reservlogix, Astra Zeneca, Intercept, Janssen, Novartis, Otsuka, Pfizer, Sanolfi, Takeda, Bristol-Myers Squibb, UCB

The following people have nothing to disclose: Rachel J. Church, David E. Kleiner, Jens C. Goepfert, Thomas Knorpp, Thomas Schindler, Shelli Schomaker

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Genome-wide association study (GWAS) to identify genetic risk factors that increase susceptibility to anti-tuberculosis drug-induced liver injury (ATDILI).

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Background: Anti-tuberculosis drugs (ATD) isoniazid, rifampicin and pyrazinamide are among the most reported causes of drug-induced liver injury (DILI). Previous candidate gene studies, focused on drug metabolising enzyme gene variant association with anti-tuberculosis DILI (ATDILI), have, however, yielded contradictory results. Aim: To identify genetic risk factors for ATDILI by a genome-wide association study (GWAS). Methods: Patients who developed ATDILI and ethnically matched controls were enrolled in Bangalore and Vellore, South India. Roussel Uclaf Causality Assessment Method was used for case adjudication. Samples were genotyped with the Illumina Human Core Exome BeadChip, data was phased by SHAPEIT and single nucleotide polymorphisms were imputed by IMPUTE2. We tested for association by logistic or linear regression models and set the genome-wide significance threshold to $5x10^{-8}$. In view of previous reports on association of ATDILI with N-acetyltransferase 2 (NAT2) phenotype, we predicted NAT2 acetylator status using genotypes for NAT*5, *6 and *7 alleles and tested for association by Fisher's Exact test. Results: We analysed 59 Indian cases (50% male; mean age 40 years) of ATDILI with 220 ethnically matched controls (65% male; mean age 40 years) including 111 patients treated with ATD without DILI and 109 healthy adults. The total DILI cohort was enriched in cases with severe liver injury with 15 (25%) patients developing acute liver failure and/or died; 22 (37%) patients had early onset of DILI (average latency was 45 days). The case-control GWAS did not demonstrate any genome-wide significant association between TBDILI and imputed or genotyped variants. The same negative outcome was obtained when we restricted the analysis to cases with acute liver failure or when we considered the latency as quantitative phenotypes in a case only study design. Regarding NAT2, we found that 40 cases (68%) were slow acetylators compared with 127 controls (58%) but this increased frequency was not statistically significant (Odds ratio 1.54 (95% CI 0.84-2.84); p=0.18). Only 60% of severe cases were slow acetylators - the group was not more enriched in NAT2 variant alleles than other groups. Conclusions: Standard GWAS analysis did not identify any high penetrance variants associated with either susceptibility or severity of ATDILI and we did not replicate the previously published NAT2 genetic association. The lack of a positive outcome could be due to the relative low case/control ratio that limits the study power and the complexity of having several culprit drugs involved in the phenotype, with each having individual drug-specific genetic risk factors.

Disclosures:

Guruprasad P. Aithal - Advisory Committees or Review Panels: Aegerion; Consulting: A Star D3, Agios, Shire

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*Multiple HLA B*57 alleles, sharing the amino acid residue Valine[97], are associated with drug-induced liver injury due to flucloxacillin in a European population.*

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Background: In Europe, flucloxacillin is a widely prescribed beta-lactam antibiotic but its use is associated with serious liver injury in approximately 1 in 12,000 patients. This liver

injury shows strong genetic susceptibility driven by the *HLA-B*57:01* allele with carriers at 80 times higher risk. **Methods**: DILIGEN/iDILIC consortia collected 197 North European flucloxacillin-induced liver injury (Flu-DILI) cases between 2005 and 2013. The cases, matched with 6835 population controls, were genome-wide genotyped and single nucleotide polymorphisms, HLA alleles and amino acid residues were predicted to confirm and further investigate risk factors located within and outside the major histocompatibility complex (MHC) region. **Results**: Our genome-wide association study confirmed that *B*57:01* is the major risk factor (odds ratio (OR) = 36.62; 95% CI [26.14-51.29]; P = $2.67*10^{-97}$) with a carriage frequency of 84%. Other HLA alleles belonging to the *B*57:01* haplotype, like *C*06:02*, *DQB1*03:03*, *DRB1*07:01*, *DQA1*02:01* and *A*01:01*, also showed a genome-wide significant risk effect while *C*07:02*, *B*07:02* and *DQB1*03:01* were protective. Haplotype analysis showed that B*57:01-containing haplotypes confer risk while *B*57:01*-omitting haplotypes seems to be protective, suggesting that *B*57:01* and no other alleles within the haplotype is the main risk factor. *B*57:01* conditional analysis revealed that *B*57:03* was the most significant independent risk factor (OR = 79.21; 95% CI [13.57-462.4]; P = $1.2*10^{-6}$). Interestingly, valine in position 97, a residue shared by several B*57 alleles including B*57:01, B*57:03 and B*57:02, had the highest effect size (OR = 38.1, 95% CI [27.07-53.62], P = $9.7x10^{-97}$). Conversely, arginine^{97} and serine^{97} have significant protective effects (OR= 0.43 P= $5.13*10^{-14}$ and OR= 0.53, P=$9.82*10^{-7}$). Serine^{97} is a characteristic residue for the protective B*07:02. No other signal was identified outside the MHC region. **Conclusion**: We found that *B*57:01* and *B*57:03* were significant independent risk determinants for Flu-DILI. The shared amino acid valine^{97} showed the strongest risk association, while arginine^{97} and serine^{97} showed a genome wide significant protective effect. Abacavir hypersensitivity is associated only with *B*57:01,* but appears to have a different mechanism from Flu-DILI involving alteration of the peptide repertoire with aspartate^{114} and serine^{116}. The novel association between Flu-DILI and valine^{97} could help explain the apparently different mechanisms involved in the adverse reactions to these two drugs.

Disclosures:

Guruprasad P. Aithal - Advisory Committees or Review Panels: Aegerion; Consulting: A Star D3, Agios, Shire

Anke H. Maitland-van der Zee - Advisory Committees or Review Panels: Astra Zeneca; Grant/Research Support: GSK

Mariam Molokhia - Grant/Research Support: SAEC

The following people have nothing to disclose: Paola Nicoletti, Sally Coulthard, Raul Andrade, Bjornsson S. Einar, Jennifer H. Martin, Mia Wadelius, Yufeng Shen, Ann K. Daly

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Sensitive detection of human drug-induced liver injury by measurement of microRNA-122 using single molecule arrays

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Background Drug-induced liver injury (DILI) is a major challenge in clinical medicine and drug development. MicroRNA-122 (miR-122) is a sensitive and specific biomarker for hepatocyte injury that has received formal regulatory support. A major roadblock to its widespread adoption is a lack of a fit-for-purpose, scalable assay that can provide quantification of circulating miR-122 with appropriate accuracy and time-to-result. We have developed a PCR-free, sensitive and highly specific assay for measuring miR-122. The assay is based on the hybridization of miR-122 to a peptide nucleic acid (PNA) probe attached to superparamagnetic beads, followed by the specific incorporation of a biotinylated nucleobase. The biotin labels are then labeled with an enzyme, and single enzymes are detected using single molecule arrays (Simoa). Methods A specific 18-mer abasic PNA probe that was complementary to miR-122 was conjugated to 2.8-micron diam. superparamagnetic beads. These beads were then incubated with sample, an aldehyde-modified cytosine base that contains biotin, and a reducing agent. The beads were then labeled with streptavidin-beta-galactosidase and analyzed on a Simoa HD-1 Analyzer to determine average number of enzymes per bead (AEB). Calibrators containing a synthetic target were also analyzed to determine the concentration of miR-122. Serum samples from patients with DILI secondary to acetaminophen and healthy controls were collected and tested using Simoa and PCR with full informed consent and appropriate ethical approval. All patients with DILI had serum alanine transaminase activity greater than 5 x ULN. Results The miR-122 single molecule assay had a limit of detection (LOD) of 500 fM, approximately 500 times more sensitive than a corresponding analog bead-based assay. The specificity of the assay to a single base mismatch in the microRNA sequence was >10^7-fold ((highest concentration of mismatched target with signal below LOD)/(LOD)). As measured by our single molecule assay, all patients with DILI had higher concentrations of miR-122 in their serum compared to healthy controls, and the concentrations of miR-122 measured correlated closely with those determined using the gold standard PCR (r^2 = 0.93; slope = 0.64). Discussion This novel single molecule assay enabled the accurate measurement of miR-122 in the serum of patients with DILI. This assay could have utility in clinical practice as it has the potential to deliver the user-independent sensitivity and time-to-result that are needed to inform pre-clinical and clinical decision making.

Disclosures:

Juan Díaz-Mochón - Board Membership: DestiNA Genomics Ltd; Stock Shareholder: DestiNA Genomics Ltd

David Duffy - Employment: Quanterix Corporation; Management Position: Quanterix Corporation; Patent Held/Filed: Quanterix Corporation; Stock Shareholder: Quanterix Corporation

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Serum MicroRNA-122 Levels Are Highly Variable in a Cohort of Healthy Volunteers

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Background: Traditional biomarkers of drug-induced liver injury (DILI) such as alanine aminotransferase (ALT) present several challenges in the clinic including a lack of organ-specificity. Newer biomarkers, such as microRNA-122 (miR-122) and glutamate dehydrogenase (GLDH) are being evaluated as highly liver-specific biomarkers for DILI; however diagnostic utility depends on an understanding of the reference interval of these biomarkers in healthy volunteers. **Methods:** The Predictive Safety Testing Consortium collected serial serum samples (fasting, in the morning, on three days) from healthy subjects (n=81) over the course of 21 days. ALT (quantified only at baseline) and GLDH were measured by standard clinical chemistry parameters. miR-122 was analyzed from isolated RNA by reverse transcription quantitative PCR. Data was normalized to mmu-miR-293. Ct values and total copy numbers/μL were calculated. A mixed effect model for log transformed data was used to obtain the variance components for inter- and intra-subject variability. The two-tailed 95th percentile is given as the biomarker reference interval. **Results:** The majority of subjects in this study were white (84%). Five subjects had ALT levels mildly above the upper limit of normal (ULN); 4 of these individuals had ALT values that were ≤1.1X ULN while one subject had an ALT level that was 1.3X ULN. In all samples, GLDH measurements were above the corresponding lower limit of quantification (LLoQ) while multiple samples returned values below the LLoQ for miR-122. The geometric means, reference intervals, and variability of ALT, GLDH and miR-122 are given in **Table 1.** Because ALT was only quantified at baseline, intra-subject variability was not assessed for ALT. Both inter- and intra-subject variability of miR-122 were surprisingly high. The high intra-subject variability of miR-122 may have been in part related to race; variability was increased in non-whites (12 blacks, 1 Hispanic; %CV=213.95%) compared to whites (%CV=143.8%). In contrast, much less variability was observed in ALT and GLDH measurements. **Conclusion:** While miR-122 is a highly liver-specific miRNA, the large inter- and intra-subject variability observed in healthy subjects may limit its clinic utility. Instead, GLDH, which is also liver-specific, displayed much less subject variability. GLDH may therefore be a better candidate for a liver-specific biomarker, compared to miR-122.

Healthy Subject Biomarker Reference Intervals

Biomarker	Geometric Mean	Reference Interval	Inter-Subject variability (%)	Intra-Subject Variability (%)
ALT (U/L)	20.8	10-48.8	48	--
GLDH (U/L)	2.81	0.99-7.97	57.16	28.13
miR-122 (copies/ul)	2406.57	415.92-13924.81	110.92	203.84

Disclosures:

Gerd A. Kullak-Ublick - Independent Contractor: Novartis Pharma

Michael Merz - Employment: Novartis Institutes for Biomedical Research

Paul B. Watkins - Consulting: Abbott, Actelion, Boerringer-Ingelheim, Cempra, Alecra, Roche, GSK, Merck, Reservlogix, Astra Zeneca, Intercept, Janssen, Novartis, Otsuka, Pfizer, Sanolfi, Takeda, Bristol-Myers Squibb, UCB

The following people have nothing to disclose: Rachel J. Church, John-Michael Sauer, Jens C. Goepfert, Thomas Knorpp, Thomas Schindler, Shelli Schomaker

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Epidemiology of Severe HDS Induced DILI

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The US Acute Liver Failure Study Group reported that the proportion of acute liver failure cases due to HDS is higher among Asians in their cohort, but Asian race has not been reported as a risk factor for liver transplantation in severe HDS induced DILI in the US population. Cases collected by the US Drug Induced Liver Injury Network (US DILIN) have suggested that the incidence of DILI from herbal and dietary supplements (HDS) has increased in the past years. We examined data on US liver transplant recipients to identify potential risk factors for severe HDS induced DILI and to assess its temporal variance. We searched the Organ Procurement and Transplantation Network (OPTN) database for liver transplants performed between 1/1/1994 and 10/31/1994. We identified patients reviewed in this study were transplanted under the primary diagnosis of "acute hepatic necrosis" (AHN) – "Drug other specify". Three subcategories were identified: "AHN: Non HDS DILI, " "AHN: etiology HDS DILI, and "AHN: unknown causes. 905 patients who received transplant due to the above diagnoses were identified. We excluded "unknown causes" group (N=260) from our analysis. The remaining patients (N=645) were grouped under two subcategories (AHN- HDS DILI and AHN-Acetaminophen DILI). Each group was evaluated on the basis of Race/Ethnicity - Asian, Black, Hispanic, White and Others (includes American Indian/Alaskan native, multiracial, and native Hawaii/pacific islander). Chi-square statistic were calculated for each comparison. HDS induced DILI was a significantly more likely cause of liver transplantation in Asians than for other races. While the proportion of HDS-DILI leading to liver transplantation was much lower in Whites, the proportion has significantly increased in recent years between 1995-2005 vs 2005-2015(P =0.022). Conclusion - The results indicate a significant relationship between race/ethnicity and HDS induced liver injury resulting in liver transplantation. The data provides further evidence of increasing severe liver injury from HDS use over time.

Race and Liver Transplantation due to HDS-DILI

Race	HDS DILI	Non-HDS DILI
Asian	21% (5)	79% (19)
Black	4% (4)	96% (105)
Hispanic	7% (4)	93% (56)
White	5% (19)	95% (412)
Others	5% (1)	95% (20)

The chi-square statistic is 13.4 and the p-value is 0.0094.

Disclosures:

Joseph A. Odin - Advisory Committees or Review Panels: Intercept Pharma.

The following people have nothing to disclose: varun kesar, Jawad Ahmad

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Exosome biogenesis in alcoholic liver disease: Dumping out trash or strategic sorting in miR-155 dependent autophagy induction and alcohol-induced exosome release?

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Background and aims: Impaired autophagy and increased exosome production were reported in alcoholic liver disease (ALD). Because autophagy and exosome biogenesis have common elements, we hypothesized that disruption of autophagy flux regulates exosome release. We also evaluated the role of miR-155 targets, mTORC, Rheb and LAMP2, in the autophagy-exosome crosstalk. Our aim was to assess whether a molecular cross-talk between miRNA-155, autophagy, and exosome release contribute to ALD. **Methods:** C57BL/6 wild type (WT) and miR-155 deficient (KO) mice received Lieber De Carli 5% alcohol or control diet for 5 weeks. Kupffer cells (KC)/macrophages and hepatocytes were used in vitro for mechanistic studies. Human liver samples were from controls and patients with ALD. Samples were analyzed by RT-qPCR, western blotting and NanoSight. **Results:** We found that chronic alcohol feeding in WT mice resulted in abnormal autophagy indicated by increased LC3II and p62 levels. In contrast, miR-155 KO mice maintained functional autophagy demonstrated by lower LC3II and decreased p62 protein levels even after alcohol feeding. This was associated with a significant increase in circulating exosomes in WT but not in miR-155 KO mice after alcohol feeding raising the question whether exosome release was related to an impaired autophagy flux. In vitro, alcohol treatment (50mM, 24h) of KCs isolated from WT mice exhibited increased cellular release of exosomes with high levels of miR-155 and LC3II in the exosome cargo. Rheb and mTORC1, upstream regulators of autophagy, are also targets of miR-155. Transfection of a miR-155 mimic into Hepa1-6 hepatocytes or RAW267.4 macrophages significantly reduced mTOR and Rheb protein expression, increased LC3II and decreased p62 protein levels demonstrating a significant role of miR-155 in autophagy induction; miR-155 over-expression also resulted in a significant increase in exosome release. Human and mouse liver samples with ALD revealed increased miR-155 and decreased Rheb and mTOR protein levels, and autophagosome accumulation reflected by increased LC3II and p62 protein expression. Both human and mouse liver samples with ALD, but not miR-155 KO alcohol-fed mouse livers, showed significantly decreased LAMP2 protein expression suggesting impaired autolysosomal degradation in chronic alcohol exposure. **Conclusions:** We found that impaired autophagy flux promotes exosome biogenesis in macrophages and hepatocytes and that miR-155 regulates key elements of cellular autophagy and exosome biogenesis. Our observations suggest a cross-talk between miR-155 and cellular autophagy in the production of exosomes in ALD.

Disclosures:

Gyongyi Szabo - Advisory Committees or Review Panels: Cyta Therapeutics, DISCUS, GALAXY Project, Nature Reviews in Gastro & Hepatology, NIH ExRNA Program, Prevent Cancer Foundation, Yale University Liver Center, University of Colorado Alcohol Center, University of Southern California Liver Center, Verlyx, University of Pittsburgh, MSTP EAB; Board Membership: ACER; Consulting: Trek Therapeutics, Novartis, Orbimed, Roviant, Salix, Tobira, Glympse Bio, Janssen Research & Development, Carlos Foundation; Grant/Research Support: NIH-NIAAA, BMS, Gilead, Genfit, Genentech, University of Florida, Intercept, Tobira, Takeda, Vertex, Shire, Allergen

The following people have nothing to disclose: Istvan Furi, Terence N. Bukong, Shashi Bala, Patrick P. Lowe, Arvin Iracheta-Vellve, Karen Kodys, Donna Catalano

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Endothelial cell Wnts regulate β-catenin signaling in murine liver zonation and regeneration

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Background: Our lab has shown that hepatocyte-specific β-catenin regulates liver regeneration in a partial hepatectomy (Phx) model, under the control of canonical Wnt signaling. This is true at early timepoints up to 40–72 hours. Using cell-specific Wntless (Wls) knockout mouse models, which lack Wls and thereby prevent Wnt secretion, we have previously determined epithelial cells are not the source of these Wnts. However, macrophage-specific Wls knockouts (MP KO) have a moderate regeneration defect at 40 hours, suggesting MP contribute Wnts during liver regeneration. Endothelial cell (EC)-specific Wls knockout was embryonic lethal, preventing us from assessing EC contributions during LR. Further, the identity of Wnts and their initiation factors during liver regeneration remain elusive. *Methods:* We generated a liver sinusoidal endothelial cell (LSEC)-specific Wls knockout (EC KO) expressing Cre Recombinase under the Lyve-1 promoter to assess contributions of LSEC-specific Wnts. To identify the Wnts responsible for β-catenin-driven liver regeneration, we isolated hepatic MP and EC pre- and post-hepatectomy and assessed changes in Wnt transcription. Finally, we asked whether shear stress is an initiating factor leading to Wnt production, and subjected endothelial cells to orbital shear stress *in vitro* and analyzed Wnt expression levels. *Results:* In EC KO, recombination in the liver was limited to endothelial cells along the sinusoids, leaving central vein endothelial cells intact. Intriguingly, EC KO demonstrate a marked reduction in zonation markers Glutamine Synthetase, Cyp2e1, and Cyp1a2 at baseline, and were protected from acetaminophen-induced liver toxicity. After Phx, EC KO display remarkably less proliferation than littermate controls until 72 hours, overall emphasizing the role of endothelial cell-specific Wnts in liver regeneration. We identified Wnt2 and Wnt9b to be highly upregulated in macrophages and endothelial cells after Phx in wildtype animals. In the presence of Rspondin 3, Wnt2 and Wnt9b can induce β-catenin activity *in vitro*. Finally, we tested whether shear stress, evident immediately after Phx due

to increased portal pressure, can initiate Wnt production. We noted an increase in Wnt2 and Wnt9b mRNA in three separate endothelial cell types including primary liver endothelial cells *in vitro*. *Conclusion:* our data suggests macrophages and endothelial cells secrete Wnt2 and Wnt9b, initiated in part by shear stress early after Phx, to temporally regulate hepatocyte-specific β-catenin activation and liver regeneration.

Disclosures:

Paul Monga - Advisory Committees or Review Panels: abbvie, dicerna; Grant/Research Support: abbvie

The following people have nothing to disclose: Morgan E. Preziosi, Hirohisa Okabe, Minakshi Poddar, Sucha Singh

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Farnesoid X Receptor/Small Heterodimer Partner (FXR/SHP) Signaling Regulates the Inositol-Requiring Enzyme 1α/X-box Binding Protein 1 (IRE1α/XBP1) Pathway *in vivo* and *in vitro*.

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Background: The unfolded protein response (UPR) is an adaptive response to endoplasmic reticulum (ER) stress that occurs in cholestasis and other forms of liver disease. The inositol-requiring enzyme 1α/X-box binding protein 1 (IRE1α/XBP1) pathway is a UPR pathway that is activated by ER stress. Phosphorylated-IRE1α (p-IRE1α) regulates expression of the transcriptionally active XBP1 spliced (XBP1s) by an unconventional splicing mechanism that removes 26 nucleotides from unspliced XBP1. The liver farnesoid X receptor/small heterodimer partner (FXR/SHP) is a bile acid responsive pathway that regulates hepatic metabolism and injury; and FXR agonists may provide novel therapies for cholestatic and fatty liver diseases. In this study, we demonstrate a novel role of the hepatic FXR/SHP pathway in the regulation of IRE1α/XBP1 signaling. Methods: Male FVB and FXR(-/-) mice were fed chow; or chow supplemented with either 0.3% sodium deoxycholate (DCA) or 2% cholestyramine for up to 7 days. C57BL/6J mice were gavaged with the FXR agonist GW4064 (150 mg/kg) for 16 hours. HepG2 cells were treated with GW4064 (2.5 μM for 4 hours) or SHP (or scramble) siRNA; or were transfected with SHP-GFP cDNA (0-2 μg). Gene and protein expression was analyzed by qPCR and western blotting. P-IRE1α splicing activity of XBP1 was measured using an XBP1 splicing-luciferase reporter construct. Results: FVB mice fed DCA for 1 or 3 days had markedly increased hepatic nuclear XBP1s protein expression, which resolved by day 7. Similarly, C57BL/6J mice gavaged with GW4064 had increased hepatic nuclear protein and gene expression of XBP1s. In contrast, FXR(-/-) mice and FVB mice fed cholestyramine to shrink the bile acid pool had diminished hepatic *Xbp1s* gene expression. HepG2 cells treated with GW4064 had markedly increased protein expression of p-IRE1α and activation of the XBP1 pathway. Luciferase activity assaying XBP1 splicing by p-IRE1α also increased 1.8-fold by GW4064 treatment ($P<0.001$). Transfection of HepG2 cells with SHP-GFP cDNA resulted in dose-dependent increases in XBP1s and p-IRE1α protein expression. Finally, HepG2 cells treated with SHP (or scramble) siRNA demonstrated that SHP knockdown attenuated both basal and GW4064-stimulated expression of p-IRE1α and XBP1s. Conclusion: FXR signaling regulates the IRE1α/XBP1 pathway of the UPR *in vivo* and *in vitro*. FXR agonist-induced XBP1s expression is associated with increased p-IRE1α expression and splicing activity. This activation is regulated, at least in part, by SHP. We speculate that this FXR/SHP regulation is important for hepatic activation of the protective UPR pathway during cholestasis and liver injury.

Disclosures:

The following people have nothing to disclose: Xiaoying Liu, Grace L. Guo, Bo Kong, Brian E. LeCuyer, Susan C. Hubchak, Antony Hsieh, Li Wang, Richard M. Green

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Hepatocellular Mitochondrial Integrity is Dependent Upon the Anti-Viral Dynamin Family Member, MxB.

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The membrane deforming dynamin family members MxA and MxB are large GTPases that convey resistance to a variety of infectious viruses including influenza, HBV, and HIV. The expression of the Mx proteins is upregulated during infection via an interferon responsive promoter. MxB has been shown to associate with nuclear pores and the cytoplasmic viral genome where it is predicted to restrict access of the invading genome into the nucleus. We have found that MxB is highly expressed in primary hepatocytes where it associates with the nucleus and, most recently, with mitochondria. From these findings, **the GOAL of this Study** was to define the role of MxB in hepatocellular mitochondrial function. We find that expressing a mutant MxB protein or reducing MxB expression levels by shRNA or siRNA treatment results in a perinuclear aggregation of mitochondria and attenuated staining with antibodies to the cytochromes of the inner membrane. Concomitant with these changes we observe a near complete loss of mitochondrial membrane potential as assessed by the vital dye Rhodamine-123, and remarkably, a loss of the mitochondrial genome (nucleoids) into the hepatocyte cytoplasm. In support of these findings, electron microscopy of these altered cells shows a striking loss of the inner cristae of these mitochondria leaving hollow, non-functional, organelles. Through molecular analysis of the MxB protein we have identified a central 15 amino acid sequence that is essential for targeting MxB to the mitochondria. Importantly, this domain exhibits significant association with mitochondrial chaperones that are key in the import of nuclear encoded proteins into the mitochondria and normal maintenance of this organelle's genome. **In Summary**, this study has identified a novel distribution and function for the anti-viral dynamin protein MxB that, in concert with mitochondrial chaperones, appears to play an essential role in maintaining the normal integrity of the mitochondrial genome in hepatocytes. Further, MxB is likely to contribute to the innate immune response during viral infection by altering mitochondrial function and viability.

Disclosures:

The following people have nothing to disclose: Hong Cao, Jing Chen, Eugene W. Krueger, Mark A. McNiven

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Endothelium derived Notch signal promotes liver sinusoidal endothelial cell senescence and blunts angiocrine to hamper liver regeneration in an eNOS-Wnt2a dependent manner

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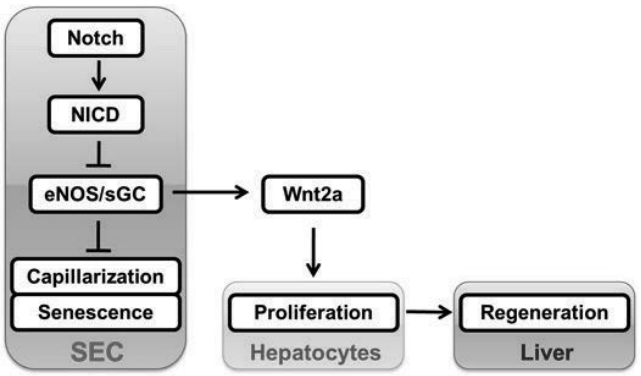
Background & Aims: Liver sinusoid endothelial cells (LSECs) play pivotal roles in liver homeostasis. Notch regulates vessel development. Disruption of Notch signaling results in hepatic vascular malformation. The current study aimed at further clarifying the roles and mechanisms of Notch signaling in LSECs in steady state and regeneration. **Methods:** Liver regeneration was induced by partial hepatectomy (PHx). Notch signaling was activated by inducible LSEC-specific expression of Notch intracellular domain (NIC) or a recombinant Notch ligand, and blocked with a g-secretase inhibitor. YC-1 was used to activate soluble guanylate cyclase (sGC). Morphological alterations were evaluated with electron microscopy, histochemistry and immunofluorescence. Gene expression was estimated with qRT-PCR and immunoblotting. **Results:** Notch signaling was reduced but reactivated later in LSECs after PHx. Reactivation of Notch coincided with increased senescence in LSECs. Pharmaceutical inhibition of Notch signaling in PHx-triggered liver regeneration reduced LSEC senescence, while Notch activation significantly increased LSEC senescence under steady state. Notch activation also induced LSEC capillarization, suggesting shared signaling pathways by LSEC senescence and capillarization. Indeed, Notch activation attenuated eNOS-sGC signaling that was essential for both of Notch activation-induced LSEC capillarization and senescence, as activation of sGC reversed these phenotypes. During PHx-triggered liver regeneration, LSEC-specific Notch activation attenuated LSEC regeneration and increased their senescence, and blunted hepatocyte regeneration as well. The latter was likely attributed to reduced Wnt2a and HGF production by LSECs in sGC-dependent and -independent ways, respectively. **Conclusions:** Notch activation in LSECs promotes LSEC capillarization and senescence by repressing the eNOS-sGC pathway, and reduces angiocrine secretion of Wnt2a and HGF that support hepatocytes.



Disclosures:

The following people have nothing to disclose: Lin Wang

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Identification of Signaling Pathways That Regulate Differentiation and Transcription in Foxl1+ Reactive Ductal Cells

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Background: Reactive ductules are frequently detected prior to and after the development of liver cancer in animal models. We have previously reported that forkhead box L1 (Foxl1)-Cre mice can be used for labeling of the epithelial components of ductular reactions, and Foxl1$^+$ cells can differentiate toward hepatocytes. One the other hand, our microarray data indicate that Foxl1$^+$ cells express multiple paracrine factors that promote angiogenesis, mitosis, and tumorigenesis, implying potential roles of Foxl1+ cells in liver regeneration and disease progression. The purpose of this study is to investigate the role of multiple signaling pathways in differentiation of Foxl1$^+$ cells and transcription of paracrine factors. **Methods:** Foxl1-Cre;RosaYFP mice (male, 6-8-week-old) were treated with the 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet for two weeks. We isolated YFP-labeled Foxl1+ cells using flow cytometry and established clonally expanded Foxl1+ cell line. Since it is well established that the Notch and Wnt pathways regulate differentiation of fetal hepatic cells during development, we investigated their relative roles in differentiation of reactive ductal cells by treating Foxl1+ cells with pharmacologic modulators of the selected pathways. **Results:** Immunostaining analyses indicated that Notch1 intracellular domain was detected in the nuclei of Foxl1+ cells while β-catenin was predominantly localized on the membrane/cytoplasm, suggesting different roles of the Notch and Wnt pathways in differentiation of Foxl1+ cells. Inhibition of the Notch pathway and activation of the Wnt pathway upregulated markers of hepatocyte differentiation. On the other hand, the conditioned medium prepared from Foxl1+ cells promoted proliferation of human umbilical vein endothelial cells and human HCC cells Hep3B. Analyses of putative cis-regulatory regions indicated that the promoters of paracrine factors expressed by Foxl1+ cells are significantly enriched for the NF-κB binding motifs. The vast majority of the paracrine factors expressed by Foxl1+ cells were upregulated in response to the NF-κB activators. **Conclusion:** Our data indicate that the Notch and Wnt signaling pathways regulate differentiation of Foxl1+ cells while the NF-κB signaling pathway regulates paracrine factor transcription. Future studies will evaluate the role of these pathways in Foxl1$^+$ cell-driven liver regeneration and tumorigenesis using animal models.

Disclosures:

The following people have nothing to disclose: Sanghoon Lee, Soona Shin

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Cardiovascular Risk in NAFLD- Not an Equal Opportunity: Implications for Women's Health

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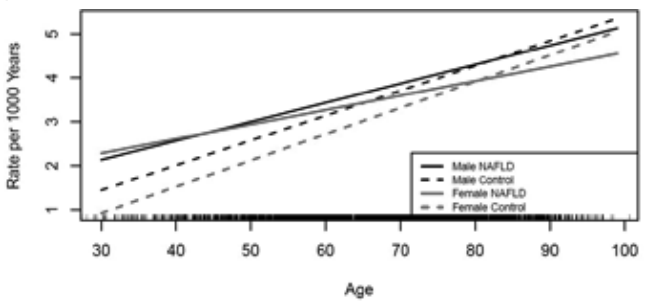
Background: Subjects with nonalcoholic fatty liver disease (NAFLD) are at higher risk for CV events. Female sex is a protective cardiovascular (CV) risk factor. We explored if sex-related differences in CV risk persist in NAFLD. **Methods:** A community cohort of all adults diagnosed with NAFLD in Olmsted County, MN between 1997-2014 was constructed using the Rochester Epidemiology Project database. Incident CV events (angina, myocardial infarction, heart failure, atrial fibrillation and stroke) after NAFLD diagnosis were identified. Using Cox regression analysis the CV risk in male (M) and female (F) NAFLD subjects referenced to a 4:1 cohort from the general population, matched for age, gender, diabetes, hypertension, hyperlipidemia, and stratified for baseline CV disease. **Results:** We identified 4,196 NAFLD subjects (median age 52, 52% women) and 15,786 controls; median follow-up was 7 (0 to 21) years. A total of 1684 CV events were identified. The risk of incident CV events was high in F (HR=1.21, 95% CI=1.08-1.36, p=0.001) but not in M (HR=1.08, 95% CI=0.97-1.21, p=0.17). In F with NAFLD the risk increased in all CV event types: angina (HR=1.55, 95% CI=1.27-1.89), myocardial infarction (HR=1.33, 95% CI=1.01-1.76), heart failure (HR=1.31, 95% CI=1.09-1.58) ($p<0.05$ for all), except atrial fibrillation and stroke. Consequently, the protective effect of F sex on CV events decreased from 24% in the general population (F:M HR =0.76, 95% CI=0.71-0.83, $p<0.001$) to 13% in NAFLD subjects (F:M HR=0.87, 95% CI=0.76-0.99, p=0.04). The CV events manifested at a younger age in NAFLD compared to controls (Figure). For example, the CV risk of a NAFLD F age 50 years is similar to that of a 58 yo M and a 62 yo F from the general population. **Conclusion:** NAFLD is associated with significantly higher incidence of subsequent CV events in women, but not in men. Compared to the general population, the CV events manifest at younger age. These findings suggest that CV risk stratification in NAFLD should consider sex-related differences, as women may require more aggressive preventative measures to avoid worse CV outcomes.



Disclosures:

Patrick S. Kamath - Advisory Committees or Review Panels: Sequana Medical, Gilead

Kymberly D. Watt - Stock Shareholder: Exel, Sgen, BMS

The following people have nothing to disclose: Alina M. Allen, Joseph J. Larson, Sharonne N. Hayes, Terry M. Therneau

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Natural history of nonalcoholic fatty liver disease (NAFLD) in children receiving standard lifestyle counseling and placebo in NASH Clinical Research Network (CRN) trials

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Background: Very little is known or published on the natural history of NAFLD in children. Since children develop NAFLD early, with histologic disease severity similar to adults, our aim was to determine significant factors related to histological outcome in children with NAFLD receiving lifestyle counseling over time. Methods: Paired liver biopsies (entry and end of treatment) were analyzed in 122 children with NAFLD, 8-17y old, enrolled in the placebo arms of two double-blind, randomized clinical trials at 10 NASH CRN centers from 2005-2015. Children received standard lifestyle counseling and placebo over 52 or 96 weeks. Histologic progression to definite NASH and increase in fibrosis ≥1 stage were analyzed with respect to baseline clinical, demographic and anthropometric data, changes in variables over time, and by adolescent (13-17y, n=62) vs. preadolescent (8-12y, n=60) age using ANCOVA and logistic regression models. Incident type 2 diabetes (T2D) was reported. Results: The cohort was 74% male, 64% white, 71% Hispanic, with mean age 13 ± 3y and BMI 32 ± 6 kg/m^2 (z-score 2.2 ± 0.4). At baseline, preadolescents had more bridging fibrosis (23% vs. 5%;p=.02) and borderline zone 1 NASH (57% vs. 13%), but less definite NASH (15% vs. 47%;p<.001) vs. adolescents. Over time, fibrosis improved in 34% and progressed in 23%, with no age/sex difference. None had cirrhosis at baseline (per protocol) or at follow-up. Borderline/definite NASH resolved in 29% (28/96). Progression to definite NASH occurred in 18% (15/84) and was associated with higher ALT (RR 3.3, 95%CI:1.3,7.9), AST (RR 6.6, 95%CI:1.3,33.0), GGT (RR 6.0, 95%CI:1.2,30.6), total cholesterol (RR 5.2, 95%CI:1.1,25.0), LDL cholesterol (RR 7.8, 95%CI:1.2,50.9) levels at baseline, and increasing BMI z-score over time (RR 3.9, 95%CI:1.0,15.0) [all $p<0.05$]. While not significant, hemoglobin A1C (RR 3.4, 95%CI: 1.0,11.4;p=.05) increased over time with NASH progression. White race (RR 3.3, 95%CI:1.1,9.3; p=.03), worsening ALT (RR 2.4, 95%CI:1.2,4.8;p=.01), GGT (1.1, 95% CI:1.0,1.1;p=.002) and A1C (RR 2.3, 95%CI:1.1,4.7;p=.03) were associated with

fibrosis progression. In all, 28% (34/122) had worsening in either fibrosis or NASH, while 7% (9/122) had both. T2D developed in 8%, an incidence rate (IR) of 44.3/1000 person years. Conclusion: Histologic severity of NAFLD worsened in almost 1/3 of children receiving standard lifestyle counseling over interval assessed. Children with NAFLD warrant close follow-up and more intensive intervention, particularly if BMI, ALT, GGT and glucose homeostasis worsen over time. High incidence of T2D, >300 fold the population IR in children, supports screening in these youth.

Disclosures:

Stavra Xanthakos - Advisory Committees or Review Panels: Target PharmaSolutions; Grant/Research Support: Raptor/Horizon Pharmaceuticals

Jean P. Molleston - Grant/Research Support: gillead, roche, vertex, none, abbvie, Shire/Lumena; Speaking and Teaching: none

Philip Rosenthal - Advisory Committees or Review Panels: Retrophin, Gilead, Alexion, Audentes, Intercept, Albireo; Consulting: Roche, Abbvie; Grant/Research Support: Roche, Bristol MyersSquibb, Gilead, Vertex, Abbvie, Roche/Genentech, Infacare, BMS; Speaking and Teaching: Retrophin

Karen F. Murray - Grant/Research Support: Gilead; Stock Shareholder: Merck

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Racial and Ethnic Disparities in Non-Alcoholic Fatty Liver Disease Prevalence, Severity, and Outcomes in the United States: A Meta-Analysis

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Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the U.S., although its burden may not be equally distributed across race/ethnicities. **Aim:** We conducted a systematic review and meta-analysis to characterize racial/ethnic disparities in NAFLD prevalence, severity, and prognosis. **Methods:** We searched MEDLINE, EMBASE, and Cochrane databases from inception through August 2016. Two investigators identified studies that reported NAFLD prevalence in population-based or high-risk cohorts, NAFLD severity including presence of nonalcoholic steatohepatitis (NASH) and significant fibrosis, and NAFLD prognosis including development of cirrhosis complications and mortality. Pooled relative risks, according to race/ethnicity, were calculated for each outcome using the DerSimonian and Laird method for a random effects model. We found no evidence of publication bias for NAFLD prevalence or severity by Egger's test or funnel plot inspection. The study was conducted in accordance with PRISMA guidelines. **Results:** We identified 34 studies with 368,569 unique patients that characterized disparities in NAFLD prevalence, severity or prognosis. Overall, Hispanics had higher risk of NAFLD than whites, with a pooled RR of 1.36 (95% CI 1.08-1.73), and whites had higher risk of NAFLD than blacks with a pooled RR of 1.48 (95%CI 1.19-1.84), although differences between groups were smaller in high-risk cohorts (range 47.6%-55.5%) than population-based cohorts (range 13.0%-22.9%). Among NAFLD patients, risk of NASH was higher in Hispanics (RR 1.09, 95%CI 0.98-1.21) and lower in blacks (RR 0.72, 95%CI 0.60-0.87) than whites. The overall pooled proportion of patients with NAFLD with significant fibrosis (stage F3-F4) was 19.5% (95%CI 18.1-20.9%). Significant fibrosis proportions were numerically highest in whites (22.3%, 95% CI 20.5-24.2%) and Hispanics (19.6%, 95% CI 16.0-23.0%) and lowest among blacks (13.1%, 95% CI 8.9-18.2%); however differences were not statistically significant (whites vs. blacks: RR of 0.91, 95% CI 0.82-1.00; whites vs. Hispanics: RR 1.02, 95%CI 0.94-1.11). Data were limited and discordant regarding racial/ethnic disparities in NAFLD prognosis. **Conclusion:** There are significant racial/ethnic disparities in NAFLD prevalence and severity in the U.S., with the highest burden in Hispanics and lowest burden in blacks; however, data are discordant regarding the presence of racial/ethnic disparities in prognosis among NAFLD patients.

Disclosures:

Neehar D. Parikh - Advisory Committees or Review Panels: Eisai, Bayer; Consulting: Bristol Meyer Squibb

Amit G. Singal - Advisory Committees or Review Panels: Roche, Bayer, Wako DIagnostics, EMD Serano, Eisai; Grant/Research Support: Gilead; Speaking and Teaching: Bayer

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Should Screening for Fatty Liver Be Conducted at Primary Care Clinics?

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Background: Fatty liver is quite prevalent among individuals living in developed countries. It correlates with the increase in prevalence of obesity, diabetes mellitus, and metabolic syndrome. Screening for fatty liver has been dependent on ultrasonography, which is not sensitive nor specific in assessing the degree of fat deposition or liver fibrosis. Over the last few years, hepatic elastography using FibroScan® has been gaining popularity in assessing hepatic fibrosis. More recently, fibroscans are capable to estimate the degree of fat deposition in the liver using controlled attenuation parameter (CAP). We initiated a screening program for fatty liver and liver fibrosis using FibroScan® in a primary care facility in Southern California. Patients attending primary care clinic who have no known history of liver disease were offered screening using the FibroScan® device. **Aim:** To estimate the prevalence of fatty liver and liver fibrosis in patients with no known history of liver disease in a primary care setting. **Methods:** Between March 6, 2017 and May 29, 2017, 1650 patients attending a primary care clinic, who have no known history of liver disease were asked to participate in the screening program. 856 individuals agreed to be screened and their demographics, past medical history and current medications were recorded, as well as their

laboratory tests. **Results:** Of the 856 patients, 554 (65%) were females; 302 (35%) were males. The mean age was 44.6 ±16.8 years. Hispanics were 77.1%, whites 6.4%, Asian 4.2% and others 1.6%. Twenty-three percent had a BMI >35 and 20% had BMI between 30-35. Diabetes was found in 12.9% and 13.4% had hyperlipidemia. 282 patients (33%) had significant fatty infiltration with a CAP >290 of which 4.3% had fibrosis score over 15 kPa; 12.0% between 7-15 kPa. **Summary:** In patients with no known history of liver disease attending a primary care clinic, 33% had significant fat infiltration of the liver; 16.7% had significant liver fibrosis. In conclusion; 1) Obesity (BMI >30) is prevalent (>40%); 2) fatty liver and liver fibrosis are prevalent (>30% and >16% respectively) among patients who are not aware of having liver disease; 3) FibroScan® is a powerful tool for screening individuals for chronic liver disease in the primary care setting.

Disclosures:

Tarek I. Hassanein - Advisory Committees or Review Panels: AbbVie Pharmaceuticals, Bristol-Myers Squibb, Trek Therapeutics; Grant/Research Support: AbbVie Pharmaceuticals, Obalon, Bristol-Myers Squibb, Eiasi Pharmaceuticals, Gilead Sciences, Merck Sharp & Dohme, NGM BioPharmaceuticals, Ocera Therapeutics, Salix Pharmaceuticals, Sundise, TaiGen Biotechnology, Vital Therapies, Tobria, Shinoghi & Co. Ltd, La Jolla Pharmaceuticals, Trek Therapeutics, Novo Nordisk, Intercept, GenFit, Norvartis, Shire; Speaking and Teaching: Baxter, Bristol-Myers Squibb, Gilead Sciences, Salix Pharmaceuticals, AbbVie Pharmaceuticals

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Prevalence and Clinical Characteristics of Nonalcoholic Fatty Liver Disease in Lean Subjects in Comparison with Overweight or Obese Individuals: A cross-sectional study

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Background/Aims: Although nonalcoholic fatty liver disease (NAFLD) is frequently diagnosed in obese or overweight subjects, approximately 10-20% of lean individuals also develop NAFLD. However, data on lean patients with NAFLD are scarce. The aim was to investigate the prevalence, clinical characteristics, and risk factors of NAFLD across populations with different body habitus. **Methods:** Out of 16,398 health check-up examinees between 2008 and 2016, we enrolled 12,002 subjects without known liver disease or significant alcohol consumption. The diagnosis of fatty liver was made using ultrasound examination. **Results:** Overall prevalence of NAFLD was 35.6% (n=4276). All subjects were categorized into three subgroups according to their body mass indexes; group 1 (BMI <23 kg/m2; n=5815), group 2 (23≤ BMI <25; n=2869) and group 3 (BMI ≥25; n=3318). The prevalence of NAFLD in each group was 13.3 % (n=772) in group 1, 42.5% (n=1231) in group 2, and 68.5% (n=2273) in group 3, respectively (*P*<0.001). Risk factors for the development of NAFLD in each group included the followings: age, female sex, less than 3 times of exercise per week, impaired fasting glucose, metabolic syndrome, hematocrit, uric acid, fat mass in group 3; age, female sex, less than 3 times exercise per week, metabolic syndrome, uric acid, fat mass in group 2; and age, female, impaired fasting glucose, HTN, metabolic syndrome, uric acid, fat mass in group 1. Among patients with NAFLD in groups, metabolic syndrome was present in 39.4% in group 1, 49.0% in group 2, and 67.8% in group 3, respectively (P <0.001). Mean C-reactive protein in group 1-3 was 1.25, 1.47, and 1.95, respectively (*P*<0.001). Mean NAFLD fibrosis score was -2.97, -2.77, and -2.46, respectively. Mean TyG index was highest in group 3 (3.89), followed by group 2 (3.83) and group 1 (3.78) (*P*<0.001). In patients who underwent transient elastography (n=161), median liver stiffness and controlled attenuation parameter were higher in group 3 (5.50 kPa; 248.1 dB/m) than in group 2 (4.18 kPa; 258.5 dB/m) and in group 1 (3.51 kPa; 280.0 dB/m), with *P*<0.001 for both parameters. For extrahepatic manifestations, mean of coronary calcium score was significantly higher in group 3 than in group 1 or group 2 (*P*<0.001); prevalence of chronic kidney disease (GFR <60 ml/min) was not different among group 1-3 (*P*=0.677*).* **Conclusions:** In this cross-sectional study, NAFLD in lean subjects (BMI <23) was not uncommon, albert less frequent than subjects with higher BMI. Noninvasive indices suggested less severe disease in terms of systemic inflammation, insulin resistance, liver fibrosis and extrahepatic manifestations.

Disclosures:

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The Long-Term Clinical Course of Histologically Advanced Nonalcoholic Fatty Liver Disease. Impact of Fibrosis Severity on Major Clinical Outcomes.

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BACKGROUND The natural course of nonalcoholic fatty liver disease (NAFLD) with advanced fibrosis has not been fully investigated. We aimed to describe the long-term clinical outcomes and influence of histological features and liver function on patients with NAFLD and advanced fibrosis. **METHODS** We conducted a multicenter, international (Spain, Australia, Hong Kong and Cuba) prospective cohort study of 458 NAFLD patients with biopsy-proven bridging fibrosis (F3=159) or compensated cirrhosis (Child-Pugh [CP] A5=222 and A6=77) who were followed between April 1995 and December 2016. Cox-proportional hazard and competing risk models were performed to define the cumulative incidence of major clinical outcomes and to identify risk factors associated with long-term prognosis. Follow-up began from the date of biopsy, and ended on the date of the last visit, death, or transplant. All analyses were adjusted by center, race/ethnicity, age, gender and fibrosis stage. **RESULTS** During a mean of 5.7 years (range, 2.7-8.2) of follow-up, there were 84 deaths/

transplants (41 deaths and 43 liver transplants), 90 initial hepatic decompensation events, 42 hepatocellular carcinoma (HCC), 14 major vascular events and 30 non-hepatic cancers (predominantly colorectal cancer [50%]). Most deaths were liver-related (35 of 43, 81%). Survival without transplant was 54% (F3, 83%; F4 + CP A5, 32%; F4 + CP A6, 0%; P<0.01). Among cirrhotic patients, a total of 115 (38%) first major events occurred, most commonly hepatic decompensation (81/115, 70%) and HCC (20/114, 17%). Among F3 patients, a total of 26 first events occurred, of which 10 (38%) and 9 (35%) were related to non-hepatic malignancies and vascular events. Cirrhosis increased the risk of death/transplant, decompensation, and HCC by 4.3, 4.5 and 3.9 folds respectively, but was associated with a lower risk of vascular events (sHR: 0.3), all P<0.05. Interestingly in the cirrhotic cohort, steatosis ≥33% but not lobular inflammation or ballooning was independently associated with better overall survival (HR: 0.44) and lower rates of liver-related complications (sHR for decompensation: 0.43; sHR for HCC: 0.32), however, it did not impact on development of vascular events. NAFLD fibrosis score (HR: 1.62 for death and sHR for HCC: 1.38) and FIB-4 index (HR: 1.34 for death and sHR for HCC: 1.17) were associated with highest risks of death/transplant and HCC, and these associations were evident even in cirrhotic patients. **CONCLUSION** The clinical course of advanced NAFLD is driven predominantly by liver-related adverse outcomes in patients with cirrhosis whereas non-hepatic malignancies and vascular events in patients with bridging fibrosis.

Disclosures:

Vincent W. Wong - Advisory Committees or Review Panels: Janssen, Allergan, AbbVie, Gilead; Consulting: Merck, NovaMedica; Speaking and Teaching: Gilead, Echosens

Jacob George - Advisory Committees or Review Panels: Pharmaxis, BMS, MSD, Gilead, Janssen, Abbvie; Grant/Research Support: MSD

Leon A. Adams - Patent Held/Filed: Quest diagnostics; Speaking and Teaching: Bayer, Nutricia

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C-BREEZE 2: Efficacy and Safety of a Two-Drug Direct-Acting Antiviral Agent (DAA) Regimen Ruzasvir 180 mg and Uprifosbuvir 450 mg for 12 Weeks in Adults With Chronic Hepatitis C Virus (HCV) Genotype (GT)1, 2, 3, 4, or 6

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Background: Ruzasvir (RZR, MK-8408, an NS5A inhibitor) and uprifosbuvir (UPR, MK-3682, an NS5B nucleotide inhibitor) are highly potent HCV DAAs. A Phase 2 trial evaluating the two-drug combination of RZR 60 mg + UPR 450 mg suggested suboptimal efficacy in certain HCV genotypes (C-BREEZE 1). The aim of this phase 2 trial was to assess the efficacy and safety of a higher dose of RZR 180 mg in combination with UPR 450 mg for 12 weeks. **Methods**: This is a Phase 2, open-label clinical trial conducted in 2 cohorts (NCT02956629). Enrollment was paused after the first 50 participants (Cohort 1); if safety and tolerability were considered acceptable during the initial treatment period, enrollment was resumed to complete Cohort 2. Treatment-naive or interferon ± ribavirin–experienced participants with or without compensated cirrhosis were enrolled in both cohorts. All participants received RZR 180 mg + UPR 450 mg once daily for 12 weeks. The primary objectives were the assessment of efficacy (proportion of participants with HCV RNA <15 IU/mL at 12 weeks after the end of study therapy; SVR12), as well as safety and tolerability. Polymorphisms within the NS5A and NS5B gene regions associated with resistance in the DAA class were assessed using next-generation sequencing (15% sensitivity threshold). **Results**: Overall, 267 participants were enrolled: 48 participants with GT1-4 infection in Cohort 1, and 219 additional participants with GT1-6 infection in Cohort 2. The distribution of genotypes in the overall study population was: 1a, n=47; 1b, n=28; 2, n=50; 3, n=61; 4, n=56; 5, n=3; 6, n=22. Fifty-nine (22%) participants had compensated cirrhosis. Results to date are based on 44 participants from Cohort 1 who have at least 4 weeks of post treatment follow-up (Table). Of those enrolled in Cohort 1, at least one baseline NS5A or NS5B resistance-associated substitution (RAS) was detected in 50% and 6%, respectively. All participants achieved SVR4. Treatment was generally well tolerated, with no reported renal safety events. The most frequent study drug-related adverse events in all participants were fatigue (8%), headache (6%), diarrhea (6%), and nausea (5%). **Conclusions**: The two-drug combination of RZR 180 mg + UPR 450 mg for

12 weeks was highly effective and well-tolerated in GT1-, 2-, 3-, or 4-infected participants with or without compensated cirrhosis treated in Cohort 1 of the trial. The presence of baseline NS5A RASs did not impact the response rate. Complete SVR12 results will be presented.

	All	GT1a	GT1b	GT2	GT3	GT4
SVR4, n/N	44/44 (100%)	13/13 (100%)	2/2 (100%)	12/12 (100%)	15/15 (100%)	2/2 (100%)

SVR4 = HCV RNA >15 IU/mL at 4 weeks after the end of study therapy.

Disclosures:

Eric Lawitz - Advisory Committees or Review Panels: AbbVie, Achillion Pharmaceuticals, Regulus, Theravance, Enanta, Idenix Pharmaceuticals, Janssen, Merck & Co, Novartis, Gilead; Grant/Research Support: AbbVie, Achillion Pharmaceuticals, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Idenix Pharmaceuticals, Intercept Pharmaceuticals, Janssen, Merck & Co, Novartis, Nitto Denko, Theravance, Salix, Enanta; Speaking and Teaching: Gilead, Janssen, AbbVie, Bristol Meyers Squibb, Merck, intercept

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Efficacy and Safety of Glecaprevir/Pibrentasvir for 8 or 12 Weeks in Treatment-Naïve Patients with Chronic HCV Genotype 3: An Integrated Phase 2/3 Analysis

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BACKGROUND: Glecaprevir (identified by AbbVie and Enanta) and pibrentasvir (coformulated as G/P) is a pangenotypic NS3/4A protease inhibitor and NS5A inhibitor combination that was well-tolerated and demonstrated high efficacy in patients with HCV genotype (GT) 3 in phase 2 and 3 clinical trials. **METHODS:** Safety and efficacy data from treatment-naïve patients with HCV GT3 infection, without cirrhosis or with compensated cirrhosis, were pooled across five phase 2 and 3 studies of 8 or 12 weeks of once-daily G/P treatment. Efficacy was measured as sustained virologic response at post-treatment week 12 (SVR12); safety (adverse events and laboratory abnormalities) was also analyzed. **RESULTS:** Among 571 patients with HCV GT3 infection, 208 and 294 patients without cirrhosis were treated with 8 and 12 weeks of G/P, respectively; 69 patients with cirrhosis were treated for 12 weeks. The majority had subtype 3a (99%; 565/571). Twenty-two percent (121/571) of patients had baseline polymorphisms in NS5A (positions 24, 28, 30, 31, 58, 92, and 93). Sixty-five percent of patients (374/571) had a history of injection drug use; 17% (95/571) were on stable opiate substitution therapy. Among patients without cirrhosis, 18% (38/208) and 11% (31/294) treated for 8 and 12 weeks had F3 fibrosis, respectively. Intent-to-treat (ITT) SVR12 rates were 95% (198/208) and 95% (280/294) in patients without cirrhosis treated for 8 and 12 weeks, respectively; modified ITT (excluding those with non-virologic failure) SVR12 rates were 97% (198/204) and 98% (280/285). Relapse rates were 2.5% (5/200) for 8 weeks and 1.4% (4/281) for 12 weeks. ITT and modified ITT SVR12 rates for patients with cirrhosis treated for 12 weeks were 97% (67/69) and 99% (67/68), respectively. Adverse events (AEs) occurring in ≥10% of patients were headache, fatigue, and nausea. There were no drug-related serious AEs. AEs leading to study drug discontinuation were rare, and there were no post nadir Grade 3 or higher alanine aminotransferase abnormalities. **CONCLUSIONS:** G/P was well-tolerated and resulted in high SVR12 rates in treatment-naïve patients with HCV

GT3 infection. Among non-cirrhotic patients, no additional benefit was associated with extension of treatment from 8 to 12 weeks.

Disclosures:

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Do resistance associated substitutions (RAS) or Ribavirin (RBV) use influence treatment success of Sofosbuvir (SOF)/Velpatasvir (VEL) in chronic hepatitis C genotype 3 (GT 3) infection? – Results from the GErman hepatitis C COhort (GECCO)

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Introduction To date EMA has approved 5 different SOF containing regimes for the treatment of GT3 with SOF/VEL being the most recent. In ASTRAL 3 study baseline NS5A RAS were associated with a higher risk of virologic failure. The worth of the addition of RBV in difficult to treat GT3 to prevent relapse is still under debate. Real-life data could further define the best use of SOF/VEL. Methods The GECCO cohort is a prospective multicenter cohort from 9 sites in Germany. For this analysis all GT3 infected patients starting SOF/VEL +/-RBV from 8/2016 were included. Baseline demographics, treatment responses and baseline RAS are reported for a subset of patients, full dataset analysis will be available at time of the conference. Results Until March 2017 n=2280 patients started in GECCO on a 2nd generation DAA regimen, n=630 with GT3 infection. N= 139 of GT3 infected started on SOF/VEL +/- RBV. Table 1 shows baseline demographics and clinical characteristics. All patients were assigned to SOF/VEL for 12 weeks, RBV was added in n= 13 (9%), n=33/139 pretreated (n=24/33 PegIFN +/- RBV, n=7/33 with SOF based regimes), n=31/139 (22%) had liver cirrhosis defined by FibroScan >12.5 kPa or APRI >2. Of those with cirrhosis n=8/31 were pretreated, for n=12/31 (39%) RBV was added to the regimen. SVR12 rates were 87% (n=85/97) (ITT) and 99% (n=85/86) (PP) for all patients. In patients with cirrhosis SVR12 was 83% (n=10/12) (ITT) and 91% (10/11) (PP) without and 88% (n=7/8) (ITT) and 100% (7/7) (PP) with RBV. One cirrhotic patient pretreated with SOF+RBV had a relapse. N=58/139 were tested for baseline NS5A RAS so far, A30K were found in n=4/58 by population sequencing. All had SVR12. Conclusion: GT3 patients treated with SOF/VEL +/- RBV in GECCO show high success rates comparable to clinical phase III studies even in the presence of baseline NS5A RAS. High LTFU are still a concern in this real world cohort.

Baseline demographics and clinical characteristics of n=139 GT3 on SOF/VEL+/-RBV

Genotype 3 n=	139
Male n= (%)	91 (66)
Median Age years (SD)	52 (10.1)
Median weight kg (SD)	77 (18.9)
Median HCV-RNA baseline IU/mL (SD)	3.641.137 (6.849.540)
Thrombocytes /µL (SD)	202.000 (69.390)
ALT U/L (SD)	75 (71.5)
eGFR ml/min/1,73m2 (SD)	108 (19.25)
HIV/HCV coinfected n= (%)	10 (7)
Patients on OST n= (%)	39 (25)
HCV pretreatment n= (%)	33 (25)
SOF/VEL + RBV n= (%)	13 (9)
Fibroscan>12,5 KPa or APRI>2 n= (%) - treated with SOF/VEL N/n= (%) - treated with SOF/VEL+RBV N/n= (%)	31 (22) 19/31 (61) 12/31 (39)
Baseline RAS available n= (%) NS5A RAS Y93H, Y93N, A30K or L31I	58/139 (42) n= 4/58 with A30K

Disclosures:

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Thomas Lutz - Grant/Research Support: Janssen Cilag, Deutsche Leberhilfe, Gilead, ViiV, MSD, BMS, GlaxoSmithKline, AbbVie

The following people have nothing to disclose: Patrick Ingiliz, Axel Baumgarten, Guenther Schmutz, Florian Berger, Martin Daeumer, Johann von Felden, Joerg Timm, Julian Schulze zur Wiesch

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Significant and Sustained Improvement of Health-Related Quality of Life (HRQL) Scores in Patients with Hepatitis C (HCV) and Sustained Virologic Response (SVR)

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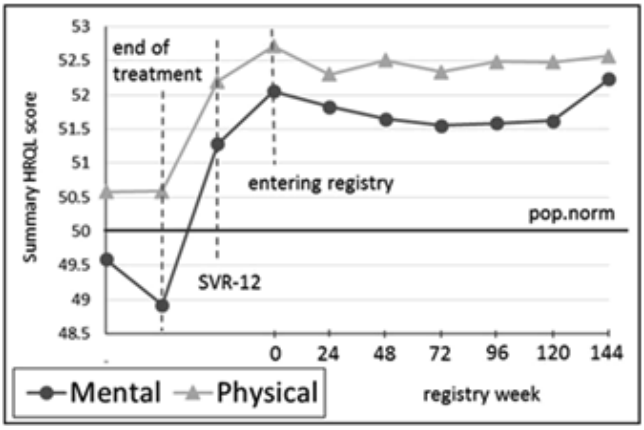
Background: Chronic HCV infection has been associated with adverse clinical outcomes (mortality related to hepatic and extrahepatic manifestations) as well as significant economic burden and impairment of patients' HRQL. Achieving SVR has been associated with clinical benefits and short-term improvement in HRQL scores. However, long-term sustainability of these HRQL improvements is unknown. **Aim:** To assess long-term changes in HRQL of subjects with chronic HCV infection who have achieved SVR. **Methods:** We included chronic HCV-infected subjects with complete baseline data who had achieved SVR with sofosbuvir-based regimens and had been enrolled in a follow-up registry. HRQL was assessed every 24 weeks for up to 144 weeks using Short Form-36v2. **Results:** Baseline data was available for 3,486 subjects with SVR-12 (age 53±10, 62.3% male, 15.6% cirrhosis, 10.1% diabetes, 62% employed). Compared to the HRQL scores prior to their initial treatment, patients experienced significant improvements of HRQL in all domains of SF-36 upon achieving SVR and entry into the registry (up to +8.2%, all p<0.0001). By week 144 of follow-up, all gains in HRQL scores were maintained (all p<0.0004) (Figure). Notably, the greatest HRQL gains were consistently observed in the General Health and Vitality domains. Furthermore, upon achieving SVR, all SF-36 domain scores in the study cohort exceeded the general population norms (all p<0.0001). In multivariate regression analysis, history of cirrhosis, depression, anxiety, and clinically overt fatigue were independent predictors of HRQL impairment (p<0.05). **Conclusions:** Improvement in HRQL after achieving SVR is maintained in long-term follow-up. These data support the comprehensive and sustainable benefit of HCV cure.



Disclosures:

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Evaluation of the efficacy and tolerability of JNJ-4178 (AL-335, odalasvir, and simeprevir) in hepatitis C virus-infected patients without cirrhosis: The Phase IIb OMEGA-1 study

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Background: The efficacy, safety, and pharmacokinetics (PK) of a 3 direct-acting antiviral (3-DAA) combination (AL-335, novel uridine-based nucleotide analog hepatitis C virus [HCV] NS5B polymerase inhibitor; odalasvir [ODV], novel HCV NS5A inhibitor; and simeprevir [SMV], approved HCV NS3/4A protease inhibitor) in HCV-infected patients (pts) have been reported previously from the Phase IIa Study AL-335-604 (NCT02569710). Sustained virologic response rates 12 (SVR12) and 24 (SVR24) weeks after end of treatment (EOT) of 100% were achieved in HCV genotype (GT)1-infected pts without cirrhosis following 6 or 8 weeks of treatment, and the regimen was well tolerated. The combination of AL-335 800 mg, ODV 25 mg, and SMV 75 mg once daily (QD) is being further evaluated as JNJ-4178 in the Phase IIb OMEGA-1 study (NCT02765490). **Methods:** This multicenter, randomized (1:1), open-label study includes treatment-naïve pts or pts previously treated with (peg)interferon (IFN) (18–70 years of age) with HCV GT1, 2, 4, 5, or 6 infection, without cirrhosis. Exclusion criteria included prior DAA treatment and HCV GT3 infection. Pts received JNJ-4178 for 6 or 8 weeks and will be followed-up for 24 weeks after EOT. The primary objective is SVR12. Secondary objectives include safety/tolerability (including adverse events [AEs] and abnormalities in laboratory parameters, electrocardiogram, or echocardiogram) and PK. SVR12 results will be compared with historical data from IFN-free, DAA regimens and tested with a non-inferiority margin of 10% (2-sided testing at 0.05 significance level). **Results**: In total, 365 pts were enrolled and randomized to 6 (N=183) or 8 weeks (N=182), the follow-up phase is ongoing. Overall 107 pts (29.3%) had GT1a, 155 (42.5%) had GT1b, 46 (12.6%) had GT2, 52 (14.2%) had GT4, 5 (1.4%) had GT5, and no pts had GT6 infection. Baseline and disease characteristics were well balanced between groups. During the treatment phase, most AEs were mild with no related serious AEs. No pts discontinued treatment due to an AE or withdrew consent. **Conclusions**: The OMEGA-1 study will provide further understanding of the efficacy and tolerability of AL-335, ODV, and SMV (as JNJ-4178) in HCV GT1, 2, 4, 5, and 6 infected-pts without cirrhosis. Treatment was well tolerated and not associated with premature discontinuation. SVR12 results will be presented at the meeting.

Disclosures:

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Rekha Sinha - Employment: Janssen Pharmaceutica Research & Development

Ronald Kalmeijer - Employment: Johnson and Johnson

Michael Biermer - Employment: Janssen Pharmaceutica BV

The following people have nothing to disclose: Susan Greenbloom, Chris Corbett, Wouter Willems

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Evaluation of an early occurrence and recurrence of hepatocellular carcinoma in chronic hepatitis C patients treated with All-Oral DAAs - propensity score-matched analysis of a prospective database -

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Aims: Viral eradication after interferon (IFN)-based therapy has been associated with a reduced risk of developing hepatocellular carcinoma (HCC) in chronic hepatitis C (CHC) patients, but the question of whether the suppressive effect of viral eradication by IFN-free regimens on hepatocarcinogenesis will be equivalent to that obtained by IFN-based regimens has remained to be solved. The aims of this study were to evaluate the occurrence and recurrence of HCC in CHC patients treated with DAAs and to identify biomarkers of HCC development after antiviral treatment. ***Patients and Methods:*** Retrospective review of a prospective database of 1897 CHC patients who were treated with IFN-based (n=1145) or IFN-free therapies (n=752). Cumulative HCC occurrence and recurrence rates were compared using propensity score-matched analysis. Predictors of HCC development after viral eradication were identified by multivariate analysis. Fib-4 index > 3.25 were evaluated as severe fibrosis in patients without histological diagnosis ***Results:*** For a comparison of HCC occurrence between IFN-based and IFN-free therapies, propensity score-matched analysis was performed

using variables identified with statistical difference factors between IFN-based and IFN-free groups: age, gender, fib4-index, and the pre-treatment albumin level. Propensity score-matched analysis showed no significant difference in HCC occurrence (3-year incidence: 3.3% in IFN-based, 1.4% in IFN-free therapy; $p = 0.49$, logrank test) and recurrence rates (5-year incidence: 54.2% in IFN-based, 45.1% in IFN-free therapy; $p = 0.54$, logrank test) among groups treated with IFN-based or IFN-free therapies. In multivariate analysis, higher levels of post-treatment α-fetoprotein (AFP) or *Wisteria floribunda* agglutinin positive Mac-2 binding protein (WFA$^+$M2BP) were independently associated with HCC occurrence and recurrence after viral eradication and only post-treatment WFA$^+$M2BP level was significantly associated with HCC occurrence and recurrence among patients without severe fibrosis. The area under the receiver operating characteristic (ROC) curve for WFA$^+$M2BP levels was greater compared with that for AFP levels in ROC analysis. ***Conclusion:*** The risks of early HCC occurrence and recurrence after viral eradication were similar between IFN-based and IFN-free therapies. Patients with high WFA$^+$M2BP level after antiviral treatment even without severe fibrosis are necessary to be followed up carefully for HCC development even after viral eradication.

Disclosures:

Mamoru Watanabe - Grant/Research Support: MSD Co., Ltd., Abbvie. GK, Chugai Pharmaceutical Co., Ltd, Dainippon Sumitomo Pharma Co., Ltd, Toray Industries, Inc, Bristol-Myers K.K., GILEAD Science, Inc

The following people have nothing to disclose: Mina Nakagawa, Yasuhiro Asahina, Hiroko Nagata, Shun Kaneko, Fukiko Kawai-Kitahata, Miyako Murakawa, Sayuri Nitta, Yasuhiro Itsui, Seishin Azuma, Sei Kakinuma, Yujiro Tanaka

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The effect of colectomy on recurrent Primary Sclerosing Cholangitis and need for re-transplantation after orthotopic liver transplantation

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Background Primary Sclerosing Cholangitis (PSC) is a chronic inflammatory disease of the intra- and extra-hepatic bile ducts of unknown etiology. PSC can lead to end-stage liver disease for which orthotopic liver transplantation (OLT) is the only therapeutic option. PSC is strongly associated with inflammatory bowel disease, especially ulcerative colitis. From a pathophysiological perspective, intestinal inflammation is suggested to influence inflammation in the biliary tree. Colectomy prior to or during OLT is suggested to prevent recurrent PSC (rPSC), although available data are ambiguous. Therefore, our aim was to investigate the potential protective effect of colectomy on rPSC and the need for re-transplantation (re-OLT) for patients with PSC. **Material and Methods** A retrospective cohort study was performed including all patients who underwent OLT for PSC between 1985 and 2015 at our Liver-unit. Data on patient and donor characteristics, rPSC, inflammatory bowel disease type, CMV-status, MELD-score, type of colectomy and indication of colectomy were collected. The Mayo Clinic criteria for the recurrence of PSC after OLT were applied. Kaplan-Meier survival analysis, Log rank tests and Pearson Chi-Square tests were performed. **Results** A total of 136 patients underwent OLT for PSC with a median age of 45.5 (range, 18-67) years at time of OLT. Most of the patients were male (71.3%) and 97 (71.3%) were diagnosed with inflammatory bowel disease, mainly ulcerative colitis (85.5%). Twenty-five patients (18.4%) underwent colectomy prior to or after OLT. The 5- and 10-year patient survival after OLT was 86% and 77% respectively with a median follow up of 10.9 (range, 0.0-29.7) years. rPSC occurred in 32 (23.5%) patients, with a median time to recurrence of 4.7 (range, 0.5-27.0) years. Patients with rPSC had a significantly increased need for re-OLT (46.9%) when compared to those without rPSC (17.3%, Chi-squared 11.641; 1df; P=0.001). The occurrence of rPSC, however, was not significantly related to overall survival (Log rank 1,783; P=0.182). Colectomy was not significantly related to rPSC (Chi-squared 0.004; 1df; P=0.951) nor to time to recurrence (Log rank 0.567; P=0.451). **Conclusion** In this observational cohort study in patients with PSC who underwent OLT, colectomy was not associated with a lower incidence of rPSC after a long follow-up time. Patients with rPSC more frequently underwent re-OLT when compared to those without rPSC, but rPSC did not have a significant effect on patient survival. Our study did not corroborate previous reports that colectomy may confer a protective effect on the occurrence of rPSC in subsequent liver grafts.

Disclosures:

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Time Trends in Major Hepatic Complications in Primary Biliary Cholangitis: incidence and transplant-free survival

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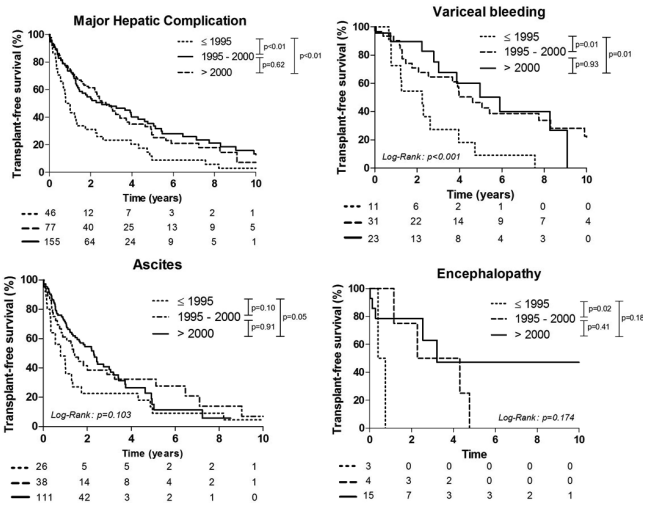
Background The prognosis of patients with primary biliary cholangitis (PBC) and the management of major non-malignant complications related to cirrhosis have improved over the past decades. We aimed to assess time trends in the incidence of these complications and the related outcomes. **Methods** Patient data from the Global PBC Study Group was used for analyses. Major hepatic complications were defined as a first occurrence of either ascites, variceal bleeding or encephalopathy. A composite endpoint of liver transplantation (LT) and death was used. Survival was assessed using Kaplan-Meier estimates. **Results** We included 3224 UDCA-treated PBC patients (91% female, 73% early biochemical disease, median follow-up 8.1 years (IQR4.4-12.7)). During follow-up, 150 patients had a LT and 337 patients died. First complications were noted in N=278. The cumulative incidence rate of first complications was 9.7 per 1000 patient-years. The overall complications rates at 5, 10 and 15 years were 3.7%, 9.1% and 14.8%. However, we found that the incidence decreased over time, with a 10-year cumulative complication rate of 13.5% for patients included before 1990, 9.3% between 1990-2000, and 5.8% after 2000 (p<0.01 for all). After occurrence of a complication, 3, 5 and 10 year transplant-free survival rates were 34.7%, 19.2%, and 10.4%, respectively (time-dependent hazard ratio 21.5; 95%CI 20.1-22.8). When stratifying according to era, particularly transplant-free survival after variceal bleeding was significantly prolonged for patients affected more recently (p=0.01) (Figure). **Conclusion** The large majority of UDCA-treated PBC patients will remain free of major hepatic complications. The incidence of these complications has decreased over the past decades while the associated prognosis has improved.



Disclosures:

Christophe Corpechot - Consulting: Intercept Pharmaceuticals

Harry L. Janssen - Consulting: AbbVie, Bristol Myers Squibb, GSK, Gilead Sciences, Innogenetics, Merck, Roche, Janssen, Medimmune; Grant/Research Support: AbbVie, Bristol Myers Squibb, Gilead Sciences, Merck, Roche, Janssen, Medimmune

Frederik Nevens - Consulting: MSD, CAF, Intercept, Gore, BMS, Abbvie, Novartis, Durect, Janssens-Cilag, Ono Pharma, Promethera Biosciences, Gilead; Grant/Research Support: Ferring, Roche, Astellas, Novartis, Janssen-Cilag, Abbvie, Gilead

Keith D. Lindor - Advisory Committees or Review Panels: Intercept, Shire

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Gideon M. Hirschfield - Advisory Committees or Review Panels: Intercept Pharma, GSK; Consulting: Cymabay, Novartis; Grant/Research Support: Falk Pharma, BioTie, Takeda

The following people have nothing to disclose: Maren H. Harms, Willem J. Lammers, Douglas Thorburn, Pietro Invernizzi, Pier Maria Battezzati, George N. Dalekos, Xavier Verhelst, Jorn C. Goet, Bettina E. Hansen, Henk R. van Buuren

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Pilot Study with IBAT Inhibitor A4250 for the Treatment of Cholestatic Pruritus in Primary Biliary Cholangitis.

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Background Pruritus is a common complication of cholestatic liver diseases Inhibition of reabsorption of bile acids (BAs) from the intestine by pharmacological inhibition of ileal bile acid transporter (IBAT/ASBT) may emerge as treatment option. **Aim** To assess in a pilot trial (NCT02360852) tolerability and effect on pruritus of the selective IBAT inhibitor A4250 in patients with primary biliary cholangitis (PBC). **Methods** Ten patients with PBC (9 female, 1male; 55±16 years) and ongoing bile acid sequestrant (BAS) treatment of cholestatic pruritus were after a two-weeks wash out of BAS treated with either 0.75 mg (n=4) or 1.5 mg (n=5) of A4250 for four weeks, followed by two-weeks wash-out and return to previous BAS treatment. One patient left the study before exposure to A4250. Patients' pruritus was assessed by Visible Analogue Scale (VAS), 5D pruritus score and the pruritus module of the PBC40 questionnaire, and stool habits by the BSFS scale. Bile acids in plasma and faeces and plasma 7a-hydroxy-4-cholesten-3-one (C4) were measured by UPLC-MS, plasma fibroblast growth factor 19 (FGF19) by ELISA, and serum autotaxin (ATX) activity by homemade assay. **Results** All nine patients exposed to A4250 reported a remarkable improvement in pruritus, until no or mild according to 5D pruritus score, and similar improvements in VAS and the pruritus mode of PBC40. Five patients finished the study prematurely, after 3 to 6 days, due to abdominal pain (5/5) and diarrhea (4/5). In those 4 patients that finished per protocol, plasma bile acids and FGF19 decreased whereas plasma C4 and fecal BAs increased, supporting the suggested mode of action of IBAT inhibition. Also, ATX activity was decreased at the end of A4250 treatment. **Conclusions** A4250 improved or even relieved from cholestatic pruritus in PBC. The high incidence of probably bile acid malabsorption-related diarrhea and abdominal pain in our BAS-pretreated population indicates that the start dose of A4250 may have been too high, which should be acknowledged in further studies with this compound.

Disclosures:

Hanns-Ulrich Marschall - Consulting: Intercept, Albireo; Grant/Research Support: Intercept

The following people have nothing to disclose: Samer Al-Dury, Annika Wahlström, Staffan Wahlin, Jacqueline Langedijk, Marcus Ståhlman

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Bilirubin is Predictive of Transplant-free Survival Even Within the Normal Range in Primary Biliary Cholangitis Patients

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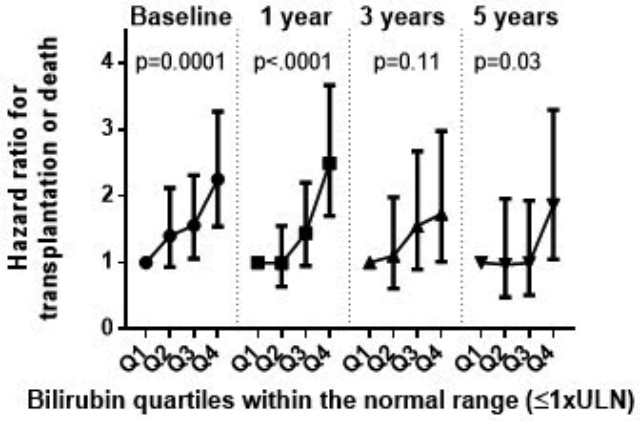
Background Due to the slowly progressing nature of Primary Biliary Cholangitis (PBC), surrogate parameters have been evaluated for their prognostic value on clinical outcomes. It is widely established that bilirubin is strongly associated with transplant-free survival and the threshold with the highest performance is at the upper limit of normal (ULN). However, its predictive value below this threshold has not been assessed. Therefore, this study aims to evaluate whether serum bilirubin concentrations within the normal range are associated with transplant-free survival. **Methods** Patient data was retrieved from the Global PBC Study group database, which comprises data from 17 centers across Europe and North America. UDCA-treated and untreated patients with normal bilirubin (≤1xULN) at either baseline, 1, 3, or 5 years were included. The influence of bilirubin was assessed as a scale and categorical variable in a Cox proportional hazards model while adjusting for sex, age at start of follow-up, year of diagnosis, ursodeoxycholic acid treatment, alkaline phosphatase (xULN), and albumin (xLLN). **Results** Four cohorts of patients were selected with normal bilirubin at baseline (n=2795), 1 year (n=3082), 3 years (n=1657), and 5 years (n=1339). Each cohort was stratified into bilirubin quartiles (Q1-Q4). The 5-year transplant-free survival for baseline Q1-Q4 was 97%, 95%, 96%, and 91%, respectively ($p<0.001$). Lower quartiles from the remaining time points also had an improved transplant-free survival. In multivariable Cox regression analyses, bilirubin quartiles remained a significant predictor of transplant-free survival, except at 3 years (Figure). In addition, higher bilirubin (per 0.1xULN increase) was associated with an increased chance for death or transplantation (baseline: HR 1.14, 1 year: HR 1.21, 3 years: HR 1.19, 5 years HR: 1.17, $p<0.05$). **Conclusion** Serum bilirubin concentrations within the normal range at baseline and follow-up are predictive of

transplant-free survival. This may imply that we must aim for the lowest possible bilirubin concentration in future intervention studies of PBC.



Disclosures:

Cyriel Y. Ponsioen - Advisory Committees or Review Panels: Takeda; Consulting: AbbVIE; Grant/Research Support: AbbVIE, Dr. Falk Pharma, Takeda

Christophe Corpechot - Consulting: Intercept Pharmaceuticals

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Tony Bruns - Advisory Committees or Review Panels: intercept; Speaking and Teaching: falk, norgine

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Keith D. Lindor - Advisory Committees or Review Panels: Intercept, Shire

Harry L. Janssen - Consulting: AbbVie, Bristol Myers Squibb, GSK, Gilead Sciences, Innogenetics, Merck, Roche, Janssen, Medimmune; Grant/Research Support: AbbVie, Bristol Myers Squibb, Gilead Sciences, Merck, Roche, Janssen, Medimmune

The following people have nothing to disclose: Carla F. Murillo Perez, Aliya Gulamhusein, Henk R. van Buuren, Jorn C. Goet, Marco Carbone, Pietro Invernizzi, Pier Maria Battezzati, George N. Dalekos, Kalliopi Zachou, Douglas Thorburn, Nicholas F. LaRusso, Raoul Poupon, Palak J. Trivedi, Xavier Verhelst, Bettina E. Hansen

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Bezafibrate improves GLOBE and UK-PBC scores and long-term outcomes in patients with primary biliary cholangitis

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Background: Bezafibrate (BF) is a widely used hypolipidemic agent and is a dual peroxisome proliferator-activated receptors (PPARs)/pregnane X receptor (PXR) agonist with potent anticholestatic efficacy. Therefore, BF is recognized as a second-line medicine for primary biliary cholangitis (PBC) that is refractory to ursodeoxycholic acid (UDCA). However, the effect of long-term BF treatment on the transplant-free survival rate is still unknown. We retrospectively studied Japanese patients with PBC who were treated with UDCA followed by BF. The actual outcomes were compared with the predicted outcomes, which were estimated during UDCA monotherapy. **Methods:** A total of 796 patients had full analysis of a dataset from a retrospective cohort from the Japan PBC Study Group (JPBCSG). Among these patients, we enrolled 100 who had received UDCA monotherapy (13–15 mg/kg/d) for at least 1 year followed by combination therapy with BF (400 mg/d) for longer than 1 year. We compared GLOBE scores just before UDCA+BF combination therapy with after combination therapy for 1 year. Additionally, the actual transplant-free survival rates of the enrolled patients that were analyzed by Kaplan–Meier plots were compared with predicted transplant-free survival rates, which were calculated by using the GLOBE scores just before combination therapy. **Results:** The median duration of UDCA monotherapy and UDCA+BF combination therapy was 2.6 years (range: 1.0–19.7 years) and 7.0 years (range: 1.1–17.7 years), respectively. The mean (± SEM) GLOBE score just before combination therapy was 0.506 ± 0.077, and this score was significantly improved after combination therapy for 1 year (0.116 ± 0.088, $p<0.0001$). The 3-, 5-, 10-, and 15-year transplant-free survival rates predicted by using the GLOBE scores just before combination therapy were 92.6% ± 0.7%, 87.4% ± 1.0%, 71.7% ± 1.8%, and 57.5% ± 2.1%, respectively. The actual 3-, 5-, 10-, and 15-year transplant-free survival rates of the enrolled patients were 97.8% ± 1.5%, 97.8% ± 1.5%, 90.7% ± 3.8%, and 90.7% ± 3.8%, respectively, which were significantly better than

those predicted by using the GLOBE scores just before combination therapy (p<0.005). We also performed the same analyses by using the UK-PBC risk score, and similar results were obtained. **Conclusion:** Addition of BF to UDCA monotherapy improves not only serum biochemistry, but also the long-term transplant-free survival rate predicted by the GLOBE score. BF is an ideal second-line medicine for treating PBC that is refractory to UDCA monotherapy.

Disclosures:

Satoshi Mochida - Grant/Research Support: A2 Health Care Co, Eisai, MSD, Tioray Medical, BMS, Dainihon Sumitomo Pharm, Ajinomoto Phamr, Abbvie, Chugai; Speaking and Teaching: MSD, Toray Medical, BMS, Tanabe Mitsubishi, Dainihon Sumitomo Pharm, Ajinomoto Pharm, Gilead, Abbvie

Ken Sato - Speaking and Teaching: Otsuka Pharmaceutical Co. Ltd

Hajime Takikawa - Grant/Research Support: MSD, Chugai, Mitsubishi Pharma, Zeria, Bayer, AbbVie, BMS, Otsuka, Kyowa-kirin, Daiichi-Sankyo

The following people have nothing to disclose: Akira Honda, Atsushi Tanaka, Atsumasa Komori, Masanori Abe, Mie Inao, Tadashi Namisaki, Hitoshi Yoshiji, Naoaki Hashimoto, Kazuhito Kawata, Atsushi Takahashi, Hiromasa Ohira, Jong-Hon Kang, Satoshi Yamagiwa, Satoru Joshita, Takeji Umemura, Jun Itakura, Akira Kaneko, Keisuke Kakisaka, Yasuhiro Takikawa, Kentaro Kikuchi, Yasushi Matsuzaki

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Effects of B-Cell depleting therapy (Rituximab) on liver biochemistry in UDCA-responsive patients: results from a randomised controlled trial of Rituximab in fatigued PBC patients (RitPBC).

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Background: Rituximab, an anti-CD20 monoclonal antibody is an effective immunotherapy agent in autoimmune diseases with a strong autoantibody response component. PBC is a chronic autoimmune liver disease in which over 95% of patients have high-titre autoantibodies directed at the mitochondrial autoantigen Pyruvate Dehydrogenase Complex (PDC) (1). Pilot studies of UDCA non-responding PBC patients treated with Rituximab have, however, only show small reductions in serum ALP despite the clear predicted action on the immune response in the disease (2-4). Rituximab therefore conforms to a pattern seen with all biological agents trialed to date in PBC with, despite their plausibility as therapies for the disease, only limited evidence of clinical efficacy. One explanation for this pattern could be limitations in the typical trial design. The most obvious potential issue is that to date trials (including Rituximab) have been in patients with UDCA non-response (i.e. in patients with established cholestasis), a "downstream" disease phenotype which might be the type least responsive to "upstream" immunotherapy. RitPBC was a trial exploring the actions of Rituximab in PBC to target fatigue. The fatigue effects are reported elsewhere, however the trial design, which included both responders and non-responders allowed us a unique opportunity to explore the effects of immunotherapy in patients who are not in a state of treatment resistant cholestasis. Methods: 57 participants (aged ≥18 years with PBC and moderate or severe fatigue {PBC-40 fatigue domain score of >33}) were randomised to receive 2 doses of either rituximab (1000mg) or saline (placebo). Labs including LFTs, AMA, Anti-PDC, TNF-alpha, IL-6 and IFN-gamma levels were taken at 3, 6, 9 and 12 months. Results: Fifty five between 34-72 years participants completed the study. Of the 92.7% patients on UDCA therapy 68% were responders as defined by Toronto criterion. Significant reduction in ALP was seen in the Rituximab but not Placebo patients, with over 90% in the active group normalising their ALP. Effect was seen in both responders and non-responders. A drop in Anti-PDC titre (measured as % from baseline) was seen in Rituximab group (70.4 and 50.4) with no change in placebo (92.6 and 97.6) at 3 and 6 months respectively. Virtually complete B-cell depletion and significant changes in cytokines levels were noted in the Rituximab arm, however neither appeared to be directly linked to liver biochemistry improvement. Conclusion: Rituximab is well tolerated and effective on biochemistry in patients in the immune phase of PBC (i.e. not UDCA non-responders). This may inform the design of future biological trials.

Disclosures:

Andrew M. Blamire - Grant/Research Support: Synageva

David Jones - Consulting: Intercept, GSK, Novartis; Grant/Research Support: Pfizer; Speaking and Teaching: Falk

The following people have nothing to disclose: Amardeep Khanna, Denise Howel, Jennifer Wilkinson

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Development and Validation of Models Predicting the Risk of Hepatocellular Carcinoma After Antiviral Treatment for Hepatitis C Virus

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Background and Aims. The vast majority of HCV-infected patients are now eligible for antiviral treatment and will likely receive DAA-based antiviral treatment in the next 3-5 years in the United States. Therefore, it is critical to develop models predicting the risk of HCC in patients undergoing antiviral treatment separately in cirrhotic and non-cirrhotic patients and incorporating whether they achieved SVR or not. Estimating the risk of HCC after antiviral treatment with or without SVR would help clinicians determine the need for ongoing HCC screening. **Methods.** We identified 62,051 patients who underwent 83,695 antiviral treatment regimens in the Veterans Affairs (VA) national healthcare system from 1999-2015, including 35,873 (57%) interferon-only regimens, 26,178 (43%) DAA±interferon regimens and 21,644 (35%) DAA-only regimens. We used Cox proportional hazards regression to develop and validate models predicting HCC risk separately in cirrhotic and non-cirrhotic patients. **Results.** We identified 2,305 incident cases of HCC diagnosed at least 180 days after initiation of antiviral treatment in 62,051 patients during a mean follow-up of 5.3 years. The incidence of HCC was highest in patients with cirrhosis and treatment failure (2.7 per 100 patient-years), followed by cirrhosis and SVR (0.93 per 100 patient-years), no cirrhosis and treatment failure (0.73 per 100 patient-years) and no cirrhosis and SVR (0.18 per 100 patient-years). The predictors of HCC after antiviral treatment were similar for IFN-ONLY, DAA±IFN or DAA-ONLY regimens. Also, the type of antiviral regimen (IFN-only vs DAA±IFN) was not significantly associated with HCC after adjusting for SVR and other predictors Models predicting HCC risk after antiviral treatment

were developed and validated separately in cirrhotic and non-cirrhotic patients consisting of the most important predictors of HCC which were: SVR, HCV genotype, serum creatinine, AST, ALT, INR, platelet count, hemoglobin, age, body mass index, gender, diabetes and HIV co-infection. The models exhibited excellent discrimination and calibration characteristics in both derivation and validation datasets. **Conclusions.** We developed and validated models to aide clinicians in stratifying HCC risk following antiviral treatment. These models, which are available as web-based tools, could help clinicians determine the need for ongoing HCC screening after antiviral treatment.

Disclosures:

George N. Ioannou - Grant/Research Support: Janssen Pharmaceuticals

The following people have nothing to disclose: Pamela Green, Kristin Berry

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Efficacy, Safety, and Pharmacokinetics of Glecaprevir/Pibrentasvir in Adults With Chronic Genotype 1-6 Hepatitis C Virus Infection and Compensated Cirrhosis: An Integrated Analysis

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Background: The direct-acting combination of glecaprevir (GLE: NS3/4A protease inhibitor, identified by AbbVie and Enanta) and pibrentasvir (PIB: NS5A inhibitor), G/P, demonstrated a pooled SVR12 rate of 97% in phase 2 and 3 studies of adults without cirrhosis or with compensated cirrhosis, infected with HCV genotypes (GTs) 1-6. We report integrated efficacy, safety, and pharmacokinetics in patients with compensated cirrhosis. **Methods:** Four multicenter, open-label, phase 2 or 3 studies included HCV GT1-6-infected adults with compensated cirrhosis, treatment-naïve or previously treated with interferon (IFN), pegylated IFN ± ribavirin (RBV), or sofosbuvir + RBV ± pegIFN, HCV protease inhibitors, or NS5A inhibitors. Cirrhosis was confirmed by liver biopsy, Metavir (or equivalent) score of 4, FibroScan score ≥14.6 kPa or FibroTest score ≥0.75 and aspartate aminotransferase (AST)-to-platelet ratio >2. The Child-Pugh score was required to be ≤6, with no current or past clinical evidence of liver decompensation. Treatment was G/P 300 mg/120 mg orally once daily, for 12 or 16 weeks. Drug exposure, adverse events (AEs), lab abnormalities, and SVR12 rates were assessed. **Results:** The phase 2 or 3 G/P studies encompassed 308 patients with compensated cirrhosis (men, 65%; white, 85%; mean age, 58 years; treatment-naive, 59%; baseline Child-Pugh score of 5, 86%; CKD stages 4 or 5, 6%; platelet count <100 x10^9/L, 23%; GT1, 40%; GT3, 38%). Eighty percent of patients received 12-week G/P treatment. Exposure to GLE in patients with compensated cirrhosis was 2-fold the exposure in noncirrhotics; exposures to PIB in these two subgroups were similar. The overall SVR12 was 96% (ITT) or 97% (mITT) (Table). Overall, 76% of patients experienced treatment-emergent AEs, most of which mild or moderate in severity. Two (0.6%) patients discontinued treatment due to AEs. The most common AEs were fatigue (19%), headache (16%), and nausea (10%). Serious AEs, none deemed related to G/P, were experienced by 9% of patients. Two patients died post-treatment, both due to an AE considered not related to treatment (cerebral hemorrhage). Post-baseline, there were 3 (1%) grade ≥3 bilirubin elevations and no grade ≥3 increases in alanine aminotransferase or AST. **Conclusions:** G/P was safe, well tolerated, and highly effective in patients with compensated cirrhosis chronically infected with HCV GTs 1-6.

HCV Genotype	SVR12, ITT % (n/N)	SVR12, mITT % (n/N)
Any GT	96 (297/308)	97 (297/305)
GT1	94 (119/126)	96 (119/124)
GT2	100 (35/35)	100 (35/35)
GT3	97 (112/116)	97 (112/115)
GT4-6	100 (31/31)	100 (31/31)

Disclosures:

Edward J. Gane - Advisory Committees or Review Panels: Alios, Merck, Janssen, Gilead Sciences; Board Membership: AbbVie; Speaking and Teaching: Gilead Sciences, Merck, Alnylam, AbbVie

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Jean-Pierre Mulkay - Grant/Research Support: GILEAD, BMS

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The following people have nothing to disclose: Fred Poordad, Susan Greenbloom, Tuan T. Nguyen, William R. Harlan, Albert Tran

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Treatment of HCV with direct acting antivirals significantly reduces liver-related hospitalizations in patients with cirrhosis

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Background: There is limited information regarding the impact of direct acting antivirals (DAA) on the frequency of inpatient admissions due to liver-related hospitalizations and potential cost savings. **Methods:** We conducted a retrospective cohort analysis comparing two cohorts

with HCV cirrhosis. The DAA cohort received DAA treatment between Jan. 2014 and Mar. 2017. The control cohort did not receive HCV therapy between Jan. 2011 and Dec. 2013. The incidence of liver-related hospitalizations, HCC, liver transplant, and all-cause mortality was measured for each cohort. Projected savings was calculated using the 95% CI for reduction in hospitalizations, the total distribution of reasons for hospitalization, and the average cost of an admission for each diagnosis at our institution, to estimate savings per patient treated per year. **Results:** Baseline characteristics between the DAA (n=196) and control (n=182) cohorts did not differ based on sex, race/ethnicity, weight, HIV or HBV coinfection, HCV genotype, HCV viral load, Child Turcotte Pugh (CTP) score distribution, or history of ascites, hepatic encephalopathy, or esophageal varices. Persons in the DAA cohort were older (59 vs. 56 years, p=0.006), had fewer patients listed for liver transplant prior to the study period (4.6% vs. 14.8%, p=0.0008), shorter mean follow-up time (17.7 vs 20.4 months, p=0.004), and a lower baseline MELD (10 vs. 11, p=0.01). The sustained virologic response rate for the treated group was 86.7%, including 89.1% (122/137) for CTP-A and 81.4% (48/59) for CTP-B/C. The DAA cohort had significantly fewer liver-related hospitalizations, and the reasons for hospitalization did not differ between the two groups (Table 1). This difference was mainly observed in patients with baseline CTP- A and CTP-B cirrhosis; however there was no difference in the incidence of hospitalizations in the CTP-C patients. During the short follow-up time, there were no differences in the incidence of HCC, liver transplant, or death between the cohorts. The greatest potential cost savings was derived from treating patients with CTP-B cirrhosis. **Conclusion**: Treatment of HCV with DAA in patients with cirrhosis reduces liver-related hospitalizations resulting in decreased healthcare utilization and reduced costs associated with liver disease. This benefit may not be realized if treating patients with CTP-C cirrhosis.

Event	Untreated group (per 100 person-years of follow-up)	DAA-treated group (per 100 person years of follow up)	Difference (95% CI) per 100 person years of follow up	P-value	Projected cost savings per patient treated per year
Hospitalizations CTP-A CTP-B CTP-C	29.1 (n=182) 12.0 (n=113) 56.6 (n=57) 94.7 (n=12)	10.4 (n=196) 2.9 (n=137) 19.7 (n=52) 96.5 (n=7)	18.7 (11.5 – 25.9) 9.1 (3.8 – 14.4) 36.9 (17.5 – 56.3) -1.7 (-78.2 - 74.8)	<0.0001 0.0008 0.0002 0.96	$3,654-$8,231 $1,208-$4,576 $5,561-$17,891 n/a
HCC (n)	5.9 (17)	5.1 (14)	0.8 (-3.1 - 4.7)	0.68	
Liver transplant (n)	4.1 (12)	1.7 (5)	2.4 (-0.45 - 5.1)	0.1	
Death (n)	5.5 (17)	3.8 (11)	1.7 (-1.7 - 5.1)	0.34	
Reasons for hospitalizations:n(%) Ascites HE Esophageal Varices SBP Other	 24 (26.7) 48 (53.3) 10 (11.1) 3 (3.3) 5 (5.6)	 4 (13.3) 14 (46.7) 6 (20.0) 2 (6.7) 4 (13.3)		 4 (13.3) 14(46.7) 6 (20.0) 2 (6.7) 4 (13.3)	

Disclosures:

Lisa M. Richards - Speaking and Teaching: Intercept, Merck, Gilead, Abbvie

The following people have nothing to disclose: Barbara Andrews, Robin Soto, Summer Collier, Alexander Kuo, Edward R. Cachay, Lucas Hill

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Randomized controlled trial of cash incentives or peer mentors to improve HCV linkage and treatment among HIV/HCV coinfected persons who inject drugs: The CHAMPS Study

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Background: Despite access to direct acting antivirals (DAAs), barriers to HCV linkage and treatment of persons who inject drugs (PWIDs) persist. The CHAMPS study aims to evaluate the impact of two innovative strategies, contingent cash incentives and peer-mentors, on HCV treatment uptake and cure in HIV+ PWIDs not engaged in HCV care. **Methods**: HCV treatment-naïve PWIDs with genotype 1 infection were eligible if they received HIV care at Johns Hopkins and had not been evaluated for HCV care within 8 months, had a CD4 count >100 mm3, and no evidence of decompensated liver disease. Eligible participants were randomized (1:2:2) to 1) Usual Care (UC, nurse supervision); 2) UC + Peer-Mentors; 3) UC + Cash Incentives. All participants were provided 12 weeks of ledipasvir/sofosbuvir (LDV/SOF) at no cost. In the Peer-Mentor group, participants engaged in structured interactions with HIV/HCV cured peers. In the Cash Incentives group, participants received escalating cash incentives (up to $220) which were contingent on attendance to clinic visits. The primary endpoint was initiation of LDV/SOF within 8 weeks of enrollment (12 weeks if a change in HIV regimen was required); secondary endpoints were sustained virologic response (SVR), drug/alcohol use, and HCV reinfection. HCV RNA was assessed at treatment weeks 4, 12, and post-treatment week 12. **Results**: 144 participants were randomized to Usual Care (n=36), Peer-Mentors (n=54) or Cash Incentives (n=54). Baseline characteristics were similar in each group. The majority of participants were >55 years old (50%), male (61%), black (93%) and infected with HCV genotype 1a (78%). Despite high levels of depression (61%) and ongoing drug (25%)/alcohol (42%) use, most (97%) were taking antiretroviral therapy with HIV RNA suppression (81%). Initiation of LDV/SOF was observed more frequently in the Peer-Mentor group (83%, 45 of 54) (p=.07) compared to the Usual Care (66%, 24 of 36) and Cash Incentives (76%, 41 of 54) groups. Overall (intention to treat), SVR was more frequently observed in the Peer-Mentor group (76%, 37 of 54) (p=.13) compared to the Usual Care (61%, 22 of 36) and Cash Incentives (68%, 37 of 54) groups. Of those that did not achieve SVR, 1 patient was reinfected, 2 relapsed, and 6 did not complete treatment. Serious adverse events not related to LDV/SOF occurred in 10% of participants. **Conclusion**: Overall, 24% of 144 coinfected PWIDs did not initiate HCV treatment despite access to expert clinicians and LDV/SOF at no cost. Access to DAAs is necessary but may not be sufficient to achieve HCV cure among populations of PWIDs; these data suggest peer-mentors may effectively increase HCV linkage and treatment.

Disclosures:

Mark S. Sulkowski - Advisory Committees or Review Panels: Merck, Janssen, Gilead, Trek, Cocrystal, AbbVie; Grant/Research Support: Merck, AbbVie, Janssen, Gilead

Sherilyn Brinkley - Consulting: Merck, AbbVie; Speaking and Teaching: Merck, Gilead, AbbVie

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Low rates of de novo or recurrent hepatocellular carcinoma in HCV cirrhotic patients treated with direct-acting antivirals (DAAs): a single-center experience

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Background and aim: Direct-acting antivirals (DAAs) for treatment of hepatitis C virus (HCV) infection have been associated with an increased incidence of hepatocellular carcinoma (HCC) in patients with and without a previous HCC history. Aim of our study was to assess the incidence of de-novo or recurrent HCC among cirrhotics treated with DAAs. **Materials and Methods:** Consecutively treated cirrhotics not listed to liver transplant (LT) who underwent DAAs at a single Center were prospectively enrolled. Cirrhosis was defined clinically, histologically (METAVIR F4) or according to liver stiffness measurement (LSM > 11.9 kPa). All patients were on regular 6-month surveillance. Hepatocellular carcinoma (HCC) was diagnosed according to International recommendations and treatment conformed to the Barcelona Clinic Liver Cancer (BCLC) criteria. **Results:** Between January 2015 and December 2016, 565 cirrhotics were treated. They were males (60%), with a median age of 65 (30-87) years, and HCV genotype was 1a in 15%, 1b in 49%, 2 in 13%, 3 in 11%, 4 in 12% and 5 in 1. At baseline, median LSM was 17.5 (10.1-75) kPa; Child-Pugh-Turcotte (CPT) score was A in 89% and B in 11%. Median baseline alpha-fetoprotein (AFP) was 9.4 (0.8-538) ng/mL. De-novo HCC occurred in 20 (4%) patients after 14-106 (median 42) weeks from treatment start, with an estimated annual incidence of 1.6%. At diagnosis, CPT was A in 80%, B in 10% and C in 10% of the patients. Cancer was either single (65%), bifocal (15%) or multifocal (20%), within Milan criteria in 16 (80%) patients. Median AFP was 6 (2-57) ng/ml. Patients were offered LT (35%), radiofrequency ablation (RFA, 30%), resection (20%), trans-arterial chemoembolization (TACE, 10%), or best-supportive care (5%). Forty-eight (8%) patients had a previous history of HCC. In 9 (19%) patients (89% CPT A) HCC recurred after 39 (5-100) weeks from DAAs start, with an annual incidence of 7.7%. Imaging was negative 8 (1-45) weeks before. At recurrence, HCC was single in 4 (44%), bifocal in 2 (22%) and multifocal in 3 (34%) patients, sized 16 (13-23) mm, with an AFP value of 9 (83-123) ng/ml. In all cases HCC was BCLC A. Patients were offered LT (34%), RFA (33%), resection (11%), or TACE (22%). Treatment options were similar in patients with recurrent or de-novo HCC (p=0.95), and no patients died of liver cancer after 9 (1-20) months from post-DAA HCC diagnosis. **Conclusions:** In a large cohort of HCV cirrhotics treated with DAAs, rates of de-novo or recurrent HCC are lower than initially reported in other cohorts. In addition, most HCC following DAA-based treatments are eligible to radical treatment options.

Disclosures:

Alessio Aghemo - Advisory Committees or Review Panels: Gilead, Abbvie, MSD, BMS, Janssen; Grant/Research Support: Gilead

Pietro Lampertico - Advisory Committees or Review Panels: Roche, Gilead, GSK, BMS, MSD; Speaking and Teaching: Roche, Gilead, GSK, MSD

The following people have nothing to disclose: Elisabetta Degasperi, Roberta D'Ambrosio, Angelo Sangiovanni, Roberta Soffredini, Stella De Nicola

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Impact of Sustained Virologic Response with Direct-Acting Antiviral Treatment on Mortality and Hepatocellular Carcinoma

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Background: Reduced all-cause mortality remains the ultimate goal of direct-acting antiviral (DAA) treatment. We evaluated the impact of sustained virologic response (SVR) achieved with oral DAAs on all-cause mortality and incident hepatocellular carcinoma (HCC) in veterans with and without advanced chronic liver disease (ACLD). **Methods**: All-cause mortality rates and incident HCC rates were determined for all veterans in the HCV registry treated with DAAs by 30 September 2016 with laboratory-confirmed SVR or No SVR. ACLD was defined by FIB-4>3.25 at DAA start. Multivariate logistic regression models were used to identify predictors of mortality. **Results**: Among 15,059 ACLD patients, 162/1,067 with No SVR and 441/13,992 with SVR died over a mean follow-up period of 1.3 years; 125/867 No SVR and 346/13,153 SVR patients had incident HCC. SVR was associated with an 80.9% reduction in mortality (12.5 deaths/100 patient years (py) No SVR versus 2.4 deaths/100 py SVR, p<0.001) and an 80.5% reduction in incident HCC (9.7 HCCs/100 py No SVR versus 2.4 HCCs/100 py SVR, p<0.001). In ACLD patients with prior HCC, death rates were 19.6 deaths/100 py with No SVR and 7.0 deaths/100 py with SVR, a 64.1% reduction (p<0.001). Among 34,493 non-ACLD patients, 52/1,330 with No SVR and 324/33,163 with SVR died over a mean follow-up of 1.2 years; 27/1,287 No SVR and 65/32,911 SVR patients had incident HCC. SVR was associated with a 79.5% reduction in mortality (3.4 deaths/100 py No SVR versus 0.8 deaths/100 py SVR, p<0.001) and a 91.3% reduction in incident HCC (1.8 HCCs/100 py No SVR versus 0.2 HCCs/100 py SVR, p<0.001). In non-ACLD patients with prior HCC, death rates were 9.0 deaths/100 py with No SVR and 3.3 deaths/100 py with SVR, a 63.1% reduction (p<0.001). In both ACLD and non-ACLD patients, SVR was strongly associated with delayed time until death (p<0.001) and until development of first HCC (p<0.001). The reductions in mortality and HCC with SVR were consistent across genotypes and were similar when considering one-year mortality and HCC rates. Reduced odds of death occurred in both ACLD and non-ACLD patients with SVR compared to No SVR (ACLD: odds ratio (OR) 0.20, 95% confidence interval (CI) 0.16-0.25, p<0.001; non-ACLD: OR 0.24, 95%CI 0.18-0.33, p<0.001). ACLD patients with a history of decompensation had increased risk of death (OR 2.92, 95%CI 2.46-3.47, p<0.001). **Conclusions**: ACLD and non-ACLD patients achieving SVR after DAA treatment had significantly lower all-cause mortality and lower incident HCC rates than those without SVR, regardless of genotype.

Disclosures:

The following people have nothing to disclose: Lisa I. Backus, Pamela S. Belperio, Troy A. Shahoumian, Larry A. Mole

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Age-dependent inflammation in liver-specific p62-mutant mice reveals the critical role of p62 in IκBα-mediated NFκB transcriptional repression

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Background: NF-kappa-B inhibitor alpha (IκBα) normally binds and sequesters NF-κB transcription factor in the cytosol. Extracellular signal triggered IκBα-phosphorylation and ubiquitin-mediated proteasomal degradation (UPD) causes unleashing and nuclear translocation of NF-κB and rapid transcriptional activation of downstream target genes including IκBα itself. The resulting *de novo* synthesized IκBα is imported into the nucleus and upon binding NF-κB, triggers its nuclear export and NF-κB-repression. Although IκBα is very stable ($t_{1/2}$ ≥8 h) when bound to NF-κB, free IκBα is rapidly degraded ($t_{1/2}$<15 min). It is unclear how newly synthesized IκBα can escape degradation and enter the nucleus. Because we previously discovered that sequestosome-1 (SQSTM1/p62) can bind and stabilize IκBα, we hypothesized that such binding could protect newly synthesized IkBa from degradation. We therefore examined the p62-domains involved in its IκBα-interaction, and its potential role in regulating NF-κB pathway. **Methods and Results**: We determined IκBα-$t_{1/2}$ in a HEK293T cell overexpression system with and without p62 co-expression. We found that p62 co-expression stabilized IκBα and extended its $t_{1/2}$ from 15 min to >4 h. Cell-treatment with UPD and calpain inhibitors revealed that p62 binding protected IκBα from degradation. Co-expression of various p62 truncation mutants with IκBα followed by co-immunoprecipitation revealed that p62-PB1 domain and the region between its ZZ and TB domains were both important for this interaction. In-cell chemical crosslinking and LC-MS/MS proteomic analyses coupled with mutagenesis-scanning analyses, successfully identified the p62 residues critical for IκBα-binding. To examine the specific role of p62 in IκBα-mediated NF-κB feedback, we employed $p62^{+/+}$ and $p62^{-/-}$ MEF cells. After TNFα-treatment, $p62^{-/-}$ cells exhibited lower content of newly synthesized IκBα relative to $p62^{+/+}$ cells, with a correspondingly prolonged NF-κB response. To determine the physiological relevance of this finding, we generated liver specific p62-mutant mice with p62 68-252-region deleted ($p62^{mut}$). Liver histology of 4, 12 and 18 month-old mice revealed significantly greater inflammation in $p62^{mut}$-mice than in wild type, which progressed dramatically with age. **Conclusions**: Our findings reveal a novel role of p62 in modulating TNFα-elicited inflammatory responses, through direct binding and stabilization of newly synthesized IκBα. Such IκBα stabilization enables its nuclear import and consequent termination of NF-κB activation. More importantly, we document that p62 is critical for limiting age-related liver inflammation *in vivo*.

Disclosures:

The following people have nothing to disclose: Yi Liu, Mike Trnka, Doyoung Kwon, James P. Grenert, Maria Almira Correia

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Extracellular pro-inflammatory ASC 'specks' released by inflammasome-mediated pyroptosis to the circulation in alcoholic hepatitis and deposition of bioactive ASC oligomers in human and murine livers are biomarkers and mediators of alcoholic hepatitis

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Background: Ethanol exposure results in NLRP3 inflammasome activation and maturation of interleukin (IL)-1β, a key pro-inflammatory cytokine in alcoholic liver disease (ALD). Oligomerization of the adaptor protein ASC (Apoptosis-associated speck-like protein containing CARD) is essential for NLRP3 inflammasome activation. Extracellular ASC 'specks' that retain bioactivity are released during pyroptosis, a caspase-1 mediated cell death. However, the role of ethanol-induced pyroptosis and extracellular ASC has not been identified in ALD. **Aim**: To investigate the role of pyroptosis and pro-inflammatory extracellular ASC 'specks' in alcoholic hepatitis (AH). **Methods:** Cell death, inflammation, and liver injury were assessed in a mouse model of alcoholic hepatitis (AH) (10 days with binge). Inhibitors for NLRP3 (MCC950), Caspase-1 (VX-765), pan-Caspases (z-VAD-fmk), and necroptosis (necrostatin-1) were administered *in vivo*. Healthy and cirrhotic human livers and plasma from patients with alcoholic hepatitis (AH) and controls were also analyzed. **Results:** We analyzed plasma of healthy volunteers and patients with acute AH for the presence of ASC protein. Acute AH patients had increased soluble ASC protein in the circulation, as measured by ELISA and confirmed with immunoprecipitation. On immunohistochemistry, all ten livers from patients with alcohol-induced cirrhosis but none of the controls had extensive ASC aggregates in extracellular spaces. Using immunofluorescence, we observed extracellular ASC aggregates and ASC oligomers with immunoprecipitation in mouse livers with AH. We then hypothesized that extracellular ASC was dependent on liver macrophages undergoing pyroptosis, a necrotic form of inflammasome-dependent programmed cell death. Using flow cytometry, we found increased ethanol-induced pyroptosis and apoptosis indicated by a decrease in AnnexinV-positive macrophages from mice pre-treated with Caspase-1 or pan-Caspase inhibitors, respectively. Pre-treatment with a necroptosis inhibitor had no effect on cell death. Finally, pre-treating mice with MCC950, an NLRP3 inhibitor, reduced levels of Caspase-1 activity, macrophage pyroptosis and steatoheatitis in AH in mice. **Conclusions:** Increased circulating ASC protein may serve as a new biomarker for alcoholic hepatitis in humans. Our novel findings in murine samples and human patients with AH demonstrate that ethanol-induced inflammasome activity results in Caspase-1-mediated pyroptosis and extracellular ASC aggregates in the liver and circulation. Pyroptosis can be abrogated by therapeutic inhibition of inflammasome components, NLRP3 or Caspase-1.

Disclosures:

Gyongyi Szabo - Advisory Committees or Review Panels: Cyta Therapeutics, DISCUS, GALAXY Project, Nature Reviews in Gastro & Hepatology, NIH ExRNA Program, Prevent Cancer Foundation, Yale University Liver Center, University of Colorado Alcohol Center, University of Southern California Liver Center, Verlyx, University of Pittsburgh, MSTP EAB; Board Membership: ACER; Consulting: Trek Therapeutics, Novartis, Orbimed, Roviant, Salix, Tobira, Glympse Bio, Janssen Research & Development, Carlos Foundation; Grant/Research Support: NIH-NIAAA, BMS, Gilead, Genfit, Genentech, University of Florida, Intercept, Tobira, Takeda, Vertex, Shire, Allergen

The following people have nothing to disclose: Arvin Iracheta-Vellve, Patrick P. Lowe, Yeonhee Cho, Charles D. Calenda, Andras Budai, Jan Petrasek, Benedek Gyongyosi, David Tornai

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TLR 2/4 & 9 mediated Inflammasome activation drives CD14+ monocyte activation and may synergize with ER stress signaling in de novo autoimmune hepatitis (DAIH).

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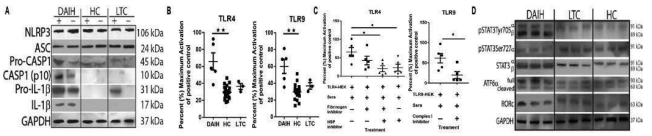
Background: Regulatory T cells (Tregs) of patients with DAIH display a phenotype of T_H1 & T_H17 cells & are functionally impaired; $CD14^+$ monocytes drive differentiation of Tregs towards T_H1-like Tregs in an IL-12 dependent manner. **Aim:** Identify mechanism(s) underlying Treg plasticity. **Methods:** Blood was obtained from liver transplanted (LT) recipients with DAIH (n=6), LT recipients without DAIH who have normal graft function (LTC) (n=16), & healthy non-transplanted children (HC) (n=13). **1)** $CD14^+$ monocytes isolated from PBMCs were stimulated with LPS for 24-hours &: (i) stained for intracellular IL-1β; (ii) RNA harvested for qRT-PCR for pro- IL-1β; (iii) protein harvested for Western Blot (WB) of inflammasome-associated proteins. **2)** Measurement of damage associated molecular patterns (DAMPs) in sera done by qRT-PCR & ELISA. **3)** To confirm TLR activation, TLR reporter cell lines cultured with patient sera +/- protein inhibitors for specific DAMPs identified above. **4)** FACS sorted Tregs ($CD127^-CD25^{hi}CD4^+$) of total PBMC's stimulated with PMA/ionomycin for 4-hrs & total RNA harvested for RNASeq & qRT-PCR; total protein harvested for WB. Comparison of quantitative variables between 2 groups performed using Wilcoxon rank-sum test. $P<0.05$ statistically significant. **Results: 1).** Inflammasome activation occurs in $CD14^+$ monocytes from patients with *D*AIH as evidenced by caspase-1 cleavage ($p<0.01$) & IL-1β secretion ($p<0.05$) (Figure 1A), & is mediated via TLR 2/4 & 9 (increased HMGB1, Fibrinogen, HSPs, mitochondrial and nuclear DNA in sera of patients with DAIH vs. HC & LTC (qPCR $p<0.005$; ELISA $p<0.05$). Moreover, DAMPs in sera of patients with DAIH activate TLR4 & 9 reporter cell lines ($p\leq0.01$ – figure 1B); & inhibition of Fibrinogen, HSPs and mitochondrial proteins in sera abrogated TLR4 & 9 activation ($p<0.05$ – Figure 1C). **2).** Enrichment of target genes of a transcriptional regulator of endoplasmic reticulum (ER) stress, (ATF6), observed in Tregs of patients with DAIH & confirmed by qPCR ($p<0.01$) & WB (cleaved ATF6a $p<0.05$). Top differentially expressed target genes of ATF6, i.e. RORC & STAT3, are transcriptionally upregulated (qPCR $p<0.01$; WB: RORC $p<0.01$, pSTAT3 $p<0.001$ – Figure 1D). **Conclusion:** Cross talk between the innate immune system & ER stress signaling may underlie Treg plasticity & disease pathogenesis in DAIH.



Disclosures:

The following people have nothing to disclose: Adam Arterbery, Mercedes Martinez, Steven J. Lobritto, Yaron Avitzur, Udeme D. Ekong

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Neutrophil Dysfunction in Acute on chronic liver failure

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BACKGROUND: Patients with acute on chronic liver failure (ACLF) often have a dysregulated immune response. Dysfunction of the innate immunity is an integral component of this immune failure. In order to better elucidate this pathway, we evaluated neutrophil dysfunction inpatients with ACLF, and its implications on 28- and 90-day mortality. METHODS: Forty-patients with chronic liver disease presenting with an acute decompensation (AD)-10 No-ACLF(ACLF-0), 10 each with grade I, II and III ACLF (CANONIC), and 10 healthy controls were prospectively evaluated for presence of neutrophil dysfunction. Neutrophil phenotype (NP) was expressed as percentage of cells positive for antibodies toboth CD16 and CD66b on flow cytometry. Neutrophil Phagocytic Capacity (NPC) was determined using flurorescein isothiocyanatelabelled IgG latex beadsphagocytosed per neutrophil and measured as Mean Fluorescence Intensity (MFI) using flow cytometer. Oxidative burstwas determined as percentage of neutrophils producing reactive oxygen species (ROS) both at rest and after stimulation with Phobol Myrisate Acetate (PMA). RESULTS: Neutrophil phenotype (NP)was better in controls compared to AD patients(CD16,66b+85.2±11.3% vs 68.3±22.4%, P=0.02). Similarly NPC was higher in controls(98.3±130.6 vs 22.5± 18.9 MFI, P=0.001). Oxidative burst (OB) at rest was more inAD patients compared to controls (Neutrophils producing ROS- 97.2±4.9% vs 91.3±9.2%, P=0.006). However, stimulated OB using PMA was more intensein controls than AD group (Neutrophils producing ROS -92±9.4% vs79.7±29.3%, P=0.003). Among patients with AD, both NPand NPCwas better in ACLF-0than ACLF-grades I-III(NP 80.7 ±12.8% vs 63.8±22.9%, P=0.034; NPC 33.6±18.4 vs 18.7±17.8 MFI, P=0.029). However, asignificant difference was not seen among different grades of ACLF.On comparing survivors vs non-survivors at 90-days, both NP (78.2±11.9% vs 62.2±24.11%, P=0.02) and NPC (33.3±22.7 vs 16.3±13.39 MFI, P=0.004) were higher in survivors. However, such a trend was not noticed at 28-days. NP(CD16,66b+) in >71.7%neutrophils had 78.6% sensitivity and 65.4% specificity in predicting 90-day survival(AUROC 0.70, 95%CI 0.55-0.90, P=0.017). NPC MFI>17.3had71.4 % sensitivity and 69.6 % specificity (AUROC 0.73, 95%CI 0.54-0.86, P=0.035) to predict 90-day survival. CONCLUSION:

Neutrophil phenotype and phagocytic capacity, and stimulated oxidative burst are impaired in patients with ACLF, despite a higher background oxidative burst in these patients. This is consistent with the impaired immune response despite higher background systemic inflammation in these patients. Neutrophil phenotype and phagocytic capacity may predict survival at 90 days.

Disclosures:

The following people have nothing to disclose: Radha K. Dhiman, Dr Kunaal Makkar, Shallu Tomer, Sahaj Rathi, Sunil Arora, Sunil Taneja, Ajay K. Duseja, Yogesh K. Chawla

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TNF-α Serves Critical Roles in Regulation of Chemokine/Cytokine/Receptor Responses Activated by Innate Immune System after Transplantation of Allogeneic Hepatocytes

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To realize benefits of liver-directed cell therapy for acute or chronic liver diseases controlling rejection of allogeneic cells is critical. Despite use of immunosuppressive drugs, innate immune system remains active and poses a major hurdle in engraftment of allogeneic cells. After transplantation of allogeneic organs, tissue or cells, cascades of chemokines, cytokines and receptors contribute in rejection. We hypothesized that identification of key regulators capable of altering chemokine/cytokine/receptor responses will be helpful for cell therapy. Approaches combining immunosuppressive and anti-inflammatory mechanisms would be particularly appropriate. Therefore, we addressed the role of host immune responses through temporal release of inflammatory cascades during rejection of transplanted cells with assays in rats. Methods/ Results: Allogeneic donor hepatocytes were isolated from healthy Long-Evans Agouti (LEA) rats and transplanted intrasplenically into MHC-mismatched DPPIV- F344 rats with noimmunosuppression (Group I) or with twice weekly dosing of mycophenolate mofetil (MMF) plus tacrolimus (Tacro) to block T cell allo responses (Group II). Tissue analysis with DPPIV staining of liver showed transplanted cells were rejected over 14d with first-order kinetics in Group I and survived in Group II, albeit less than syngeneic hepatocytes. We then analyzed gene expression by qRT-PCR arrays for 84 chemokines/cytokines/receptors 6h and 3,,7 or 14d after cell transplantation. In Group I, inflammation due to tissue injury, neutrophil, monocyte or lymphocyte activation, including TNF-α and IL-6 expression was noted within 6h and up to 14d. In Group II, inflammation decreased or normalized over time but TNF-α expression remained elevated. To define role of TNF-α in rejection, we blocked it by etanercept (ETN) alongside MMF and Tacro before transplanting cells (Group III). In this case, TNF-α and IL-6 expression normalized within 6h and more cells engrafted. In additional studies, activation of Kupffer cell phagocytosis, myeloperoxidase + neutrophils and CXCR2 protein expression also decreased. Moreover, liver repopulation assays in retrorsine/partial hepatectomy model showed significantly superior engraftment and proliferation of transplanted cells in Group III. Conclusions: Rejection of allografted hepatocytes was accompanied by early and persistent activation of chemokines/cytokines/receptors associated with innate immune responses. TNF-α served a major role in regulating this immune response with involvement of neutrophils and Kupffer cells. This principle of cytokine interference should help improve outcomes in cell therapy and organ transplantation.

Disclosures:

The following people have nothing to disclose: Fadi L. Jaber, Yogeshwar Sharma, Sorabh Kapoor, Sanjeev Gupta

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Exercise dramatically improves age-related, inflammation-driven liver damage and cancer.

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Background Whilst there are several mouse models to study the potential of therapeutic interventions in chronic liver disease (CLD) and hepatocellular carcinoma (HCC) most lack one of the major risk factors associated with HCC development 'ageing'. Ageing is associated with chronic low-grade inflammation, increased hepatic lipid accumulation, loss of the regenerative potential of hepatocytes and an increased risk of fibrosis. In this study we are utilizing the aged NF-kB1$^{-/-}$ model characterised by spontaneous premature ageing, systemic progressive low-grade inflammation, hepatic steatosis, fibrosis and HCC (Wilson *et al, Nature Commun* 2015) to investigate the impact of a feasible lifestyle therapy 'moderate exercise' on age-related CLD and HCC development. **Methods** 16 month old NF-kB1$^{-/-}$ with established chronic inflammation and spontaneous liver damage were treadmill exercised 3 times/week 20cm/sec or remained as sedentary controls. Behavioural tests were performed at 16, 17 and 18 months. Animals were sacrificed at 19 months and serum and organs removed for IHC analysis, cytokine array and liver enzyme and lipid quantification. **Results** The development of spontaneous liver disease including inflammation, steatosis and the development of liver tumours was dramatically reduced in the exercise group. IHC analysis revealed a striking reduction in various immune cells including neutrophils, B cells and T cells (FOXP3^{+} and CD8^{+}) in the exercised livers. Cytokine array in serum and liver protein also revealed a significant reduction in the chemokines CXCL9 and CXCL10 in both blood and liver from the exercised group. Whilst there was no difference in the ALT and AST levels between groups there was a clear reduction in steatosis shown by H&E and circulating LDL and cholesterol in the exercised mice. Furthermore, cognitive function and activity tests revealed that exercise positively impacts behaviour and activity of the mice. **Conclusion** We have shown that a simple moderate exercise regime in an aged group of mice with chronic low grade inflammation and CLD significantly improves liver health and the development of cancer. This striking finding has major implications as an easily translatable low impact therapy for an increasingly aged population with incrementally increasing NASH and HCC numbers.

Disclosures:

The following people have nothing to disclose: Arianna Bianchi, Dina Tiniakos, Derek Mann, Caroline Wilson

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Frailty After Liver Transplantation (LT)

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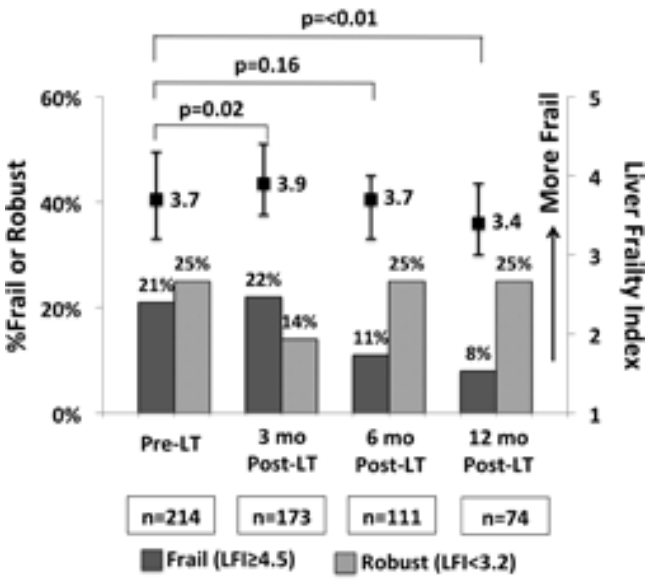
Background: We developed the Liver Frailty Index (LFI) using grip strength, chair stands, and balance to capture "frailty" in cirrhotic patients. In contrast to metrics specific to liver fxn such as jaundice and ascites, which reverse soon after LT, little is known of what happens to frailty *after* LT. **Methods**: We analyzed data on 214 adult LT recipients who had >=1 assessment using the LFI [= (sex-adjusted grip*-0.33)+(chair stands*-2.53)+(balance*-0.04)+6] at 3, 6, *or* 12 months (mo) post-LT [higher values=more frail]. "Frail" and "robust" were defined using previously established cut-offs (>=4.5 and <3.2). Odds ratios assessed associations with post-LT robustness. **Results**: At a median of 2.4 mo pre-LT, median(IQR) LFI=3.7(3.2-4.3); 21% were frail pre-LT (Fig). After LT, median(IQR) LFI scores worsened at 3mo but improved at 6 and 12 mos post-LT (Fig). **Among those who were robust pre-LT (53/214), 61% remained robust post-LT. In contrast, among those who were frail pre-LT (44/214), only 4% became robust at any time post-LT.** In univariable analysis, each point improvement in pre-LT LFI (=*less* frail) was associated with an increased odds of being robust after LT at 3mo (OR 17.7; 95%CI 5.6-55.6), 6mo (OR 14.7; 95%CI 4.8-45.0), and 12mo (OR 3.8; 95%CI 1.8-8.2), which did not change w/ multivariable analysis. Pre-LT frail pts had higher post-LT length of stay (9 vs. 7d; p<0.01), and a trend toward more ICU days (3vs.2d; p=0.07) and 90d readmissions (1vs.0; p=0.06). **Conclusions**: Frailty worsens 3mo post-LT but improves modestly by 12mo. But only 1 in 4 pts are robust post-LT. Among the pre-LT frail pts, achieving robustness after LT is rare (<5%). Aggressive interventions aimed at *preventing* frailty pre-LT or intervening early post-LT are urgently needed to optimize LT-benefit.

Figure. Liver Frailty Index (LFI) scores and %frail /robust before and up to 12 months after liver transplant.



Disclosures:

Jennifer C. Lai - Consulting: Third Rock Ventures

Sandy Feng - Grant/Research Support: Novartis, Quark; Stock Shareholder: Amgen, Glaxo Smith Kline, Pfizer

The following people have nothing to disclose: Dorry L. Segev, Charles E. McCulloch, Jennifer L. Dodge

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New insights from the SILVER study; a 50 % reduction in Calcineurin Inhibitors at day 30 does not impact on long-term renal function after liver transplantation.

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Background: Renal dysfunction after liver transplantation (LT) is common and the long term use of calcineurin inhibitors (CNI) is associated with nephrotoxicity. The 50% reduction in CNI at 4 - 6 weeks post transplantation during the SILVER study enabled evaluation of this early reduction and its impact on long term renal function. **Methods**: An immunosuppressive strategy with a 50 % reduction of CNI and introduction of the mTOR inhibitor Sirolimus within 4 to 6 weeks after LT (group B, n=249) was compared to standard CNI-based mTOR-free immunosuppression (group A, n=254). Data was retrieved from the randomised controlled multi-centre trial of Sirolimus in Liver Transplant Recipients with HCC (SiLVER) study in an intention-to-treat approach over a study period of 5 years. **Results** The two groups were well-matched for recipient, donor and transplant procedure characteristics. Median pre-transplant recipient MELD was 10 (7 - 15) in group A vs 11 (8 - 15) in group B. A total of 38.5% vs 39.3% and 3.6% vs 2.6% were low MELD and high MELD recipients, respectively (MELD cut-off 24). Early CNI reduction was achieved as stipulated in the protocol with median CNI reduction of 11% vs 56% and 20% vs 54% for CNI trough and dose at 3 month post-transplantation in group A vs group B, respectively. The Sirolimus trough target of 4 - 10 ng/ml was achieved in 63% - 82% in group B over the study period. Baseline renal function as measured by eGFR was similar for both groups. A temporary renal sparing effect was observed at 3 months in the Sirolimus arm [67 (55 - 85) vs 74 (59 - 95) ml/min, p=0.0051) but renal function was not significantly different at all later time points (6 months, 12 months, and yearly intervals up to 5 years). Using generalized estimating equations with post-transplant day 28 as baseline, no difference was found for eGFR between the groups with further sub-stratification into CyclosporinA and tacrolimus as CNI. **Conclusion:** This analysis suggests that a 50% reduction in CNI dose at 4 - 6 weeks in low MELD patients with HCC does not have a protective effect on long term renal function.

Disclosures:

James W. Ferguson - Advisory Committees or Review Panels: Astellas, Novartis

Andreas Schnitzbauer - Advisory Committees or Review Panels: Sequana, Novartis, Chiesi; Speaking and Teaching: Atellas

Edward Geissler - Speaking and Teaching: Novartis, Astellas, Pfizer

The following people have nothing to disclose: Bettina M. Buchholz, Peter Nightingale, Darius Mirza

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Cardiovascular Disease Outcomes Related to Early Stage Renal Impairment Following Liver Transplantation

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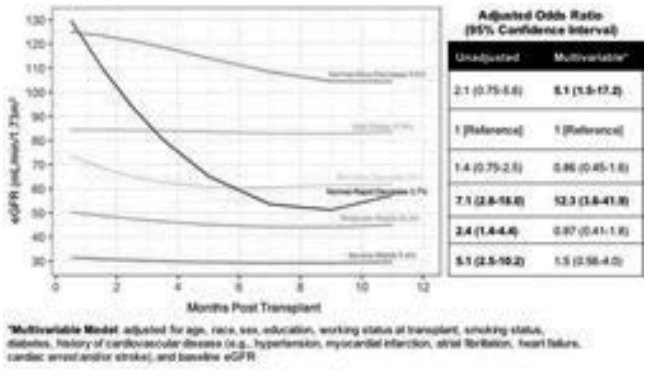
Background: Renal and cardiovascular disease (CVD) are major complications after liver transplant (LT). In the general population, even mild renal disease is associated with increased mortality, mainly from CVD. Whether this is true in LT recipients is unknown. **Methods:** This was a retrospective cohort study of 37,322 LT recipients (2002-2012) using Vizient data linked to the United Network for Organ Sharing. The Modification of Diet in Renal Disease (MDRD4) equation estimated glomerular filtration rate (eGFR). The primary endpoint was mortality. Latent mixture modeling identified trajectories in eGFR within a year of LT in a subsample of 671 recipients from an academic center. Logistic regression assessed associations between eGFR trajectory and 1-year CVD complication, defined as death/hospitalization from myocardial infarction, heart failure, atrial fibrillation, cardiac arrest, pulmonary embolism or stroke. **Results:** Mean(SD) eGFR was 72.1(45.7) ml/min/1.73m^2. The risk of mortality increased with lower baseline eGFR. Each 5-unit lower eGFR was associated with a 2% and 5% higher hazard of all-cause and CVD mortality, respectively ($p<0.0001$). Six distinct eGFR trajectories were identified (**Figure)**: qualitatively Normal-Slow Decrease (4% of cohort), Normal-Rapid Decrease (4%), Mild-Stable (18%), Mild-Slow Decrease (35%), Moderate-Stable (30%), and Severe-Stable (9%). In multivariable models, compared to the Mild-Stable group, the greatest odds of a CVD complication were in the Normal-Rapid Decrease group even when adjusted for confounders and baseline eGFR (**Figure**). **Conclusion:** Even mild renal disease is a risk factor for mortality after LT. The pattern of decline in eGFR early after LT, particularly when more rapid, correlates with the risk of adverse CVD outcomes, further highlighting the need for early renal preservation strategies.



eGFR Trajectories by Months from Liver Transplant in the Northwestern Medicine Enterprise Data Warehouse (NMEDW) and Adjusted Odds Ratio for the Association of Trajectory Group with a Cardiovascular Disease Complication within 1 Year of Transplant, Total N=671

Disclosures:

Lisa B. VanWagner - Grant/Research Support: Novartis; Speaking and Teaching: Salix

Norrina Allen - Grant/Research Support: Novartis

John J. Friedewald - Consulting: Transplant Genomics, Inc.; Grant/Research Support: Shire, Pfizer; Speaking and Teaching: Novartis, Sanofi

Josh Levitsky - Consulting: Transplant Genomics Incorporated; Grant/Research Support: Novartis; Speaking and Teaching: Gilead, Salix, Novartis

The following people have nothing to disclose: Samantha E. Montag, Donald M. Lloyd-Jones, Arighno Das, Samuel Hohmann

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Center-level Impact of Early Liver Transplantation for Alcoholic Hepatitis

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Background: Early liver transplantation (LT) for severe alcoholic hepatitis (AH) can be a rescue therapy in highly selected patients not responding to medical therapy and with favorable psychosocial profiles. Given the prevalence of alcoholic liver disease and the lack of medical therapies for AH, many centers are now considering performing LT for this emerging indication. The center-level impact of performing early LT for AH is unknown. **Purpose:** The purpose of this study is to examine the center-level impact of implementing a program of early LT for AH. **Methods:** Using our previously published candidate selection process, our center implemented a strategy of early LT for severe AH in January 2012. We reviewed 176 patients with severe AH hospitalized at our center from 7/2011 to 7/2015, including psychosocial evaluation data. We also examined a prospectively maintained database of consecutive referrals for adult inpatient hospital transfers to our LT center. Patients seen by a hepatologist at our center prior to transfer and repeat transfers of the same patient were excluded. The primary outcome for analysis was number of AH transfers. Secondary outcomes were LT and time spent on psychosocial evaluations. **Results:** Over a 4-year period, 1126 patients were transferred to our center, 526 (47%) of which were never seen previously (new-to-center). Of these, 81 (15%) were transferred for severe AH. These patients had a mean age of 44 years, median MELD 33, median discriminant function 73 and a slight male predominance (54%). In the 6 months prior to implementing our early LT for AH program, 1 patient was transferred for AH. After program initiation, about 2 AH patients per month were transferred to our center. The mortality rate of transferred AH patients was 42%. Overall, expedited psychosocial evaluations of 147 AH nonresponders were performed by our psychosocial team, comprised of 1 LT psychiatry fellow and attending and 1 LT social worker. A total average time of 2 hours were spent on each psychosocial evaluation and 15 minutes/candidate at our weekly transplant candidacy meeting. Two transplant psychiatrists and 4 full-time LT social workers are

employed by our center. During the study period, 27 (18%) candidates had favorable profiles and were accepted for early LT, 18 (12%) survived to waitlisting and 14 (9.5%) underwent early LT for AH. **Conclusions:** The center-level impact of early LT for AH is significant and affects many aspects of a LT center. Centers should consider the significant downstream effects of program implementation: an 11-fold increase in hospital transfers for AH and 331 hours of psychosocial evaluations during the study period.

Disclosures:

The following people have nothing to disclose: Alexander Vogel, Jonathan Nahas, Akhil Shenoy, Zorica Filipovic-Jewell, Laura Ford, Margot Wankoff, Lindsey Zelus, Simone Safyer, Sander S. Florman, Thomas Schiano, Gene Y. Im

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Induction of mixed chimerism and prolonged liver allograft survival off immunosuppression in cynomolgus monkeys

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Background: Spontaneous liver tolerance occurs only in highly selected patients with stable liver function for several years. There is an unmet need for a safe and reliable regimen allowing rapid immunosuppression (IS) withdrawal. Herein, we investigated if tolerance to liver allografts can be achieved using a mixed chimerism (MC) approach. **Methods:** Nine combined liver and bone marrow transplantations (CLBMT) were performed in cynomolgus macaques, using three different non-myeloablative conditioning regimens (group A, B, C). All animals received total body irradiation (1.5Gy), thymic irradiation (7Gy), and ATGAM prior to CLBMT. Rituximab, anti-CD40, and anti-CD2 monoclonal antibodies were given according to Table 1. Two additional livers (group D) were transplanted with the same protocol as in group C, but excluded the BMT. All IS was completely discontinued by day 55. **Results:** All recipients developed multilinege mixed chimerism. Group A showed severe rejection with CD8+ T effector memory (Tmem) cell expansion. Improved pretransplant host Tmem depletion using anti-CD2 in group B resulted in prolonged multlineage MC and graft acceptance. Animal B(2), however, developed grade 1 GVHD in skin and rectum. The addition of post-transplant anti-CD2 in group C also depleted donor Tmem, thereby preventing GVHD, while permitting prolonged MC and extended IS-free graft survival. With the exclusion of BMT (group D), passenger leukocytes promoted transient MC. Even when chimerism was lost, and memory cells recovered, group D maintained normal graft function. The percentage of Tregs in total CD4 were elevated over baseline during the period of stable graft function in Groups C and D animals. **Conclusions:** This is the first proof of concept study demonstrating prolonged liver allograft survival in nonhuman primates using mixed chimerism to facilitate rapid IS withdrawal. The results demonstrate a need to control Tmem responses and suggest even low-level MC that occurs with liver only transplants is associated with prolonged graft survival after IS withdrawal.

Group (animal#)	MHC mismatch	Induction IS	Anti-CD2	Chimerism (days)	Peak chimerism	Survival (days)	Outcome
A(1)	4/6	CyA	None	33	65%	42	Severe AMR/TCMR
A(2)	5/6	CyA Rituximabx1	None	40	17%	69	Severe TCMR
A(3)	Haplo	CyA Rituximabx1	None	22	21%	57	Severe TCMR
B(1)	Full	Tacrolimus Anti-CD40 Rituximabx2	Day-4,-3	〉61	30%	61	Arrhythmia, diarrhea No rejection
B(2)	5/6	Tacrolimus Anti-CD40 Rituximabx2	Day-4,-3	〉54	93%	54	GvHD grade 1 No rejection
C(1)	4/6	Tacrolimus Anti-CD40 Rituximabx1	Day-3,+1	117	85%	317	Arrhythmia Transient moderate rejection
C(2)	Full	Tacrolimus Anti-CD40 Rituximabx1	Day-3,+1	〉69	93%	69	Pneumonia No rejection
C(3)	Haplo	Tacrolimus Anti-CD40 Rituximabx1	Day-3,+1	127	87%	〉260	Ongoing Rejection crisis
C(4)	Haplo	Tacrolimus Anti-CD40 Rituximabx1	Day-3,+1	〉25	33%	25	Volvulus No rejection
D(1)	Haplo	Tacrolimus Anti-CD40 Rituximabx1	Day-3,+1	34	6%	〉134	Ongoing Stable graft function
D(2)	4/6	Tacrolimus Anti-CD40 Rituximabx1	Day-3,+1	〉76	21%	〉78	Ongoing Stable graft function

Disclosures:

The following people have nothing to disclose: Erik Berglund, Jeffrey Stern, Sulemon Chaudhry, Paula Alonso-Guallart, Mercedes Martinez, Genevieve Pierre, Diane Ordanes, Joshua Weiner, Dilrukshi Ekanayake-Alper, Alina Iuga, Yojiro Kato, Megan Sykes, Adam D. Griesemer

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Gut Microbial Functional Changes After Liver Transplant Can Modulate Infection Risk And Increase Atherogenic Metabolites

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There is pre-liver transplant (LT) gut dysbiosis, which improves after LT. Pre-LT dysbiosis is linked with infections e.g. C.difficile & intestinal barrier dysfunction due to lower gut bile acids (BAs, total, secondary & iso-BA). Post-LT pts are also prone to atherogenesis, which in non-cirrhotics are associated with microbial-mammalian metabolite trimethylamine oxide (TMAO) increase. **Aim**: Define the effect of LT on gut microbial function related to infections & TMAO generation. **Method**: Cirrhotics on the LT list underwent blood, urine & stool collection for gut microbiota structure(16SrRNA) &functionality (stool BA using GC/MS, serum endotoxin LAL assay, urine metabolomics NMR). These were re-analyzed 6 mths post-LT. Pts unable to consent/provide samples, with rejection/infections post-LT in 3 mths, re-LT or HIV were excluded. Microbiota function: BA profile included total, conjugated, unconjugated, primary, secondary, iso & oxo BAs. Of these conjugated BAs are produced by the liver, which undergo transformations related to deconjugation, conversion of secondary BAs & conversion of native to iso-BAs, which make them less toxic to the intestinal barrier. **Results**: 35 pts (MELD 28, 12 HCC, mean 56 yrs, 21 Hep C) underwent LT 4±3 mths post-enrollment & re-eval-

uated 6±3 mths post-LT. All were on regular tacrolimus & Bactrim. 24hr dietary recall remained similar. Microbiota structure: A significant increase in diversity with relatively higher beneficial taxa, *Lachnospiraceae, Ruminococcaceae* &Clostridiales XIV &lower *Enterobacteriaceae* were seen post-LT. Microbial function: There was a significant reduction in endotoxin levels (0.78±0.3 vs 0.09±0.4, p=0.001). There was a 2.4 fold increase in urinary TMAO levels post-LT. BA profile changed with a significantly higher total, primary, secondary BAs & iso-BAs (Table) without change in deconjugation. **Conclusions**: After LT, there is a significant improvement in the gut bacterial milieu likely induced by a resumption of BA flow encouraging the growth of microbiota that can generate secondary and iso-BAs. These bacterial changes generating TMAO could accelerate atherogenesis. Bacterial functional alterations post-LT could mediate both beneficial and adverse effects on the host.

Micromoles/gm of stool	Pre-LT	Post-LT
Total BAs	3.44±4.62	10.43±9.78*
Total Primary BAs	1.73±2.21	4.31±5.8*
Total Secondary BAs	1.07±2.82	6.23±7.10*
Secondary/Primary BA ratio	1.54±2.4	15.1±15.6*
Total conjugated BAs	0.29±0.54	0.12±0.16
Total Oxo-BAs	0.47±0.74	2.16±2.72*
Total Iso-BAs	0.44±0.57	1.37±1.7*

Disclosures:

Jasmohan S. Bajaj - Consulting: Abbott, Norgine, Valeant; Grant/Research Support: Salix, Grifols

Douglas M. Heuman - Consulting: Bayer, Grifols, Genzyme; Grant/Research Support: Exilixis, Novartis, Bayer, Bristol Myers Squibb, Scynexis, Ocera, Mannkind, Salix, Globeimmune, Roche, SciClone, Wyeth, Otsuka, Ikaria, UCB, Celgene, Centocor, Millenium, Osiris, AbbVie, Gilead; Speaking and Teaching: Otsuka, Astellas; Stock Shareholder: General Electric

William M. Pandak - Employment: Virginia Commonwealth University, Veterans Affairs Medical Center

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Advanced machine learning techniques to identify a panel of biomarkers that identify nonalcoholic steatohepatitis

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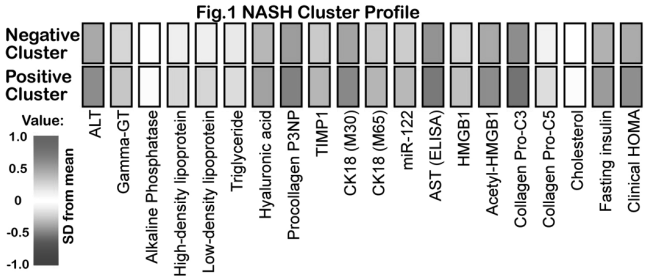
Background Around 25% of the adult population globally has non-alcoholic fatty liver disease (NAFLD), among those 7-60% have nonalcoholic steatohepatitis (NASH) which may progress to cirrhosis and liver cancer, leading to decompensation, transplantation and death. Although non-invasive markers of liver fibrosis have come into routine clinical practice, non-invasive markers that correlate with histopathological changes of NASH are desirable tools to stratify patients for interventions and to monitor disease progression. **Methods** A prospective cohort of patients characterised by demography, anthropometry, clinical, radiological and laboratory parameters as well as histology were collated from several separate studies in which serum analytes had been determined. 374 UK patients with biopsy-proven NAFLD were included. Histological parameters were scored using the Clinical Research Network NAFLD Activity Score (NAS) scale. A total of 44 variables were analysed using machine learning techniques. **Results** Clustering based on Principal Component method on mixed data identified the optimal number of clusters as 2 for histological variables of steatosis, ballooning and lobular inflammation and 2 NAS clusters: 1-4 and 5-8. The NASH 'positive cluster' identified comprised NAS>4, steatosis>2, ballooning>1, lobular inflammation>1 (Fig.1). Recursive Feature Elimination on Naïve Bayes identified 7 variables required for effective classification of NASH: Acetyl-HMGB1, Aspartate Aminotransferase (AST), Collagen Pro-C3, cytokeratin CK18 (M30) fragments, Procollagen III N-terminal peptide (P3NP), PNPLA3 rs738409, and Alanine transaminase. The Naïve Bayes model based on this panel gave an area under the curve of 0.87, 71% sensitivity, 87% specificity prediction performance. Regression analysis revealed significance for P3NP, AST, acetyl-HMGB1, CK18 (M30), and possession of PNPLA3 variant. **Conclusions** We describe a machine learning approach which identified a panel incorporating key biomarkers that would reliably identify NASH among patients with NAFLD. Negative predictive value 84% suggests a potential use of the identified panel in clinical

practice screening NAFLD patients to identify those likely to have NASH for appropriate intervention.



Disclosures:

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Risk prediction for the development and progression of nonalcoholic fatty liver disease with genetic markers

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Background & Aims: The genetic background affecting the natural history of nonalcoholic fatty liver disease (NAFLD) including the development of nonalcoholic steatohepatitis (NASH) and NASH-derived hepatocellular carcinoma (NASH-HCC) is still unknown. Our aim was to identify genetic factors for the development of NAFLD, NASH and NASH-HCC, and to establish risk estimation models. **Patients & Methods**: 936 histologically proven NAFLD patients were recruited for this study, who were histologically classified into 4 types according to the classification of Matteoni et al. Genome-wide association (GWA) studies were conducted for 902 patients including 476 NASH and 58 NASH-HCC against 7,672 controls. Risk estimations for NAFLD, NASH and NASH-HCC were performed using SNPs showing significant associations in the GWA studies. **Results**: Rs2896019 in *PNPLA3* (p=2.3x10^{-31}, OR(95%CI)=1.85(1.67-2.05)), rs1260326 in glucokinase regulator (*GCKR)* gene, (p=9.6x10^{-10}, OR(95%CI)=1.38(1.25-1.53)) and rs4808199 in GATA Zinc Finger Domain Containing 2A (*GATAD2A*) gene (p=2.3x10^{-8}, OR(95%CI)=1.37(1.23-1.53)) were significantly associated with NAFLD. The associations of *PNPLA3* and *GATAD2A* were lost when Matteoni type 1 to type 3 patients were used as cases. On the other hand, they became stronger when only type 4 and NASH-HCC patients were used for rs2896019 and for rs4808199, respectively, but the association of *GCKR* was lost. We newly identified rs17007417 in dystrophy-associated fer-1-like protein (Dysferlin or *DYSF)* gene (p=5.2x10^{-7}, OR(95%CI)=2.74(1.84-4.06)) being associated with NASH-HCC. The risk for NAFLD showed a multiplicative increase with the accumulation of risk alleles in *PNPLA3*, *GCKR* and *GATAD2A* (OR(95%CI)=40.1(10.9-222.9) for those carrying six risk alleles as compared to those without the risk alleles. The number of risk alleles in *PNPLA3* and *GATAD2A* was much higher in Matteoni type 4 (NASH) patients than type 1, type 2 and type 3 NAFLD patients, and the number in *PNPLA3* and *DYSF* was much higher in NASH-HCC than in NASH. **Conclusions**: We demonstrated that Matteoni type 4 NASH was genetically and clinically different from the other NAFLD subgroups. We also established risk estimation models for NAFLD, NASH and NASH-HCC, respectively, using multiple genetic markers. These results will improve the accuracy of NAFLD diagnoses and treatment decisions for patients.

Disclosures:

The following people have nothing to disclose: Takeshi Okanoue, Toshihide Shima, Masayuki Mizuno, Yasuhide Mitsumoto, Yoshihiro Kanbara, Miwa Kawanaka

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Development and Validation of the Collagen Neo-Epitope Biomarker Pro-C3 "FIB-C3 Score" for Detection and Staging of Advanced Non-Alcoholic Fatty Liver Disease in a Large International Multi-Centre Patient Cohort

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Background There is an urgent need for accurate non-invasive biomarkers to detect advanced fibrosis in NAFLD. Biomarkers that measure collagen turnover are appealing candidates but data from well characterised NAFLD cohorts to support their use are scarce. Pro-C3 is a direct marker of fibrogenesis that is released by ADAMTS2 during type III collagen maturation. Using a large, histologically characterised international patient cohort we assess the utility of the Pro-C3 collagen neo-epitope biomarker and develop a tractable panel that may be used to accurately diagnose advanced NAFLD fibrosis (F≥3) in the clinic. **Methods** A cohort of 433 well characterised patients with biopsy proven NAFLD across the full disease spectrum from steatosis to NASH-cirrhosis (F0: N=90; F1: 88; F2: 99; F3: 94; F4: 62) were studied. All biopsies were scored using the NASH CRN Score by an expert pathologist. NASH was defined by the presence of each component of the NAFLD Activity Score (NAS). Plasma Pro-C3 levels were determined using a competitive ELISA. After initial exploratory analysis, the cohort was divided into a Discovery group (n=320) and a Validation group (n=113) and logistic regression performed to establish a diagnostic panel for clinical use, "FIB-C3". Performance was compared against the FIB4 Score. **Results** Plasma Pro-C3 levels correlated with histological NAS (r_s=0.27, p<0.0001) and fibrosis stage (r_s=0.46, p<0.0001). In the discovery set, Pro-C3 had a similar performance to the FIB4 score for detection of F≥3 fibrosis (AUROCs 0.76 and 0.77 respectively). Based on logistic regression analysis, the optimised FIB-C3 diagnostic panel comprising age, BMI, T2DM, platelets and Pro-C3

was developed and validated (Discovery AUROC 0.86, Validation AUROC 0.85). An optimum threshold for F≥3 fibrosis (FIB-C3 ≥ -0.28) was determined using the Youden Index. The FIB-C3 panel consistently outperformed the FIB4 in both the Discovery and Validation cohorts. **Conclusion** Plasma Pro-C3 levels correlate with disease activity and fibrosis stage in NAFLD. The FIB-C3 panel has been validated as a highly accurate tool that maintains both sensitivity and specificity for the identification of NAFLD patients with active disease and advanced fibrosis (F≥3).

Test	Cohort	Sensitivity % (95%CI)	Specificity % (95%CI)	PPV % (95%CI)	NPV % (95%CI)
FIB4 (≥2.67)	Discovery (N=320)	25.2 (17.9-33.7)	91.1 (86.3-94.7)	64.0 (51.0-75.2)	66.1 (63.6-68.5)
FIB-C3 (≥ -0.28)		77.0 (68.7-84.0)	80.4 (74.1-85.8)	71.8 (65.4-77.5)	84.3 (79.5-88.2)
FIB4 (≥2.67)	Validation (N=113)	29.0 (14.2-48.0)	86.8 (78.1-93.0)	42.9 (25.9-61.6)	78.2 (73.9-82.0)
FIB-C3 (≥ -0.28)		76.7 (57.7-90.1)	75.9 (65.3-84.6)	53.5 (42.8-63.9)	90.0 (82.3-94.6)

Disclosures:

Jörn M. Schattenberg - Advisory Committees or Review Panels: Intercept Pharmaceuticals, AbbVie, Gilead Sciences, Boehringer Ingelheim; Consulting: Genfit SA, Novartis; Grant/Research Support: Yakult Deutschland GmbH; Speaking and Teaching: MSD Sharp & Dohme

Mette J. Nielsen - Grant/Research Support: Nordic Bioscience A/S

Morten A. Karsdal - Management Position: Nordic Bioscience; Stock Shareholder: Nordic Bioscience

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Gender and menopause significantly modify effects of metabolism-related SNPs on fibrosis stage in adult patients with nonalcoholic fatty liver disease (NAFLD)

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Background and Aims: Various bio-physiological attributes affecting NAFLD risks are significantly influenced by sex and sex hormones (i.e., regional fat distribution, lipid metabolism, adipocyte biology). We hypothesize that metabolism-related SNPs may influence hepatic fibrosis risk in sex- and/or menopausal status specific manners. **Methods:** We analyzed 636 adults who had a histologic diagnosis of NAFLD and whose blood had previously been analyzed using the Cardio-MetaboChip (Illumina), which includes 137,130 SNPs associated with metabolic syndrome and cardiovascular disease. We grouped subjects by gender and menopausal status according to demography and self-reported reproductive information. First, significant interaction was screened by ordinal logistic regression models including fibrosis stage (stage 0, 1, 3, and 4) as an outcome, each of SNPs and the gender/menopause categories as predictors, an interaction term (SNP*gender/menopause), and race/ethnicity and age as covariates. The Benjamini-Hochberg procedure was used to control for multiple comparisons with a target FDR of 0.3 to accommodate this exploratory step. Then, SNPs associated with a significant interaction term were further analyzed using separate ordinal logistic regression models for men, premenopausal women, and postmenopausal women. Cumulative odds ratio (COR) and 95% confidence interval of each SNP was computed for each gender/menopause category. **Results:** The study population includes 219 men, 223 postmenopausal women, and 194 premenopausal women (age ± SD: 48±11, 56±7, 40±8, respectively). There were significant differences in race/ethnicity among the gender/menopause categories (P<0.02). We found 47 SNPs that interacted significantly with the gender/menopause categories (P<0.05). The majority of the SNPs (N=39) were correlated with fibrosis severity only in postmenopausal women; 37 SNPs increased fibrosis risk (e.g., RS332136, RS11024158, RS7946010) while 2 others (RS2740487, RS742748) decreased the risk. One SNP (RS60296118) reduced fibrosis risk only in premenopausal women while 6 others (CHR1_1202719, RS1034716, RS12265666, RS332182, RS12501548, and RS4659248) showed different effects on fibrosis depending on gender/menopause. Of note, SNPs related to NOTCH2 showed women-specific effects on hepatic fibrosis while SNPs related to PIK3C2A and ABCA1 showed significant effects on fibrosis only in postmenopausal women. **Conclusions:** Gender and menopause modify the impact of several gene variants on NAFLD-related fibrosis. Further characterization of identified interaction is warranted to delineate gender disparities in NAFLD pathobiology.

Disclosures:

Cynthia A. Moylan - Grant/Research Support: TaiwanJ Pharmaceuticals, Madrigal Pharmaceuticals

Manal F. Abdelmalek - Advisory Committees or Review Panels: Tobira, NGM Pharmaceuticals; Consulting: BHV Pharma, TaiwanJ Pharma; Grant/Research Support: Tobira, Gilead Sciences, NIH/NIDDK, Genfit Pharmaceuticals, Immuron, Galmed, TaiwanJ Pharma, Intercept, NGM Pharmaceuticals, BMS Pharma, Conatus, Galactin; Speaking and Teaching: Alexion

The following people have nothing to disclose: Kara Wegermann, Jiayin Zheng, Shein-Chung Chow, Cynthia D. Guy, Anna Mae Diehl, Ayako Suzuki

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Serum-Based Biomarker Accurately Stratifies Hepatic Fibrosis in Patients with Nonalcoholic Steatohepatitis

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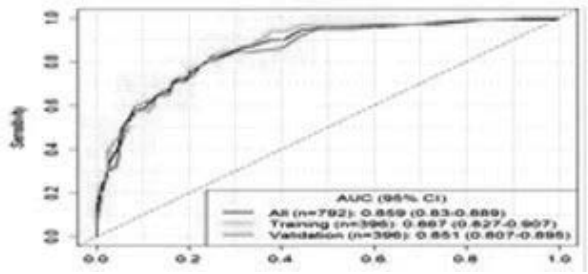
Background: Nonalcoholic steatohepatitis (NASH) affects 25 million adults in the United States and can lead to cirrhosis and liver cancer. Liver biopsy remains the "gold standard" for staging hepatic fibrosis in patients with NASH. A non-invasive, blood-based diagnostic test is required for population-based screening to identify those patients with NASH and advanced hepatic fibrosis. We aimed to develop and validate a serum biomarker for NASH capable of stratifying fibrosis severity. **Methods**: The concentrations of three biomarkers (α2-macroglobulin, hyaluronic acid and tissue inhibitor of metalloproteinase-1; FIBROSpect Test) were measured in serum collected on same day as liver biopsy from 792 patients with NASH. Liver biopsies were graded and staged according to NASH CRN

criteria. The samples were randomly split into a training (n=396) and a validation set (n=396) under constraints that no bias existed between the two sample sets on liver fibrosis, gender, age, ethnicity, race, or biomarker concentrations ($p \geq 0.52$). A logistic regression model was developed from the training cohort utilizing 10-fold cross validation and 10,000 bootstrapping iterations and then tested on the validation cohort. Area under the receiver operating characteristic curve (AUROC), sensitivity for correctly diagnosing patients with severe liver fibrosis (F3/F4) and specificity for correctly diagnosing patients with mild liver fibrosis (F0/F1/F2) were used to validate the model. **Results**: On the training samples, the model had an AUROC of 0.867 (95% CI: 0.827-0.907) (Figure 1), a sensitivity of 84.4% (95% CI: 75.5-91.0%) and a specificity of 72.3% (95% CI: 66.9-77.3%). On the validation samples, the model had an AUROC of 0.851 (95% CI: 0.807-0.895), a sensitivity of 81.1% (95% CI: 71.7-88.4%) and a specificity of 73.8% (95% CI: 68.4-78.6%). The model correctly classified 76.5% to 100% of F0, F1, F3 and F4 patients. **Conclusion**: We validated a serum-based biomarker test which correctly classifies fibrosis severity and demonstrates good diagnostic performance characteristics for the prediction of advanced histological fibrosis stage in patients with NASH.

Figure 1. ROC Curve of F0-F2 vs. F3-F4 in NASH patients.



Disclosures:

Manal F. Abdelmalek - Advisory Committees or Review Panels: Tobira, NGM Pharmaceuticals; Consulting: BHV Pharma, TaiwanJ Pharma; Grant/Research Support: Tobira, Gilead Sciences, NIH/NIDDK, Genfit Pharmaceuticals, Immuron, Galmed, TaiwanJ Pharma, Intercept, NGM Pharmaceuticals, BMS Pharma, Conatus, Galactin; Speaking and Teaching: Alexion

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Diagnosing NASH and Assessing NASH Disease Severity with HepQuant-STAT, a Simple Quantitative Liver Function Test

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Background: It is difficult to diagnose NASH and assess disease severity. Biopsy is invasive and can exhibit 40% variability in staging (Ratziu, et al, 2005). The HepQuant-STAT test (HQ-STAT) test is a simple quantitative liver function test. The goal of this pilot study was to determine if STAT could diagnose NASH and assess NASH disease severity. **Methods**: There were 50 healthy controls, 30 of normal weight (BMI 18.5 - 25), 16 overweight (BMI 25 - 30), and 4 obese (BMI >30). NASH patients from the University of Colorado Denver (N = 16) and Baylor University Medical Center Dallas (N = 15) included 27 with biopsy-diagnosed NASH and 4 with cryptogenic cirrhosis, concurrent obesity, and presumed late stage NASH. By Brunt-Kleiner fibrosis stage, there were 4 at F1, 4 at F2, 5 at F3, and 18 at F4 (cirrhosis). Of the 18 cirrhotic patients, 9 were decompensated. Clinical manifestations of NASH disease severity were captured from patient histories and included any-size varices (N = 14), large varices (N = 9), and cirrhosis (N = 18). The test requires only a single blood draw at 60 min after oral administration of 40 mg tetra-deuterated cholic acid (d4-CA). The serum concentration of d4-CA, determined by LCMS, and normalized to body weight, is the HepQuant-STAT test result. The ability to diagnose NASH and to assess NASH disease severity was evaluated by AUROC analyses (c-statistic) and by the diagnostic performance (sensitivity, specificity, PPV, NPV) at the optimum cutoffs which were defined by the maximum Youden Index (J). **Results:** A HepQuant-STAT > 0.50 μM could differentiate NASH patients from healthy control subjects, even overweight and obese controls, with high c-statistic and excellent sensitivity and NPV (Table). Within the NASH cohort, increasing HepQuant-STAT test values were associated with increasingly severe disease manifestations, any-size varices at STAT > 1.08 μM, cirrhosis at STAT > 1.25 μM, and large varices at STAT > 2.40 μM. The cutoffs exhibited good sensitivity and NPV for the risk of any-size varices and large varices, and excellent specificity and PPV for the risk of cirrhosis. **Conclusions**: HepQuant-STAT, a simple quantitative liver function test, could be a minimally-invasive alternative to biopsy for the diagnosis of NASH. HepQuant-STAT could assess NASH disease severity by identifying patients at risk of any-size varices, cirrhosis, and large varices.

Diagnostic Performance of HepQuant-STAT

	AUROC c-statistic	optimum cutoff	Sensitivity	Specificity	PPV	NPV	Youden Index (J)
Diagnosing NASH	0.93	STAT >0.50 μM	94%	76%	71%	95%	0.70
Diagnosing NASH Varices	0.90	STAT >1.08 μM	86%	88%	86%	88%	0.74
Diagnosiing NASH Cirrhosis	0.86	STAT >1.25 μM	67%	100%	100%	68%	0.67
Diagnosing NASH Large Var.	0.93	STAT >2.40 μM	89%	95%	89%	95%	0.84

Disclosures:

Steve M. Helmke - Employment: HepQuant, LLC

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The following people have nothing to disclose: John D. Marr, Shannon Lauriski, Michael W. Cookson, Jennifer DeSanto, James F. Trotter

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Phase 2 open-label efficacy and safety study of the apical sodium-dependent bile acid transporter inhibitor maralixibat in children with progressive familial intrahepatic cholestasis: 48-week interim efficacy analysis

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Background Maralixibat is a potent, selective and minimally absorbed ileal apical sodium-dependent bile acid transporter (ASBT) inhibitor. Pharmacological inhibition of enterohepatic circulation may offer an alternative to surgical disruption in patients with progressive familial intrahepatic cholestasis (PFIC). **Methods** This was a pre-specified interim analysis of 48-week data from a phase 2 multi-center study of maralixibat (SHP625; LUM001) in children with PFIC. Patients with liver transplants, surgically disrupted enterohepatic circulation or decompensated cirrhosis were excluded. Maralixibat doses were escalated from 14 to 280 μg/kg/day over 13 weeks (depending on tolerability) and maintained for ≤59 weeks. Change from baseline in serum bile acid (sBA) levels was the primary efficacy measure. Secondary efficacy measures included serum alanine transaminase (ALT), bilirubin and 7α-hydroxy-4-cholesten-3-one (C4) levels. Caregivers reported pruritus severity on a 5-point scale (0, no itch; 4, most severe itch) using the Itch Reported Outcome (ItchRO) twice-daily e-diary and completed the Pediatric Quality of Life Inventory (PedsQL). **Results** The 33 enrolled participants had a median age of 3.0 years (range, 1–13) and 14 (42.4%) were male. Efficacy data were available in 26/29 participants who reached week 48 (PFIC1, n=6; PFIC2, n=20), of whom 23 were receiving maralixibat 280 μg/kg/day. Overall trends in key efficacy measures indicated improvement from baseline to week 48 (Table 1). In 6/20 participants with PFIC2 (30%), efficacy profiles over 48 weeks indicated clinically significant response to maralixibat: sBA levels normalized to ≤8.5 μmol/L (n=4) or reduced by ≥70% (n=2); ItchRO scores showed no pruritus (n=2) or improved by ≥1.0 points (n=4); and transaminase and/or bilirubin levels normalized in 2 participants with baseline elevations. Treatment-emergent adverse events (TEAEs) were reported in all 33 participants, were judged related to maralixibat in 22, serious in 15 and led to discontinuation in 1. The most frequently reported TEAEs were pyrexia (n=15), diarrhea (n=14), cough (n=13), abdominal pain (n=10) and vomiting (n=10). **Conclusion** Inhibition of ileal bile acid reuptake with maralixibat may provide therapeutic benefit for a subset of children with PFIC. These findings support further clinical studies of ASBT inhibitors as pharmacotherapies for PFIC.

Table 1. Key efficacy measures at baseline (n=33) and week 48 (n=26)

	sBA (μmol/L)	ALT (UI/L)	Total bilirubin (mg/dL)	C4 (ng/mL)	ItchRO score	PedsQL total score
Baseline, mean (range)	352.16 (34.39, 602.14)	108.1 (13, 438)	2.88 (0.1, 15.1)	4.16 (0.1, 47.3)	2.27 (0.14, 3.79)	61.49 (18.1, 85.9)
Change from baseline to week 48, mean (95% CI)	−32.17 (−110.42, +46.08)	−11.5 (−35.8, +12.9)	+0.79 (−0.12, +1.70)	+5.96 (−0.58, +12.49)	−1.01* (−1.40, −0.63)	+8.17* (+0.71, +15.64)

*Confidence intervals exclude zero, indicating nominal statistical significance.

Disclosures:

Richard J. Thompson - Consulting: Alexion, Alberio, Arcturus, GSK, Retrophin; Grant/Research Support: Shire; Speaking and Teaching: Shire

Patricia McClean - Grant/Research Support: Shire Development LLC

Alexander G. Miethke - Consulting: Shire plc; Grant/Research Support: Shire plc

Thomas Jaecklin - Employment: Shire

The following people have nothing to disclose: Deirdre A. Kelly, Nisreen Soufi, Christine Rivet, Irena Jankowska, Cara L. Mack, Palaniswamy Karthikeyan, Joan Gu, Robert H. Squires, Kathleen M. Loomes

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Improvement in GGT predicts event-free survival in primary sclerosing cholangitis regardless of ursodeoxycholic acid treatment: data from the Pediatric PSC Consortium

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Background: Ursodeoxycholic acid (UDCA) is commonly used to treat primary sclerosing cholangitis (PSC) in children. Randomized-controlled trials in adults with PSC demonstrate that UDCA does not improve survival with native liver. We evaluated the effect of UDCA on clinical and biochemical outcomes in children with PSC using a large, multicenter cohort. Methods: The pediatric PSC consortium is a research collaboration between 36 international centers. We recorded liver biochemistries at diagnosis and 1 year, and UDCA treatment status. Survival analysis from PSC diagnosis to several clinical events was performed: 1) portal hypertensive complications (ascites, encephalopathy, varices), 2) biliary complications (dominant stricture requiring dilation, stenting or drainage), 3) liver transplantation (LT), 4) cholangiocarcinoma (CCA) or 5) liver-related death. A biochemical response (BR) was defined as GGT >50 U/L at diagnosis that was <50 at 1 year. The probability of event-free survival at 5 years was calculated among four groups: UDCA treated (T) and untreated (U) patients, with or without BR. Results: The cohort consisted of 309 patients, 40% female, mean age at diagnosis 11.4 years, with 2026 person-years of follow-up (mean 6.6 years). Inflammatory bowel disease (IBD) occurred in 84%, autoimmune hepatitis (AIH) in 39%, and large duct involvement in 74% studied patients. UDCA was used in 81% patients at a mean dose of 17 mg/kg/day. T and U patients had similar mean liver biochemistries at diagnosis [GGT 314 vs. 300 U/L; ALT 239 vs. 175 U/L (p=NS)], lower values at 1 year [GGT 99 vs. 175 (p=0.002); ALT 63 vs. 96 (p=0.008)], and greater reduction in values over the first year [GGT decreased by 215 vs. 125 (p=0.039) and ALT decreased by 175 vs. 79 (p=0.037)]. BR occurred in 45% overall. Patients with and without BR had similar baseline liver biochemistries, AST to platelet ratio index, age and prevalence of IBD, AIH and large duct involvement (all p=NS). Despite biochemical improvement, T and U patients had similar rates of adverse events: portal hypertensive complications 19 vs. 18%, biliary complications 6 vs. 8%, LT 11 vs. 12%, CCA 1 vs. 0%, and death 1 vs. 0% (all p=NS). The 5-year event-free survival was 91% in patients with BR (90% in T vs. 100% in U, p=0.45) and 67% in patients without BR (66% in T vs. 69% in U, p=0.96), p<0.001. Conclusions: UDCA treatment was associated with improvement in GGT and ALT, but not reduction in rates of adverse clinical outcomes. Patients with GGT <50 at one year did markedly better than those with GGT >50, regardless of UDCA treatment. These data support GGT as a surrogate marker of clinical outcomes in pediatric PSC.

Disclosures:

Katryn Furuya - Advisory Committees or Review Panels: Alexion; Speaking and Teaching: Alexion

M. K. Jensen - Consulting: GLG Consultation

Binita M. Kamath - Consulting: Retrophin; Speaking and Teaching: Shire

Parvathi Mohan - Grant/Research Support: Bristol Meyers Squib, Vertex

Miriam B. Vos - Advisory Committees or Review Panels: Aegerion, Immuron, Target Pharmasolutions; Consulting: Shire, Boehringer Ingelheim, Intercept, Bristol Myers Squibb; Grant/Research Support: Resonance Health, AMRA-Advanced MRI Analytics AB

The following people have nothing to disclose: Mark R. Deneau, Mansi Amin, ACHIYA Z. AMIR, Fateh Bazerbachi, Annemarie Broderick, Albert Chan, Matthew Di Guglielmo, Wael El-Matary, Mounif El-Youssef, Federica Ferrari, Madeleine Gottrand, Frederic Gottrand, Nitika A. Gupta, Matjaz Homan, Kaija-Leena Kolho, Anastasia Konidari, Raffaele Iorio, Cara L. Mack, Mercedes Martinez, Alexandra Papadopoulou, Amanda Ricciuto, Lawrence J. Saubermann, Pushpa Sathya, Eyal Shteyer, Vratislav Smolka, Atsushi Tanaka, Pamela L. Valentino, Raghu Varier, Bernadette Vitola, Jason Y. Yap, Tamir Miloh

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FXR Agonist GW4064 Prevents Parenteral Nutrition Associated Cholestasis (PNAC) in Mice

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Background: Parenteral nutrition (PN) associated cholestasis (PNAC) complicates the care of patients with intestinal failure. In mice and humans, phytosterol (PS)-containing PN synergizes with intestinal injury to suppress FXR signaling and promote PNAC. Here we hypothesized that pharmacological activation of FXR would prevent PNAC. **Methods and Results:** Combining intestinal injury (oral dextran sulfate sodium (DSS)) with continuous infusion of PS-containing PN (DSS/PN mice) x 14 days resulted in PNAC (increased serum bilirubin, bile

acids, AST, ALT), with reduced hepatic *Nr1h4, Abcb11*, and *Abcc2* mRNA relative to control mice. *Cyp7a* mRNA (rate limiting for bile acid synthesis) was not suppressed, and *Nrbo1* mRNA (encoding the FXR target *Shp* that suppresses *Cyp7a*) was not increased as would have been expected in cholestasis. LC-MS revealed that DSS/PN mice had increased taurine-conjugated, hydrophobic and FXR antagonistic bile acids in serum. Admixing FXR agonist GW4064 (30mg/kg/day) in the PN solution (days 3-14; DSS/PN-GW4064) prevented PNAC and reversed these alterations in gene expression and serum bile acids. Chromatin-immunoprecipitation (ChIP) showed that GW4064 increased FXR binding to the *Abcb11* promoter *in vivo*. Furthermore, in contrast to DSS/PN mice, which had suppressed liver mRNA for the canalicular PS transporter *Abcg5/8* and the phospholipid transporter *Abcb4* (which is required for PS transport), DSS/PN-GW4064 mice had increased mRNA of these genes and serum PS levels were significantly lower than in DSS/PN mice. ChIP showed that GW4064 also increased FXR binding to the *Abcg5/8* promoter *in*[Office1] *vivo.* Intestinal inflammation and ileal FXR target gene mRNA levels (*Shp, Fgf15* and *Osta*) were not different between DSS/PN and DSS/PN-GW4064 mice, indicating that the effect of GW4064 was limited to the liver. GW4064 prevented DSS/PN-induced hepatic macrophage accumulation *in vivo*, hepatic macrophage cytokine transcription in response to LPS *in vitro*, and hepatic expression of genes associated with macrophage activation (*IL1b, Ccr2, Cd11b, Ly6C*). Finally, while IL1β exposure *in vitro* suppressed FXR activity and expression of *BSEP, MRP2, SHP* in human HepG2 cells and in primary mouse hepatocytes, this effect was ameliorated with GW4064 treatment. **Conclusion:** FXR agonist prevents PNAC in mice through restoring FXR signaling, resulting in increased expression of canalicular bile, sterol and phospholipid transporters (which decreases hepatocyte PS concentrations) and in suppression of macrophage recruitment and inflammatory cytokine levels. These data support a rationale for increasing FXR activity as a therapeutic strategy to alleviate PNAC.

Disclosures:

Ronald J. Sokol - Consulting: Alnylam, Retrophin, Alexion; Grant/Research Support: Giliad, Shire/Lumena, FFF Enterprises

The following people have nothing to disclose: Karim C. El Kasmi, Swati Ghosh, Aimee Anderson, Michael W. Devereaux, Julie Reisz, Natarajan Balasubramaniyan, Linda Johnson, Frederick J. Suchy, Angelo D'Alessandro

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Heat shock chaperone STIP1 as a modulator and genetic risk factor of extra-hepatic biliary atresia

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Biliary atresia (BA) is a neonatal cholangiopathy that is the leading indication for pediatric liver transplantation. There is increasing evidence that BA is a complex disease that occurs as a result of environmental trigger(s) in genetically susceptible hosts. Using an *in vivo* zebrafish biliary assay, we have isolated biliatresone, a novel plant isoflavonoid with selective extrahepatic biliary toxicity that is responsible for BA outbreaks in newborn Australian livestock. This toxin-mediated BA model recapitulates the cardinal features of human BA. Expression profiling of mRNA recovered from cholangiocytes and total liver of biliatresone-treated zebrafish larvae showed early up-regulation of multiple evolutionarily conserved stress responses, including the heat shock response. Interestingly, exome sequencing of BA patients from the NIDDK-funded Childhood Liver Disease Research Network identified a child with isolated BA to be heterozygous for a *de novo* nonsense mutation in the *STIP1/HOP* gene, a stress-induced HSP90 co-chaperone. STIP1 transfers client proteins from HSP70 to HSP90, thus promoting client maturation. Its expression is upregulated 2.5 fold in the extrahepatic cholangiocytes (EHC) of biliatresone treated larvae, suggesting that it may play a role in the toxin-mediated BA model, and by analogy human BA. Supporting this idea, expression of the human STIP1 isoform targeted by the BA mutation was upregulated 3-fold in biliatresone treated HepG2 cells, whereas the expression of other STIP1 isoforms were minimally changed. We used the CRISPR/Cas9 technology to introduce a frameshift mutation into Exon 1 of the zebrafish *stip1* gene. We found that the EHC of *stip1* heterozygous larvae were highly sensitive to a low dose of biliatresone that is inactive in wild type larvae. Similarly, pharmacological inhibition of Hsp90 in wild type larvae using 17-*N*-allylamino-17-demethoxygeldanamycin (17-AAG), a derivative of the antibiotic geldanamycin, sensitize both the EHC and the otherwise resistant intrahepatic cholangiocytes (IHC) to low dose biliatresone. In contrast, activation of Hsp70 via treatment of larvae with the small molecule BGP-15 did not attenuate biliatresone toxicity, thus arguing that biliatresone specifically targets Hsp90 function. Collectively, these data identify aberrant proteomic stress response as a key mediator of biliatresone-induced biliary injury and suggest that germline inactivating mutations in *STIP1* and potentially other heat shock pathway genes may be a risk factor for BA.

Disclosures:

The following people have nothing to disclose: Xiao Zhao, Ramakrishnan Rajagopalan, Kathleen M. Loomes, Nancy B. Spinner

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Characteristics and Outcomes of Pediatric Cholestasis in Alagille Syndrome in the Modern Era: Results of a Multi-Centre Prospective Observational Study

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Background: Alagille syndrome (ALGS) is an autosomal dominant, multisystem disorder with substantial variation in the penetrance of clinical features. To date, only retrospective cohort studies from the 1990's and earlier, each with heterogeneous inclusion criteria, exist. The lack of prospective data describing current characteristics and outcomes of cholestasis in ALGS undermines clinical management, prognostic capabilities and evaluation of novel therapies. **Methods:** We ascertained a cohort of children with ALGS (clinically +/- genetically defined) and native liver, from a multi-center prospective observational study within the Childhood Liver Disease Research Network (ChiLDReN). All patients had documented cholestasis, or a history of cholestasis, as defined by at least one of the following: total bilirubin > 2mg/dl, serum bile acids or GGT > 3x ULN for age, fat soluble vitamin deficiency or intractable pruritus. Survival analysis and competing risk analysis were used to calculate cumulative incidence rate over time for complications with native liver, liver transplant and death. **Results:** 272 cholestatic ALGS subjects (2 months-25 years) with a median follow-up of 2.3 years (0-8.5yrs) were included. Genetic data are currently available for 157 subjects. In this group 135/157 (86%) had *JAGGED1* mutations, 5/157 (3%) had *NOTCH2* mutations and 17/157 had no mutations detected in either *JAGGED1* or *NOTCH2*. Mean (SD) total bilirubin, ALT and GGT at baseline were 5.7mg/dL (6.6), 175U/L (136), and 526U/L (606) respectively. Growth at baseline was significantly delayed compared to norms, with median height Z-score -1.9 (interquartile range [IQR] -2.7, -1.0) and weight Z-scores -1.8 (IQR -2.9, -0.8). Amongst cholestatic ALGS subjects, 50% reported ascites and almost 25% had one or more episode of variceal bleeding by the age of 25 years. 38% experienced bone fractures with the majority of these occurring before the age of 13 years. 70% of cholestatic ALGS children had undergone liver transplantation by 17 years of age and 10% had died (multiple causes, only 3 clearly liver-related). **Conclusions:** This study represents a comprehensive assessment of ALGS children with cholestasis in the largest prospective cohort ever described. Survival to early adulthood with native liver is only 20% with a high rate of liver-related complications. The burden of hepatic disease in ALGS is substantial and underpins the need for close medical monitoring and novel therapies.

Disclosures:

Binita M. Kamath - Consulting: Retrophin; Speaking and Teaching: Shire

Rene Romero - Grant/Research Support: BMS, Gilead

James E. Heubi - Consulting: Cystic Fibrosis Foundation, Retrophin, Alynlam, Nordmark; Stock Shareholder: Asklepion Pharmaceuticals LLC

Daniel H. Leung - Grant/Research Support: Bristol-Meyers Squibbs, CF Foundation, Gilead, Roche, Abbvie

Saul J. Karpen - Consulting: Intercept Pharmaceuticals, Albireo, Retrophin, Inc., Regulus Therapeutics

Jean P. Molleston - Grant/Research Support: gillead, roche, vertex, none, abbvie, Shire/Lumena; Speaking and Teaching: none

Karen F. Murray - Grant/Research Support: Gilead; Stock Shareholder: Merck

Philip Rosenthal - Advisory Committees or Review Panels: Retrophin, Gilead, Alexion, Audentes, Intercept, Albireo; Consulting: Roche, Abbvie; Grant/Research Support: Roche, Bristol MyersSquibb, Gilead, Vertex, Abbvie, Roche/Genentech, Infacare, BMS; Speaking and Teaching: Retrophin

Jeffrey Teckman - Consulting: Dicerna, Ionis Pharmaceuticals, Genkyotex, The Alpha-1 Project, RxCelerate, Editas, Intelia, AstraZenica, Vertex, Proteostasis; Grant/Research Support: Alnylam, Arrowhead, Alpha-1 Foundation, Gilead

The following people have nothing to disclose: Wen Ye, Nathan P. Goodrich, Kathleen M. Loomes, Nancy B. Spinner, David A. Piccoli, Kasper S. Wang, Averell H. Sherker, John C. Magee

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Biliatresone, a toxic model of biliary atresia in animals, causes damage to extrahepatic bile ducts, and its toxicity is related to WNT and NOTCH signaling pathways

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Background & Aims: Biliary atresia (BA) is a fibrotic disease of unknown etiology affecting the extrahepatic bile ducts (EHBDs) of newborns. The isoflavonoid biliatresone, which causes rapid decreases in reduced glutathione (GSH), leads to a BA-like disease in newborn livestock and larval zebrafish as well as to EHBD obstruction in neonatal mouse EHBD explants. As biliatresone represents a new model of BA, our goal was to understand the nature of toxin-associated injury. We have previously shown that biliatresone decreases Sox17 and that silencing Sox17 mimics the effects of biliatresone. We aimed to further understand the molecular mechanisms involving injury in the EHBD. **Methods:** We investigated molecular pathways

by treating cholangiocytes in culture with biliatresone, Buthionine Sulfoxamide (BSO) to decrease GSH, or DMSO. We then performed real time PCR and immunofluorescence staining of cholangiocytes in culture in different time points. Cholangiocyte organoids with open lumens were generated by growth in 3D culture. We used plasmids for overexpressing proteins. Target proteins were assessed in human livers by comparing immunostaining of human BA and normal liver tissues. **Results:** Biliatresone caused an increase in RhoU and Hey2. Both overexpression of RhoU and Hey2 resulted in lumen obstruction of cholangiocyte organoids in 3D culture. Both biliatresone and BSO caused increased expression of RhoU and Hey2 after 3 hours of treatment, measured by immunofluorescence stain intensity. Staining human biliary atresia livers demonstrated increased RhoU and Hey2 staining of intrahepatic cholangiocytes at the time of BA diagnosis compared to non-BA liver biopsies. **Conclusion:** The molecular pathway of biliatresone toxicity includes decreases in GSH and Sox17 and increases in RhoU and Hey2. Decreased GSH likely plays a central and primary role in cholangiocyte injury. In vivo, this is a potential cause of lumen obstruction and may be an important part of the pathophysiology of BA.

Disclosures:

The following people have nothing to disclose: Sophia Fried, Alyssa Kriegermeier, Pierre-Marrie Lavrut, Pierre Russo, Raanan Shamir, Rebecca G. Wells, Orith Waisbourd-Zinman

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Acute-on-chronic alcoholic steatohepatitis and fibrosis in FRGN mice with humanized liver.

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The mechanisms contributing to alcohol induced liver disease (ALD) are not completely understood. Most studies to uncover ALD pathogenesis have been performed in murine models, which allowed the identification of potential targets for ALD treatment. However, the efficacy of this approach for ALD patients has been dissapointing, reflecting our incomplete understanding of human ALD pathogenesis. As the biology and metabolism of mice and humans differ, the limited effectiveness of currently available therapy for human ALD illustrates the pitfalls in translating the knowlegde derived from mice to humans. To enhance the potential translational applicability of experimental ALD research to humans, our aim was to explore the feasibility and characterization of an alcoholic steatohepatitis (ASH) model in FRGN ($Fah^{-/-}$/$Rag2^{-/-}$/$IL1rg^{-/-}$ in NOD background) mice, a genetically engineered model that allows the repopulation of the murine liver with human adult hepatocytes and hematopoietic cells. **Methods**: FRGN mice were repopulated with human adult hepatocytes (>80%) as judged by the human albumin levels in serum and expression of human Fah. FRGN mice were subjected to an acute-on-chronic ASH model or fed a control diet. Liver histology, clinical biochemistry, ER stress and signs of inflammation and fibrosis were examined in FRGN control or alcohol treated mice. In some cases, FRGN mice were humanized with hepatocytes plus CD34+ human stem cells. **Results**: FRGN mice fed alcohol exhibited hepatomegaly, liver steatosis and release of ALT in serum (5-7 fold) compared to hFRGN mice fed control diet. Alcohol feeding increased the expression of human CYP2E1 levels and the upregulation of StARD1 expression that paralelled the increase in mitochondrial cholesterol levels and mitochondrial GSH depletion. hFRGN mice fed alcohol exhibited increased ER stress markers CHOP and XPB1 as well as the expression of proinflammatory markers, MCP-1, IL-1b and increased expression of procaspase-1 and ASC, indicating inflammasome activation. Immunohistochemistry analyses revealed increased MPO staining in hFRGN mice fed alcohol. Furthermore, Sirius Red staining revealed liver fibrosis that was accompanied by increased hepatic hydroxyproline levels and expression of Col1a, a-SMA and TGFb levels. The inflammatory response and fibrosis phenotype induced by alcohol feeding were enhanced in FRGN mice with double humanization of both hepatocytes and hematopoietic cells. **CONCLUSIONS**: These findings illustrate the potential relevance of this particular model to design studies aimed to uncover molecular players and novel therapeutic targets of significance for human ALD.

Disclosures:

The following people have nothing to disclose: Cristina Alarcon-Vila, Susana Nuñez, Raquel Fucho, Juan Caballeria, Carmen Garcia-Ruiz, Jose Fernandez-Checa

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Alcohol Abuse in Patients with Cirrhosis is associated with Widespread Mucosal Dysbiosis and Intestinal Biliary Profile Alterations

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Alcohol abuse in cirrhotics can worsen the pro-inflammatory state. Prior studies have demonstrated increase in stool secondary bile acids (BA), which could be due to increased hepatic BA synthesis that could potentiate this pro-inflammatory milieu. **Aim**: Define the effect of alcohol abuse on intestinal BA profile, microbiota & inflammatory markers in cirrhosis. **Methods**: Cirrhotics abstinent for >6mths (NAlc) were age & MELD-matched to actively drinking (AUDIT>8, Alc) cirrhotics. Age-matched healthy controls (Ctrl) were also included. All subjects underwent EGD/colonoscopy with duodenal, terminal ileal (TI) & sigmoid biopsies for measuring inflammatory cytokine levels by RT-PCR, & microbiota using 16SrRNA sequencing. BA profile from stool and duodenal samples was performed using HPLC/GC-MS & reported as total, conjugated/unconjugated, primary/secondary BA levels. **Results**: 58 age-matched subjects (20 Alc, 18 Ctrl, 28 NAlc) were enrolled. MELD scores were similar (14.3 vs 13.9, p=0.42). Alc pts had higher systemic inflammation & endotoxemia (Table). BA profile: Alc pts had higher total and secondary BAs in both duodenal and stool compared to NAlc and Ctrl. Microbiota: TI and sigmoid colon showed lower autochthonous taxa (*Ruminococcaceae, Lachnospiraceae*) and higher endotoxin-producing taxa (members of *Proteobacteria*) were seen in Alc compared to the other groups. Similarly, Alc pts had higher *Proteobacteria* taxa in the duodenum. Intestinal expression: mRNA expression of TNF, MCP-1 & IL-6 were significantly higher

(X3 fold) in TI of Alc compared to the other groups but not in the duodenum or sigmoid colon. **Conclusions**: Active alcohol abuse in human cirrhosis is associated with higher secondary bile acids in the duodenal contents and stool, and widespread mucosal dysbiosis resulting in endotoxemia. This could potentiate systemic and intestinal inflammation. This increase in total BA and secondary BA in duodenal contents could signify a higher hepatic synthesis and enterohepatic BA circulation induced by alcohol even in cirrhotic pts. Altering this BA profile could potentially ameliorate this inflammatory milieu.

Mean values	Controls	Abstinent cirrhosis	Actively drinking cirrhosis
Endotoxemia EU/ml	0.08	0.07	0.89*
serum TNF pg/ml	0.01	1.8	3.0*
Duodenal content biliary profile(all conjugated, mmol/L)			
Total BA	0.36	1.37	20.86*
Total Primary BA	0.28	1.35	18.27*
Total Secondary BA	0.07	0.02	2.59*
Fecal Biliary Profile (mostly unconjugated, μM/gm)			
Total BA	5.4	2.5	8.9*
Total Primary BA	0.08	0.46	0.16*
Total Secondary BA	4.2	2.4	7.6*

*p<0.05 on ANOVA between groups

Disclosures:

William M. Pandak - Employment: Virginia Commonwealth University, Veterans Affairs Medical Center

Douglas M. Heuman - Consulting: Bayer, Grifols, Genzyme; Grant/Research Support: Exilixis, Novartis, Bayer, Bristol Myers Squibb, Scynexis, Ocera, Mannkind, Salix, Globeimmune, Roche, SciClone, Wyeth, Otsuka, Ikaria, UCB, Celgene, Centocor, Millenium, Osiris, AbbVie, Gilead; Speaking and Teaching: Otsuka, Astellas; Stock Shareholder: General Electric

Michael Fuchs - Grant/Research Support: Gilead, Abbvie, Intercept, Conatus, Galectin, Allergan; Speaking and Teaching: Falk Founddation

Jasmohan S. Bajaj - Consulting: Abbott, Norgine, Valeant; Grant/Research Support: Salix, Grifols

The following people have nothing to disclose: Genta Kakiyama, Derrick Zhao, Andrew Fagan, Hiroshi Nittono, Hajime Takei, Runping Liu, Phillip Hylemon

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SREBP-2 promotes the transition from alcohol-induced steatosis to alcoholic steatohepatitis

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Alcohol induced liver disease (ALD) comprises a spectrum of disorders beginning with steatosis, a reversible stage of the disease that can progress to alcoholic steatohepatitis (ASH), an advanced form of the disease characterized by steatosis, inflammation and liver damage. The molecular drivers that promote the transition from alcoholic steatosis to ASH are not completely understood. As cholesterol has emerged as a key factor in the progression of nonalcoholic steatohepatitis, we hypothesized that SREBP-2, the master transcription factor that controls cholesterol synthesis, would prime the liver for ASH development. Thus, we analyzed the sensitivity of transgenic SREBP-2 (Tg-SREBP-2) mice overexpressing a dominant-positive truncated form of human SREBP-2 to ASH development and to explore potential therapies for treatment. **Methods**: Tg-SREBP-2 mice and wild type (WT) mice were fed the Lieber-DeCarli alcohol liquid diet (36% of calories) or a control (dextrin maltose) diet for 4 weeks. Liver histology, clinical biochemistry, mitochondrial cholesterol (mt-chol) and ER stress were examined. In some cases, Tg-SREBP-2 mice were treated with atorvastatin (20mg/kg), TUDCA (500mg/kg) or GSH ethyl ester (GSHEE, 1.25mmol/kg) every 12 h for the last 15 days of feeding. **Results**: Tg-SREBP-2 mice had higher basal liver cholesterol content than WT mice and ethanol feeding further increased this effect. This outcome was accompanied by higher mt-chol loading and StARD1 expression in Tg-SREBP-2 mice fed alcohol vs alcohol-fed WT and subsequent mitochondrial GSH (mGSH) depletion. Serum ALT, H&E and Sirius red analysis indicated increased liver injury, steatosis and fibrosis in Tg-SREBP-2 mice fed alcohol compared to alcohol-fed WT, which developed steatosis and mild liver injury but not fibrosis. Further, Tg-SREBP-2 fed alcohol exhibited increased hepatic expression of TNF, MCP-1 and MPO staining, indicative of neutrophil infiltration, compared to WT mice fed alcohol. In addition, alcohol feeding increased the expression of ER stress markers, CHOP and XBP-1, that was more pronounced in the Tg-SREBP-2 mice. Atorvastatin treatment ameliorated alcohol-induced mt-chol loading, mGSH depletion, liver injury and steatosis in Tg-SREBP-2 mice fed alcohol. Moreover, TUDCA and GSHEE treatment restored mGSH levels and protected Tg-SREBP-2 mice from alcohol-induced ER stress, steatosis and liver injury. **Conclusions**: These findings indicate that increased cholesterol synthesis sensitizes to ASH by accelerating mt-chol accumulation leading to mGSH depletion. Strategies regulating cholesterol homeostasis and targeting mGSH recovery could be promising approaches for the treatment of ALD.

Disclosures:

The following people have nothing to disclose: Carmen Garcia-Ruiz, Susana Nuñez, Laura Conde de la Rosa, Montserrat Elena, Juan Caballeria, Jose Fernandez-Checa

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CD40L enrichment of hepatic exosomes augment IL-17A production of γδ T cells by regulating hepatic stellate cells and Kupffer cells in alcoholic liver disease

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Background: Hepatic exosomes deliver diverse types of cargos to activate various signaling pathways in the neighboring cells. Our previous study shows that damage-induced self-ligands in hepatic exosomes activate toll-like receptor 3 (TLR3) in hepatic stellate cells (HSCs), which leads to enhanced liver fibrosis by amplifying interleukin (IL)-17A production of recruited γδ T cells. However, the mechanism by which hepatic exosomes are internalized into HSCs is not fully understood. Thus, we investigated the endocytic mechanism of hepatic exosomes and their effects on alcoholic liver disease (ALD). **Methods:** Wild type (WT), TLR3 knockout (KO), and CD40 KO mice were administrated with 4 g/kg of ethanol for binge or they were fed with liquid ethanol diet for 8 weeks. To identify major source of IL-1β, resident Kupffer cells were eliminated by clodronate injection. Isolated liver mono-

nuclear cells (MNCs) were subjected to flow cytometry and PCR for IL-17A analyses. *In vitro*, ethanol-induced hepatic exosomes were incubated with HSCs or Kupffer cells in the presence or absence of CD40L neutralizing antibody or dynasore. **Results:** In binge and chronic ethanol treatments, major IL-17A-producing cells were γδ T cells, whereas their production were significantly decreased in TLR3 KO and CD40 KO mice compared to WT mice. Indeed, depletion of Kupffer cells attenuated IL-17A production and IL-1β expression in γδ T cells and liver MNCs, respectively, compared to controls. In vitro, ethanol treatment increased expression of CD40L in hepatocytes, reflecting CD40L enrichment at exosomes. Treatment of ethanol-induced hepatic exosomes increased expression of CCL20 expression, a chemokine for γδ T cell recruitment, and CD40 in HSCs, whereas its expression was remarkably decreased in HSCs of TLR3 KO mice. In treatments with CD40L neutralizing antibodies or dynasore, almost internalization of hepatic exosomes into HSCs was inhibited, suggesting CD40L/CD40-mediated endocytosis of hepatic exosomes. In contrast, CD40L-enriched exosomes stimulated IL-1β expression of Kupffer cells, while CD40L neutralizing antibody reversed its expression. **Conclusion:** CD40L-enriched hepatic exosomes by ethanol activate TLR3 in HSCs through CD40L/CD40-mediated endocytic uptake and, rather than endocytosis, they stimulate IL-1β expression of Kupffer cells via CD40-mediated signaling pathway, subsequently, leading to recruitment and augmentation of IL-17A production of γδ T cells, respectively, in ALD. Therefore, interactions between CD40L-enriched hepatic exosome and its target cells expressing CD40 might be a novel target for ALD.

Disclosures:

The following people have nothing to disclose: Jun-Hee Lee, Myung-Ho Kim, Hee-Hoon Kim, Sunglae Cho, Young-Ri Shim, Wonhyo Seo, Won-Mook Choi, So Yeon Kim, Won-Il Jeong

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Galectin3 Mediates Alcoholic Liver Injury via the Inhibition of the Aryl Hydrocarbon Receptor

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Galectin3, a profibrogenic lectin family member was shown to be increased in the serum of alcoholic patients. However, its role in alcoholic liver disease (ALD) has not been elucidated. Aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, mediates FGF21 transcription, and FGF21 is protective in ALD. Thus, we **hypothesized** that galectin3 may affect the expression or function of AhR, thereby exacerbating alcoholic injury via the inhibition of the AhR/FGF21 axis. **Methods**: Galectin3$^{-/-}$, AhR$^{-/-}$ mice and their wt controls were pair-fed with Lieber-DeCarli alcohol or control diet for 16 days with a last day binge (NIAAA). Liver tissues were processed for RT-qPCR to analyze the expression of galectin3, AhR, FGF21, TNF-α, IL-6, MCP1 and CXCL1. Triglyceride (TG) content was measured. Wt and galectin3$^{-/-}$ hepatocytes were treated with ethanol (EtOH), and knockout cells were also treated with recombinant galectin3. The transcripts of AhR and FGF21 were assessed by RT-qPCR. Hepatocytes treated with EtOH were pre-incubated with a physiological AhR inducer, chrysin. Apoptosis was analyzed by active caspase-3 staining. Wt and galectin3$^{-/-}$ macrophages were treated with acetaldehyde; AhR and the expression of inflammatory transcripts were examined. **Results:** Compared to the wt EtOH-treated group, galectin3$^{-/-}$ mice had lower ALT, less inflammation, and TG content ($p<0.05$); while the AhR$^{-/-}$ mice showed significantly higher ALTs ($p<0.05$) and ASTs. Galectin3$^{-/-}$ mice displayed lower TNF-α, IL-6 and CXCL1. AhR$^{-/-}$ mice had higher levels of TNF-α, IL-6, MCP-1 and lower FGF21. AhR and FGF21 mRNAs were significantly reduced in EtOH-fed wt mice ($p<0.05$, $p<0.05$), but significantly less reduction was seen in galectin3$^{-/-}$ mice ($p<0.05$, $P<0.05$). *In vitro* we showed that AhR and FGF21 ($p<0.05$) mRNAs were downregulated in wt hepatocytes by EtOH, but no reduction was seen in galectin3$^{-/-}$ cells. AhR ($p<0.05$) and FGF21 ($p<0.05$) were significantly reduced by recombinant galectin3. With AhR induction by chrysin, FGF21 mRNA increased and caspase-3 activation decreased in hepatocytes. Galectin3$^{-/-}$ macrophages had increased AhR transcript, and decreased inflammatory markers (TNFα. MCP1, IL-6 and CXCL1) when treated with acetaldehyde. **Conclusion:** Alcoholic liver injury was attenuated in galectin3$^{-/-}$ but exacerbated in AhR$^{-/-}$ mice. Galectin3$^{-/-}$ mice had improved AhR/FGF21 signaling sugesting galectin3 may mediate ALD via the inhibition of AhR/FGF21 axis. Therefore, galectin3 inhibition and AhR physiologic induction can be potential therapies.

Disclosures:

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Transferrin Is Downregulated in Alcoholic Hepatitis and Predicts Short-term Survival

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Background: Iron is an essential and potentially toxic micronutrient linked to oxidative stress, bacterial infection and systemic inflammation. The liver plays a central role in iron homeostasis through synthesis of the iron serum transporter, transferrin, and the iron-regulating hormone, hepcidin. Dysregulation of iron parameters is common in liver disease. In particular, serum transferrin has been associated with outcome in patients with decompensated alcohol-related cirrhosis. Therefore, we examined the relationship between parameters of iron metabolism and outcome in patients with severe alcoholic hepatitis (AH). **Methods**: Ferritin, transferrin, iron, transferrin saturation (TSAT), soluble transferrin receptor and hepcidin were measured in serum samples from 825 patients with severe AH recruited prospectively via the STOPAH trial. Hepatic transferrin mRNA expression was assessed in a further cohort of 29 AH patients. **Results**: AH patients had diminished serum transferrin (median 93 mg/dl), but

ferritin (median 625 ng/dl) and TSAT (median 70%) was increased. Among iron parameters, transferrin showed the strongest association with prognostic scores: It was negatively correlated with MELD score, Glasgow Alcoholic Hepatitis score (GAHS) and Maddrey's discriminant function (DF) (*rho*=-.34, rho=-.31, and rho=-.23, respectively; all *p*<.0001). AH patients who died after 28 days had lower transferrin as well as higher hepcidin, ferritin and TSAT levels than AH survivors (all *p*<.001). The ability of transferrin to predict 28-day survival (AUROC 0.68, 95% CI 0.61-0.76) was comparable to MELD, GAHS or DF (AUROCs 0.74, 0.76 and 0.69, respectively). Its usefulness was even higher in patients without acute bleeding (AUROC 0.72, 95% CI 0.67-0.78). Adding transferrin to MELD improved the predictive ability (AUROC 0.78, 95% CI 0.73-0.82). Hepatic transferrin mRNA was significantly reduced in AH patients compared to healthy controls. Moreover, hepatic mRNA levels demonstrated marked negative correlation between transferrin and inflammation-associated genes (i.e., IL8, CXCL5 and DR6; all *p*<.05), while HNF4a seems to drive constitutive transferrin expression. Additionally, a prominent negative correlation between transferrin mRNA levels and the hepatic venous pressure gradient was observed (*rho*=-.54, *p*=.004). **Conclusion**: Parameters of iron metabolism, particularly transferrin as a negative acute phase reactant, are strongly associated with outcome in severe AH. The performance of transferrin alone in predicting 28-day mortality is comparable to commonly used scoring systems. Hence, it may be a useful parameter alone or combined with currently used scores.

Disclosures:

Ramon Bataller - Speaking and Teaching: Echosens

Christian Trautwein - Advisory Committees or Review Panels: Abbvie, MSD, BMS, Gilead, Bayer, AstraZeneca; Speaking and Teaching: Falk, MSD, Abbvie, BMS, Gilead

Mark R. Thursz - Advisory Committees or Review Panels: Gilead, BMS, Abbvie, CN-Bio, Altimmune, Novartis; Grant/Research Support: Vital Therapeutics, GSK

The following people have nothing to disclose: Karim Hamesch, Igor Spivak, Joaquin Cabezas, Josepmaria Argemi, Pavel Strnad

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Human bile contains stem cells which can be cultured in vitro as 3D organoids.

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Background: Primary sclerosing cholangitis (PSC) is a rare, heterogeneous, and progressive fibro-inflammatory cholangiopathy. Although PSC is an autoimmune mediated disease, immunomodulation therapy has not been effective. Translational advances in the field require a better understanding of cholangiocyte pathophysiology. However, obtaining human cholangiocytes for studies remains a significant challenge, with most primary cholangiocytes drawn from late-stage explant tissue. **Aim:** To determine whether stem cells can be isolated from the bile of PSC patients, cultured as 3D organoids, and used as a sustainable cell system to study immune regulation of the cholangiocyte. **Methods:** Bile was collected from PSC and non-PSC controls during ERCP, diluted with cold PBS containing antibiotic and antifungal agents, filtered, and centrifuged several times to wash and pellet stem cells. The resulting material was resuspended in Matrigel, plated in a 24 well plate and cultured with established organoid medium containing Wnt3a and R-spondin conditioned medium. Organoids were passaged every 7 days and either frozen down to establish a biobank of PSC and non-PSC control organoids, or mRNA was isolated for gene analysis by RT qPCR. Immunofluorescence was used to visualize cholangiocyte markers. Immune responsiveness of organoids to LPS treatment was assayed by ELISA. **Results:** Organoids were successfully grown from bile of PSC and non-PSC patients. Although fewer stem cells were present in control bile vs PSC bile, the morphology and growth rate of the organoids were similar between the groups. Gene expression and immunofluorescence labeling confirmed a biliary lineage (e.g. KRT19, EpCAM, and SOX9 positive) of the organoids. Importantly, organoids from bile of PSC bile had increased expression of key genes (e.g. IL-8, FUT2, LCN2 and ITGB2) versus control bile. Furthermore, organoids from PSC bile secreted IL-8 after 24-hour treatment with LPS. The bile from the PSC patients also had higher IL-8 levels than non-PSC bile. **Conclusions:** Stem cells can be isolated from human bile and cultured as 3D organoids that express a cholangiocyte genotype. Organoids from bile of PSC patients express key immune responsive genes and, when stimulated, secrete the pro-inflammatory cytokine IL-8. This is a novel translational method that permits the study of biliary progenitor cells from PSC patients at earlier disease stages, and is also a potential cell system for personalized pharmacotherapeutic studies.

Disclosures:

James L. Boyer - Consulting: Allergan, Alnylam; Grant/Research Support: Gilead, Intercept pharma

The following people have nothing to disclose: Carol J. Soroka, Leina Alrabadi, Albert Mennone, David N. Assis, Randolph de la Rosa Rodriguez

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Acute severe autoimmune hepatitis: prognostic factors of steroid therapy response

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Background: In acute severe autoimmune hepatitis (AS-AIH), the role of steroid therapy and the timing of liver transplantation (LT) are still a challenge. The aim of the present study was to establish AS-AIH predictive factors of steroid response. **Methods:** Multicenter French retrospective study (2010-2015). Inclusion criteria: 1- No medical history of AIH 2; Histological proven AIH; 3- IAIHG (International AutoImmune Hepatitis Group) score definite or probable 4. INR>1.5 any time since admission. Fibrosis was evaluated according to METAVIR score. The steroid response was defined by LT-free survival. **Results:** One-hundred-fourteen patients (pts) were included, 81 (71%) female, median age: 52 [18-89] years. At admission: 12 (10%) pts had hepatic encephalopathy. Total bilirubin: 254 [16-748] μmol/L, ALT: 940 [41-3581] IU/L, INR: 2 [1-15] and creatinine 62 [50-369] μmol/dL. Median MELD score was 27 [15-40]. Antinuclear and anti-smooth muscle antibodies > 1:80 were detected in 55 (60%) and 48 (51%) of pts, respectively. Median IgG were 21 [1-63] g/L. Cirrhosis was diagnosed in 17 (22%) of pts. Infection episodes occurred in 31 (27%) pts. Forty-two (37%) pts underwent LT. Median follow-up was 71 [0-220] months. Overall survival, at 90 days, was 88%. Hundred-three (90%) pts were treated with steroids with a median time since admission of 5 [0-29] days. Among them, 32 (30%) underwent LT (median time of 12 [0-41] days since steroid therapy). Sixty-one (59%) pts responded to steroids. Factors significantly associated with steroid response were: INR (p=<.0001), MELD (p<.0001), platelet count (p=0.033) at admission, absence of infection (p=0.035), the improvement of bilirubin (p=0.0001) and MELD (p=0.0133) at day 3 of therapy and the improvement of bilirubin (p=0.0143), INR (p=0.0001) and MELD (p=0.001) at day 7 of therapy. In multivariate analysis, factors independently associated with steroid response were: INR (HR 2.780 [1.467-5.270], p=<.0001), bilirubin (HR 1.012 [1.005-1.019], p=0.021) and platelet count (HR 0.983 [0.967-0.999], p=0.028) at admission and the improvement of bilirubin at day 7 after steroid introduction (HR 1.008 [1.001-1.015], p=0.002). **Conclusion:** In AS-AIH early LT was required in 37% of pts. Steroid therapy was effective in 59% of pts. Factors independently associated with steroid response were low INR and bilirubin and higher platelet count at admission and the improvement of bilirubin at day 7 of steroid therapy. These findings will help in the selection of pts who should pursuit the therapy and pts who should be referred for LT.

Disclosures:

Jean-Marie Peron - Board Membership: BAYER; Consulting: BMS, GILEAD, BOSTON SCIENTIFIC

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Jerome Boursier - Consulting: Echosens; Speaking and Teaching: Gilead, Intercept

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Serum and Tissue Leptin Serve as Inflammatory Activity Markers and Predict Steroid Responsiveness in Autoimmune Hepatitis

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Introduction: Adipokines, especially leptin is involved in wide spectrum of pro-inflammatory functions. Leptin inhibits T regulatory cell (Tregs) proliferation/ functions and increases the Th17 cell proliferation and functions, in *in vitro* models. The role of Tregs and Th17 in pathogenesis of autoimmune hepatitis (AIH) is well known. We investigated the relationship between leptin, Tregs, Th17

in AIH patients. **Patients and Methods**: We prospectively analyzed leptin levels in treatment naïve autoimmune hepatitis (AIH, n=34) patients and compared with primary biliary cirrhosis (PBC, n=15), chronic hepatitis B (CH, n=15) and healthy controls (HC, n=15). AIH patients were treated with steroids and >50% reduction in AST/ALT at week 4 was considered as response, decrease but >2 times ULN as partial and >2 times ULN as non-response. We checked relation of circulatory leptin over liver tests, disease activity, Tregs and TH17 in the liver biopsies. Tregs IHC specific antibodies – CD25 and Fox P3+ and Th17 were done to look for the helper T cells in the disease activity in liver biopsies of all above 4 groups and counted by Image-J. **Results:** Serum leptin level was significantly higher in AIH in comparison to PBC, chronic hepatitis and healthy controls {AIH: 337.6(49.2-744.15), PBC: 205(71.3-449.2), Chronic hepatitis: 41.5(0-97.5) and healthy controls: 54 (36.9-117.3) pg/ml with p=0.004}. In AIH cases; serum leptin levels correlated with hepatic activity index (HAI) (r=0.922; p<0.001); serum transaminases (AST: r=0.568, p=0.001, ALT=0.537, p=0.002). It inversely related with presence of Tregs in liver biopsies (p=-0.789, p<0.001) and positively with Th17 cells (r= 0.811, p<0.001). Tregs negatively correlated with severity of disease activity (r=-0.757, p<0.001) and positively with Th17 (r=0.742, p=0.001). Of 34 AIH cases 13 were negative for leptin (<200ng/ml) and 21 were positive for leptin. Leptin positive cases had higher IgG (20.5 ±6.3 vs 15.5±5.9, p=0.05) and more ANA sero-pososity (15 vs 4, p=0.047), while no difference noted in absolute neutrophil and eosinophil count. Leptin positivity was found to be associated with steroid responsiveness at 3 months after therapy {leptin negative- response/partial response/ no response: 10/3/0 vs leptin positive: 7/7/7; p=0.02 with an odds ratio of 9.28 (1.57-54.76)}. **Conclusion:** Leptin is a potential prognostic biomarker for the severity of autoimmune hepatitis and reduced Treg activity. Leptin positivity in hepatic tissue correlates with steroid responsiveness and could help select appropriate patients.

Disclosures:

The following people have nothing to disclose: Chhagan Bihari, Deepika Lal, Archana Rastogi, Nirupma Trehanpati, Shiv K. Sarin

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Association between disease-predisposing HLA DR3/DR7/DR13 genes with numeric impairment of immunoregulatory T-cells in patients with autoimmune hepatitis/sclerosing cholangitis

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Background: Autoimmune hepatitis (AIH) and autoimmune sclerosing cholangitis (ASC) are chronic liver diseases characterized by hepatocyte damage, and in ASC also by bile duct abnormalities. AIH and ASC hallmarks include seropositivity for anti-nuclear and/or smooth muscle antibodies, impaired immunoregulation and presence of disease-predisposing genes such as HLA-DR3/DR7/DR13. The mechanisms leading to persistent autoimmune liver damage and impaired immunoregulation remain elusive. **Aim:** To define the association between HLA genes and regulatory T-cell (Treg) frequency in AIH/ASC patients and their first-degree relatives (FDRs). **Subjects and Methods:** Peripheral blood mononuclear cells (PBMCs) were obtained from 57 patients (40 AIH, 17 ASC) and 79 FDRs, immunophenotyped by FACS and compared with PBMCs from 50 healthy subjects (HS). Genomic DNA was extracted from PBMCs using Qiagen DNA isolation kit. HLA-class I/II alleles genotyping was performed using PCR/sequence specific primers (Biotest kit). **Results:** The frequencies of total CD4 T-cells in AIH/ASC patients (42.3%) and FDRs (43.3%) were similar, being higher in FDRs than in HS (37.7%, p<0.05). No differences were noted in the frequency of CD8 T-cells and in the CD4/CD8 T-cell ratio among the groups. The frequencies of immunoregulatory protein PD-1 expression by CD4 T-cells were lower in patients (1.67%) and FDRs (1.78%) than in HS (2.44%, p<0.01); whereas PD-1 expression on CD8 T-cells was lower in patients (1.11%), but not in FDRs, compared to HS (2.08%, p<0.01). The proportions of $CD4^+CD25^+CD127^-FOXP3^+$ Tregs in both patients (3.4%) and FDRs (3.7%) were reduced compared to HS (4.38%, p<0.05). By analyzing the association between HLA genes and Treg frequency, we found that 91% of patients and 86% of FDRs who had the HLA DR3/DR7/DR13 genes displayed Treg frequencies (patients: 3.19%, FDRs: 3.66%) lower than non-DR3/DR7/DR13 FDRs (4.68%, p<0.05). 44% of AIH/ASC patients and 28% of FDRs possessed the A1-B8-DR3 haplotype (p=0.08). The frequency of Tregs in A1-B8-DR3 positive patients was lower in those without this haplotype (2.79% vs 3.58%, p<0.05). However, Treg frequency was similar in FDRs with or without this haplotype (3.83% vs 3.86%). **Conclusion:** 1) AIH/ASC patients and FDRs have higher frequencies of total CD4 T-cells, but lower proportions of $PD\text{-}1^+CD4^+$ cells and Tregs than HS; 2) There might be an association between the presence of disease-predisposing HLA genes and impaired immunoregulation in AIH and ASC. This association, however, seems not strong enough to break tolerance in FDRs, implicating the involvement of non-HLA genes or environmental factors in the development of tissue damage.

Disclosures:

Nedim Hadzic - Consulting: Alnylam; Speaking and Teaching: Synageva

Michael A. Heneghan - Consulting: Novartis; Speaking and Teaching: Falk Pharma

The following people have nothing to disclose: Pengyun Wang, Muhammed Yuksel, Stella Gabeta, Jonathon Graham, Munther J. Hussain, Laura J. Blackmore, Xiaohong Huang, Marianne Samyn, Tassos Grammatikopoulos, Wayel Jassem, Rodrigo Liberal, Maria Serena Longhi, Diego Vergani, Giorgina Mieli-Vergani, Yun Ma

113

A small molecule inhibitor of Macrophage Migration Inhibitory Factor (MIF) significantly reduces liver injury in an antigen- and liver-specific pre-clinical model of autoimmune hepatitis (AIH)

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Background: The pathophysiology of AIH is incompletely understood, and steroid-sparing regimens are needed but lacking. MIF is a proinflammatory cytokine that counteracts the immunosuppressive effect of glucocorticoids, is associated with autoimmunity, and mediates T-cell activation. High-expression *MIF* polymorphisms correlate with more severe disease and higher steroid requirements. Both MIF and its receptor CD74 are highly expressed in the liver of AIH patients as previously published (Assis *et al* 2014, 2016). We hypothesized that pharmacologic MIF inhibition can reduce liver injury caused by antigen-specific T-cells. **Aim:** Evaluate the effect of MIF inhibition *in vivo* using the OVA HEP model of AIH. **Methods:** OVA HEP transgenic mice express ovalbumin$^{139\text{-}385}$ on the hepatocyte surface. Adoptive transfer of OT1 T-cells, expressing an OVA-specific TCR, into OVA HEP host mice results in antigen-specific T-cell mediated liver injury. OVA HEP or OVA HEP MIF KO male mice received purified OT1 or OT1 MIF KO T-cells (OVA HEP+OT1) ± small molecule antagonist MIF098 (40 mg/kg b.i.d., i.p.). Mice were studied at day 4 for serum ALT, liver histology, and portal vein blood cytokines (LEGENDplex). **Results:** OVA HEP+OT1 mice developed hepatitis (median ALT 250 U/L vs. baseline 80 U/L, p=0.0078) while OVA HEP MIF KO+OT1 MIF KO mice had much less injury (120 vs. 40 U/L, p=0.1). OVA HEP MIF KO+OT1 mice developed more injury vs. OVA HEP MIF KO+OT1 MIF KO mice (205 vs. 120 U/L, p=0.05), suggesting a key role of MIF in pathologic T-cell activation. Importantly, administration of MIF098 to OVA HEP+OT1 mice prevented hepatitis (ALT 70 vs. baseline 40 U/L, p=0.13) and injury was significantly attenuated in OVA HEP+OT1+MIF098 vs. OVA HEP+OT1 mice (ALT 70 vs. 250, p=0.0004). Reduction of interface hepatitis by pharmacologic MIF inhibition was confirmed by histology. Surprisingly, MIF098 treatment of OVA HEP+OT1 mice significantly reduced liver injury compared to OVA HEP MIF KO+OT1 MIF KO mice (ALT 70 vs. 120 U/L, p=0.02), suggesting added targets of MIF antagonism. Finally, portal vein blood TNF-α levels were reduced in the MIF098 group (3.38 vs. 5.86 pg/mL, p=0.01) with similar trends for IL-4 and IFN-γ. **Conclusions:** MIF is necessary for antigen-specific T-cell inflammation in the OVA HEP model of AIH and the novel small molecule antagonist MIF098 significantly reduced liver injury. These results support a key mechanistic role of MIF in AIH and suggest that pharmacologic MIF antagonism, which is in clinical development, may be a steroid-sparing alternative to standard immunosuppression. Such a therapeutic approach may be especially beneficial in high genotype *MIF* expressers.

Disclosures:

James L. Boyer - Consulting: Allergan, Alnylam; Grant/Research Support: Gilead, Intercept pharma

Richard Bucala - Patent Held/Filed: Yale University

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Gene-disease associations identify a connectome with shared molecular pathways in human cholangiopathies

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Background: Cholangiopathies are a diverse group of progressive diseases whose primary cell targets are cholangiocytes. The anatomical sites of injury obey a reproducible pattern focused predominantly either on intrahepatic bile ducts (as seen in primary biliary cholangitis [PBC]), extrahepatic ducts and intrahepatic bile ducts (as in primary sclerosing cholangitis [PSC]), or extrahepatic bile ducts with secondary involvement of intrahepatic cholangiocytes (as in biliary atresia [BA]). The anatomical continuity and functional integrity of intra- and extra-hepatic bile ducts are key to the homeostasis of bile production, modification and excretion. Based on the common cellular target in the biliary epithelium, we hypothesized that these cholangiopathies have common genes and gene groups that relate, at least in part, to shared pathogenic mechanisms of disease. **Methods:** To identify shared pathogenesis and molecular connectivity among BA, PBC and PSC, we built a comprehensive platform of published data on gene variants, gene expression and functional studies, and applied bioinformatics algorithms and network-based analytics in search for shared molecular circuits. **Results:** We found 276, 387 and 166 human genes associated with BA, PBC and PSC, respectively. Mining the data platform with largest connected component and interactome analyses, we validated previously reported associations for individual diseases and identified essential- and hub-genes for each cholangiopathy (Fisher exact test, $P < 0.05$). Analyzing disease-specific modules, we found a substantial overlap of disease neighborhoods (BA vs PBC, z-score = -8.30; BA vs PSC, z-score = -5.39; PBC vs PSC, z-score = -7.24), and uncovered a unique group of 34 core genes that are shared by the three diseases. These core genes were enriched for immune processes (Hypergeometric test, P = 2.28E-21) and abnormal intestine/hepatobiliary mouse phenotypes (Hypergeometric test, P = 5.10E-17). Within this core, 14 genes were prominently placed in a regulatory connectome related to cellular immunity, pro-inflammatory response and fibrosis, with inter-relationship among *STAT3, IL6, TNF* and *FOXP3* genes. By enrichment analysis, we identified a shared, previously unrecognized AGE-RAGE pathway (Hypergeometric test, P = 1.41E-10) that activates IL6/STAT3, TNF and the pro-fibrogenic signaling. **Conclusions:** Human cholangiopathies share pathways enriched by immunity genes and a molecular connectome that links different pathogenic features of BA, PBC and PSC. Within the connectome, the AGE-RAGE pathway link to IL6/STAT3, TNF and fibrosis may be a potential novel mechanism of pathogenesis shared among the cholangiopathies.

Disclosures:

Jorge A. Bezerra - Grant/Research Support: Molecular Genetics Laboratory, CHMC, Gilead, Shier

The following people have nothing to disclose: Zhenhua Luo, Anil G. Jegga

115

HCV infection induces ISG56 in hepatocytes and suppresses HBV replication.

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Background and aim: Recently, HBV reactivation during IFN-free DAA therapy in patients with HBV/HCV coinfection has been reported. The purpose of this study was to investigate whether host innate immunity suppresses coinfected HBV. **Methods:** In vitro, primary human hepatocytes (PHHs) isolated from humanized liver chimeric mice were inoculated with HBV and HCV. In vivo, humanized liver chimeric mice were inoculated with HBV and HCV derived from patients' sera. We measured mRNA levels of ISG56 in human liver samples from chronic hepatitis B(CHB), chronic hepatitis C (CHC) and non-B non-C patients. **Results:** PHHs were inoculated with HCV (50 Multiplicity of infection) 20 days after inoculated with HBV (500 Genome Equivalent/cell) in HBV/HCV inoculated group. On the other hand, PHHs were inoculated with HBV alone in HBV inoculated group. HBV DNA, HBs antigen and HBe antigen levels in HBV/HCV inoculated group were significantly lower than HBV inoculated group at day 28 post HBV inoculation. At day 23 after HBV inoculation, mRNA levels of RIG-I, IRF3, ISG15, and ISG56 in HBV/HCV inoculated group were significantly higher than HBV inoculated group. At the same time, mRNA levels of pgRNA in HBV/HCV inoculated group were significantly lower than HBV inoculated group. Next, we knocked down ISG56 in PHHs using ISG56 siRNA followed by inoculation HCV and HBV. PHHs were treated with siISG56 or siNegative control (siNC) 3 days before inoculated with HCV. At day 3 after HCV inoculation, we inoculated the PHHs with HBV. HBV DNA levels in siISG56-treated PHHs were significantly higher than siNC-treated PHHs at day5 post HBV inoculation. At day 1 after HBV inoculation, mRNA levels of pgRNA in siISG56-treated PHHs were significantly higher than siNC-treated PHHs. In vivo, humanized liver chimeric mice were infected with HCV and then infected with HBV. HCV was eliminated by DAA therapy after 12 weeks post HCV infection. Mice infected with HBV/HCV showed significantly lower serum HBV DNA levels than mice infected with HBV alone. mRNA levels of RIG-I, ISG15, and ISG56 in liver tissue in mice infected with HBV/HCV were higher than mice infected with HBV alone. At the same time, mRNA levels of pgRNA in liver tissue in mice infected with HBV/HCV were lower than mice infected with HBV alone. Serum HBV DNA levels increased after HCV elimination by DAA therapy. In human liver tissue, the expression levels of ISG56 in the liver tissue were significantly higher in CHC patients than in non-B non-C patients or CHB patients. **Conclusion:** HCV infection during HBV/HCV coinfection induced ISG56, resulted in inhibition of the HBV replication.

Disclosures:

Hayato Hikita - Grant/Research Support: Bristol-Myers Squibb; Speaking and Teaching: Bristol-Myers Squibb, EA Pharma Co., Ltd

Ryotaro Sakamori - Grant/Research Support: MSD; Speaking and Teaching: Gilead, SYSMEX

Tetsuo Takehara - Grant/Research Support: Janssen pharmaceutical Co., AbbVie, Gilead Sciences, Chugai Pharmceutical Co., MSD K.K., Bristol-Meyer Squibb, Mitsubishi Tanabe Pharma Corparation, Toray Industories Inc.; Speaking and Teaching: Bristol-Meyer Squibb, Gilead Sciences, AbbVie, MSD K.K.

The following people have nothing to disclose: Kazuhiro Murai, Yugo Kai, Tasuku Nakabori, Hiroshi Suemizu, Takahiro Kodama, Tomohide Tatsumi

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A global perspective on HBV-related SNPs and evolution during human migration

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Background: Genome-wide association studies have indicated that HLA-DP and -DQ play roles on persistent hepatitis B virus (HBV) infection in Asian. To understand the evolution of HBV-related single nucleotide polymorphisms (SNPs) and to correlate these SNPs with chronic HBV infection among different populations, we conducted a global perspective analysis on hepatitis-related SNPs. **Methods:** We selected 12 HBV-related SNPs on the HLA locus, and two HBV and three hepatitis C virus immune-related SNPs for analysis. Five nasopharyngeal carcinoma-related SNPs served as controls. All SNP data from 26 populations all over the world were downloaded from 1000 genomes. **Results:** We found a dramatic difference in the allele frequency in most of the HBV- and HLA-related SNPs in East Asia among the other continents (Figure). A sharp change of allele frequency in 8/12 SNPs was found between Bengali populations in Bangladesh and Chinese Dai populations in Xishuangbanna, China ($p < 0.001$), which represents the junction of South and East Asia. For the immune-related SNPs, significant changes were found after leaving Africa. Most of these genes shifted from higher expression genotypes in Africa to lower expression genotypes in either Europe or South Asia ($p < 0.001$). **Conclusion:** During this two-stage adaptation, the immunity was adjusted toward weak immune response, which could be a survival strategy during human migration to East Asia. The prevalence of chronic HBV infection in Africa is as high as in Asia. However, the HBV-related SNPs genotypes are not present in Africa. The genetic mechanism of chronic HBV infection in Africa will need further exploration.

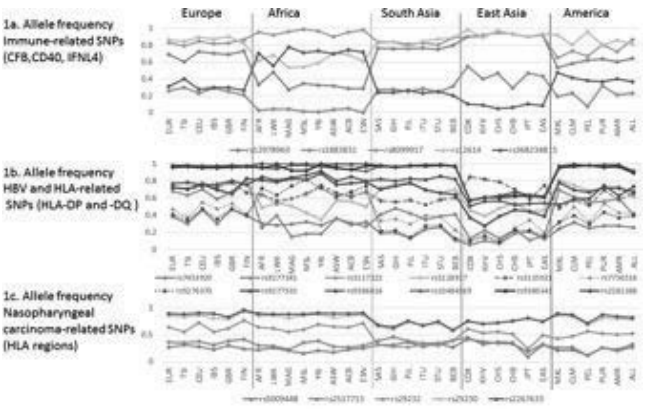

Figure 1a. Significant allele type differences between African and European populations, and between African and South Asian populations in all immune-related SNPs. Figure 1b. Significant allele type differences between South and East Asian populations in 8/12 HLA-related SNPs, and between African and South Asian populations in 3/12 SNPs. Figure 1c. No significant difference among different populations in five NPC-related SNPs.

Disclosures:

The following people have nothing to disclose: Dar-In Tai, Chun-Yen Lin

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A novel culture model for coinfection of hepatitis B and hepatitis C viruses using human induced pluripotent stem cell–derived hepatic cells for analyses of changes in host-innate immune responses

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Background/Aim: Interferon (IFN)-free regimens for hepatitis C virus (HCV) infection using direct acting antivirals (DAAs) have dramatically improved the treatment efficacy of antiviral therapy for HCV-related chronic liver diseases. On the other hand, several cases suffering from reactivation of hepatitis B virus (HBV) during DAAs treatment against HCV have been reported, which were coinfected with HBV and HCV: however, mechanism of HBV reactivation in such cases remains unknown. As *in vitro* model, we have recently demonstrated that human induced pluripotent stem (iPS) cell-derived hepatocyte-like cells (iPS-Heps) are susceptible to HBV, and exhibit innate immune responses in host cells against HBV. Objective of this study is to reveal the mechanisms of HBV reactivation during HCV DAAs treatment for hepatocytes coinfected with HBV and HCV. Methods: Several human iPS cell lines were differentiated into hepatic lineage using modified 4-step protocol as previously described (Kamiya, 2013). HBV and HCV were obtained from the culture supernatant of HepG2.2.15 and Huh7.5.1 transfected with HCV-RNA (JFH1 clone), respectively. HBV-related products and responses of IFN-stimulated genes (ISGs) were analyzed using culture system of iPS-Heps coinfected with HBV and HCV under the culture condition with or without sofosbuvir (SOF). Results: Quantitative RT-PCR analysis demonstrated that the expression levels of host factors related to HCV life cycle in iPS-Heps were significantly higher relative to Huh7.5.1. The concentrations of HBe antigen (Ag) and HCV core Ag in culture supernatant of coinfected iPS-Heps were increased in a time-dependent manner after HBV and HCV coinfection. HCV infection induced the expression of ISGs, such as MxA, ISG15, and PKR, in host cells, whereas the expression levels of ISGs were relatively lower in HBV-infected host cells. Expression levels of intracellular ISGs were significantly lower in the coinfected cells relative to cells infected with HCV alone. Both HCV-core Ag in the culture supernatant and intracellular HCV-RNA were significantly decreased in the coinfected cells after SOF treatment. On the other hand, HBeAg in the supernatant, intracellular HBV-cccDNA, and pregenomic RNA were significantly increased in SOF-treated cells relative to vehicle-treated cells. Furthermore, the expression levels of intracellular ISGs were significantly decreased in SOF-treated cells relative to vehicle-treated cells. Conclusion: Our data revealed that *in vitro* system of iPS-Heps could be a novel research model for coinfection of HBV and HCV, and suggest that HBV reactivation during HCV DAAs treatment is induced due to suppression of ISGs expression.

Disclosures:

Mamoru Watanabe - Grant/Research Support: MSD Co., Ltd., Abbvie. GK, Chugai Pharmaceutical Co., Ltd, Dainippon Sumitomo Pharma Co., Ltd, Toray Industries, Inc, Bristol-Myers K.K., GILEAD Science, Inc

The following people have nothing to disclose: Shun Kaneko, Sei Kakinuma, Yasuhiro Asahina, Akihide Kamiya, Masato Miyoshi, Tomoyuki Tsunoda, Emi Inoue-Shinomiya, Sayuri Nitta, Ayako Sato, Yu Asano, Hiroko Nagata, Fukiko Kawai-Kitahata, Miyako Murakawa, Yasuhiro Itsui, Mina Nakagawa, Seishin Azuma

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Identification of a Triple Mutation that Confers Tenofovir Resistance in Chronic Hepatitis B Patients

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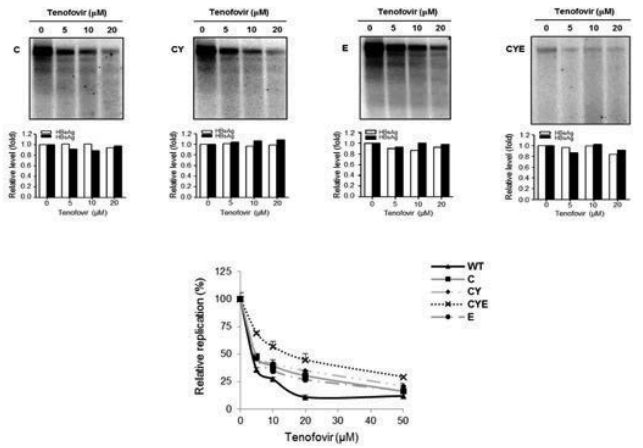
Background: Tenofovir disoproxil fumarate (TDF) is the most potent nucleoside analogue for the treatment of chronic hepatitis B virus (HBV) infection. This study aimed to characterize HBV mutations that confer tenofovir resistance, which has not yet been reported. **Methods:** Two consecutive patients with viral breakthrough during treatment with TDF-containing regimens were prospectively enrolled. The gene encoding HBV reverse transcriptase (RT) was sequenced. Nine HBV clones harboring a series of mutations in the RT gene were constructed by site-directed mutagenesis. Drug susceptibility of each clone was determined by Southern blot analysis and real-time PCR. Relative frequency of mutants were evaluated by ultra-deep sequencing **Results:** Seven mutations (rtS106C [C], rtH126Y [Y], rtD134E [E], rtV173L, rtL180M, rtM204V, and rtL269I) were commonly found in viral isolates from both patients after viral breakthrough; C, Y, and E were novel mutations. An HBV mutant harboring all three mutations (CYE) was resistant to tenofovir. The IC_{50} values for wild-type HBV and the CYE mutant were 3.8±0.6 μM and 14.1±1.8 μM, respectively (Figure 1). Ultra-deep sequencing showed that CYE mutant was dominant than any other mutant in both patients. All tenofovir-resistant mutants had similar susceptibility to a core inhibitor, NVR 3-778 (IC_{50}<0.4 μM), compared with wild-type (IC_{50}=0.4). **Conclusion:** Our study reveals that a novel triple mutation (CYE) is associated with tenofovir-resistance. These results demonstrate that tenofovir-resistant HBV mutants can emerge, although the genetic barrier is high. A novel core inhibitor might be a potential rescue therapy for tenofovir-resistant HBV.

Figure 1



The tenofovir susceptibility assay was done by Southern blot after the transfection of Huh7 cells with the indicated HBV1.2mer mutants. The levels of secreted HBsAg and HBeAg were determined by ELISA to assess transfection yield. (*, P <0.05)

Disclosures:

Yoon Jun Kim - Grant/Research Support: Bristol-Myers Squibb, Roche, JW Creagene, Bukwang Pharmaceuticals, Handok Pharmaceuticals, Hanmi Pharmaceuticals, Yuhan Pharmaceuticals; Speaking and Teaching: Bayer HealthCare Pharmaceuticals, Gilead Science, MSD Korea, Yuhan Pharmaceuticals, Samil Pharmaceuticals, CJ Pharmaceuticals, Bukwang Pharmaceuticals, Handok Pharmaceuticals

Jung-Hwan Yoon - Grant/Research Support: Bayer HealthCare Pharmaceuticals, Daewoong Pharmaceuticals, Bukwang Pharmaceuticals

Fabien Zoulim - Advisory Committees or Review Panels: Janssen, Gilead, Abbvie, Arbutus, Transgene; Consulting: Roche, Contravir, Assembly; Grant/Research Support: Gilead, Roche, Assemblypharm, Janssen, Contravir, Arbutus, Sanofi; Speaking and Teaching: Gilead

The following people have nothing to disclose: Jeong-Hoon Lee, Yun Bin Lee, Hyeki Cho, Young Youn Cho, Eun Ju Cho

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Transcriptome profiles of NK and T cells regulating immune control in chronic hepatitis B, proximate to patients treated with Pegylated Interferon alpha-Nucleos(t)ide analogue (NA) sequential therapy compared to de novo NA therapy.

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BACKGROUND & AIMS: Strategies to achieve functional cure in Chronic Hepatitis B (CHB) are the focus of rigorous investigation. We recently demonstrated that PegIFNα priming, prior to nucleot(s)ide analogue (NA) use, expands a population of functional NK cells resulting in HBsAg decline *(Gill et al., 2016).* Based on these findings further exploration of sequential/combined therapeutic approaches are warranted to better define mechanisms of HBsAg decline and loss. To comprehensively analyse innate and adaptive immune responses we studied the transcriptome of treated patients; sequential NA therapy (following PegIFNα failure) and de novo NA therapy; and compared it with that found in inactive carriers (IC) with ostensible immune control. PATIENTS & METHODS: Sorted fractions of total NK and CD8 T cells from 40 patients were analysed using NanoString, for mRNA transcription of 600 immune genes. Ten PegIFNα patients who progressed to sequential NAs were compared with de novo NA therapy patients (n=10). A further 20 patients classified as IC's (eAg-, normal ALT, low HBV DNA) were also analysed to determine if the cohorts of treated patients were similar to those with immune control. RESULTS: Patients clustered together according to their treatment regime. Sequential NA therapy patients differentially expressed 17 genes on NK cells and 13 genes on T cells that were significantly upregulated, in comparison to de novo NA treated patients. On NK cells, the KIR activating and CCR1 gene, involved in NK cell and chemokine activation respectively, were the most enhanced, with the CCR1 and EGR1 (implicated in IFNα/β signalling) genes most highly expressed on T cells. More differentially expressed genes were noted between IC patients and de novo NA treated, compared to sequential NA treated patients. The most significantly upregulated genes in ICs, on both NK cells (KLRG2, MCL-1, NCR2) and T cells (KLRG2, LILRA3, ICOSLG) potentially associated with more robust immune control, were seen at higher levels in sequential NA compared to de novo NA treated patients. CONCLUSIONS: Sequential NA therapy is associated with greater declines in HBsAg. We show that patients primed with PegIFNα prior to viral suppression with NA's exhibit an immune transcriptome profile that is more consistent with that observed in IC patients. A step-wise hierarchy towards ostensible immune control was noted; de novo NA then sequential NA followed by IC patients, suggesting PegIFNα priming may be important in immune restoration. Further study of immune cell interactions and ingenuity pathway analysis is mandated to better define mechanisms of functional cure in CHB patients.

Disclosures:

Mala K. Maini - Advisory Committees or Review Panels: Roche; Board Membership: Gilead; Consulting: Arbutus, Immunocore; Grant/Research Support: Gilead, Roche

Patrick T. Kennedy - Grant/Research Support: Gilead; Speaking and Teaching: Roche, BMS, Gilead

The following people have nothing to disclose: Upkar S. Gill, Nina Le Bert, Damien Tan, Laura Rivino, Wei-Chen Huang, Dimitra Peppa

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Hepatitis B virus deregulates cell cycle control to promote viral replication and a premalignant phenotype

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Background: Hepatitis B virus (HBV) infection is a major risk of developing hepatocellular carcinoma (HCC). The pathogenic mechanisms of HBV-associated HCC remain elusive. The aim of the study is to assess whether HBV infection directly affects infected hepatocyte and promotes HCC. Methods: Human growth factor antibody array was performed in HBV-infected primary human hepatocytes (PHHs). To obtain a genome-wide host response to HBV infection, transcriptome profile was analyzed by

microarray. Human cell cycle RT^2 profiler PCR array was performed to verify certain genes in the signaling. Flow cytometer was used to study cell cycle alteration. Inhibitors of cell cycle were tested on HBV infection. Expression of several proviral host factors were evaluated in HBV infected PHHs as well as the liver of HBV infected chimpanzees. Results: HBV-infected PHHs produced significantly less transforming growth factor (TGF)-β but more insulin-like growth factor (IGF) binding proteins. Revealed by microarray, many genes involved in carcinogenesis are affected including the genes in the cyclin D1 signaling pathway, which is a major pathway in cell cycle control. Human cell cycle RT^2 profiler PCR array demonstrated down-regulation of 21 and up-regulation of 3 cell cycle genes in HBV-infected PHHs. Analysis of cell cycle by flow cytometer showed that HBV infection shifted cell cycle from G_0/G_1 to G_2/M phase. Inhibitors of cell cycle at the G_1 or G_1/S phase but not G_2/M phase restricted HBV infection. Interestingly, proviral host factors, such as PPARA, RXRA and CEBPB, were also up-regulated upon HBV infection. Consistent with the *in vitro* data, HBV infection resulted in elevation of IGF and CEBPB in the liver of HBV infected chimpanzees. Conclusion: These results support the hypothesis that HBV deregulates cell cycle control to foster an environment with high levels of proviral factors. In addition, IGF-1 activates the PI3K/AKT pathway that is known to promote tumorigenesis, whereas the deregulated TGF-β pathway can result in the loss of tumor suppressors. Collectively, HBV infection, by disturbing cell cycle regulation of infected cells, creates a premalignant phenotype that predisposes to malignant transformation.

Disclosures:

The following people have nothing to disclose: Yuchen Xia, Xiaoming Cheng, Yao Li, Weiping Chen, T. Jake Liang

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Continued Increase in Incidence of De Novo Hepatocellular Carcinoma Among Liver Transplant Registrants with Hepatitis C Virus Infection

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Background: There is widespread optimism that direct acting anti-viral agents (DAA) for the treatment of HCV will lead to a reduction in hepatitis C (HCV)-related liver complications. The aim of this study was to describe the incidence of de novo HCC among waitlist (WL) registrants for liver transplantation (LT) in the United States (US) over time. **Methods:** A population-based cohort of all WL registrants in the US was created using the Scientific Registry of Transplant Recipients database. Eligible patients included adults with cirrhosis listed for primary LT from January 2013 to December 2015, without HCC at registration or an HCC-MELD exception within 180 days of WL. Patients were followed until December 2016. Patients were divided into 3 eras based on the initial date of LT listing: interferon (IFN) era (2003-2010), protease inhibitor (PI) era (2011-2013), and DAA era (2014-2015). De novo HCC was defined as the presence of an HCC MELD exception > 180 days after registration. Incidence rates (IR) of de novo HCC were calculated, and incident rate ratios (IRR) were compared by era using the Mantel-Haenszel method. Cox proportional hazards regression methods were used to evaluate the association between era of LT WL and de novo HCC by etiology after adjustment for confounders. **Results:** There were 48,158 eligible patients — 62% were male, 42% had HCV, and the median age was 55 years (IQR 49-60). A total of 3,112 (6.5%) patients developed de novo HCC after a median follow-up of 493 days. In individuals listed with HCV, the IR of HCC was 49% higher in the DAA era (IR 6.6/100 person-years[py], 95% CI 5.6-7.9) vs. the IFN era (4.5/100 py, 95% CI 4.2-4.7; IRR 1.49, 95% CI 1.24-1.79, $p < 0.001$). In multivariate Cox regression adjusting for age, sex, Child-Pugh score, race, and diabetes, individuals with HCV in the DAA era had a 22% higher hazard of de novo HCC compared to the IFN era (HR 1.22, 95% CI 1.01-1.48, $p = 0.04$). There was no association between era of WL and de novo HCC in patients with other etiologies of cirrhosis, including hepatitis B, non-alcoholic steatohepatitis, or alcohol-related cirrhosis ($p > 0.05$ for all). **Conclusion**: In this population-based cohort of LT registrants in the US, the incidence of de novo HCC in HCV patients has continued to increase despite availability of DAA. On the patient level, vigilant surveillance for HCC remains important, while on the policy level, the ongoing discussion regarding LT allocation for HCC should incorporate consideration of these patients.

Disclosures:

W. Ray Kim - Advisory Committees or Review Panels: Intercept, Gilead Sciences, Merck, Abbvie; Consulting: Conatus

The following people have nothing to disclose: Allison J. Kwong, Jennifer A. Flemming

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Hepatitis B Reactivation and Outcomes in Persons Treated with Directly Acting Antiviral Agents Against Hepatitis C Virus: Results from ERCHIVES

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Background: Higher risk of hepatitis B reactivation has been reported in patients with hepatitis C treated with newer directly acting antiviral agents (DAAs). We sought to determine the proportion of persons who develop HBV reactivation and its clinical consequences. **Methods:** In persons treated with a newer DAA regimen, we calculated the proportion of persons who developed HBV viral reactivation (new detectable HBV DNA or increase of >1log$_{10}$); serum alanine aminotransferase flare (>10 times baseline); all-cause mortality and hepatic decompensation. We used Cox proportional hazards analysis to determine factors associated with HBV flare/reactivation. **Results:** Among 43,137 persons initiated on DAA therapy, HBV viral reactivation was observed in 12 out of 37,295 (0.03%) persons with available HBsAg test at baseline (5 out of 4,413 [0.11%] HBsAg+ and 7 out of 32,882 [0.02%] HBsAg- persons). Flares of ALT were observed in 0.26% of HBsAg+ and 0.20% of HBsAg- persons. **(Table)** Black race, male sex, alcohol use or dependence, and detectable HBV DNA at baseline were associated with a higher risk of flare/reactivation while treatment with a DAA regimen was associated with a lower risk. Kaplan-Meir survival curves demonstrated a significantly quicker time to first hepatic decompensation event and death for persons with HBV reactivation compared with those without HBV reactivation. **Conclusions:** HBV flare up or reactivation are relatively uncommon after DAA therapy in HCV infected

persons. When observed, the risk is higher among those with baseline HBsAg+ and are associated with a higher risk of hepatic decompensation and death.

Table. Rates of HBV reactivation in specified subgroups.

		ALT flare up				HBV DNA/viral reactivation			
Baseline variable	N available at baseline	ALT flare	N available for follow up	% of N at baseline	% of N available for follow up	HBV viral reactivation	N available for follow up	% of N at baseline	% of N available for follow up
HbsAg									
Positive	4413	11	4262	0.25%	0.26%	5	56	0.11%	8.93%
Negative	32882	62	31766	0.19%	0.20%	7	275	0.02%	2.54%
HBcAb (HBsAg- at baseline)									
Positive	8136	14	7885	0.17%	0.18%	5	174	0.06%	2.87%
Negative	18859	43	18184	0.23%	0.24%	2	90	0.01%	2.22%
HBeAg (HBsAg- at baseline)									
Positive	78	0	74	0.00%	0	0	4	0.00%	0
Negative	1882	5	1837	0.27%	0.27%	2	79	0.11%	2.53%
HBV DNA									
Positive	2636	9	2553	0.34%	0.35%	11	316	0.42%	3.48%
Negative	523	3	521	0.57%	0.56%	6	36	1.15%	16.67%
ALT at baseline									
Normal (Men <31; Women <20)	6252	30	6018	0.48%	0.50%	4	70	0.06%	5.71%
High	36859	55	35634	0.15%	0.15%	13	282	0.04%	4.61%
SVR									
Yes	20693	19 (3)		0.09%		3 (1746)		0.01%	
No	1766	44 (m)		2.49%		9 (2057%)		0.51%	

Disclosures:

Adeel A. Butt - Grant/Research Support: Gilead, Merck

The following people have nothing to disclose: Obaid S. Shaikh, Abdul-Badi Abou-Samra

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The Declining Burden of HCV on the Liver Transplant Waitlist associated with the DAA era

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Background: We aim to compare liver transplant (LT) waitlist trends and outcomes prior to and following the availability of direct-acting antiviral (DAA) agents for the treatment of hepatitis C virus (HCV) in the United States (US). **Methods:** Utilizing the United Network for Organ Sharing (UNOS) database, we analyzed LT waitlist outcomes in adult HCV registrants without hepatocellular carcinoma (non-HCC HCV) initially listed during the 36 months before (pre-DAA) and after (DAA) the approval of second-generation DAA agents (November 2013). 90-day rate for waitlist mortality was compared in each era. Cox regression analysis was performed to determine the impact of DAA era on 90-day waitlist mortality and was adjusted for clinical demographics (age, gender, ethnicity), Model for End-Stage Liver Disease score, portal hypertension complications, and UNOS Region. **Results:** From November 2010 to December 2016, the percentage of non-HCC waitlist additions with HCV declined 35% in the DAA era (pre-DAA n=8620, 34.0%. *vs.* DAA n=5579, 21.8%, p <0.001). Moreover, a significant decline in the monthly HCV waitlist addition rate (per 100 non-HCC waitlist additions) was seen in DAA era (p <0.001) (Figure). Among HCV waitlist additions, the DAA era was associated with lower 90-day waitlist mortality (pre-DAA 8.1% vs. DAA 7.3% p <0.001). A similar reduction in mortality was seen among decompensated HCV waitlist additions (pre-DAA 12.3% vs DAA 10.2%, p <0.001). In addition, the DAA era was associated with a 16% reduction in the risk for 90-day waitlist mortality (HR 0.85, p <0.001). **Conclusion:** US national trends in LT waitlist additions and mortality in non-HCC HCV patients suggest DAA agents have reduced the previous HCV burden placed on our LT allocation system.

Monthly HCV waitlist addition rate (per 100 non-HCC additions) in the United States from 2010-2016.

Disclosures:

Sanjaya K. Satapathy - Advisory Committees or Review Panels: Gilead, Abbvie; Board Membership: Intercept; Grant/Research Support: Genfit, Gilead, Biotest, Conatus, Intercept, Shire; Speaking and Teaching: Intercept, Alexion

Aijaz Ahmed - Consulting: Bristol-Myers Squibb, Gilead Sciences Inc., Roche, AbbVie, Shire, Janssen pharmaceuticals, Intercept; Grant/Research Support: Gilead Sciences Inc., Intercept

The following people have nothing to disclose: George Cholankeril, Katherine L. March, Eric R. Yoo, Andrew A. Li, Rosann Cholankeril, Ryan B. Perumpail

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Increasing Primary Incidence of Hepatitis C Among HIV-Infected Men Who Have Sex with Men in San Diego; a Pooled Analysis of Two Large Clinics from 2000-2015

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Background: Our recent analyses found increasing hepatitis C virus (HCV) incidence among HIV-infected men who have sex with men (HIV+ MSM) attending the largest HIV clinic in San Diego, but generalizability was unclear. We perform a retrospective pooled analysis of HCV incidence among HIV+ MSM attending two of the largest HIV clinics in San Diego, California. Methods. We performed a retrospective cohort analysis of incident HCV infection among HIV+ MSM attending two of the largest HIV clinics in San Diego (UCSD Owen Clinic and the San Diego Veterans Affairs (VA) Hospital) from 2000-2015. Incident HCV infection was assessed among HIV+ MSM with a baseline negative anti-HCV test between 2000 and 2015, and defined as any new positive anti-HCV or HCV-RNA test after the start of follow-up. Group risks were defined as individ-

uals who ever reported a history of injecting drug use (IDU) or methamphetamine (meth) use. Results. A total of 2,768 MSM, who were initially HCV uninfected and had at least 1 subsequent test during a median of 4.3 years of follow-up (IQR 1.9-8.0), were included in the analysis. Overall, 172 HCV seroconversions occurred over 14,455 person-years(py) of follow-up, giving an incidence rate of 1.19/100py (95%CI 1.02-1.38) with statistical no difference between sites. There was a significant increase in incidence over time, from 0.68/100py in 2000-2003 rising to 1.50/100py in 2012-2015 (p=0.022). HCV incidence was three-fold higher among HIV+ MSM reporting ever meth use only compared to those with no history of meth use or IDU (1.49/100py vs 0.49/100py, p<0.001) with a significant increase over time (p<0.001). Compared to those with no history of IDU or meth use, HCV incidence was also significantly higher among HIV+ MSM reporting a history of IDU only (Incidence Rate Ratio [IRR] =5.7 [1.1-18.8], p=0.003), or combined IDU and meth use (IRR=4.6 [2.3-8.9]. p<0.001). Conclusions. These congruent results from the 2 largest HIV clinics in San Diego reinforce our findings that HCV incidence is high and increasing among HIV+ MSM in San Diego, similar to other major European cities. Further studies are needed to monitor the epidemic trajectory and evaluate prevention measures to reduce the risk of HCV infection among HIV+ MSM.

Disclosures:

David L. Wyles - Advisory Committees or Review Panels: Gilead, Merck, AbbVie; Grant/Research Support: Gilead, Merck, AbbVie

Susan J. Little - Advisory Committees or Review Panels: ViiV; Grant/Research Support: Gilead Sciences

Natasha K. Martin - Grant/Research Support: Gilead; Speaking and Teaching: AbbVie, Merck, Gilead

The following people have nothing to disclose: Antoine Chaillon, Edward R. Cachay, David Looney, Richard L. Garfein, Christy M. Anderson, Thomas C. Martin, Sonia Jain, Sanjay R. Mehta

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The impact of hepatitis C diagnosis on substance-use behaviors in patients engaged in opioid substitution therapy

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Introduction: Opioid misuse is a public health crisis in many populations. In Canada, the province of Ontario has more than 50,000 opioid-dependent persons who are engaged in opioid substitution therapy (OST), using mainly methadone and suboxone. Hepatitis C Virus (HCV) infection, with an estimated prevalence of 0.3%-0.9% among all Canadians, is more common in this population. Many experts advocate for testing all OST patients for chronic HCV infection. To date, the impact of HCV infection diagnosis on the substance use behaviors of OST patients is unknown and we aim to explore that here. Methods: We conducted a retrospective cohort analysis using the electronic health data, urine toxicology and antibody-based HCV infection screening information from a network of 43 addiction treatment clinics in Ontario from 2000 to 2013. We used a logistic regression analysis on the data to determine the impact of HCV infection diagnosis on substance-use behaviors for patients engaged in OST. *Results:* 2406 individuals were identified amongst the 43 clinics who were screened for HCV infection. 527 (21.9%) individuals tested positive for anti-HCV Ab. Substance use was evaluated one year following the HCV testing. Those who screened positive for HCV were 53.1% more likely to significantly alter their substance-use behaviors and reduce their consumption of non-prescribed opioids according to urine toxicology after the anti-HCV Ab screening when compared to those who screened negative for Hepatitis C after controlling for age, sex and geographic location (aOR=0.653; $CI_{95\%}$=0.803-0.531; p<0.001). Patients who were diagnosed with HCV infection subsequently had a significantly lower proportion of positive urine drug screens, including non-prescribed opioids (aOR=0.743), benzodiazepines (aOR=0.631), and cocaine (aOR=0.625). **Conclusion:** We have demonstrated that HCV infection screening can have a positive impact on substance-use treatment outcomes among patients engaged in OST. Expansion and universal screening of OST clients for HCV infection should be encouraged.

Disclosures:

Jordan J. Feld - Advisory Committees or Review Panels: Merck, Gilead, AbbVie, Bristol Myers Squibb, Contravir; Grant/Research Support: AbbVie, Janssen, Gilead, Merck

Hemant A. Shah - Advisory Committees or Review Panels: Abbvie, Merck; Consulting: Gilead, Intercept; Grant/Research Support: Boehringer-Ingelheim; Speaking and Teaching: Lupin

The following people have nothing to disclose: Hooman Farhang Zangneh, Graham Gauthier, David C. Marsh

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A high prevalence rate of a positive screen for neurocognitive dysfunction in patients with chronic Hepatitis C infection in an Irish clinic.

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Objective: Our objective is to determine the prevalence of cognitive dysfunction in patients attending our institution with chronic hepatitis C infection. **Background** The reported prevalence of neurocognitive dysfunction in chronic hepatitis C (HCV) infection is 30%. In Ireland, between 30,000 – 50,000 people are believed to be infected with HCV but there is no Irish data on HCV related neurocognitive deficits. **Methods:** Since August 2016 we have been undertaking a prospective, cross-sectional, assessment of patients attending the hepatology service at St. James's Hospital, (Dublin, Ireland) using the Brief NeuroCognitive Screen (BNCS). Inclusion criteria are as follows: age ≥ 18, hepatitis C virus DNA positive and capable of giving informed consent. Pertinent exclusion criteria include: co-infection with HIV and/or hepatitis B, visual/motor impairment, language barriers and end-stage liver disease/cirrhosis. Those who screen positive for impairment are then recruited to the CANDI project, a longitudinal study employing neuropsychological tests and neuroimaging techniques to investigate the impact of exercise and viral eradication with direct acting anti-virals (DAA) on cognitive dysfunction. **Results:** Between August 2016 and April 2017, 321 eligible subjects were tested and 49% screened positive for cognitive dysfunction. The majority were male (64.5%), the median age was 41 years and the median duration of formal education completed was 11 years. Most subjects (66%) were born in Ireland.

The most common method of HCV acquisition was from previous intravenous drug abuse (86%) and 31.3% are currently prescribed methadone. **Conclusion:** The interim results of this ongoing study highlight the necessity for screening for cognitive dysfunction in non-cirrhotic HCV infected patients. Further analyses will help to determine factors associated with the development of cognitive dysfunction. The potential role of DAA therapy or exercise intervention in ameliorating cognitive dysfunction needs to be investigated further.

Disclosures:

The following people have nothing to disclose: Damien Ferguson, Orla Strahan, Paul Dockree, Robert Coen, Suzanne Norris

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Normothermic *ex vivo* liver perfusion prevents platelet sequestration and platelet induced sinusoidal cell injury in the liver after liver transplantation

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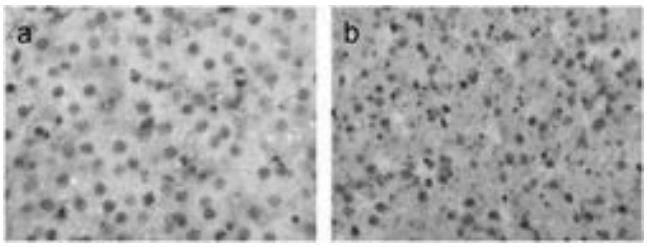
Background. Platelets are mediators of preservation injury by inducing hepatic sinusoidal endothelial cell injury after ischemia and reperfusion. We investigated the impact of normothermic *ex vivo* liver perfusion (NEVLP) vs cold storage on platelet sequestration following pig liver transplantation (LT). **Methods.** Pig LT with heart-beating donor (HBD) grafts were performed in 30kg pigs. Livers were either preserved by static cold storage (SCS) for 8hrs (HBD+SCS-group) or subjected to 3hrs SCS and 5hrs NEVLP (HBD+NEVLP-group; n=5 each). Liver injury parameters and platelet counts were evaluated during a 3-day survival-period. Liver biopsies obtained 3hrs after LT were stained for the platelet marker CD61 and for CD31 as marker of endothelial cell injury. Platelet-factor-4 (PF-4) was measured in platelet-free plasma before, 3hrs after LT and at post-operative-day (POD) 1. **Results.** Both groups had comparable results for postoperative prothrombin time, INR and haemoglobin levels. The HBD+NEVLP-group showed significantly lower AST levels on POD1 and POD2 compared to the HBD+SCS-group (581 vs. 1675 U/L, p=0.003 and 190 vs. 1198 U/L, p=0.005, respectively). Following LT, platelet counts recovered significantly faster in the HBD+NEVLP-group (HBD+NEVLP vs. HBD+SCS (% from baseline): 12hrs after LT: 67% vs. 31%; p=0.019, 24hrs after LT: 72% vs. 21%; p=0.007). CD61 staining of liver biopsies 3hrs after LT revealed significantly more aggregation and trapping of platelets in liver sinusoids of the HBD+SCS-group, compared to HBD+NEVLP-pigs (Mean number of clumps/5 HPF: HBD+NEVLP-group: 4 ±1.6 vs. HBD+SCS-group: 70 ±46, p=0.039 Figure 1a HBD+SCS 1b: HBD+NEVLP). Furthermore, endothelial cell injury was significantly worse in the HBD+SCS-group compared to the HBD+NEVLP-group (rated between 1=mild to 4=severe; mean: 3.1 ±1 vs. 1.4 ±0.2; p=0.0018). There was a trend towards higher postoperative PF-4 levels in HBD+SCS-pigs compared to HBD+NEVLP-pigs (3hrs: 102% vs. 108%, 24hrs: 106% vs. 150% of baseline, respectively). **Conclusion.** NEVLP prevents platelets sequestration in the liver following reperfusion allowing a faster recovery of the platelet count. Furthermore, NEVLP may protect liver sinusoidal endothelial cell injury induced by platelets.



Disclosures:

The following people have nothing to disclose: Dagmar Kollmann, Ivan Linares Cervantes, Roizar Rosales, Claudia Bruguera, Matyas Hamar, Juan Echeverri, Oyedele Adeyi, Markus Selzner, Nazia Selzner

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Safe long-term repopulation of uninjured livers by regulated activation of Yap in primary adult hepatocytes transplanted into DPPIV$^-$ rats and the Gunn rat model of Crigler-Najjar Syndrome type 1

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Background: Hepatocyte transplantation is an attractive alternative to liver transplantation, but to date extensive liver repopulation by adult primary hepatocytes has required genetic, physical or chemical injury to the host liver. Here we aimed to repopulate uninjured livers of DPPIV$^-$ rats and UGT1A1-deficient Gunn rats (model of Crigler-Najjar Syndrome type 1) by regulated activation of the Yap gene in transplanted adult primary hepatocytes. **Methods:** Wildtype primary hepatocytes were lentivirally transduced with the YapERT2 fusion protein before transplantation into the liver of congeneic DPPIV$^-$ or Gunn rats. Nuclear translocation/function of YapERT2 was induced by dietary tamoxifen administration. Repopulation was assessed by histochemical, immunohistochemical (IHC) and Western blot (WB) analysis, and serum bilirubin measurement in Gunn rats. To identify genes and signaling pathways involved in repopulation, hepatocytes repopulating DPPIV$^-$ livers were FACS-sorted and their transcriptomic profile was compared with that of the host hepatocytes by RNASeq analysis. **Results:** Liver repopulation progressed over one year in tamoxifen-fed

DPPIV- rats transplanted with DPPIV+ YapERT2-transduced hepatocytes. The repopulating hepatocytes exhibited normal morphology, incorporation into the hepatic plates, expression of hepatocyte-specific genes at levels comparable with host hepatocytes, and showed no dysplasia, de-differentiation or tumorigenicity one year after cell transplantation. Serum bilirubin declined in the Gunn rat by 50-80% by 6 months and remained at that level for one year. Without YapERT2 transduction and/or tamoxifen feeding, serum bilirubin was reduced by only 10-20%. YapERT2/tamoxifen-dependent proliferation of donor cells in Gunn rat livers was demonstrated by WB, as well as UGT1A1-positive clusters of hepatocytes by IHC. RNASeq analysis showed a Yap signature in the repopulating hepatocytes. Induced expression of cell proliferation and anti-apoptotic genes recapitulated the cell competition mechanism for liver repopulation that we previously observed with transplanted fetal liver stem/progenitor cells. **Conclusions:** Tamoxifen-regulated nuclear translocation of YapERT2 enabled long-term repopulation of uninjured rat livers without tumorigenicity. Repopulation and serum bilirubin reduction could be initiated by starting tamoxifen administration 6 months after cell transplantation, demonstrating the ability to control repopulation by cycles of tamoxifen administration. This cell transplantation strategy may offer a potential therapeutic option for the majority of inherited monogenic liver diseases that do not cause liver injury.

Disclosures:

The following people have nothing to disclose: Esther A. Peterson, Zsuzsanna Polgar, Gnanapackiam S. Devakanmalai, Yanfeng Li, Fadi L. Jaber, Wei Zhang, Xia Wang, Niloy J. Iqbal, John W. Murray, Namita Roy-Chowdhury, Leslie E. Rogler, Liang Zhu, Jayanta Roy-Chowdhury, David A. Shafritz

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Biliary reconstruction following transplantation of a bio-engineered bile duct incorporating primary human cholangiocyte organoids

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Background: The treatment of common bile duct disorders such as biliary atresia or ischemic strictures is restricted by the lack of biliary tissue suitable for surgical reconstruction. Here, we report a novel method for the isolation and propagation of human extrahepatic biliary epithelium in the form of Extrahepatic Cholangiocyte Organoids (ECOs) and demonstrate the application of this platform for the generation of bioengineered biliary tissue. **Methods:** Primary adult human cholangiocytes were isolated by mechanical dissociation from deceased organ donors with appropriate ethical approval and informed consent (n=8). Propagation of the cells was achieved using our established culture conditions. Microarrays were performed using the Illumina HumanHT-12 v4 Expression BeadChip Array. Biliary tissue was generated by seeding ECOs on biodegradable scaffolds. Biliary reconstruction in immunodeficient (NSG) mice was achieved by replacing part of the gallbladder wall with an ECO populated PGA-scaffold patch (ECO-patch; n=8), or replacing a length of the native common bile duct (CBD) with ECO populated collagen tubes (ECO-tubes) through end-to-end anastomosis (n=11). Fibroblast-populated (n=5 PGA scaffolds; n=4 collagen tubes) or acellular scaffolds (n=2, PGA) were used as negative controls. Mouse serum samples were routinely processed for markers of cholestasis by the University of Cambridge Core biochemical assay laboratory (CBAL). **Results:** ECOs closely correlate with primary cholangiocytes in terms of transcriptomic profile (r:0.92), and functional properties (ALP, GGT, bile acid transfer) and expand exponentially in culture maintaining their genetic stability. Following kidney capsule transplantation in NSG mice, ECOs self organize into tubular structures expressing biliary markers (CK7). When seeded on scaffolds, ECOs form tissue-like structures, maintaining their function (ALP, GGT) and biliary markers (CK7, CK19, HNF1B). This bioengineered tissue was used to reconstruct the gallbladder wall and replace the native CBD, in NSG mice. Animals transplanted with ECO-scaffolds exhibited prolonged survival vs. controls (ECO-patch, P=0.0027; ECO-tubes, P=0.0082; log-rank test), with ECO integration in the biliary epithelium, normal levels of serum cholestasis markers (up to one month post transplantation) and a patent lumen confirmed on MRCP and cholangiogram. All fibroblast reconstructions failed and the biliary epithelium was replaced by fibrotic tissue occluding the lumen. **Conclusion:** ECOs can organize in bioengineered tissue and reconstruct the biliary tree providing the first proof-of-principle for organ regeneration using human primary cells expanded in vitro.

Disclosures:

The following people have nothing to disclose: Fotios Sampaziotis, Olivia Tysoe, Stephen Sawiak, Edmund M. Godfrey, Sara Upponi, Miguel Cardoso de Brito, Natalie L. Berntsen, Graeme J. Alexander, Tom H. Karlsen, Espen Melum, Kourosh Saeb-Parsy, Ludovic Vallier

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Evaluation of Clinical Perfusates for Normothermic Ex-Vivo Liver Perfusion in a Porcine Donor after Circulatory Death Model

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Introduction European and North American clinical trials have used two different perfusate solutions for normothermic ex vivo liver perfusion (NEVLP): Gelofusine™ (bovine derived gelatin-based solution) and STEEN (Human albumin-based solution). We aimed to compare both perfusate solutions and their effects on the outcome of pig liver transplantation. **Material and Methods:** Porcine livers were retrieved 30 minutes after circulatory death. Grafts were perfused for 5 hours and transplanted into recipient pigs. Two groups were compared based on the perfusate solution (Gelofusine vs STEEN, n=6, respectively). Hemodynamic variables, liver injury and function were evaluated during perfusion and after transplantation. Plasma levels

of AST, alkaline phosphatase (ALP), total bilirubin and INR were measured during perfusion and until the 3rd post-operative day (POD). **Results:** Hepatic artery flow during perfusion was higher in the STEEN vs Gelofusine group and within physiologic range since the beginning of perfusion (238±90 vs 97±33; p=0.015). Electrolytes and pH from STEEN but not the Gelofusine group were within physiologic limits from the start of the perfusion time. Both perfusate groups cleared lactate reaching normal levels by the end of perfusion (Gelofusine: 0.78±0.30 vs STEEN: 0.68±0.60; p=0.80**)**. At POD1, levels of AST (Gelofusine: 1495±612 IU/L vs STEEN: 1160±564 IU/L; p=0.23), ALP (Gelofusine: 177±128 IU/L vs STEEN: 145±47 IU/L; p=0.49) and total bilirubin(Gelofusine: 11±4 vs STEEN: 5±1umol/L; p=0.05) were lower in the STEEN vs Gelofusine. Post transplantation, liver function recovery based on INR values was significantly faster in the STEEN vs Gelofusine group (**Figure 1**). All animals in the STEEN group survived while one pig from the Gelofusine group died on POD1 due to primary nonfunction. **Conclusion** The characteristics from the STEEN perfusate resembled more to the serum physiologic conditions compared to Gelofusine. The use of STEEN perfusate decreases hepatic injury and improves liver function recovery in pig livers retrieved after circulatory death.

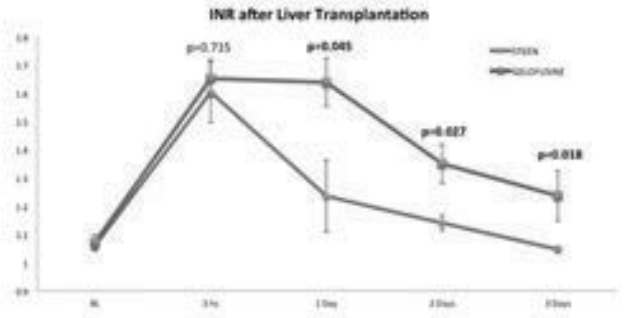

Figure 1

Disclosures:

The following people have nothing to disclose: Ivan Linares Cervantes, Dagmar Kollmann, Juan Echeverri, Moritz Kaths, Roizar Rosales, Claudia Bruguera, Matyas Hamar, Peter Urbanellis, Paul Yip, Oyedele Adeyi, Markus Selzner, Nazia Selzner

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Live image and mechanistic analysis of Mesenchymal stem cells (MSCs) and induced bone marrow derived macrophages (id-BMMs) combination therapy for liver cirrhosis in mice

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Background & Aims: Decompensated liver cirrhosis often progresses even after treatment. Thus, novel therapeutic approaches are urgently needed. Herein, we describe a novel therapeutic approach for decompensated cirrhosis using mesenchymal stem cells (MSCs) and induced bone marrow-derived macrophages (id-BMMs) and analyzed the mechanisms underlying liver fibrosis improvement and regeneration. **Methods:** Mouse MSCs and id-BMMs were cultured from mouse bone marrow. The mRNA expression and changes after addition of serum from liver-damaged mice were analyzed by microarray and real-time PCR, respectively. Co-culture and id-BMMs phagocytosis assays were performed. MSCs, id-BMMs, and a combination therapy using MSCs and id-BMMs (50/50) were administered to CCl4-induced liver cirrhosis mice, and fibrosis regression and liver regeneration were evaluated. The behavior of administered cells was evaluated by live imaging. Host migrated cells in the liver were quantified by immunohistochemistry and flow cytometry. **Results:** Highly enriched MSCs and id-BMMs were cultured. These cells expressed variety of their own factors for liver fibrosis regression and pro-regeneration. The characteristics of these cells changed significantly after adding serum. In co-culture, MSCs induced the switch of id-BMMs toward the M2 phenotype. M2 polarized id-BMMs gained high phagocytic ability. The combination therapy dramatically increased liver fibrosis regression (27.3% regression compared to control, $p < 0.01$) and PCNA positive cells, and decreased serum levels of hepatobiliary enzymes. Live imaging detected that a large number of id-BMMs, which phagocytized hepatocyte debris, and few MSCs migrated to the fibrotic area in the liver. A large number of MSCs in the lung expressed anti-inflammatory factors which may affected the behavior and characteristics of macrophages. Host macrophages and neutrophils gathered after combination therapy and produced anti-fibrotic MMP, followed by producing the pro-regenerative factors from phagocytizing id-BMMs. **Conclusions:** MSCs and id-BMMs synergistically improved liver cirrhosis in mice. This concept paves the way for new treatments for decompensated liver cirrhosis.

Disclosures:

The following people have nothing to disclose: Yusuke Watanabe, Atsunori Tsuchiya, Takayuki Watanabe, Kenya Kamimura, Satoshi Yamagiwa, Shuji Terai

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Impact of Xeno-Compatibility on Outcomes of Bioengineered Humanized Liver Grafts

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Background: In an effort to develop human transplantable organs, Human Umbilical Vein Endothelial Cells (HUVECs) have been proposed as the vital vascular network in bioengineered organs. However, little is known about their immune-compatibility under *in-vivo* conditions. Therefore, we aimed to study the xeno-compatibility of bioengineered grafts composed of HUVECs in a porcine model of auxiliary liver transplantation. **Methods:** Cryopreserved HUVEC cells were obtained from a commercial vendor (Lonza ®). Blood group type of HUVEC cells was determined by DNA qPCR. Samples of naïve pig sera were obtained from ten donor domestic pigs. Three other pigs were transplanted with a bioengineered liver graft composed of HUVEC cells and followed post-operatively for at least 7 days. Blood group compatibility and xeno-compatibility between pig sera and HUVECs grafts were determined by a complement-dependent cytotoxicity assay and fluoroquench vital staining. Cytotoxicity was further characterized by immunoglobulin class; immunoglobulin levels were quantified by ELISA. Independent sample t-test was used for

comparison. **Results:** All 13 naïve pig sera were cytotoxic to HUVECs with mean cell death of 83.2±14.4%. Deactivation of IgM by heat and Dithiothreitol significantly reduced cell death to 22.2±11.8% and 6.15± 4.4%, respectively ($p<0.01$), suggesting high levels of anti-HUVEC IgM in naïve pig serum. The cytotoxicity profile of pig sera to HUVEC cells rose further after transplantation (95.7 ± 3.4%) at day 7, and remained high even after IgM deactivation (85 ± 8.9%, $p< 0.01$) suggesting the appearance of new anti-HUVEC IgG. The relative contribution of IgG and IgM to cytotoxicity in day 7 samples increased by ~35% and ~460% ($p=0.047$), respectively, compared to baseline and corresponded with the loss of perfusion to the implanted liver grafts. Blood group incompatibility between pigs and HUVECs had no measurable contribution to the level of cytotoxicity. **Conclusion:** Pigs harbor naturally occurring anti-human antibodies targeted against HUVECs. In addition, an adaptive immune response ensues within 7 days after exposure to HUVEC-derived grafts. Measures to enhance xeno-compatibility of HUVEC-derived grafts must be considered for their successful preclinical testing in pigs.

Disclosures:

Jeff Ross - Employment: Miromatrix Medical Inc.

Anne Young - Employment: Miromatrix Medical Inc.

Scott L. Nyberg - Board Membership: Liver Cell Therapies, Liver Cell Technologies

The following people have nothing to disclose: Mohammed F. Shaheen, Yi Li, Raymond D. Hickey, Harvey S. Chen, DongJin Joo, Bruce Amiot, Manish Gandhi

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The combination of entecavir and peginterferon alfa-2a in HBeAg-positive immune-tolerant (IT) children and adolescents with chronic hepatitis B virus (HBV) Infection: Results of the HBRN pediatric IT trial.

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Background/Aim: The immune tolerant (IT) phase of chronic HBV infection is defined by high levels of HBV DNA in serum but normal ALT values and no or only mild liver inflammation and damage. Many children with chronic HBV remain in the IT phase during much of childhood. Treatment with interferon in such children is rarely effective; loss of HBV DNA or HBeAg occurring in <10% of those treated. More encouraging results have been reported with combination antiviral therapy. The aims of this open-label, single-arm study, were to evaluate the safety and efficacy of a lead-in phase of 8 weeks of entecavir monotherapy followed by 40 weeks of its combination with peginterferon in children in the IT phase of chronic HBV infection. Methods: Entecavir was given once daily in a dose of 0.015 mg/kg (0.5 mg maximum) for 48 weeks while peginterferon alfa-2a (180 μg/1.73m2 subcutaneously) once weekly was added after 8 weeks. Endpoints included sustained loss of HBsAg and HBeAg 48 weeks after stopping therapy with a primary endpoint being the lack of detectable HBeAg with HBV DNA levels below 1,000 IU/mL. Adverse events (AE) were monitored prospectively during and after therapy. Results: 60 children/adolescents (75% female), mean age 10.9 (min-max 3.4-17.9) years, were enrolled. Most (90%) were Asian and most had HBV genotypes B (53%) or C (37%). All were positive for HBsAg and HBeAg and had high levels of HBV DNA (median 170 million IU/mL) with normal or minimally elevated ALT levels (median 39 U/L in boys and 26 U/L in girls). Fifty-five participants completed the 48 weeks of entecavir and the 40 weeks of peginterferon therapy. At 48 weeks after treatment, two participants (3%) were HBsAg-negative, lost HBeAg, and achieved the primary endpoint of lack of HBeAg and HBV DNA levels less than 1000 IU/mL. Overall, serum ALT levels were similar to baseline and median HBV DNA levels were 174 million IU/mL. The most common of the 75 AEs reported were hematologic (n=16, 21%), infection (n=12, 16%), and hepatic (elevated bilirubin or ALT) (n=8, 11%). No serious AEs were reported. Z-scores for weight, height, & BMI tended to be lower at the end of treatment than at baseline, as did height 48 weeks after treatment. Conclusions: The combination of entecavir and peginterferon given for up to 48 weeks rarely led to loss of HBeAg with sustained suppression of HBV DNA levels and was associated with frequent but not serious AEs. More potent and more broadly targeted regimens against HBV are needed to treat children in the IT phase of chronic HBV infection.

Disclosures:

Philip Rosenthal - Advisory Committees or Review Panels: Retrophin, Gilead, Alexion, Audentes, Intercept, Albireo; Consulting: Roche, Abbvie; Grant/Research Support: Roche, Bristol MyersSquibb, Gilead, Vertex, Abbvie, Roche/Genentech, Infacare, BMS; Speaking and Teaching: Retrophin

Simon C. Ling - Grant/Research Support: Bristol Myers Squibb

Karen F. Murray - Grant/Research Support: Gilead; Stock Shareholder: Merck

Sarah J. Schwarzenberg - Advisory Committees or Review Panels: ABBVIE; Grant/Research Support: Bristo-Myers Squibb Pharm.

Jeffrey Teckman - Consulting: Dicerna, Ionis Pharmaceuticals, Genkyotex, The Alpha-1 Project, RxCelerate, Editas, Intelia, AstraZenica, Vertex, Proteostasis; Grant/Research Support: Alnylam, Arrowhead, Alpha-1 Foundation, Gilead

Kathleen B. Schwarz - Advisory Committees or Review Panels: Alexion; Grant/Research Support: Bristol-Myers Squibb, Gilead, Roche/Genentech, Roche

The following people have nothing to disclose: Steven H. Belle

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Baseline liver echotexture in children with cystic fibrosis predicts changes over time in non-invasive biomarkers of fibrosis and portal hypertension

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Background: Cirrhotic CF liver disease (CFLD) is the third leading cause of death in CF and affects only 5-7% of

patients. Identification of children with progressive CFLD at an early stage would enable targeted study of preventative therapies. Methods: We studied all 251 eligible children enrolled in an ongoing multicenter study of abdominal ultrasonography (US) to predict development of cirrhosis (PUSH study, NCT01144507). Children age 3-12 years with pancreatic insufficient CF and heterogeneous liver pattern on baseline US (HTG, n=63) were matched 1:2 with children with CF and normal US (NL, n=125). We also included children with nodular (NOD, n=24) and bright homogeneous (HMG, n=39) liver patterns at baseline. We standardized US spleen size by age to yield a spleen size age-adjusted z-score (SSAZ). Blood work was taken annually and US biennially for up to 6 years. We used longitudinal mixed effects models to assess relationships between baseline US pattern and rate of change in biomarkers of liver disease severity, including GGT, ALP, AST, ALT, albumin, platelet count, AST to platelet ratio index (APRI) and fibrosis index based on 4 factors (FIB4). Results: Median follow-up was 3.8 y (range 0 to 6.1 y), including a mean of 4 (range 1 – 7) annual blood draws per child. Baseline US pattern was associated with different baseline values of biomarkers including GGT, AST, ALT, ALP, platelet count, SSAZ, APRI and FIB4 (Ling SC et al, AASLD 2016). Compared to NL, baseline HTG predicted more rapid fall in platelet count and rise in FIB4 and SSAZ, and NOD grade predicted more rapid rise in APRI, FIB4 and SSAZ (Table). Change in AST, ALT, GGT, ALP, albumin did not differ between US groups. Rates of change in biomarkers in HTG were intermediate between NL and NOD. HMG did not differ from NL in rate of biomarker change. Conclusion: Baseline US grade predicts rate of change of certain biomarkers of severity of liver disease. Rates of biomarker changes in HTG are intermediate between those of NL and NOD. Our findings are supportive that US patterns correlate with the severity of liver disease. Our future studies will explore associations between baseline US grades, biomarker changes and final US grade at 6-year follow-up.

Estimated slope of change in biomarkers per year, with 95% confidence intervals

Biomarkers	Baseline US grade			
	NL (n=63)	HTG (n=125)	NOD (n=24)	HMG (n=39)
Platelet count (x10^9/L per year	-5.0(-8.4, -1.1)	-12.1(-17.3, -7.0) *	-14.4(-23.4, -5.5)	-12.0(-19.2, -4.8)
APRI (units/year)	-0.01(-0.02, 0.01)	0.02(-0.00, 0.04)	0.04(0.00, 0.01) *	0.01(-0.02, 0.04)
FIB4 (units/year)	0.01(0.01, 0.02)	0.03(0.02, 0.04)*	0.06(0.04, 0.08) **	0.03(0.01, 0.05)
SSAZ (units/year)	0.3(0.2, 0.4)	0.5(0.4, 0.6) **	0.7(0.5, 0.8) **	0.4(0.2, 0.5)

Comparison with NL *p<0.05, **p<0.001

Disclosures:

Simon C. Ling - Grant/Research Support: Bristol Myers Squibb

Daniel H. Leung - Grant/Research Support: Bristol-Meyers Squibbs, CF Foundation, Gilead, Roche, Abbvie

Michael R. Narkewicz - Consulting: Vertex, AbbVie; Grant/Research Support: Gilead, AbbVie; Stock Shareholder: Johnson and Johnson, Express Scripts, United Healthcare

The following people have nothing to disclose: Wen Ye, Wikrom Karnsakul, Alvin J. Freeman, John C. Magee

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Development of an *in vitro* model of hereditary tyrosinemia type 1 using patient-derived induced pluripotent stem cells

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Background: Hereditary type 1 tyrosinemia (HT1) is a severe inborn error of liver metabolism caused by the deficiency of fumarylacetoacetate hydrolase (FAH). The long-term effects of the only effective treatment available (the herbicide NTBC) are unknown. No in vitro model of HT1 is currently available. **Aim:** to develop an *in vitro* model of HT1 using hepatocyte-like cells (iHeps) differentiated from patient-derived induced pluripotent stem cells (iPSCs). **Methods:** Peripheral blood mononuclear cells from HT1 patients were reprogrammed into iPSCs, cultured in strict feeder-free and xeno-free conditions, and extensively characterized. iHeps were generated from iPSCs using our *in vitro* differentiation protocol mimicking liver development, and fully characterized for the expression of hepatocytes' markers and functions, and for the accumulation of toxic compounds. iHeps derived from iPSCs reprogrammed from healthy subjects served as control. **Results:** Of the 10 iPSC populations reprogrammed from 2 patients carrying 2 different *FAH* mutations, 2 highly-pure populations were fully characterized (HT1-iPSCs). FAH mRNA expression was very low in both healthy and HT1-iPSCs, while the protein was not detectable. HT1-iPSCs' self-renewal potential was not affected by the absence of NTBC supplementation. Succinylacetone (SA), a toxic by-product of tyrosine metabolism, was undetectable in HT1-iPSCs' conditioned media even in the absence of NTBC. Hepatocytes differentiated from HT1-iPSCs (HT1-iHeps) expressed markers and performed functions typical of neonatal hepatocytes. mRNA-Seq profile of HT1-iHeps was not different from healthy iHeps. FAH and all the enzymes involved in the tyrosine degradation pathway were progressively expressed upon differentiation into iHeps (with only FAH being absent only in HT1-iHeps). SA accumulated in HT1-iHeps' conditioned media, significantly increasing upon supplementation with L-tyrosine or homogentisic acid (51±5 nM v. 259±28 nM without and with supplementation of 300 μM homogentisic acid for 24h; $p<.05$). Apoptosis (caspase-3/7 levels quantified by live cell imaging) and cell mortality progressively increased with higher doses and longer exposures. NTBC treatment (50-300 μM) prevented SA accumulation upon high-dose supplementation with L-tyrosine, with resulting negligible cell death and absent apoptosis. SA was undetectable in iHeps derived from healthy iPSCs. **Conclusions:** To our knowledge, this is the first representative human *in vitro* model of HT1. HT1-iHeps faithfully replicate HT1 phenotype, allowing to study the pathophysiology of the disease and the effect of NTBC on a human model under controlled conditions, for the first time.

Disclosures:

Massimiliano Paganelli - Grant/Research Support: Gilead Sciences

The following people have nothing to disclose: Quang Toan Pham, Marie-Agnès M'Callum, Paula Waters, Ugur Halac, Chenicka-Lyn Mangahas, Claudia Raggi

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Liver Transplantation in Pediatric Acute Liver Failure (PALF): Practices and Patient Characteristics

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Background: Liver transplant (LT) decisions in PALF are complex and ultimately based on the alignment of physician experience, clinical assessment, and organ availability. We interrogated the PALF study group data to characterize those participants listed and not listed for LT. **Methods**: The PALF registry contains demographic, clinical, laboratory, and outcome data on 1144 participants (age 0-17 years) enrolled between Dec 1999 and Dec 2014. Three 5 yr phases were interrogated to examine differences between those listed for and ultimately receiving LT. **Results**: In Phase 1 (P1; n=522), Phase 2 (P2; n=464), and Phase 3 (P3; n=158), a decrease in the cumulative incidence rate for listing (P1=43.6%, P2=34.9%, P3=30.1%; p<0.005) and receiving (P1=24.7%, P2=22.2%, P3=17.1%; p<0.05) LT occurred. For those listed, cumulative incidence of LT within 7d of enrollment (P1=47.5%, P2=51.6%, P3=48.4%) and death (P1=3.6%, P2=5%, P3=2.3%) was similar across phases. The median time to listing after enrollment was similar across phases (1 day; Q1-Q3=0-2). Clinical and biochemical differences between those listed and not listed for LT were noted (Table 1). Maximum coma grade was higher in listed patients (p<0.0001). The most frequent reasons for not listing in the first 7 days were "not sick enough" and "medically unsuitable"; irreversible brain damage was uncommon (P1=3/19, 7.7%; P2=2/75, 2.7%; P3=1/27, 3.7%). Patients deemed medically unsuitable differed from those that were listed by being younger (0.2 vs. 5.6 yr, p<0.0001) and more likely to have an identified diagnosis of viral infection, shock/ischemia, HLH, and other (p<0.001). Additionally, medically unsuitable patients were more likely to have received ventilator (62% vs. 22%, p < 0.0001) or pressor support (57% vs. 9%, p<0.0001). Among those listed, an indeterminate diagnosis was most frequent (55.1%). In P3, cadaveric liver offers were made and accepted in all age groups. **Conclusions**: During the PALF study, the frequency of listing for and receiving LT decreased over time without an increase in frequency of death. If listed, the frequency of LT within 7 d of enrollment was similar. Age, having an established or indeterminate diagnosis, and requirement for cardiopulmonary support appear to influence decisions for listing. Optimizing listing decisions in PALF may reduce the frequency of LT without increasing the frequency of death.

Table 1

Clinical Feature	Listed	Not Listed	p-value
Age, median (Q1-Q3)	5.6 (1.6-12.6)	3.8 (0.4-13.8)	<0.01
INR, median (Q1-Q3)	3 (2.3-4.6)	2.3 (1.9-3.3)	<0.0001
Total Bilirubin, median (Q1-Q3)	15 (7.5-20.2)	4.9 (2.0-12.4)	<0.0001
Venous NH3, median (Q1-Q3)	63 (41-102)	57 (36-87)	0.01

Disclosures:

The following people have nothing to disclose: James E. Squires, David A. Rudnick, Simon P. Horslen, Vicky L. Ng, Estella M. Alonso, Steven H. Belle, Robert H. Squires

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Increasing split liver transplantation in the U.S. could decrease pediatric deaths on the liver transplant waiting list

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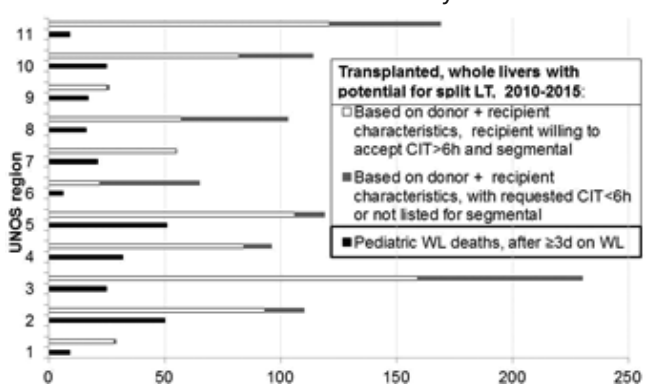
Background: In the United Kingdom, defaulting to split liver transplantation (LT) with suitable deceased donor grafts has virtually eliminated pediatric waitlist (WL) mortality. In the US, only <2% of LTs are split, but 1 in 10 infants die on the WL. **Methods:** Using UNOS STAR data, livers for potential split LT were identified from all transplanted, deceased-donor livers 2010-15 who fit strict criteria: age 18-40y, BMI<30, recovered in US after donor brain death, 0-1 vasopressors, Na<155meq/L, AST/ALT<100IU/L, bilirubin<3mg/dL, <7d hospitalized, cardiac arrest≤30min, HBV/HCV neg, not CDC high-risk, steatosis≤10% if biopsied, not multi-organ transplant, and no bloodstream infection. Livers allocated to patients high-risk for split LT were also removed: status 1A or MELD/PELD≥40 at WL removal, re-transplant, in the ICU, BMI>34, or >300mi from donor hospital. Pediatric WL deaths included deaths and removals for too sick to transplant, never relisted. **Results**: Of 35,461 livers transplanted 2010-15, 6.7% were potentially utilizable for split LT based on donor characteristics. Of these, 95% were transplanted whole (n=2,253). 50% went to recipients deemed possibly high-risk for split LT. This left 1,116 potential livers for split LT (FIGURE); 78% of their primary recipients were listed as willing to accept a segmental liver, and 97% to accept cold ischemia time≥6h (CIT, median 12h). Median donor risk index for this subset was 1.06 (max 1.67). During the same 5y, 261 children died after ≥3d on the WL (median 57d, IQR 15-161)—87% of all pediatric WL deaths. Of these, 56% were <2y of age, 26% 2-12y, 18% 13-18y. Median weight was 9.2kg (IQR 5.9-29.4kg). 36% died at centers that reported doing no pediatric split LTs (15%) or ≤1/year (22%). **Conclusions**: Increased utilization of split LT could decrease US pediatric WL mortality—without decreasing LT access for adults. Barriers are significant, but changes to allocation policy, increasing centers with splitting experience, and splitting on normothermic perfusion could increase access and reduce WL mortality.



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Disclosures:

John P. Roberts - Consulting: medSleuth; Stock Shareholder: Gilead, Alexion

The following people have nothing to disclose: Emily R. Perito, Garrett Roll, Jennifer L. Dodge, Sue Rhee

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Clinical consequences of cardiomyopathy in children with biliary atresia requiring liver transplantation

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Background: Myocardial dysfunction is a known complication of end stage cirrhotic liver disease (ESLD). We aimed to characterize the clinical course and perioperative complications associated with cardiomyopathy in children with biliary atresia (BA) listed for liver transplantation (LT). We hypothesized that cardiomyopathy in patients with BA is associated with adverse peri-transplant outcomes. **Methods:** A total of 75 patients with BA [median age 11 mo (9-21); 58% females] were listed for LT at our institution (2011-2017) and had a pre-operative 2-dimensional echocardiography (2DE). Three patients with congenital heart disease were excluded. In the 72 patients included, an abnormal 2DE was defined as a shortening fraction >2 Z-scores, left ventricular relative wall thickness (RWT) >0.41 cm, and/or left ventricular mass index (LVMI) >110 g/m^2. Primary [mortality and graft failure] and secondary outcomes [perioperative ICU and hospital lengths of stay (LOS), duration of mechanical ventilation (MV), and use of dialysis and vasopressors] were compared between those with abnormal and normal 2DE who survived to LT. Statistics: Continuous variable data analyzed with Wilcoxon rank sum [median (range 10-90%)]; Fisher's exact test for contingency [OR (95% CI)]; Pearson's analysis for correlation. **Results:** An abnormal 2DE was seen in 36/72 (50%) patients with BA. Five of 72 (7%) died before LT, all of whom had an abnormal 2DE. Of the 67 patients who survived to LT, those with an abnormal 2DE had higher median ICU [26 (5-102) vs. 5 (2-22), p<0.001] and hospital LOS [49 (12-139) vs. 21 (8-40), p<0.001] versus those with normal 2DE. Though there was no difference in mortality or graft failure between the two groups, an abnormal 2DE was associated with an increased odds of dialysis use [3.88 (1.24-10.78), p=0.018] and vasopressor support [3.41 (1.08-9.38), p=0.034], and more median days on MV [42 (14-64) vs. 8 (2-21), p<0.001], dialysis [33 (23-54) vs. 20 (2-40), p=0.015] and vasopressors [15 (7-35) vs. 6 (6-19), p=0.015]. LVMI correlated with ICU (r=0.40, p<0.001), hospital LOS (r=0.40, p<0.001), MV (r=0.43, p<0.001) and dialysis days (r=0.36, p<0.01). Serum bilirubin (r=0.36, p<0.01), INR (r=0.35, p<0.01) and platelet count (r= -0.45, p<0.001) correlated with LVMI. Cardiomyopathy resolved post-LT in 14 (67%) of the 21 patients who received a post-LT 2DE, with a median time to resolution of 40 days (22-321). **Conclusion:** Cardiomyopathy in BA with ESLD is highly prevalent and, despite its reversibility, is associated with significant morbidity. Therefore, to improve outcomes, there is an urgent need to recognize, prevent, systematically monitor and treat this disease.

Disclosures:

Ayse Arikan - Consulting: Baxter

Jorge Coss-Bu - Speaking and Teaching: Nestle USA

The following people have nothing to disclose: Noelle Gorgis, Kathleen Thompson, Curtis Kennedy, Fong Lam, Tamir Miloh, John A. Goss, Moreshwar S. Desai

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Decrease in Alpha-fetoprotein from >1000 to <500 ng/mL in Waitlisted Patients with Hepatocellular Carcinoma Resulted in Improved Post-Transplant Survival and Reduced Risk of Tumor Recurrence: Validation of the Current National Policy

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Background: High alpha-fetoprotein (AFP) >1000 ng/mL is associated with poor outcome after liver transplant (LT) for hepatocellular carcinoma (HCC). A new national policy has been implemented for HCC with AFP > 1000, requiring a decrease in the AFP to <500 before LT, but there is a paucity of data on the optimal AFP threshold before LT. **Study Aim:** To evaluate the effects of a reduction in AFP from >1000 to different AFP thresholds before LT on survival and HCC recurrence after LT. **Patients and Methods:** We identified in the UNOS registry 390 patients transplanted between 1/2005 and 9/2015 who had AFP >1000 at least once prior to LT and had tumor stage initially within Milan criteria (93.6%) or beyond Milan criteria but within the University of California, San Francisco downstaging inclusion criteria (6.4%). The last AFP within 90 days before LT was >1000 in 293 (75.1%), decreased from >1000 to 101-499 in 39 (10%), and to ≤100 in 58 patients (14.9%). There were very few patients (n=17) with AFP of 500-1000 before LT and they were excluded from the analysis. **Results:** The median MELD score was 11 (IQR 8-14) and 16.5% had Child's C cirrhosis. Local regional therapy was not performed in 45.4% of patients with AFP > 1000 at LT versus 12.8% of those with AFP of 101-499 and 10.3% of those with AFP decreased to ≤100 at LT (p< 0.01). The median time for the decrease in AFP from >1000 to 101-499 and to ≤100 was 88 days (IQR 81-181) and 181 days (IQR 91-344), respectively. The Kaplan-Meier 5-year post-LT survival for those with AFP >1000 at LT was 48.8%, versus 67.0% for those with decrease in AFP to 101-499 (p<0.0001) and 88.4% for those with decrease in AFP to ≤100 before LT (p<0.0001). The difference in survival between the groups with AFP of 101-499 and AFP ≤100 at LT was not statistically significant (p=0.09). The probability of HCC recurrence at 5 years was 35% for patients with AFP >1000 versus 13.3% for those with decrease in the AFP to 101-499 (p=0.0006) and 7.2% for those with a decrease in AFP to ≤100 ng/mL (p<0.0001). The difference in HCC recurrence rates between the latter two groups was not statistically significant (p=0.38). In multivariable analysis, a decrease in the AFP to 101-499 was associated with a >2 fold reduction in post-transplant mortality (HR 0.46; 95% CI 0.26-0.83, p=0.01) and a nearly 3 fold reduction in HCC recurrence (HR 0.35; 95% CI 0.14-0.86, p=0.04) compared to AFP >1000 at LT. **Conclusion:** Our results demonstrated significantly improved post-LT survival and decreased HCC recurrence when restricting LT to patients with a reduction in AFP from >1000 to

<500 ng/mL before LT, thus validating the recently implemented national policy.

Disclosures:

John P. Roberts - Consulting: medSleuth; Stock Shareholder: Gilead, Alexion

The following people have nothing to disclose: Francis Yao, Jennifer L. Dodge, Ryutaro Hirose, Neil Mehta

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A risk prediction score based on magnetic resonance cholangiopancreatography (MRCP) accurately predicts disease progression in patients with primary sclerosing cholangitis (PSC)

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Background: Although MRCP is the primary method of PSC diagnosis, data describing its prognostic utility are limited. Our objective was to prospectively evaluate the association between biliary disease severity on MRCP and disease progression in PSC patients in a clinical trial. **Methods:** MRCP was performed at baseline (BL) in 234 PSC patients enrolled in a phase 2b, placebo-controlled trial of simtuzumab (SIM). Consensus reading of MRCPs by two radiologists was performed to characterize 18 hepatobiliary features (modified from Ruiz, *et al*. Hepatology 2014) including the presence and severity of stricturing and dilatation, intraductal stones, hepatic dysmorphy (e.g. lobulated liver surface, atrophic lobe, caudate lobe hypertrophy), portal hypertension, and peri-hepatic lymph nodes. The associations between these features and PSC-related clinical events (e.g. decompensation, ascending cholangitis, cholangiocarcinoma, transplantation) were determined using Cox regression and an MRCP risk score (MRCP-RS) was derived based on factors with independent prognostic value. The discrimination of the risk score for predicting clinical events was determined using the c-statistic. **Results:** The median age was 45 years, 64% were male, 48% had ulcerative colitis, 62% were on ursodeoxycholic acid, and the median alkaline phosphatase (ALP) was 260 U/L (IQR 129-401). At BL, 40% of subjects had bridging fibrosis and 11% had cirrhosis. Over a median follow-up of 23.0 months (range, 23.0-23.1), 47 patients (20%) developed a PSC-related clinical event (ascending cholangitis [n=27], jaundice [n=10], cholangiocarcinoma [n=3], ascites [n=2], encephalopathy [n=2], variceal hemorrhage [n=2], sepsis [n=1]). In multivariate analysis, PSC-related events were associated with BL hepatic dysmorphy (HR 3.11; 95% CI 1.22, 7.92), signs of portal hypertension (HR 2.31; 1.28-4.17), and peri-hepatic lymph nodes (HR 2.14; 1.20-3.81). Based on the model coefficients, an MRCP-RS assigning 1-point for each of the 3 variables was derived (range, 0-3), which accurately predicted clinical events (c-statistic 0.71; 95% CI 0.63-0.79). During follow-up, the risk of clinical events increased according to BL MRCP-RS: 0 (6% [3/48]), 1 (14% [14/101]), 2 (30% [21/69]), and 3 (56% [9/16] (log-rank p<0.001). The association between the MRCP-RS and clinical events remained significant (HR 2.09; 95% CI 1.44-3.04) after adjustment for baseline serum ALP (p=0.006) and ELF score (p=0.28). **Conclusions:** A simple MRCP risk prediction model based on signs of hepatic dysmorphy, portal hypertension, and peri-hepatic lymph nodes accurately predicts PSC-related disease progression in the clinical trial setting over 96 weeks.

Disclosures:

Andrew J. Muir - Advisory Committees or Review Panels: Merck, Abbvie, BMS, Gilead, Janssen; Consulting: Shire, Inovia Pharmaceuticals, Portola Pharmaceuticals; Grant/Research Support: Janssen, Merck, Hologic, Intercept, NGM Biopharm, Roche, Abbvie, BMS, Gilead

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Zachary D. Goodman - Grant/Research Support: Gilead Sciences, Galectin Therapeutics, Intercept, Alexion, Conatus, Cempra, Allergan, Exalenz

Michael P. Manns - Consulting: Falk, Novartis, Roche, Gilead, GlaxoSmithKline, AbbVie, Boehringer Ingelheim, Intercept, Bristol Myers Squibb, Janssen, Medgenics, Eiger, Enyo, Merck; Grant/Research Support: Merck, Falk, Novartis, Roche, Gilead, GlaxoSmithKline, Intercept, Boehringer Ingelheim, Janssen, Eiger, AbbVie; Speaking and Teaching: Falk, Gilead, Roche, AbbVie, Bristol Myers Squibb, Janssen, Merck

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Clare Tempany-Afdhal - Advisory Committees or Review Panels: Profound Medical; Consulting: echosens; Employment: Sprinkbank Pharmaceuticals; Grant/Research Support: Gilead

The following people have nothing to disclose: Elmira Hassanzadeh, Nisha Sainani, Bertus Eksteen, Roger W. Chapman

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Nivolumab in Sorafenib-Naive and -Experienced Patients With Advanced Hepatocellular Carcinoma (HCC): Survival, Hepatic Safety, and Biomarker Assessments in CheckMate 040

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Background: Many patients (pts) with advanced HCC progress on standard-of-care therapy. Nivolumab is a fully human anti–PD-1 IgG4 mAb that demonstrated durable responses in pts with advanced HCC in the CheckMate 040 study (NCT01658878; El-Khoueiry and Sangro et al. 2017). Here we present updated survival, hepatic safety, and biomarker analyses with extended follow-up in both sorafenib-naive and -experienced pts with advanced HCC in CheckMate 040. **Methods:** Pts naive to or previously treated with sorafenib received nivolumab in phase 1/2 dose-escalation (ESC; 0.1–10 mg/kg) and -expansion (EXP; 3 mg/kg) cohorts Q2W regardless of PD-L1 status. Primary endpoints were safety/tolerability (ESC) and objective response rate (ORR; EXP). ORR was reported by blinded independent central review (BICR) and investigator assessment using RECIST v1.1. Secondary endpoints included overall survival (OS), duration of response (DOR), and disease control rate. Exploratory analyses of on-treatment HCV and HBV viral kinetics and α-fetoprotein (AFP) levels were performed. **Results:** Pts (N=262) had median follow-up durations of 14–16 mo. Overall, 98% of pts (258/262) had Child-Pugh scores of 5–6, and 68% (178/262) had extrahepatic metastases. The 18-mo OS rate was 57% in sorafenib-naive pts (ESC+EXP; n=80) and 46% and 44% in sorafenib-experienced pts in the ESC (n=37) and EXP (n=145) phases, respectively (Table). Overall, ORRs (BICR) across the cohorts were 14%–20%, and median DORs were 16.59–19.35 mo. AFP levels at baseline were not associated with response (BICR); however, AFP levels in responders appeared to decrease on treatment. Frequencies of grade 3/4 treatment-related ALT/AST elevations were 5%–9% in sorafenib-naive pts and 3%–4% in sorafenib-experienced pts (ESC+EXP; n=182). No drug-related deaths due to hepatic AEs occurred; 2 pts had hepatic failure unrelated to treatment. No new safety signals were observed. **Conclusion:** Nivolumab demonstrated long-term survival, durable tumor responses, and manageable overall and hepatic safety profiles, regardless of prior sorafenib treatment in pts with advanced HCC with extended follow-up.

Table. Survival and selected hepatic AEs with nivolumab

Parameter	Sorafenib Naive	Sorafenib Experienced	
Overall survival	ESC + EXP (n=80)	ESC (n=37)	EXP (n=145)
Median OS (95% CI), mo	28.6 (16.6–NE)	15.0 (5.0–28.1)	15.6 (13.2–18.9)
12-mo OS rate (95% CI), %	73 (61.3–81.3)	58 (40.2–72.2)	60 (51.4–67.5)
18-mo OS rate (95% CI), %	57 (44.3–67.1)	46 (29.5–61.7)	44 (35.3–51.9)
Select grade 3/4 treatment-related hepatic AEs	ESC + EXP (n=80)	ESC + EXP (n=182)	
ALT increased, n (%)	4 (5)	5 (3)	
AST increased, n (%)	7 (9)	7 (4)	
Blood bilirubin increased, n (%)	1 (1)	0	

NE, not estimable.

Disclosures:

Bruno Sangro - Advisory Committees or Review Panels: Bayer Schering Pharma, BMS, Astra Zeneca, Adaptimmune, Onxeo

Ignacio Melero - Advisory Committees or Review Panels: Pfizer, Bristol Myers Squibb, Merk Serono; Consulting: Digna Biotech

Thomas Yau - Consulting: Bayer, Pfizer, Novartis, Bristol-Myers Squibb

Masatoshi Kudo - Consulting: Eisai, Bayer HealthCare; Grant/Research Support: Astellas Pharma, Abbvie, Daiichi Sankyo, Bristol Myers Squibb, Taiho, Chugai, Otsuka, Medico's Hirata; Speaking and Teaching: Bayer HealthCare, Eisai

Jorg Trojan - Advisory Committees or Review Panels: BMS, Bayer

Timothy Meyer - Advisory Committees or Review Panels: Bayer, BMS, Eisai; Grant/Research Support: Bayer, BTG

Yoon-Koo Kang - Advisory Committees or Review Panels: Bayer, Pfizer; Grant/Research Support: Bayer; Speaking and Teaching: Pfizer

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Todd S. Crocenzi - Grant/Research Support: Bristol-Myers Squibb

The following people have nothing to disclose: Chiun Hsu, Theodore H. Welling, Winnie Yeo, Akhil Chopra

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Eradication of HCV induced by direct-acting antivirals is associated with a 79% reduction in HCC risk.

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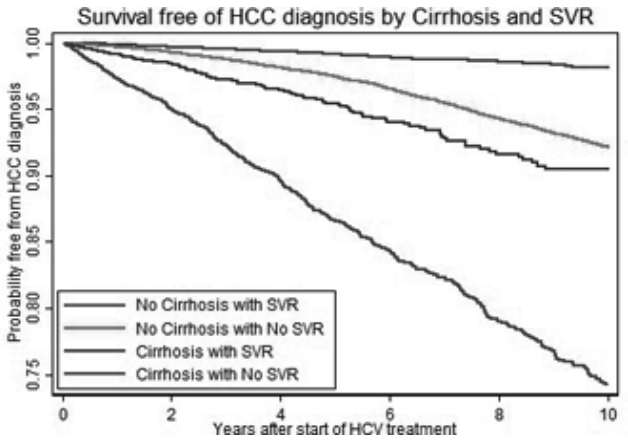
Background and Aims. It is unclear whether direct-acting antiviral (DAA) treatment-induced sustained virologic response (SVR) reduces the risk of hepatocellular carcinoma (HCC) in patients with hepatitis C virus (HCV) infection. We wanted to determine the extent to which eradication of HCV with DAA-based treatments was associated with reduction in the risk of HCC, and whether this association was different for SVRs achieved by DAA versus interferon-based regimens. **Methods.** We identified 62,051 patients who underwent 83,695 antiviral treatment regimens in the Veterans Affairs (VA) national healthcare system from 1999-2015, including 35,873 (57%) interferon-only regimens, 26,178 (43%) DAA±interferon regimens and 21,644 (35%) DAA-only regimens. We used Cox proportional hazards regression to determine the association between SVR and HCC risk after adjusting for a large number of potential confounders. **Results** The incidence of HCC was highest in patients with cirrhosis and treatment failure (2.7 per 100 patient-years), followed by cirrhosis and SVR (0.93), no cirrhosis and treatment failure (0.73) and no cirrhosis and SVR (0.18) (see Figure). Among all patients, SVR was associated with a 70% reduction in the risk of HCC (adjusted hazard ratio [AHR] 0.30, 95% CI 0.26-0.35). SVR was associated with a similar proportional decrease in HCC risk in patients with cirrhosis (AHR 0.34, 95% CI 0.26-0.43) and patients without cirrhosis (AHR 0.29, 95% CI 0.24-0.34). SVR was associated with a similar and significantly decreased risk of HCC in multivariable

models irrespective of whether the antiviral treatment was IFN-ONLY (AHR 0.32, 95% CI 0.27-0.37), DAA±IFN (AHR 0.21, 95% CI 0.13-0.32) or DAA-ONLY (AHR 0.21, 95% CI 0.09-0.53). **Conclusions** DAA-induced SVR is associated with an 79% reduction in HCC risk.



Disclosures:

George N. Ioannou - Grant/Research Support: Janssen Pharmaceuticals

The following people have nothing to disclose: Pamela Green, Kristin Berry

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Efficacy And Safety Of Carvedilol In Patients Of Acute-On-Chronic Liver Failure With Small Or No Esophageal Varices – A Placebo Control Open Label Randomised Trial (NCT02583698)

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Background and Aims: ACLF is a serious and often progressive clinical syndrome with high mortality. The portal and systemic hemodynamics is distinct in ACLF than cirrhosis as the dynamic component related to liver failure and cytokine release plays a major role. Rapid rise in portal pressure (PP) probably underlies the portal hypertension (PHT) related complications, like ascites, hepatorenal syndrome and variceal bleed. Role of PP reduction, safety and efficacy of beta-blockers has not been studied in ACLF. We investigated the efficacy of carvedilol in ACLF patients to prevent growth of varices and PHT related complications. **Methods**: ACLF patients with no/small esophageal varices and HVPG≥12 mmHg were randomized to carvedilol(Gr.A, n=66, 43.29±10.1yr, M:93.9%) or placebo(Gr.B, n=70, 44.7±10.5yr, M:91.4%) and followed till death or 90 days to assess reduction in PP, prevention of progression of varices and outcomes at day 28 and 90. **Results**:280 consecutive ACLF patients were screened; 136 fulfilling selection criteria were randomized to Gr.A or B. Baseline clinical parameters{alcoholic hepatitis 69% and 70%, mean MELD 24.62±4.12 and 25.06±3.3) [p=0.49]and hemodynamic parameters were comparable {mean HVPG 19.7±5.2 and 18.6±4.9 respectively[p=0.18]}. Median maximum tolerated daily dose of carvedilol administered was 12.5 mg(3.13–25). The incidence of bleed at 90 days was comparable [n=8(12.1%) vs. n=4(5.7%), p-0.18], with no difference in baseline HVPG in bleeders and non-bleeders [n=12(19.7±4.2 mm Hg) and n=124(19.1±5.1mm Hg), [p-0.69]. Repeat HVPG at 90 day was done in 63 of 100(63%) survivors, with 32(50.7%) showing ≥20% reductions;{Gr. A=18, GrB=14} with lower variceal growth in those with HVPG reduction by ≥20%[3/32, 9.4% vs. 14/31, 45.2%, p=0.01]. HVPG reduction with carvedilol [18/34(52.9%) in Gr.A- 34/63 (53.9%)] or spontaneous[14/29 (48.3%) in Gr.B- 29/63(46.1%)] was comparable. The PVRI increased with carvedilol[28/34(82.4%) vs. 16/29(55.2%), p=<0.01] from 112.1±38.5 to 134.4±44.2. Carvedilol compared with placebo, reduced the incidence of AKI [9(13.6%) vs. 25(35.7%), p=0.03] and sepsis[10 (15.2%) vs. 33(47.1%), p=0.01] at day 28 with reduced mortality [7(10.6%) vs. 17(24.3%), p=0.03] though not at day 90[16 (24.2%) vs. 20 (28.6%), p=0.69]**. Conclusion:** Use of carvedilol in modest dose, reduces incidence of sepsis and AKI with improved survival at 28 days. HVPG reduces by ≥ 20% in nearly 50% patients with ACLF, with reduction in growth of varices. Addition of carvedilol did not delay growth of varices or increased HVPG reduction, but it improved pulmonary hemodynamics and reduced complications due to rapid rise in PP with survival benefit at day 28.

Disclosures:

The following people have nothing to disclose: sumeet kainth, Manoj Kumar, Ashok k. Choudhury, Lovkesh Anand, Ankur Jindal, Guresh Kumar, Priyanka Jain, Shiv K. Sarin

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Results of ITCH, A Multi-center Randomized Double-blind Placebo-controlled Trial of Maralixibat, an Ileal Apical Sodium-dependent Bile Acid Transporter Inhibitor (ASBTi), for Pruritus in Alagille Syndrome (ALGS)

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Background: Medically refractory, severe, cholestasis-induced pruritus in ALGS may be improved by surgical interruption of the enterohepatic circulation (biliary diversion or ileal exclusion). This trial tested the hypothesis that pharmacologic interruption of the enterohepatic circulation of bile acids (BA), using the ASBTi maralixibat (previously LUM001; SHP625), would reduce pruritus in ALGS (NCT02057692). **Methods**: 37 children with ALGS (age 6.8 ± 4.5 yr) were randomly assigned to 1 of 4 treatment groups: once daily placebo, 70, 140 or 280 μg/kg of maralixibat for 13 weeks. Pruritus was assessed using a novel pediatric, observer version of the Itch Report Outcome (ItchRO™[Obs]), range 0 – 4 [severe], entry criteria ≥ 2) and by clinician report (clinician scratch scale, CSS, range 0 – 4 [severe]). Liver chemistries, serum BA (sBA) and 7-α-hydroxy-4-cholesten-3-one (C4, a bile acid biosynthesis marker) were measured serially. The primary outcome was the mean change from baseline to wk 13 in ItchRO™(Obs). A priori, the first statistical test of efficacy pooled subjects with the two highest tolerated doses (140 + 280 μg/kg/d indicated by*) compared to placebo. **Results**: Primary outcomes are in the Table. The % of subjects with change from baseline to wk 13 of ≤-1 was higher in maralixibat* vs placebo for ItchRO (65% vs. 25%, p=0.06) and CSS (76% vs. 25%, p=0.01). Of those with baseline CSS of ≥3, improvement of ≥3 occurred in 6/11 maralixibat* and 0/9 placebo subjects. Maralixibat* yielded statistically non-significant decreases in total and direct bilirubin (-0.7 [0.39] and -0.4 [0.21] mg/dL, p=0.09 and 0.06, respectively) and an increase in ALT (+33 [18.6] IU/L, p=0.08). sBA changed minimally, presumably due to a compensatory increase in C4, supporting the biological activity of maralixibat (%Δ sBA* -37% (48%) p = 0.44, %Δ C4* +401% (199%) p=0.15). Adverse events were similar between maralixibat and placebo. **Conclusions**: Although the pre-specified primary analyses of ItchRO were not all statistically significant, the data suggest that maralixibat was safe and may reduce pruritus in ALGS. Determination of optimal dosing and further assessments of safety and efficacy in children with cholestasis are warranted.

	Placebo N=12	maralixibat dose (μg/kg/day)				
		70 N=8	140 N=11	280 N=6	140+280* N=17	All active N=25
ItchRO[Obs], Wk 13 – baseline^	-0.58 (0.24)	-1.47 (0.30)	-1.49 (0.26)	-0.62 (0.36)	-1.05 (0.21)	-1.19 (0.18)
Difference from placebo^ p-value (ANCOVA)	-----	-0.89 (0.40) 0.032	-0.91 (0.35) 0.014	-0.04 (0.44) 0.930	-0.47 (0.33) 0.159	-0.61 (0.31) 0.055

^mean or mean difference (SE)

Disclosures:

Benjamin L. Shneider - Consulting: Bristol Myers Squibb; Stock Shareholder: Bristol Myers Squibb

Binita M. Kamath - Consulting: Retrophin; Speaking and Teaching: Shire

Alexander G. Miethke - Consulting: Shire plc; Grant/Research Support: Shire plc

Jean P. Molleston - Grant/Research Support: gillead, roche, vertex, none, abbvie, Shire/Lumena; Speaking and Teaching: none

Karen F. Murray - Grant/Research Support: Gilead; Stock Shareholder: Merck

Philip Rosenthal - Advisory Committees or Review Panels: Retrophin, Gilead, Alexion, Audentes, Intercept, Albireo; Consulting: Roche, Abbvie; Grant/Research Support: Roche, Bristol MyersSquibb, Gilead, Vertex, Abbvie, Roche/Genentech, Infacare, BMS; Speaking and Teaching: Retrophin

Saul J. Karpen - Consulting: Intercept Pharmaceuticals, Albireo, Retrophin, Inc., Regulus Therapeutics

Daniel H. Leung - Grant/Research Support: Bristol-Meyers Squibbs, CF Foundation, Gilead, Roche, Abbvie

Ronald J. Sokol - Consulting: Alnylam, Retrophin, Alexion; Grant/Research Support: Giliad, Shire/Lumena, FFF Enterprises

The following people have nothing to disclose: Cathie Spino, John C. Magee, Peter F. Whitington, Cara L. Mack, Robert H. Squires, Kathleen M. Loomes, Stephen L. Guthery, Danny Thomas, Averell H. Sherker

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Polyprenoic acid, the first-discovered hepatocyte nuclear factor 4 alpha-activating ligand, inhibits liver carcinogenesis.

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[**Background**] Hepatocyte nuclear factor 4 alpha (HNF4a) is one of the most ancient nuclear receptors known as a master regulator of hepatocytes in lipids, carbohydrates, and drug metabolism. It also works as a tumor suppressor to regulate cell cycle in hepatocellular carcinoma (HCC), but the ligand activating HNF4a has not yet been discovered thus far. We evaluated the effect of polyprenoic acid (PA), formerly known as an acyclic retinoid, on HNF4a activation in dysplastic nodule and HCC *in vitro* and *in vivo*. [**Methods**] Human HCC cell lines Huh7, HLE, and HLF as well as HCC cells established from surgically resected HCCs were cultured and used for the evaluation of the effect of

PA on HNF4a. PDGF-C transgenic (Tg) mice was evaluated by Gd-EOB-DTPA-enhanced MRI to examine the effect of PA on HCC development *in vivo*. Whole exome sequence analysis was performed using Illumina Hiseq 2000 system. Contrast enhanced CT scan data of patients enrolled in previous phase II/III randomized placebo-controlled study were collected and analyzed (n = 124 for PA and 127 for placebo). [**Results**] PA treatment regressed the liver dysplastic nodule and a subset of HCCs in PDGF-C Tg mice evaluated by MRI. This tumor regression was accompanied with the reduction of accumulated gene mutations, inactivation of cell cycle, and transcriptional activation of HNF4a-target genes. The binding of PA on ligand binding domain of HNF4a was shown by docking simulation *in silico* and immunoprecipitation-mass spectrometry analysis *in vitro*. Transcriptional activation of HNF4a by PA was also verified using a luciferase reporter assay and binding of HNF4a on DNA binding elements. PA treatment in HCC cells immediately resulted in the degradation of HNF4a by ubiquitin-proteasome system with activation of DNA damage responses when cultured in lipid depleted medium. *In vivo* knockdown of *HNF4A* cancelled the effect of PA on regression of liver tumor growth in PDGF-C Tg mice. PA treatment (600 mg/day) suppressed the HCC development from dysplastic nodule compared with placebo in human with statistical significance (HR 0.41; 95% CI, 0.17~0.98, P = 0.046). [**Conclusions**] Taken together, our data indicated that PA is the first-discovered HNF4a ligand to activate its function. PA activates the DNA damage responses and suppresses the accumulation of gene mutations especially in dysplastic nodule, warranting the future prospective study to evaluate the effect of PA on suppression of HCC development from dysplastic nodules in human.

Disclosures:

Hikari Okada - Employment: Kanazawa University

Shuichi Kaneko - Grant/Research Support: MDS, Co., Inc, Chugai Pharma., Co., Inc, Toray Co., Inc, Daiichi Sankyo., Co., Inc, Dainippon Sumitomo, Co., Inc, Ajinomoto Co., Inc, Bristol Myers Squibb., Inc, Pfizer., Co., Inc, Astellas., Inc, Otsuka Pharmaceutical, Co., Inc, Eizai Co., Inc, Bayer Japan, Eli lilly Japan, Takeda., Co., Inc

The following people have nothing to disclose: Taro Yamashita, Masao Honda

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Leukotriene B_4 Generated by Alveolar Macrophages Drive Hepatocellular Carcinoma Lung Metastasis

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Background: Hepatocellular carcinoma (HCC) is frequently complicated with lung metastasis, thereby leading to an extremely poor prognosis. Thus, it is necessary to establish a novel therapeutic strategy against HCC lung metastasis, based on the elucidation on its cellular and molecular mechanism. Arachidonic acid (AA)-derived mediators; leukotrienes (LTs), prostaglandins (PGs) are individually reported to be increased in cancer development and metastasis process. However, their cellular source and pathophysiological contribution in lung metastasis of HCC remain to be delineated. We herein extensively analyzed mouse HCC lung metastasis model to elucidate the key eicosanoids contributing the lung metastasis and its cellular source. **Methods:** A mouse HCC cell line, BNL, was injected into tail vein of BALB/c mice to induce the lung metastasis and the lungs were collected 21 days after injection. LC-MS/MS-based lipidomic analyses were performed for comprehensive analysis of eicosanoids in lungs. The mice were administered intraperitoneally with 5-lipoxygenase (LOX) or cyclooxygenase (COX) inhibitor after BNL cell injection. To selectively deplete alveolar macrophages (AMs) but not interstitial macrophages (IMs), the mice were intratracheally administered clodronate liposomes (CLLs). **Results:** Comprehensive determination of AA metabolite levels revealed increases in almost all LTs and PGs in the lungs with metastasis compared with untreated lungs ($P<0.05$). A LOX inhibitor but not a COX inhibitor significantly reduced the numbers of metastatic foci (0.5±0.5 v.s. 6.7±1.4; means±SD; $P<0.01$). A major 5-LOX metabolite, LTB_4, augmented *in vitro* cell proliferation of human HCC cell lines as well as BNL cells ($P<0.05$). Moreover, in this lung metastasis course, macrophages were the predominant cell present in metastatic foci, and AMs exhibited a higher expression level of the 5-LOX than IMs. Consistently, 5-LOX-expressing AMs significantly increased in the lungs of human HCC patients with lung metastasis, compared with those without lung metastasis (342.6±144.3 v.s. 62.8±42.3; numbers/field; $P=0.03$). Furthermore, intratracheal CLL injection selectively depleted AMs, together with reduced LTB_4 content (1168.0±529.1 v.s. 2695.7±1266.5; pg/lung; $P=0.03$) and metastatic foci numbers in this lung metastasis process (0.5±0.5 v.s. 3.0±1.6; $P<0.01$). **Conclusions:** We provided the first definitive evidence to indicate that LTB_4 produced by lung AMs directly augmented the proliferation and metastasis of HCC cells. Thus, AM-derived 5-LOX and its metabolites, LTB_4, may be a novel target to treat and/or prevent HCC metastasis to lungs.

Disclosures:

The following people have nothing to disclose: Takuto Nosaka, Tomohisa Baba, Tatsushi Naito, Masahiro Ohtani, Katsushi Hiramatsu, Tomoyuki Nemoto, Yasunari Nakamoto

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Obesity-driven hepatocellular carcinoma adapts to lipid-rich conditions by CPT2 down-regulation and promotes carcinogenesis through acylcarnitine accumulation

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Background: Metabolic reprogramming of tumor cells that allows for adaptation to their local environment is a hallmark of cancer. Interestingly, obesity- and nonalcoholic steatohepatitis (NASH)-driven hepatocellular carcinoma (HCC) mouse models commonly exhibit strong steatosis in tumor cells as seen in human steatohepatitic HCC, which may reflect a characteristic metabolic alteration. However, its pathogenesis has yet to be elucidated **Methods:** Non-tumor and HCC tissues obtained from diethylnitrosamine-injected 8-month-old mice fed either a normal or a high-fat diet (HFD) were subjected to comprehensive metabolome analyses. Moreover, the serum levels of the accumulated metabolites were measured in 250 patients with NAFLD with and without HCC. **Results:** Metabolome analyses showed the extensive accumulation of

acylcarnitine species in HCC tissues and in the serum of HFD-fed mice. The accumulation of acylcarnitine could be attributed to the down-regulation of carnitine palmitoyltransferase 2 (CPT2), a common feature of other mouse models of obesity- and NASH-driven HCC. CPT2 down-regulation induced the suppression of fatty acid β-oxidation in HFD-HCC tissues, which would account for the steatotic changes seen in HCC. CPT2 knockdown in mouse HCC cells resulted in their resistance to lipotoxicity via the reduced production of reactive oxygen species and JNK activation. Additionally, oleoylcarnitine enhanced sphere formation by HCC cells via STAT3 activation, suggesting that acylcarnitine accumulation was not only a surrogate marker of CPT2 down-regulation but directly contributed to hepatocarcinogenesis. HFD feeding and carnitine supplementation synergistically enhanced acylcarnitine accumulation and HCC development *in vivo*. The increased level of acylcarnitine was also found in the serum of patients with NASH-HCC and high acylcarnitine level was an independent risk factor for the presence of HCC even after adjusting for other factors such as presence of advanced fibrosis and alpha fetoprotein by using a multivariable logistic regression analysis. **Conclusion:** In obesity- and NASH-driven HCC, metabolic reprogramming mediated by the down-regulation of CPT2 not only enables HCC cells to escape lipotoxicity but also promotes hepatocarcinogenesis via the acylcarnitine-mediated gain of stem cell properties by the tumor cells. Serum acylcarnitine level is a potential marker of HCC.

Disclosures:

Kazuhiko Koike - Speaking and Teaching: Gilead, MSD, Abbvie

The following people have nothing to disclose: Naoto Fujiwara, Hayato Nakagawa, Kenichiro Enooku

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MicroRNA-206 Prevents the Development of Hepatocellular Carcinoma Via Modulation of *cMet* and *Cdk6* Expression

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Background: Hepatocellular carcinoma (HCC) is one of the most lethal cancers worldwide and therapeutic agents for this malignancy are lacking. MicroRNAs (miRNAs) play critical roles in carcinogenesis and present a significant therapeutic potential. However, the detailed mechanism by which miRNAs prevent hepatocarcinogenesis is poorly understood. **Methods:** FVB/N mice (wild-type) were hydrodynamically injected with AKT/Ras or cMyc with Sleeping Beauty to induce HCC. Chemically-modified miR-206 mimics or a mini-circle expression vector of miR-206 was injected into AKT/Ras and mice to determine the therapeutic effect of miR-206 on HCC. Livers were collected at the different time points for pathological and molecular analysis. The role of miR-206 in carcinogenesis was analyzed by soft-agar colony formation, cell cycle analysis, and xenograft tumor assay. **Results**: Here we report that miR-206 was undetectable in livers of AKT/Ras and cMyc HCC mice and significantly reduced in human individuals bearing HCC and human HCC cell lines. Combining bioinformatic prediction and experimental approaches, we identified *cMET* (Met proto-oncogene), *CCND1,* and *CDK6* as functional targets of miR-206. By inhibiting expression of *cMET*, *CCND1* and *CDK6*, microRNA-206 delayed cell cycle progression, induced apoptosis and impaired proliferation of three distinct human HCC cell lines. Colony formation assay showed that miR-206 robustly prevented growth of three HCC cell lines (Figure 1A). Systemic administration of miR-206 completely prevented HCC development in both cMyc and AKT/Ras HCC mice, while 100% of control mice died from lethal tumor burdens (Figure 1B). Conversely, re-introduction of *cMet* or *Cdk6* into livers of cMyc and AKT/Ras HCC mice recovered growth of HCC inhibited by miR-206. These results strongly suggested that *cMet* and *Cdk6* were two functional targets that mediated the inhibitory effect of miR-206 on the development of HCC. MiR-206 overexpression demonstrated a profound therapeutic effect on HCC in xenograft and cMyc HCC mice. **Conclusion**: This study for the first time identified a miRNA that fully prevents the development of HCC, and suggests its use as a potential therapeutic strategy for this malignancy.

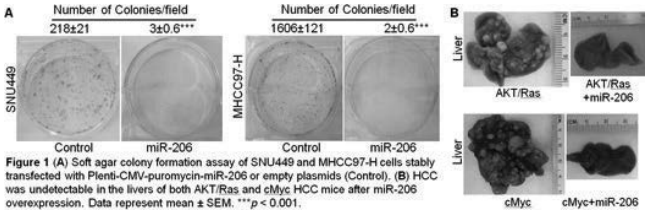

Figure 1 (A) Soft agar colony formation assay of SNU449 and MHCC97-H cells stably transfected with Plenti-CMV-puromycin-miR-206 or empty plasmids (Control). (B) HCC was undetectable in the livers of both AKT/Ras and cMyc HCC mice after miR-206 overexpression. Data represent mean ± SEM. ***$p < 0.001$.

Disclosures:

The following people have nothing to disclose: Guisheng Song, Tianpeng Zhang, Xin Chen, Clifford J. Steer

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Epigenetic and paracrine control of hepatic cancer stemness

Manlio Vinciguerra, Oriana Lo Re; *This work was supported by the European Social Fund and European Regional Development Fund - Project MAGNET (No. CZ.02.1.01/0.0/0.0/15_003/0000492).;* *Center for Translational Medicine (CTM), International Clinical Research Center (ICRC), Brno, Czech Republic*

Background and Aims: Hepatocellular cancer (HCC) is an aggressive disease with a poor outcome. Cancer stem cells (CSCs) are responsible for tumor relapse and chemoresistance. The epigenetic bases of self-renewal of CSCs are not well understood, and what are the paracrine mechanisms by which CSCs influence the behavior of surrounding cancer cells (CC) is unknown. MacroH2A1 is a variant of histone H2A1 that is a robust marker of differentiated HCC (Borghesan M et al, Cancer Res 2016); conversely, absence of macroH2A1 transforms HCC cells in CSCs, characterized by resistance to chemotherapy, enhanced potential to generate bigger and undifferentiated tumors, enhanced glycolytic metabolism and resilience to hypoxia (Lo Re O et al, in revision). The aim of this study was to understand if epigenetically-modified CSCs may secrete specific factors influencing HCC growth and response to therapy. Methods: We used as a model system HCC Huh-7 cells knocked down (KD) for macroH2A1 expression, xenograft nude mice and human HCC biopsies. Metabolomics, immunoblotting, ELISA, gene expression (qPCR), immune assays and immunohistochemistry were used to dissect signaling pathways involves in cancer cell stemness. Results: Mass spectrometry coupled to ultra-high performance liquid chromatography (UHPLC-MS) was used to profile the metabolism of Huh-7 cells KD for macroH2A1: enhanced glycolytic flux, response to hypoxia and expression of redox carriers was found compared to control cells, consistent

with CSCs features. Conditioned medium (CM) of Huh-7 KD for macroH2A1 was strikingly depleted of cytokines IL-6, IL-8 and MIP-1d. Exposure of Huh-7 cells to CM from Huh-7 KD cells protected them from chemotherapy-induced senescence *in vitro* and *in vivo* in nude mice. CM of KD cells had no influence on hepatic stellate cells activation, but failed to activate human T cells, whereas normal cell medium led to both CD4+ and CD8+ cell activation. Immunohistochemistry analyses of human HCC biopsies revealed that undifferentiated parts of tumors expressed less IL-6/IL-8 levels and CD8+ infiltrates compared to differentiated areas. Conclusions: HCC cells devoid of histone macroH2A1 acquire a CSC-like phenotype and secrete a cytokine depleted medium which renders normal HCC resistant to chemotherapy and able to escape immune surveillance. These data uncover a new potential mechanisms by which CSCs tweak the cellular environment to favor cancer growth. This work was supported by the European Social Fund and European Regional Development Fund - Project MAGNET (No. CZ.02.1.01/0.0/0.0/15_003/0000492).

Disclosures:

The following people have nothing to disclose: Manlio Vinciguerra, Oriana Lo Re

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Clinicopathological characteristics and mutational profile of PD-L1 positive hepatocellular carcinoma

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Background and Aims: Programmed cell death 1 ligand 1 (PD-L1) is a member of immune checkpoint molecules involved in the regulation of immune responses. Several reports showed that PD-L1 is expressed in a subset of human hepatocellular carcinoma (HCC), which reflect an immunosuppressive environment of tumors. However, clinicopathological characteristics of PD-L1-positive HCC and their molecular background are still controversial. *Methods:* 56 HCCs and their non-cancerous livers were analyzed for PD-L1 and HIF-1α expression of HCC cells and infiltration of CD8$^+$ lymphocyte in tumors. Expression of stem cell markers, EpCAM, CK19, and SALL4 were also examined by immunohistochemical analysis. The antibody used for immunohistochemistry were clone 28-8 (abcam), C8/144B (Invitrogen), H1α67 (Novus Biologicals), mAb8(-Millipore), RCK108(Dako), 6E3 (Abnova) for PD-L1, CD8, HIF-1α, EpCAM, CK19, and SALL4, respectively. Percent of positive cells for immunohistochemical analysis was evaluated using BioZero(KEYENCE). We also analyzed somatic mutations of 409 kinds of known cancer-related genes and TERT promoter by Ion AmpliSeq Comprehensive Cancer Panel using Ion Proton^{TM} sequencer. HCCs were classified based on the alterations of genes involved in 7 kinds of oncogenic pathways; alteration of Wnt/β-catenin pathway, p53/RB pathway, PI3K/RAS pathway, chromatin remodeling, oxidative/ER stress, DNA repair, and TERT promoter. Clinical backgrounds of the patients and overall survival (OS) were also examined. *Results:* 33% (19/56) of tumors showed positive for PD-L1 expression of >5% in HCC cells. PD-L1-positive HCCs was significantly more frequent in HCCs with portal vein thrombosis (p = 0.0013), moderately/poorly differentiated phenotype (p = 0.0019), with infiltration of CD8$^+$ lymphocyte (p = 0.0001), and CK-19 and SALL-4 expression (p = 0.0141 and 0.1049, respectively). Patients with PD-L1-positive tumors showed shorter OS than those with PD-L1-negative. Interestingly, expression of PD-L1 was associated with gene alterations involved in PI3K/RAS pathway (p = 0.0491) and expression of HIF-1α in tumors (p = 0.1036). Conclusion: Positive for PD-L1 was associated with advanced tumor characteristics with stem/progenitor feature as well as infiltration of CD8$^+$ lymphocytes. In addition, constitutive activation of PI3K/RAS pathway by somatic mutation and HIF-1α expression might induce abnormal expression of PD-L1 in HCC.

Disclosures:

Masatoshi Kudo - Consulting: Eisai, Bayer HealthCare; Grant/Research Support: Astellas Pharma, Abbvie, Daiichi Sankyo, Bristol Myers Squibb, Taiho, Chugai, Otsuka, Medico's Hirata; Speaking and Teaching: Bayer HealthCare, Eisai

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A novel mechanism involving NKT17 and NKT1/2 subsets in different phases of non-alcoholic fatty liver disease

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Background: Non-alcoholic fatty liver disease (NAFLD) is a major cause of liver cirrhosis. Although, lipid metabolism plays an important role in the development of NAFLD, the role of lipid-reactive, CD1d-restricted type I natural killer T (NKT) cells and their subsets secreting different cytokines, NKT1 (IFNγ), NKT2 (IL-4/13) and NKT17 (IL-17/22) is not known. Our **Aim** was to determine their role during progression of non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) in both mice fed choline-deficient amino acid-enriched diet (CDAA) and in humans with NAFLD. **Methods and Results:** We have used lipid/CD1d-tetramers and intracytoplasmic staining to determine the frequency, activation and cytokine phenotype of type I NKT subsets in B6 mice fed CDAA diet for 20 weeks. Interestingly, while NKT17 cells predominate during the beginning of steatosis, IFNγ/IL-13-secreting NKT1/2 cells predominate in the fibrosis phase, indicating differential activation of type I NKT cells. A critical role for activated type I NKT cells in mediating NAFLD was demonstrated by a significantly reduced steatosis and fibrosis in Jα18-/- mice deficient in type I NKT cells. Notably, infiltration of CD8$^+$ T cells into liver was also blunted in Jα18-/- mice. Furthermore, inhibition of type I NKT cells with a RAR-γ agonist also resulted in a significant decrease in steatosis, fibrosis and CD8$^+$ T cells in liver. The RT-PCR analysis showed a reduction in the expression of several pro-inflammatory cytokines and chemokine genes. Interestingly, flow cytometric analysis indicated that type I NKT activation is associated with CD1d-dependent accumulation of plasmacytoid DC (pDC) into liver. Accordingly, type I NKT activation was significantly inhibited in mice deficient in pDC using anti-PDCA-1 treatment or following Diphtheria toxin-mediated depletion in BDCA2-DTR transgenic mice. Since tetramers cannot be used to analyze type I NKT cells in liver biopsies, we have analyzed the frequency and cytokine secretion profiles of type I NKT cells in PBMCs derived from patients with NAFL (n=5) and NASH (n=20) and compared to healthy donors (n=20). Remarkably, type I NKT cells in NASH patients showed a significant increase in IFNγ and T-bet but not IL-4 in comparison to NAFL patients or healthy donors

suggesting their NKT1 phenotype. Also, expression of the activation marker CXCR3 was significantly elevated only in NASH patients. Similar to the murine data, frequency of pDC was also elevated in NASH patients. **In summary**, these studies suggest a novel type I NKT-centered mechanism mediating steatosis and fibrosis in a murine model as well as their potential key role in NASH in humans.

Disclosures:

Rohit Loomba - Advisory Committees or Review Panels: Galmed Inc, Tobira Inc, Arrowhead Research Inc; Consulting: Gilead Inc, Corgenix Inc, Janssen and Janssen Inc, Zafgen Inc, Celgene Inc, Alnylam Inc, Inanta Inc, Deutrx Inc; Grant/Research Support: Daiichi Sankyo Inc, AGA, Merck Inc, Promedior Inc, Kinemed Inc, Immuron Inc, Adheron Inc

Vipin Kumar - Board Membership: GRI bio; Consulting: GRI bio; Stock Shareholder: GRI bio

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Lipolysis and lipophagy pathways work in tandem to catabolize lipid droplets in hepatocytes.

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The initial stages of fatty liver disease are marked by the hepatocellular accumulation of fat-storage organelles known as lipid droplets (LDs) that are degraded by two pathways: lipolysis that involves the recruitment of cytosolic lipases (ATGL, HSL) to the LD surface, and lipophagy that relies on autophagic membranes to target and traffic LDs for lysosomal degradation. Importantly, the mechanisms underlying a cooperation between these seemingly distinct pathways are not well understood and are perhaps viewed as controversial. Previous studies have shown that the engulfment of autophagic cargo such as mitochondria is size-limited. Because of this, we **hypothesize** that lipolysis and lipophagy occur as sequential processes initiated by cytosolic lipases to reduce LD size and allow for subsequent engulfment by autophagic membranes. **Methods**: In AML12 hepatocytes, immunofluorescence microscopy and biochemical approaches were used to determine the enrichment of lipolytic vs autophagic machinery on large vs. small LDs. Live-cell microscopy was used for the first time to monitor LDs transitioning from lipolysis to lipophagic engulfment. **Results**: Confocal microscopy of AML12 cells immunolabeled for adipose triglyceride lipase (ATGL) revealed a preferential association with large LDs with an average area of 4.0 μm^2, seven-fold larger than LDs not associated with ATGL. In striking comparison, LDs associated with components of the autophagic machinery (LC3) were two-fold smaller in area (0.39 μm^2). Consistent with this observation was the finding that siRNA knockdown of ATGL resulted in the persistence of large LDs (2.8 μm^2), whereas knockdown of the lysosomal acid lipase (LAL) left cells filled with many LDs of a smaller size (1.5 μm^2) These observations were mimicked by inhibitors of ATGL (atglistatin) and lysosomal acidification (chloroquine). In support of these findings, density gradient centrifugation of AML12 homogenates revealed two distinct population of LDs: a light buoyant LD fraction enriched in cytosolic lipases and a second, heavier, collection of LDs enriched in lysosomal enzymes such as Lamp2A. Most interesting are time lapse movies of live cells showing engulfment of small LDs by multivesicular bodies positive for the tetraspanin protein CD63, suggesting that these organelles play a central role in the targeting and trafficking of LDs for lysosomal degradation. **Conclusion:** Hepatocytes appear to catabolize LDs in a tandem stepwise process that first utilizes cytosolic lipases to substantially reduce LD size, thereby allowing for subsequent engulfment by autophagosomes as well as late endosomes and lysosomes.

Disclosures:

The following people have nothing to disclose: Micah B. Schott, Shaun Weller, Ryan J. Schulze, Mark A. McNiven

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Identification of a novel transcriptional mechanism by which obeticholic acid upregulates hepatic SR-BI expression in hyperlipidemic hamsters

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Background and Aim: The farnesoid X receptor (FXR) plays critical roles in hepatic and plasma cholesterol metabolism, in particular HDL-C homeostasis. Obeticholic acid (OCA) is a FXR agonist being developed for treating various chronic liver diseases including PBC and NASH. Utilizing hyperlipidemic and normolipidemic hamster models, our previous studies demonstrated that OCA reduces plasma HDL-C levels and promotes transhepatic cholesterol efflux in hamsters via a mechanism involving upregulation of hepatic SR-BI under hyperlipidemic state but not under normolipidemic conditions. The aim of this current study was to examine the role of hepatic cholesterol in OCA-induced SR-BI transcription. **Methods**: Various in vitro studies including genomic sequence analysis, reporter assays and direct DNA binding assays were performed to identify cis-regulatory elements involved in OCA-activated SR-BI gene transcription. For in vivo studies, male hamsters fed a normal chow diet were orally administered OCA (10 mg/kg), liver X receptor (LXR) agonist GW3965 (30 mg/kg) or the combination for 10 days and fasting serum lipids and hepatic SR-BI mRNA and protein levels were assessed. **Results:** We identified a regulatory region in the first intron of the hamster SR-BI gene that contains a functional FXRE motif and a LXRE site separated by 57 basepairs. The nucleotide sequence within this region is highly conserved between hamster and mouse with identical binding sequences and the spacing between FXRE and LXRE. Hamster promoter reporter activities were increased 15-fold by OCA, 5-fold by GW3965 and 30-fold by OCA and GW3965 cotreatment, indicating a synergistic activation of SR-BI gene transcription by the two nuclear receptors. We further demonstrate that this transcriptional activation synergy indeed exists in hamster liver tissue as hepatic SR-BI mRNA and protein levels did not change by individual treatments that is consistent with our previous study. In contrast to the single treatment, in hamsters cotreated with OCA and GW3965, we detected a 1.7-fold ($p<0.001$) increase in hepatic SR-BI mRNA levels and a 1.8-fold ($p<0.001$) increase in SR-BI protein levels ($p<0.001$) compared to control hamsters treated with vehicle. **Conclusion:** We have identified a critical regulatory region in hamster SR-BI gene that mediates SR-BI gene transcription upon FXR activation by OCA treatment under the LXR activated states either by endogenous agonist sterols or by synthetic agonist GW3965, consequently leading to

plasma HDL-C reduction. Our novel findings shed new light for a better understanding of potential mechanisms of FXR-mediated HDL regulation in dyslipidemic patients.

Disclosures:

The following people have nothing to disclose: Bin Dong, Amar B. Singh, Grace L. Guo, Jingwen Liu

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OX40 mediates crosstalk between intrahepatic innate and adaptive immunity and promotes nonalcoholic steatohepatitis (NASH) development

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Background: Both innate and adaptive immune cells are involved in the pathogenesis of NASH. The interactions of these immune cells are most important for the development and progression of NASH. However, the crosstalk between innate and adaptive immunity in sustaining hepatic inflammation is still largely unknow. In this study, we aimed to determine the role of OX40 in the regulation of hepatic inflammation and NASH development. **Methods:** WT or OX40-/- mice consumed a high fat diet (HFD) for 16 weeks or methionine and choline deficient diet (MCD) for 4 weeks. NASH development was evaluated by liver sample HE and Oil Red O staining. The activation, differentiation and proinflammatory cytokines secretion of liver infiltrated immune cells were compared in each group. **Results:** After 16 weeks HFD or 4 weeks MCD, WT mice showed significantly increased OX40 levels on CD4 T cells. The plasma soluble OX40 concentrations in HFD and MCD mice were also significantly higher than those in control mice. Compared with WT mice, OX40-/- mice exhibited significantly less weight gain, lowered plasma ALT and fasting glucose levels, reduced liver infiltrated inflammatory immune cells, decreased liver fat accumulation, lobular inflammation and focal necrosis. Mechanistically, OX40 deficiency in HFD or MCD fed mice remarkably suppressed T cells activation, survival and Th1/Th17 differentiation. OX40 knockout also markedly decreased liver infiltrated $CD11b^{int}F4/80^{low}$ liver monocytes with downregulated CCR2 expression, TNF-α secretion and MHC II, CD86, TLR4 expression. Interestingly, these changes were not found on $CD11b^{low}F4/80^{hi}$ Kupffer cells. Furthermore, repopulation of lymphocyte-free B6. Rag2/Il2rg double KO mice with WT T cells but not OX40 deficient T cells provoked liver monocytes migration, antigen-presentation and M1 polarization, promoted NASH development. Finally, recombinant soluble OX40/Fc stimulation *in vitro* could also upregulated antigen presentation associate molecules (MHC II, CD40, CD86 and TLR4), chemokine receptors (CCR2, CCR5 and CCR9) and proinflammatory cytokines expression (TNF-α, IL-1β, IL-6 and IFN-γ) in monocytes/macrophages but not on Kupffer cells. **Conclusion:** OX40 is key molecule that mediates crosstalk between intrahepatic innate and adaptive immunity, generates two-way signals, promotes both proinflammatory monocytes/macrophages and T cells function, causes NASH development and progression. OX40 could serve as a diagnostic index and a therapeutic target of NASH.

Disclosures:

The following people have nothing to disclose: Guangyong Sun, Chunpan Zhang, Hua Jin, Tianqi Wang, Wen Shi, Yue Tian, Xinmin Li, Dong Zhang

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CM-101, a novel CCL24 blocking monoclonal antibody ameliorates hepatic injury in NASH induced mouse model

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Introduction: The chemokine system plays a key role in the development of chronic hepatic inflammation and fibrosis in NASH. We have recently detected that chemokine CCL24 was significantly overexpressed in NASH and that CCL24 was associated with the progression of NASH hepatic inflammation and fibrosis in mice. Aim: To evaluate the role of the CCL24 in NASH and to explore the impact of a novel monoclonal antibody CM101, a blocking human CCL24 agent developed by ChemomAb, on the hepatic inflammation and fibrosis progression in a NASH induced mouse model. Methods: C57BL/6 (WT) mice were fed with methionine choline-deficient (MCD) diet for 8 weeks to induce NASH. On day 10 mice were administered with CM101 or vehicle intraperitoneally, twice weekly for 6 weeks. CCL24 knockout mice, generated via CRISPR/CAS technology, were also fed with MCD diet to induce NASH. Level of serum liver enzymes, liver collagen concentration (Sircol kit), histological NAFLD activity score (NAS) and RNA analysis using affymetrix CHIP were assessed. Results: CCL24 knockout mice following the induction of NASH exhibited a significant reduction in fibrosis stage, inflammatory grade, NAS and liver damage compared with NASH induced in WT mice. The administration of CM101 5mg/kg to WT mice attenuated MCD induced NASH by ameliorating liver fibrosis and inflammation. ALT, AST and bilirubin levels were reduced significantly in CM101 treated group compared to vehicle (reduction of 62%, 33% and 48% respectively) ($P \geq 0.05$). Liver collagen level, was reduced by 95% in NASH induced WT mice treated with CM101 compared with vehicle, reversing the liver collagen level into its baseline normal level. In addition, the NAS was reduced by 35% in mice treated with CM101 compared to vehicle treated mice. A full RNA expression analysis revealed a significant change in the RNA level of NAFLD related genes, in the CM101 treated group compared to vehicle treated group that could further support the antibody's mechanism of action. Conclusions: We provide for the first time evidence that the chemokine CCL24 is a critical mediator of NASH and that blockade of CCL24 by CM-101, a novel human CCL24 blocking antibody, was associated with an effective reduction in the hepatic injury in NASH induced mice. CM-101 may potentially serve as a novel therapeutic approach in NASH.

Disclosures:

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Significant anti-fibrotic efficacy of EDP-305, a highly potent and selective farnesoid X receptor (FXR) agonist, in a rat model of thioacetamide-induced liver fibrosis and cirrhosis

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BACKGROUND: EDP-305, a selective and potent small molecule FXR agonist, is currently in clinical development for the treatment of non-alcoholic steatohepatitis (NASH) and primary biliary cholangitis (PBC). We have evaluated the *in vivo* antifibrotic effects of EDP-305 in rats with ongoing fibrosis or established cirrhosis due to thioacetamide (TAA)-induced injury. This model induces progressive fibrosis that has reduced spontaneous regression and which has predicted drug efficacy in humans for several compounds such as Ocaliva and Cenicriviroc. **METHODS:** Fibrosis and cirrhosis were induced in male Sprague-Dawley rats by intraperitoneal administration of TAA (150 mg/kg 3 X/week for 8 wks). Rats (n=6–9/group) received EDP-305 10 mg/kg/day, EDP-305 30 mg/kg/day, or vehicle control, concurrently with TAA during Weeks 5–8 (Group 1; therapeutic intervention in ongoing fibrosis) or during Weeks 9–12 following completion of TAA administration (Group 2; cirrhosis reversal). Biochemical, gene expression, and histologic evaluations of the liver were conducted. **RESULTS**: When treatment was started 4 weeks after TAA-induced injury (Group 1), EDP-305 at 10 mg/kg and 30 mg/kg significantly reduced fibrosis by 50% and 55%, respectively ($p<0.001$), as assessed by collagen morphometry. Pathology scores (including Scheuer, Ishak and ductular reaction scores) also decreased in parallel to collagen morphometry. Significantly decreased aspartate aminotransferase levels (by 50% - 60%; $p<0.01$) were observed for EDP-305 at 10 mg/kg and 30 mg/kg. Alpha-smooth muscle actin (α-SMA) and collagen protein were reduced by 50% and 16%, respectively, for EDP-305 in the 10 mg/kg group. For the cirrhosis reversal treatment arm (Group 2), EDP-305 reduced fibrosis in a dose-dependent manner. A 25% decrease in collagen deposition by morphometry was observed for EDP-305 at 30 mg/kg, which was consistent with decreases in collagen and α-SMA protein levels (18%↓ and 72%↓, respectively, with $p<0.01$). **CONCLUSIONS**: EDP-305 exhibits excellent anti-fibrotic efficacy in rats with ongoing TAA-induced fibrosis. The compound also decreases collagen deposition in rats with established cirrhosis, but to a lesser extent. These results warrant further clinical development of EDP-305 for the treatment of NASH and PBC.

Disclosures:

Li-Juan Jiang - Employment: Enanta Pharmaceuticals, Inc

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Scott L. Friedman - Consulting: Ironwood Pharmaceuticals, Glycotest, Allergan Pharmaceuticals, Madrigal Pharmaceuticals, Metacrine, Conatus Pharm, Jecure Therapeutics, Genfit, Galectin Therapeutics, Exalenz Biosciences, Abbvuiew PHarmaceuticals, Revive Therapeutics, Morphic Rock, Nitto Corp., Scholar Rock, Xtuit Therapeutics, Kowa Pharmaceuticals, Blade Therapeutics, DeuteRx, Abbvie, Novartis, BirdRock Bio, Arbutus, Sanofi Aventis, Chemocentryx, Nimbus Therapeutics, Bristol Myers Squibb, Karos Pharmaceuticals, Gemphire Pharm., Galmed, Northern Biologics, Takeda Pharmaceuticals, Gilead Pharm., Glympse Bio, Fractyl Bioscience, Dicerna Pharmaceuticals, Dignity Biosciences; Grant/Research Support: Enanta Pharm., Zafgen Pharmaceuticals; Stock Shareholder: Intercept Pharma

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Phenotype and genotype analysis of metabolic liver disease in half million US Veterans enrolled in the Million Veteran Program (MVP) megabiobank

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Background: Nonalcoholic fatty liver disease (NAFLD) is an increasing cause of morbidity that impacts 30% of Americans in association with obesity, insulin resistance, type 2 diabetes (T2D) and dyslipidemia. A number of genome wide association studies have associated genetic variations in PNPLA3, GCKR, TM6SF2, APOC3 and ChREBP with outcomes related to NAFLD. In this study, we embarked on phenotype and genotype analysis of NAFLD among US Veterans in the Million Veteran Program (MVP). **Methods:** NAFLD, defined as ALT > upper limit of normal (ULN) at two time points at least 6 months apart AND at least one of the following: BMI ≥ 30, T2D, or hypertension, was assessed in 510,167 US Veterans enrolled in MVP. Veterans with alcohol use disorders and viral hepatitis (B and C) were included in comparator phenotypes: alcoholic liver disease (ALD), simultaneous metabolic and alcohol liver disease (SMALD), viral hepatitis with metabolic liver disease (VH+NAFL), and viral hepatitis with one metabolic co-factor (VH+M). The association between 657,459 directly genotyped DNA variants and NAFLD was assessed in 353,323 participants with genotype data. Genome-wide association testing, controlling for BMI and T2D, was performed separately in individuals of European, African, and Hispanic ancestry and the results were combined using inverse variance weighted meta-analysis. **Results:** Among 510,167 Veterans enrolled in MVP, we identified 80,082 (16%) with NAFLD. Subjects with NAFLD had the following characteristics: median age 63, 88% male, 76% white, 16% black, 61% with BMI ≥30, 52% with T2D, 72% with dyslipidemia and median BMI 31.6. Additionally, 14,597 (3%) of the MVP cohort had SMALD, and 5,058 (1%) with VH-NAFL. 42% of VH patients had at least 1 metabolic cofactor. Notably, nearly one quarter of the entire 510,167 patient MVP cohort had a phenotypic profile that included metabolic liver disease. Trans-ethnic

genetic analysis in participants with available genotype data confirmed previously observed risk loci at PNPLA3 ($p=8.0 \times 10^{-107}$), TRIB1 (1.9×10^{-38}), CPN1-ERLIN1-CHUK (4.0×10^{-33}), TM6SF2 (5.7×10^{-20}), HSD17B13 (5.7×10^{-14}), and PPP1R3B (2.3×10^{-10}). Novel associations at 5 loci met genome-wide significance including GPT (1.6×10^{-31}) and SERPINA1 (6.6×10^{-29}), FADS1, SUGP1, MYRF and GPAM. **Conclusion**: 16% of over half million MVP enrollees meet clinical criteria for NAFLD without alcoholic or viral co-factors. GWAS confirmed previously NAFLD-associated genes demonstrating the robustness of both those loci and the clinical phenotyping in MVP. Novel NAFLD risk-loci highlight opportunities for further investigation and ongoing discovery.

Disclosures:

David E. Kaplan - Grant/Research Support: Bayer Pharmaceuticals, Inovio Pharmaceuticals

Daniel J. Rader - Stock Shareholder: Aegerion Pharmaceuticals

Kyong-Mi Chang - Advisory Committees or Review Panels: Arbutus Inc; Consulting: Alnylam

The following people have nothing to disclose: Marina Serper, Jung-Jin Lee, Scott Damrauer, Donald R. Miller, Qing Shao, Peter W. Wilson

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Next-Generation Sequencing Improves the Detection of Malignant Biliary Strictures: A Large Prospective Study of Bile Duct Brushings/ Biopsies

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Background: Despite advancements in imaging and tissue sampling, determination of whether a biliary stricture is benign or malignant in etiology can be challenging. Recently, next-generation sequencing (NGS) has identified recurrent genetic alterations among pancreatobiliary neoplasms. Moreover, NGS has the capability of detecting small quantities of mutant DNA within biliary brushings/ biopsies obtained during ERCP and, thus, the potential to be used as a diagnostic test to screen for malignancy. A prospective study was performed to evaluate the accuracy of NGS in detecting malignant biliary strictures. Methods: Within 30 months, 111 bile duct brushings/ biopsies obtained during ERCP to evaluate indeterminate biliary strictures were prospectively submitted to detect alterations in 28 genes associated with pancreatobiliary neoplasms. Molecular findings were correlated with clinical presentation, CA19-9, stricture location, preoperative cytologic/histologic evaluation of biliary brushings/biopsies, and follow-up. A stricture was designated as benign or malignant on the basis of diagnostic surgical pathology, subsequent malignant brushings/biopsies, death due to a malignancy, or clinical course at > 6 months. Results: Genetic alterations were identified in 47 of 111 (42%) biliary specimens, and primarily found in TP53 (53%), KRAS (51%), SMAD4 (15%), GNAS (11%), and CDKN2A (11%). While the presence of mutations correlated with an elevated CA19-9 and malignant preoperative brushings/biopsies ($p < 0.001$), no association was seen with primary sclerosing cholangitis, jaundice or location of the biliary stricture ($p > 0.05$). Eighty-nine (80%) patients had > 6 months follow-up or a final pathologic diagnosis, and consisted of 61 patients with a malignant stricture and 28 with a benign stricture. The sensitivity and specificity of NGS for malignancy was 74% and 100%, respectively (AUC 0.87, $p < 0.001$). In comparison, cytologic/histologic evaluation of biliary brushings/biopsies was associated with a sensitivity of 44% and specificity of 100% (AUC 0.72, $p = 0.001$). However, the combination of NGS and cytologic/histologic evaluation had 84% sensitivity and 100% specificity for a malignant biliary stricture (AUC 0.92, $p < 0.001$). Conclusions: Although larger studies are needed, these findings suggest the detection of genetic alterations by NGS is highly specific for malignancy. Further, NGS improved the sensitivity of cytologic/histologic evaluation of biliary brushings/biopsies for malignant biliary strictures, and maintained a high specificity. Combining NGS with routine cytology/histology achieved an accuracy of 92%.

Disclosures:

Melissa Hogg - Grant/Research Support: sages, intuitive

Adam Slivka - Grant/Research Support: Mauna Kea Technology, Boston Scientific, Wilson Cook

The following people have nothing to disclose: Aatur Singhi, Asif Khalid, Georgios I. Papachristou, Mordechai Rabinovitz, Kevin McGrath, J Wallis Marsh, Allan Tsung, Amer Zureikat

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Identification Of Novel Viral Single Nucleotide Variants Associated With Baseline HBV DNA Levels And HBeAg Status In Chronic Hepatitis B Patients.

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BACKGROUND: Deep sequencing of chronic hepatitis B patients has the potential to identify viral genetic variation that may provide insight into clinical outcomes. Our goal was to identify associations of baseline HBV diversity with patient clinical characteristics. **METHODS:** Whole genome HBV amplicons from 1098 patients enrolled in two global phase 3 studies of Tenofovir Alafenamide (TAF) versus Tenofovir Disoproxil Fumarate (TDF) for the treatment of HBeAg-Negative and Positive chronic hepatitis B (GS-US-320-0108 and GS-US-320-0110; Chan et al., 2016; Buti et al., 2016) were sequenced on Illumina MiSeq (150bp paired-end reads) to a depth of ~32,000-fold. Genetic diversity was measured using Shannon entropy and intra-host single-nucleotide variant rate. Viral diversity was measured at a single nucleotide level and across 15 nucleotide overlapping sliding-windows. Generalized linear model was used to test statistical associations and machine learning was implemented to build classifica-

tion models to investigate how well viral variation alone predicts clinical features. **RESULTS:** Patient population contained HBV genotypes (GT) A (N=71), B (N=203), C (N=547), D (N=265), E (N=7), and F (N=5) with 767 HBeAg+ and 331 HBeAg- patients. HBV diversity across all GT was higher in HBeAg- patients compared to HBeAg+ patients ($p<0.001$). We identified novel viral variants that significantly associated with HBeAg status ($p<1x10^{-14}$) and serum HBV DNA ($p<1x10^{-10}$). Twelve HBV variants across precore, core, and surface regions showed significant association with HBeAg status at $p<0.001$ level. Given these variants, patient's HBeAg status could be predicted with ~95% accuracy (AUC=0.98), suggesting HBeAg status is strongly associated with a mutation process. Patients harboring viral variants C1817T and A1838G at higher than 15% frequency showed ~3 log reduction in baseline HBV DNA ($p<0.001$), regardless of HBeAg status and HBV genotype. These findingsvalidate the variants detected in our previous independent study of n=365 chronic HBV patients (EASL 2017: ILC2017-RS-2810). **CONCLUSION:** Whole-genome HBV sequencing analysis identified novel variation within and outside of PC/BCP region to be significantly associated with baseline levels of HBV DNA and HBeAg status, respectively, and validate the results of our previous study. Given global HBV cure efforts, high quality HBV genomic data can provide novel insights into genomic dynamics of HBV and its relationship to patient clinical characteristics.

Disclosures:

Kosh Agarwal - Advisory Committees or Review Panels: Merck, AbbVie, Gilead; Grant/Research Support: BMS, Gilead; Speaking and Teaching: Gilead, Merck, Abbvie

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Cellular Bioenergetics: A Promising Biomarker in Management of Patients with Alcoholic Liver Disease

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Background and Aims: Alcoholic hepatitis (AH) is associated with 40-50% mortality at 1 month. Liver biopsy is often needed especially for uncertain clinical diagnosis. Corticosteroids (CS) provide 50% survival benefit with response evaluable only at 1 week. Defects in bioenergetics or mitochondrial oxygen consumption rate (OCR) in peripheral cells are shown in diseases associated with systemic inflammation like diabetes and sepsis. We tested our hypothesis that AH patients with severe bioenergetics defects will progress to liver failure and be non-responsive to CS (NRS). **Methods**: After informed consent, 20 mL blood was collected from ALD patients (with or without AH) and healthy controls. Second 20 mL sample was collected at 1 wk. from AH patients receiving CS. Monocytes were isolated within 30 min using CD14 antibodies. Cellular bioenergetics and OCR (pmol/min./mcg protein) were obtained using XF96 analyzer (*Seahorse Biosciences*). **Results**: Of 86 ALD patients (39 with AH), 78 without concomitant HCV and 40 healthy controls were analyzed. ALD with AH (n=37) compared to 41 without AH were younger (44±11 vs. 53±9 yrs.) with higher white blood cell count (16±10 vs. 6±9) and MELD score (30±10 vs. 16±10), $P<0.0001$ for all. Compared to 35 healthy controls, OCR differed among 63 ALD patients for basal (3.1±1.6 vs. 2.5±0.8, P=0.002), proton leak (0.6±0.4 vs. 0.5±0.2, P=0.03), non-mitochondrial (1.5±0.6 vs. 1.4±0.4, P=0.03), and oxidative burst in monocytes (8.5±5.2 vs. 6.3±3.7, $P<0.05$). OCR among ALD with AH (n=28) compared to 35 without AH differed for basal (2.5±1.3 vs. 3.5±1.8, P=0.02) and proton leak (0.4±0.3 vs. 0.7±0.5, P=0.02) linked OCR. After controlling for age, WBC, and MELD score, basal and ATP linked OCR predicted diagnosis of AH with OR (95% CI) of 0.5 (0.3-0.9, P=0.04) and 0.4 (0.2-0.9, P=0.04) respectively. Bioenergetics in monocytes improved at one week among five responders to corticosteroids, but not in three NRS. Of these 63 ALD patients, serum cytokines were measured using the standard milliplex commercial kits in 32 (14 with AH) patients. Chemotactic chemokine CCL20 levels (mean ± SEM) were significantly elevated among AH as compared to patients without AH (1256±57 vs. 434±36 pg/mL, P=0.032). Bioenergetics measurements available in 14 of these 32 patients showed negative correlation with basal OCR in monocytes (R= -0.59, P=0.043). **Conclusions**: Baseline cellular bioenergetics seems a promising biomarker for personalized medicine in ALD patients for a) diagnosis of AH and b) predicting response to CS and outcome on follow up. Data in larger multicenter population are needed before accepting use of this novel biomarker in clinical practice.

Disclosures:

The following people have nothing to disclose: Ashwani Singal, Balu Chacko, Victor Darley-Usmar

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Genomic and transcriptomic analysis reveal tumor suppressive effect of TGF-β signaling in liver cancer are associated with impaired DNA damage repair pathways

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Background/Aims: Hepatocellular Cancer (HCC) is the second leading cause of cancer deaths worldwide, with lower than an 11% five-year survival for advanced cases. The dismal prognosis could be attributed to multiple factors including an absence of an integrated approach combining bioinformatic analyses with functional validation of genomic/pathway associated mutations, copy number changes, and animal models that support effective targeted therapies for this common and lethal cancer. Of the key pathways involved, the TGF-β is a potent regulator of liver inflammation, tumor suppression, as well as tumor progression. However, to date, the role of TGF-β members at these specific stages in HCC remains poorly delineated. Here, we utilized an integrated approach to identify and validate specific temporal events and populations that reflect the complex components of the TGF-β signaling pathway in HCC towards identifying new targets. **Methods:** A comprehensive whole genome analysis of a large liver cancer cohort within The Cancer Genome Atlas (TCGA) was performed to validate the etiological factors of liver cancer, and elucidate the functional role of the TGF-β signaling pathway, with prognostic signatures of 488 cases. Further functional validation was performed on one of the most commonly altered TGF-β members, Smad adaptor β2SP. **Results:** We observed alterations in at least one of the TGF-β pathway members in 38% of HCC samples. These alterations correlate with oncogenic genome aberrations, irrespective of known risk factors such as HBV, HCV and alcohol. Unsupervised clustering of the whole transcriptome sequencing data delineated two groups of TGF-β–related genes: Increased levels of TGF-β–related genes that we refer to as an "activated group," potentially reflecting ongoing inflammation and fibrosis during tumor progression; The "inactivated" group reveals decreased levels of TGF-β–related genes, associated with loss of TGF-β tumor suppressor function and a significantly poorer survival as compared to the "activated" TGF-β signature (p=0.0027). Interestingly, we observe strong correlations between the TGF-β signature and DNA damage response pathway and Sirtuin pathways. Furthermore, β2SP D1089Y mutant, provides an association with sensitivity to DNA crosslinking agents in this recalcitrant cancer. **Conclusions:** Our findings provide new insight into how disruption of the TGF-β pathway correlates with fibrosis, immune modulation, tumor suppression and tumor progression that drives HCC. The TCGA analyses support current clinical trials targeting TGF-β in specific patient populations, and further therapeutic intervention in the context of the pathway.

Disclosures:

The following people have nothing to disclose: Shuyun Rao, Jian Chen, Patricia Farci, Kirti Shetty, Xifeng Wu, Ray-Chang Wu, Shulin Li, Cuiying Xiao, Chu-xia Deng, Rehan Akbani, Lopa Mishra

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Progression of alcoholic liver disease to severe alcoholic hepatitis is characterized by decreased activity of transcription factors implicated in hepatocyte differentiation

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Background: Alcoholic liver disease (ALD) is the main cause of cirrhosis worldwide and the main driver of health expenditure in hospitalized patients with liver disease in the US. The development of targeted therapies is hampered by a poor knowledge of the underlying mechanisms. It is unknown why some patients develop severe forms (i.e. alcoholic hepatitis -AH-) characterized by poor hepatocyte function. We hypothesize that global changes in the transcription factor activity may play a role in the development of severe forms. To test this hypothesis, we performed a functional analysis of RNAseq data from livers of patients with different ALD phenotypes compared to normal livers and cirrhosis. **Methods:** Liver biopsy specimens were collected from ALD patients in different stages of disease progression: early compensated ASH (N=12), non severe AH (N=11), severe AH non responders to steroids (N=9), severe AH responders (N=9) and liver explants from transplanted patients with severe AH (N=11). Compensated HCV cirrhosis (n=10) and fragments from normal

livers (N=10) were also studied. High-throughput RNA sequencing (RNA-Seq) was done. Unsupervised clustering (Bioconductor), gene ontology analysis (GSEA) and upstream regulator prediction (Ingenuity Pathway Analysis and Opossum) were performed. **Results:** Clustering analysis showed a specific transcriptome pattern across different ALD phenotypes. Major changes in ALD progression included the inhibition of hepatocyte biosynthetic pathways, drug metabolism, hepatocyte differentiation and the activation of cell proliferation, ECM deposition, inflammation and hypoxia. Importantly, AH mortality was associated with inhibition of cell responses to hypoxia and inflammation. Analysis of upstream regulators revealed profound transcription factor reprogramming. Changes in the transcriptome along disease progression predicted a marked inhibition of nuclear factors responsible for hepatocyte differentiation (i.e. HNF4A, HNF1A and FOXA1), while factors implicated in cell damage and inflammation (i.e. NFATC2, STAT1 and NFKB1) were significantly activated. Bioinformatic prediction showed an involvement of EGFR signaling in disease progression. Studies in cultured hepatocytes demonstrated the inhibitory effect of EGFR activity on HNF4A expression and stability. **Conclusion:** Progression to severe forms of ALD including AH is characterized by decreased activity of transcription factors implicated in hepatocyte differentiation. These results suggest that targeting transcription factors that maintain normal hepatocellular function represent a potential novel therapeutic strategy in these patients.

Disclosures:

Philippe Mathurin - Board Membership: MSD, Janssen-Cilag, BMS, Gilead, Abvie, Verlyx; Consulting: Roche, Bayer

Ramon Bataller - Speaking and Teaching: Echosens

The following people have nothing to disclose: Josepmaria Argemi, Joaquin Cabezas, Veronica L. Massey, Meritxell Ventura-Cots, M. Ujue Latasa, Constantino Fondevila, Peter Starkel, Laurent Dubuquoy, Alexandre Louvet, Gemma Odena, Juan Caballeria, Pau Sancho-Bru, Carmen Berasain, Matias A. Avila

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The Rising Healthcare Burden of Alcoholic Cirrhosis in the United States

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Background: Alcoholic cirrhosis is a major cause of liver-related morbidity and mortality both in the United States and worldwide, and rising rates of alcohol use disorders are predicted to lead to further increases. Recent studies have focused on the economic and healthcare burden of liver disease due to HCV and NAFLD. The aim of this study was to use a large, nationally-representative cohort to determine the prevalence, healthcare utilization and costs of alcoholic cirrhosis in the United States. **Methods**: We collected data on the prevalence, admissions, readmissions, and costs from patients aged 18-65 with alcoholic cirrhosis (identified by ICD-9/ICD-10 codes) enrolled in the Truven Analytic MarketScan Commercial Claims and Encounters database (2008-2015). We determined yearly prevalence trends, weighted to the national employer-sponsored insured population (approximately 120 million people). Using competing risk analysis, we estimated event rates for portal hypertensive complications and determined the effect of alcoholic cirrhosis on total and per-person costs as well as admissions and readmissions. **Results:** 294,561 enrollees had cirrhosis in 2015 (0.27% prevalence nationally), with 43% attributed to alcohol (0.12% prevalence). Mean age at diagnosis was 54 years. National prevalence of cirrhosis and alcoholic cirrhosis rose from 0.20% to 0.27% between 2008 and 2015 for cirrhosis overall, and 0.09% to 0.13% for alcoholic cirrhosis. Compared to non-alcoholic cirrhosis, alcoholic cirrhosis patients were significantly more likely to be decompensated at diagnosis (ascites: 20% vs 7%; hepatic encephalopathy 6% vs 1%; variceal bleeding: 4% vs 1%; $p<0.05$ for all). Cirrhosis and alcohol-related admissions were higher for alcoholic cirrhosis patients (22.8 excess cirrhosis admissions per 100 patients) as were 30-day readmissions (29.2 excess readmissions per 100 patients). Per-person healthcare costs in the first year after index diagnosis were nearly double for alcoholic cirrhosis (per-person: 69,489 US$ vs 37,129 US$, $p<0.05$). Total direct healthcare costs for the first year after diagnosis for all ALD were 11 billion US$. **Conclusion:** In an analysis of a nationally representative cohort of privately insured cirrhosis patients, almost half had alcoholic cirrhosis. Patients with alcoholic cirrhosis were sicker at presentation, had more admissions and readmissions, and total healthcare costs were nearly twice that of non-alcoholic cirrhosis. Early diagnosis and aggressive alcohol cessation efforts may help improve outcomes and healthcare utilization in these patients.

Disclosures:

Robert J. Fontana - Consulting: Abbvie, CLDF, Alnylam; Grant/Research Support: BMS, Gilead

Michael Volk - Consulting: Best Doctors

Anna S. Lok - Grant/Research Support: Gilead, Target Solutions, BMS

The following people have nothing to disclose: Jessica L. Mellinger, Kerby Shedden, Elliot B. Tapper, Gerald S. Winder, Megan A. Adams, Frederic Blow

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Use of an Innovative Telehealth Platform to Reduce Readmissions and Enable Patient-Centered Care in Cirrhotic Patients

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Background: Cirrhosis affects > 600,000 US adults, with > 150,000 hospitalizations annually. 30-day readmissions range from 20-40%; 20% being potentially avoidable in studies nationwide, and 90-day readmission rates are 53% from the NACSELD consortium. At Penn, the 30-day readmission rate for the cirrhosis service is 29% and it has the highest percentage of potentially preventable readmissions (PPRs) of any service (23.3%), with 50% of PPRs returning within 10 days of discharge. We aimed to pilot a wireless mobile device monitoring system to detect early symptoms & signs, thereby preventing readmissions and keeping patients engaged and feeling cared for on a daily basis. Methods: Patients were provided with a 4G tablet, wireless blood pressure monitor, pulse oximeter

and weighing scale to monitor: medication adherence, hepatic encephalopathy, fluid overload, bleeding, and infections. Biometric parameters, medication administration & symptom questionnaires were transmitted wirelessly to RNs in the Penn E-lert center. Tablets were kept for 90 days by each patient. RNs & MDs could intervene by phone or video chat. Cirrhotics admitted to the hepatology service from 5/1/2015 to 7/31/16 in zip codes in Philadelphia serviced by Penn E-lert were eligible for inclusion and offered enrollment in telehealth monitoring. Enrolled patients were compared to controls admitted during the same time who fit enrollment criteria (same zip codes, home bound status). Results: 19 cirrhotics were enrolled in the telehealth program and compared to 143 controls. Telehealth patients had a median age of 58 years, were mostly non-Hispanic white (52.6%) and female (52.6%). 53% had hepatitis C as the etiology of liver disease. 13 (68.4%) had ascites, 7 (36.8%) had portal hypertensive bleeding, and 14 (73.7%) had hepatic encephalopathy (HE). Median MELD-Na on admission was 19, 57.9% were Child Pugh (CP) score B and 31.6% CP C. There were no significant differences in demographics of telehealth and control patients. 15.8% of telehealth patients were readmitted in 30 days compared to 21% in the control population, which was numerically but not statistically significant. PPRs were not significantly different at 30 days, but were at 90 days with 33.8% of readmissions in the control group being due to HE or volume overload compared to 0% in the telehealth group (p=0.02). Conclusions: A telehealth platform reduces readmissions due to potentially preventable causes (HE and volume overload) & improves patient satisfaction. We plan to move forward with a large randomized controlled trial to determine its effectiveness at reducing readmission rates in larger and more diverse cohorts.

Disclosures:

The following people have nothing to disclose: Vandana Khungar, Marina Serper, Diane Peyton, Shivan Mehta, Anne Norris, Ann Huffenberger, Shazia M. Siddique, Kimberly A. Forde-McLean

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Liver Cirrhosis and Cancer: Comparison of Mortality

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Background/Aims*:** The survival rate of liver cirrhosis is well known to be low and its assessment in relation to other fatal diseases will help us design better health policy. This study estimates the mortality of liver cirrhosis and compares it with that of five major cancers (lung, colorectal, stomach, liver, and breast cancers). ***Methods: We used both the Cause of Death Statistics and National Health Insurance Service-National Sample Cohort (NHIS-NSC) database to study nationally representative mortality across the diseases. The NHIS-NSC provides a cohort data of 1,025,340 representative sample for the 46,605,433 population in Korea from 2002 to 2010. Liver cirrhosis is carefully defined using ICD-10 codes (K702, K703, K704, K717, K720, K721, K729, K740-K746, K761, K766-K767, R18, I850, I859, I864, I868, I982, I983). Its eight-year mortality from 2002 to 2010 was compared with that of five major cancers [lung (C33-C34), colorectal (C18-21), stomach (C16), liver (C22), and breast cancer (C50)]. ***Results:*** According to the NHIS-NSC, 800 out of 2,609 liver cirrhosis patients in 2002 died during the following eight years while 1,316 out of 4,852 five major cancer patients died. When we estimated the mortality of liver cirrhosis in relation to five major cancers using the NHIS-NSC, the relative mortality of liver cirrhosis was greater [hazard ratio=1.47 (95% CI: 1.28-1.67), after age, gender, area of residence, type of insurance, insurance premium level (proxy for income level) and comorbidities were adjusted for]. When sensitive analysis was done to check the robustness of this result excluding patients with both liver cirrhosis and five cancers, the relative mortality was still greater for liver cirrhosis [hazard ratio=1.27 (95% CI: 1.10-1.47)] when mortality related factors were adjusted for. We conducted another analysis by limiting the liver cirrhosis patients to those with decompensated liver cirrhosis (ICD-10 codes; K720, K721, K729, R18, I850, I983) and found that the relative mortality of decompensated liver cirrhosis was even greater than five cancers [hazard ratio=1.82 (95% CI: 1.51-2.20)]. ***Conclusions:*** The mortality of liver cirrhosis is greater than that of five major cancers. This implies that we need to prioritize development of appropriate health interventions for liver cirrhosis.

Disclosures:

The following people have nothing to disclose: Dong Joon Kim, Ki Tae Suk, Sang Hyun Choi

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Significant Declines in the Proportion of Advanced Tumor Stage at Diagnosis Among Adults with Hepatocellular Carcinoma Reflect the Success of Cancer Screening and Surveillance Programs in the U.S.

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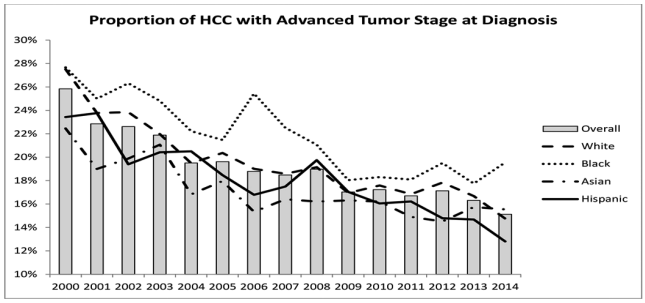
Background: Effective surveillance programs for hepatocellular carcinoma (HCC) aim to improve early tumor stage diagnosis, which improves options for curative therapy. **Aim:** To evaluate tumor stage-specific trends in annual HCC incidence and stage-specific distribution of HCC burden **Methods:** We retrospectively evaluated U.S. adults with HCC using the most recent 2000-2014 population-based Surveillance, Epidemiology, and End Results (SEER) cancer registry, to analyze tumor stage-specific trends in HCC incidence using SEER staging systems (localized vs. advanced). Annual incidence rates were standardized to year 2000 population. Proportion of localized vs. advanced stage HCC at time of diagnosis was stratified by year, sex, and race/ethnicity and compared using chi-square methods. **Results:** From 2000 to 2014, incidence of localized HCC increased from 2.3 to 5.1 per 100,000/y (+122%), while incidence of advanced HCC remained stable at 1.4 per 100,000/y. When stratified by race/ethnicity, incidence of localized HCC increased from 1.7 to 3.8 per 100,000/y (+124%) in non-Hispanic whites, 2.5 to 5.8 per 100,000/y (+132%) in blacks, 4.2 to 8.6 per 100,000/y (+105%) in Hispanics, and 6.2 to 7.9 per 100,000/y (+27%) in Asians. For advanced HCC, incidence remained unchanged at 1.1 per 100,000/y in non-Hispanic whites and 1.9 per 100,000/y in Hispanics, whereas incidence increased from 1.9 to 2.4 per 100,000/y (+26%) in blacks and decreased from 3.2 to 2.1 per 100,000/y (-34%) in Asians. Overall the proportion of HCC patients with advanced tumor stage at diagnosis decreased from 25.8% in 2000 to 15.1% in 2014 (Figure). When stratified by race/ethnicity, all groups demonstrated

a significant decline in the proportion of advanced stage HCC, and in 2014 blacks had the highest proportion of HCC with advanced stage (19.5%), followed by Asians (15.5%), non-Hispanic whites (14.8%), and Hispanics (12.8%), p<0.01. **Conclusion:** Over the past 15 years, the proportion of HCC patients with advanced tumor stage has significantly declined, reflecting the success of HCC surveillance programs. However, significant race/ethnic disparities persist with nearly 20% of blacks having advanced HCC at time of diagnosis.



Disclosures:

Robert J. Wong - Advisory Committees or Review Panels: Gilead; Consulting: Gilead; Grant/Research Support: Gilead; Speaking and Teaching: Gilead, Salix, Bayer

The following people have nothing to disclose: Jennifer Wang, Benny Liu, Taft Bhuket

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Market Competition Standardizes Liver Transplant Waitlist Management Practices

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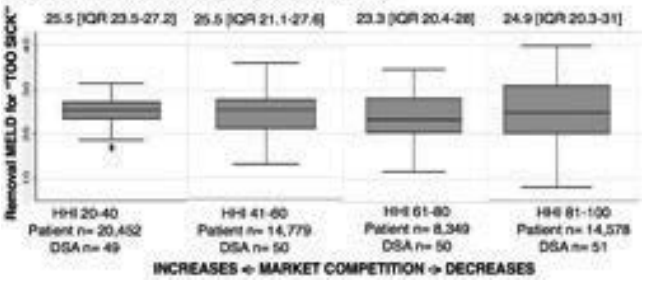
Background: Geographic inequity in access to liver transplantation associated with market competition (MC) has been previously characterized. However, waitlist management practices at the donor service area (DSA) level with respect to delisting for "too sick to transplant" are unknown. We hypothesized that market forces may influence decisions to delist, as well. Methods: We obtained delisting, transplant, and other demographic data from the Scientific Registry of Transplant Recipients for 58,158 adult patients listed for liver transplantation, excluding status 1 and exception point patients from 2002 to 2012. Linear regression analysis was used to assess the effect of medical and non-medical factors on Model for End Stage Liver Disease at removal for "too sick to transplant" (TS). MC was measured with the Herfindahl-Hirschman Index (HHI). F-test of equity of variances was used to assess variation. Results: On adjusted analysis, less MC predicted higher TS. For every unit decrease in MC, the TS increased by 0.03 points (p <0.001). Among single center DSAs, TS ranged from 8 to 40, while variation in highly competitive DSAs was much smaller (all p <0.001) (Figure 1). Conclusions: TS increases with MC, while variation decreases. In other words, increased MC is associated with more aggressive acceptance of patients for transplantation. More importantly, the definition of "too sick to transplant" converges with increasing MC, suggesting that transplant center practices may naturally standardize without policy implementation and simply by including more transplant centers in DSAs.

Figure 1. Market Competition Standardizes Removal MELD for "too sick to transplant". *HHI, Herfindahl-Hirschman Index; DSA, Donor Service Area*



Market Competition Standardizes Removal MELD for "too sick to transplant". HHI, Herfindahl-Hirschman Index; DSA, Donor Service Area

Disclosures:

The following people have nothing to disclose: Yanik J. Bababekov, Ya-Ching Hung, David Chang, Joel T. Adler, Elizabeth Pomfret, James Markmann, Heidi Yeh

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Trends in pregnancy in women with liver disease: 1983 - 2017

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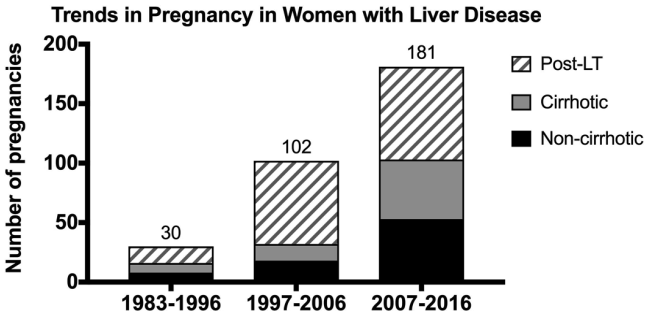
Background The overall burden of liver disease is increasing worldwide. Moreover, the proportion of women of reproductive age diagnosed with chronic liver disease (CLD) or undergoing liver transplantation (LT) is also rising. This has to led to a growing need for multidisciplinary input into the management of such pregnancies. Since 2014, we have offered a combined hepatology & obstetrics clinic. We aimed to review the changing trends in pregnancy rates & outcome in this population over the past 3 decades. **Methods** Retrospective analysis of all pregnancies occurring in women with CLD or following LT between 1983-2017 at King's College Hospital. Patients were identified from a prospectively collated database & data was extracted from patient records. Data was analyzed using GraphPad Version7. We compared the results of pregnancies occurring in 3 time periods: 1983-1996, 1997-2006, and 2007-2017. **Results** 327 conceptions occurred in 193 women: 80 in 48 cirrhotic women, 85 in 52 non-cirrhotic women & 162 in 93 women post-LT. Aetiology of liver disease included autoimmune hepatitis (35%), metabolic/congenital (18%), drug toxicity (7%), PBC/PSC (7%), biliary atresia (6%), seronegative liver failure (7%) & viral hepatitis (6%). Data was available from 313 conceptions for comparison. The number of pregnancies had increased from 30 pre-1997, to 102 between 1997-2006 & to 181 since 2007 (Figure 1). Median age at conception was similar; 28years for 1983-1997 (range 23-37), 29years for 1997-2006 (17-49) & 29years since 2007 (16-42). The corresponding live birth rates were 73%, 70% & 87% respectively. The prematurity rate (gestation <37weeks) had reduced significantly; 67%, 31% & 26% (p=0.013). The corresponding rates of elective terminations were 10%, 10% & 4%; miscarriages 17%, 20% & 7%; and stillbirths 0%, 1% & 1%. For those with available data (n=218), the rate of caesarean section was 80%, 33% & 50%. **Conclusion** The number of pregnancies in women with CLD or

following LT has risen over the past 3 decades. The proportion of pregnancies reaching term has increased significantly. Moreover, pregnancy outcomes were favourable for the majority of women.



Disclosures:

Mary Cannon - Advisory Committees or Review Panels: Gilead; Speaking and Teaching: Gilead

Michael A. Heneghan - Consulting: Novartis; Speaking and Teaching: Falk Pharma

The following people have nothing to disclose: Tiong Yeng Lim, Enoka Gonsalkorala, Stella Gabeta, Leonie Penna

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Serum soluble programmed cell death 1 level as a key predictor for spontaneous functional cure of chronic hepatitis B patients

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Background and aims: The functional cure, seroclearance of hepatitis B surface antigen (HBsAg), is a desired goal for patients with chronic hepatitis B (CHB), who often show weak or absent virus-specific immunity due to programmed cell death 1 (PD-1)-associated T cell exhaustion. We aim to assess soluble PD-1 (sPD-1) as a novel seromarker for spontaneous HBsAg seroclearance. **Method:** Baseline and follow-up serum level of sPD-1 was evaluated in all 1,046 patients from the REVEAL-HBV study who were hepatitis B e antigen-seronegative with serum HBV DNA undetectability until HBsAg seroclearance or the end of follow-up. sPD-1 levels were categorized in four groups (≥4000, 536-3999, 125-535, <125 pg/mL) according to upper and lower limits, and median level of quantification. We used multiple regression analyses to assess associations of baseline sPD-1 level with HBsAg decline during follow-up, and of baseline sPD-1 and its reduction with spontaneous HBsAg seroclearance. Odds ratio (OR) and rate ratio (RR) with 95% confidence interval (95% CI) were used to estimate magnitudes of associations. **Results:** A total of 390 patients achieved spontaneous HBsAg seroclearance during a mean 5.4 years of follow-up. Baseline sPD-1 levels were correlated with qHBsAg levels (P<0.0001), and associated with decline in HBsAg during follow-up (OR (95% CI) for ≥0.5 log annual decline= 4.7 (1.7-12.8), P=0.002 for baseline sPD-1 <536 vs. ≥4000 pg/mL). Incidence rates of spontaneous HBsAg seroclearance increased with decreasing levels of baseline sPD-1 from 11.5, 61.7, 96.7, to 151.0 per 1000 person-years for sPD-1 levels ≥4000, 536-3999, 125-535, and <125 pg/mL, respectively (P_{trend}<0.0001). After adjustment for gender, age, and alanine aminotransferase level, the RR (95% CI) of HBsAg seroclearance was 5.7 (3.8-8.6), 8.9 (6.0-13.2), and 14.6 (9.9-21.6), respectively, for baseline sPD-1 levels of 536-3999, 125-535, and <125 pg/mL compared to sPD-1 levels of ≥4000 pg/mL. Among patients with baseline sPD-1 levels ≥4000 pg/mL, those who reduced sPD-1 to <125 pg/mL during follow-up had an OR (95% CI) of HBsAg seroclearance of 68.6 (13.2-357.4) compared to those who persistently had sPD-1 >125pg/mL. Four SNPs near the *SDC4* gene were associated with baseline sPD-1 levels and subsequent HBsAg seroclearance in a genome-wide association analysis. **Conclusion:** Reduced sPD-1 levels strongly predict greater chance of HBsAg decline and HBsAg seroclearance in HBeAg-seronegative CHB patients with HBV DNA undetectability. This seromarker may help in patient stratification, and might be considered as a promising target for achieving functional cure for CHB patients.

Disclosures:

The following people have nothing to disclose: Hui-Han Hu, Mei-Hung Pan, Chia-Ling Chang, Rachel Wen-Juei Jeng, Yen-Tsung Huang, Chien-Yu Su, Sheng-Nan Lu, Li-Yu Wang, Li-Rung Huang, Chien-Jen Chen, Hwai-I Yang

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Serum HBV RNA level predicts response to peginterferon add-on therapy for HBeAg-positive chronic hepatitis B

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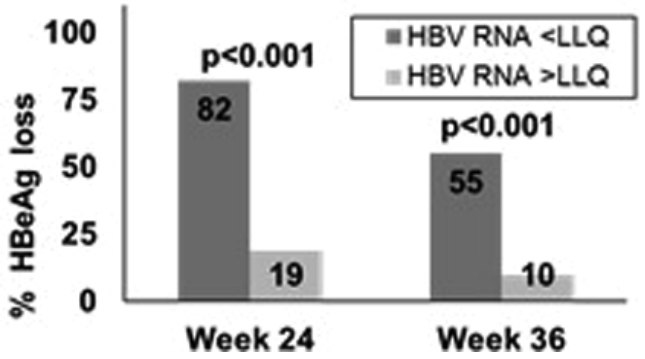
Background Hepatitis B RNA (HBV RNA) is a new serum marker that is a direct product of the cccDNA and is associated with response to nucleos(t)ide (NA) and peginterferon (PEG-IFN) monotherapy. We aimed to study if HBV RNA can predict response to PEG-IFN add-on therapy. **Methods** HBV RNA levels were measured in stored serum samples of 175 HBeAg-positive chronic HBV patients who were treated within an international trial (ARES study). All patients started ETV 0.5 mg/day and were after 24 weeks allocated to ETV monotherapy (n=90) or addition of 24 weeks of PEG-IFNα2a 180 mcg/week (n=85). All patients were followed until week 72. HBV RNA was measured at week 0, 12, 24, and 36 using a RACE-PCR technique (LLQ

800 c/mL). We studied the association with HBeAg loss at week 72. **Results** The mean age was 32 (SD 10) years, 125 (71%) were male, and HBV genotype distribution was 7/19/42/31% for genotype A/B/C/D. The mean HBV RNA at baseline was 6.1 (1.1) $\log_{10}$ c/mL. Correlations with serum HBV DNA and qHBsAg were r=0.8, p<0.001 and r=0.6, p<0.001, resp. After 24 weeks of ETV, mean HBV RNA had declined to 4.7 (1.5) log c/mL, and HBV RNA was <LLQ in 30 (17%) patients. After randomization, mean HBV RNA in the PEG-IFN add-on arm decreased, in contrast to HBV RNA in ETV monotherapy (week 36: -0.97 vs. +0.30 log, p<0.001). At week 72, HBeAg was negative in 27/85 (32%) of the PEG-IFN add-on group vs. 16/90 (18%, p=0.04) in ETV monotherapy group. In patients allocated to add-on, HBeAg loss occurred in only 12/64 (19%) with HBV RNA > LLQ at week 24 vs. in 9/11 (82%) with HBV RNA <LLQ (p<0.001). HBV RNA level at randomization was independently associated with HBeAg loss at week 72 (OR 0.4, CI-95% 0.2-0.6, p<0.001, adjusted for therapy allocation, HBV genotype, presence of PC or BCP, and ALT). **Conclusion** PEG-IFN add-on after 24 weeks of ETV pretreatment accelerated HBV RNA decline. HBV RNA level at the end of ETV, before PEG-IFN addition, was associated with subsequent HBeAg loss. Serum HBV RNA testing may help to select NA-treated HBeAg-positive patients who are most likely to benefit from PEG-IFN add-on treatment.



Disclosures:

Robert J. de Knegt - Advisory Committees or Review Panels: Norgine, Janssen Cilag, AbbVie, Roche; Grant/Research Support: Janssen Cilag, BMS, AbbVie, Philips, Roche; Speaking and Teaching: Roche, Janssen Cilag, AbbVie, Gilead

Andre Boonstra - Advisory Committees or Review Panels: Gilead; Grant/Research Support: BMS, Janssen Pharmaceutics, Merck, Roche, Gilead, Fujirebio

Thomas Berg - Advisory Committees or Review Panels: Gilead, BMS, Roche, Tibotec, Vertex, Jannsen, Novartis, Abbott, Merck, Abbvie, Intercept, Norgine; Consulting: Gilead, BMS, Roche, Tibotec; Vertex, Janssen; Grant/Research Support: Gilead, BMS, Roche, Tibotec; Vertex, Jannssen, Merck/MSD, Boehringer Ingelheim, Novartis, Abbvie; Speaking and Teaching: Gilead, BMS, Roche, Tibotec; Vertex, Janssen, Merck/MSD, Merck, Bayer, Abbvie, Novartis, Norgine, Intercept, SIRTEX, Falk

Harry L. Janssen - Consulting: AbbVie, Bristol Myers Squibb, GSK, Gilead Sciences, Innogenetics, Merck, Roche, Janssen, Medimmune; Grant/Research Support: AbbVie, Bristol Myers Squibb, Gilead Sciences, Merck, Roche, Janssen, Medimmune

The following people have nothing to disclose: Margo J. van Campenhout, Florian van Bömmel, Willem Pieter Brouwer, Qing Xie, Fehmi Tabak, Adrian Streinu-Cercel, Jiyao Wang, Anneke J. van Vuuren, Bettina E. Hansen

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REAL-B (Real-world Effectiveness from the Asia Pacific Rim Liver Consortium for HBV) – A Risk Score for the Prediction of Hepatocellular Carcinoma (HCC) in Chronic Hepatitis (CHB) Patients Treated with Oral Anti-HBV Therapy

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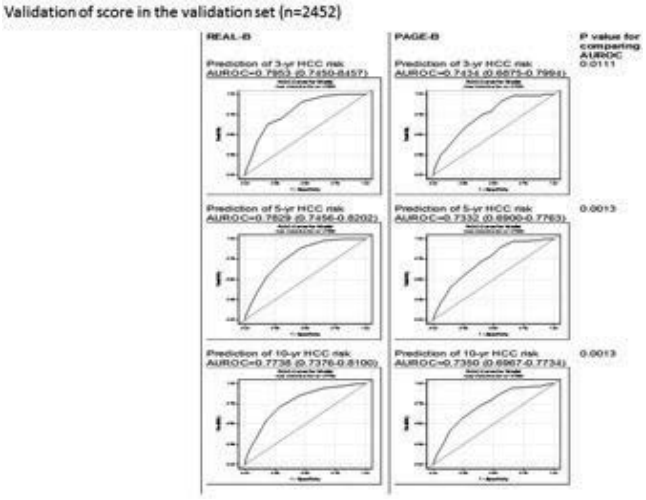
Background: The PAGE-B score was developed to predict HCC risk in treated Caucasian CHB patients. This study developed, validated and compared a non-invasive risk score (REAL-B score) to predict HCC in a large consortium of Asian Americans and Asia Asians with CHB on oral antiviral therapy. **Methods:** Data were from the REAL-B consortium database (n=9106 treated CHB patients; 6 US and 11 centers from 5 Asia Pacific countries). Patients excluded (n=1752) had HCC at baseline, within 1 year from study entry or treatment date before study. For risk score development, patients were randomly divided into 2 groups: derivation group [(DG), n=4902; 368 HCC cases] and validation group [(VG) n=2452; 173 HCC cases]. Cox regression analyses determined HCC predictors. **Results:** Overall, median age 49.0 (39.2-57.0) years; majority male (70%), no cirrhosis (82%) or decompensated cirrhosis (92%) at baseline, HBeAg negative (65%), HBV genotype B (59%), CPT class A (82%), median MELD score 6.3 (6.0-7.4) There were no significant differences in demographics between DG/VG groups. In the DG, cirrhosis at baseline (HR: 2.59, 95% CI: 2.00-3.36, p<0.0001; score=2) and platelet count <100 (HR: 2.24, 95% CI 1.54-3.25, p<0.001; score=2) were strongest HCC predictors followed by age, male gender, diabetes mellitus, lower platelet count and AFP >20; variables formed a 13-point REAL-B score to predict 10-year HCC risk range 0.004 to 0.83. In VG cohort, PAGE-B score produced an area under the curve (AUROC) of 0.74-0.73 predicting HCC risk at 3, 5, 10 years respectively. The REAL-B score

performed significantly better than PAGE-B predicting risk of 3, 5, 10-year HCC development with AUROC of 0.80-0.77 (p=0.0111-0.0013), respectively (Figure). Using the REAL-B score, the projected cumulative incidence of HCC at the 10th year in VG was 4.1%, 17.4%, and 63.6% for low, medium, and high score groups, respectively. **Conclusion:** The REAL-B score is an updated model based on five physiologic variables and the presence of diabetes mellitus and has a high 10-year predictability for HCC development in Asian CHB patients on treatment.



Disclosures:

Mindie H. Nguyen - Advisory Committees or Review Panels: Dynavax Laboratories, Gilead Sciences, Inc., Alynam Pharmaceuticals, Intercept Pharmaceuticals; Grant/Research Support: Gilead Sciences, Inc., Bristol-Myers Squibb, Janssen Pharmaceuticals

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Huy N. Trinh - Grant/Research Support: Intercept, Abbvie, merk, Gilead; Speaking and Teaching: Gilead; Stock Shareholder: Gilead

Jian Q. Zhang - Speaking and Teaching: Gilead Sciences

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Yoshiyuki Ueno - Grant/Research Support: Gilead Sciences, MSD, Abbvie; Speaking and Teaching: BMS

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Norihiro Furusyo - Grant/Research Support: MSD K.K., Gilead Sciences K.K., Bristol-Myers Squibb, Janssen Pharmaceutica

Ming-Lung Yu - Advisory Committees or Review Panels: ABBOTT, MSD, ABBVIE, GILEAD, J&J, ROCHE, BMS; Consulting: MSD, ABBVIE, GILEAD, J&J, ROCHE, BMS; Grant/Research Support: ABBOTT, ROCHE, MSD, ABBVIE, GILEAD, ABBVIE, GILEAD, ROCHE, BMS; Speaking and Teaching: ABBOTT, ROCHE, MSD, GILEAD, BMS, GSK

The following people have nothing to disclose: Hwai-I Yang, Ming-Lun Yeh, Grace L. Wong, Chien-Hung Chen, Ka-Shing Cheung, Tung-Hung Su, Ritsuzo Kozuka, Eiichi Ogawa, Jiayi Li, Chris Wong, Joseph Hoang, Chia-Hsin Lin, Huichun Xing, Masaru Enomoto, Jia-Horng Kao, Man Fung Yuen

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Polymorphism of HLA-DRB1 3'UTR (rs9272105), GRIK1 (rs455804), KIF18 (rs17401966) are associated with spontaneous HBsAg seroclearance in HBeAg seronegative chronic hepatitis B carriers.

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BACKGROUND: The role of polymorphisms on HLA immune region in predicting long term outcome of patients with chronic hepatitis B (CHB) is unclear. This study aims to examine whether polymorphisms in HLA region have predictive effects on spontaneous HBsAg seroclearance among HBeAg seronegative CHB patients. **METHODS**: A total of 2672 HBeAg seronegative CHB patients were enrolled in this study. Eleven SNPs, rs2856718 (HLA-DQB1 3'UTR), rs7453920 (HLA-DQB2), rs3077 (HLA-DPA1), rs9277535 (HLA-DPB1), rs7574865 (STAT4), rs17401966 (KIF1B), rs455804 (GRIK1), rs9275319 (HLA-DQB1 3'UTR), rs9272105 (HLA-DRB1 3'UTR), rs3957146 (HLA-DQA2), and rs17615141(HLA-DQB1) were genotyped by TaqMan genotyping assay. Associations between the candidate SNPs and HBsAg seroclearance were assessed by two steps cox regression analysis (step one: SNP only; step two: multivariate analyses adjusted for known predictive factors). **RESULTS:** Among 2672 HBeAg seronegative CHB patients, 507 (19%) achieved spontaneous HBsAg seroclearance. Among the 11 SNPs analyzed in step one, rs17401966 GG genotype (MAF: 0.299) and rs9272105 GA/GG genotype (MAF: 0.494) are significantly associated with lower likelihood of HBsAg seroclearance with adjusted hazard ratio (HR) and 95% confidence interval (CI) of 0.59 (0.44-0.78), P=0.0002 and 0.81 (0.70-0.93), P=0.0025, respectively; while rs9277535 AA genotype (MAF:0.256) and rs455804 CA/AA genotype (MAF: 0.32) had borderline association with HBsAg seroclearance [HR (95% CI)= 1.36 (1.08-1.70), P=0.008 and 1.17 (1.03-1.33), P=0.0135, respectively]. In step two, after adjusting gender, cirrhosis, smoking, alcohol, HBV DNA, and HBsAg, patients who carried rs17401966 GG genotype and rs9272105 GA/GG genotype had significantly lower chance to achieve HBsAg seroclearance [HR (95% CI)= 0.60 (0.46-0.80), P=0.0004 and 0.87 (0.76-1.00), P=0.0466, respectively], and patients who carried rs45584 CA/CC genotype had higher chance of HBsAg seroclearance [HR (95% CI)= 1.15 (1.02-1.32), P=0.0233]. **CONCLUSIONS:** HBeAg seronegative CHB patients carried the GA/GG genotype of rs9272105 (HLA-DRB1 3' UTR,) and the GG genotype of rs17401966 (KIF1B) had lower likelihood of achieving spontaneous HBsAg seroclearance; while those carried the CA/CC genotype of rs455804 (GRIK1) were prone to achieve HBsAg seroclearance during follow-up.

Disclosures:

The following people have nothing to disclose: Rachel Wen-Juei Jeng, Hui-Han Hu, Jessica Liu, Yu-Ju Chu, Chia-Ling Chang, Mei-Hsuan Lee, Jaw-Ching Wu, Chien-Jen Chen, Hwai-I Yang

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Prevalence of hepatitis D viremia among injection drug users in San Francisco

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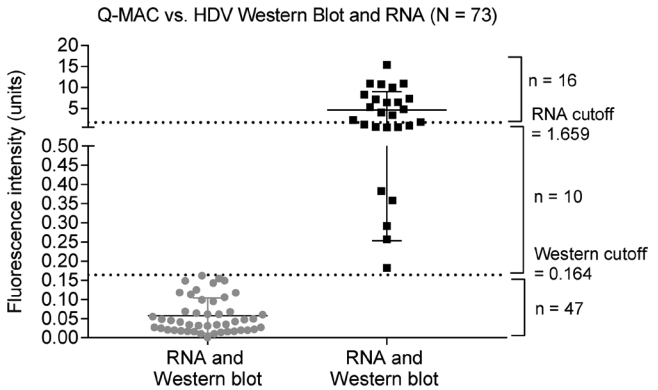
Background: People who inject drugs (PWID) have a high risk of acquiring infection with HDV, which needs HBV for its life cycle. HDV RNA positive people are more likely to transmit the virus, however, most studies that evaluated HDV prevalence have focused on anti-HDV measurement. Herein, we report prevalence of HDV viremia in PWID and evaluate performance of a new quantitative microarray antibody capture (Q-MAC) assay. **Methods:** We included PWID enrolled in San Francisco (1998-2000) and previously tested for HBV and HCV infection. Plasma samples from subjects who were positive for hepatitis B surface antigen (HBsAg) were tested with Q-MAC, HDV Western blot and HDV RNA assays. **Results:** Among 2,296 PWID, 73 (3.2%) were HBsAg-positive of whom, 26 were positive by HDV Q-MAC. All 26 also tested positive by both Western blot and HDV RNA assays. The figure shows performance characteristics of the Q-MAC assay in predicting anti-HDV or RNA positivity (based on previously suggested fluorescent intensity cut-offs). The prevalence of HDV viremia was 1.1% in the overall cohort, 1.5% among participants who were ever infected with HBV (HBV core antibody positive; n=1,764) and 35.6% in HBsAg-positive. Among PWID who were ever infected with HBV, HDV viremia was more common in those with resolved HCV compared to those with chronic HCV infection (5.1% vs. 0.6%; adjusted odds ratio, 9.8; p<0.0001). **Conclusion:** The novel Q-MAC assay performed well in predicting HDV infection in PWID. HDV viremia was very frequent in PWID who were positive for HBsAg, but uncommon overall due to the low prevalence of HBV infection. HCV viremia was inversely associated with HDV viremia.



Prediction of:	Sensitivity	Specificity	PPV	NPV
Western Blot	100%	100%	100%	100%
HDV RNA	61.5%	100%	100%	82.5%

Disclosures:

Fan-Chen Tseng - Employment: United BioPharma Inc.

The following people have nothing to disclose: Parag Mahale, Peter V. Aka, Xiaohua Chen, Sabrina Chen, Thomas O'Brien

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The Characteristics and Predictors of Postpartum Hepatitis Flares in Women with Chronic Hepatitis B

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Background We aimed to characterize postpartum disease flares among mothers with chronic hepatitis B (CHB). Mothers with CHB were compared to non-infected mothers in terms of postpartum alanine aminotransferase (ALT) abnormalities. **Methods** Healthy and CHB mothers were screened for current study. The inclusion criteria were test results of a liver panel available within 8 weeks before delivery, at delivery, and during at least one follow-up with test results available within 16 weeks after delivery. Mothers who had coinfection or received antiviral therapy were excluded. Data including demographic, virological, and biochemical parameters were collected up to postpartum week 16. ALT flares and exacerbations were defined as ALT levels 5–10-times and > 10-times the upper limit of normal, respectively. Outcome assessments included ALT flares/exacerbation and their predictive parameters. **Results** Among 4,236 patients enrolled, 869 were non-infected (group A) and 3,367 had CHB (group B). Infected mothers were further stratified into two subgroups by the presence (B1, n = 1,928) or absence (B2, n = 1,439) of viremia (based on the lowest levels of quantitation of hepatitis B virus [HBV] DNA [500 copies/mL]). A significantly higher frequency of ALT abnormalities was observed in group B (20.37%, vs. 28.27%, p < 0.001). ALT events mainly occurred in patients in group B1, with flare and exacerbation rates of 5.96% (115/1,928) and 2.96% (57/1,928), respectively. A bimodal pattern was observed in ALT levels in these patients, with the peaks at postpartum weeks 3–4 and 9–12 (Figure 1). In multivariate analysis, elevated ALT levels and detectable levels of HBV DNA at delivery were independent risk factors for postpartum disease flares. Further subgroup analysis in group B1 demonstrated that a cut-off HBV DNA level of 5.0 log10 IU/mL at delivery predicts ALT events with a positive predictive value of 14.4% and negative predictive value of 98.2%. ***Conclusion*** ALT flares/exacerbations are mainly observed in mothers with elevated ALT or HBV DNA levels ≥ 5log10 IU/mL at delivery.

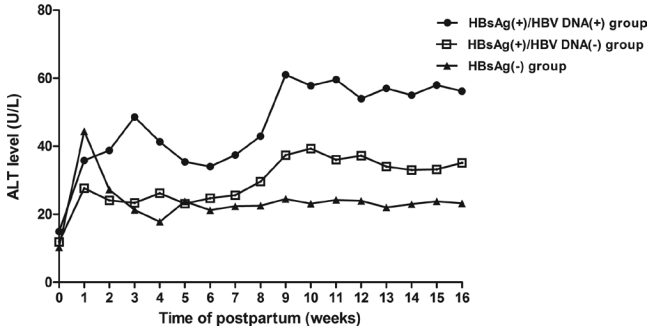

Figure 1. Postpartume ALT changes

Disclosures:

Calvin Q. Pan - Advisory Committees or Review Panels: Gilead; Consulting: BMS, Gilead; Grant/Research Support: BMS, Gilead, Merck; Speaking and Teaching: synergy, Gilead, Salix, Allergan, Abbvie

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LncRNAH19-containing exosomes from cholangiocytes promote cholestatic liver injury in Mdr2 knock out mice

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Background: Extracellular vesicle (EV) plays a critical role in regulating various physiological and pathological processes. Exosomes (Exs) are small EVs (40-150 nm) with a multivesicular endosomal origin. We recently reported that aberrant expression of the long non-coding RNA (LncRNA) H19 was associated with significant down-regulation of nuclear receptor small heterodimer partner (SHP), which was closely correlated with the severity of liver injury in Mdr2$^{-/-}$ mice. Both taurocholic acid (TCA) and estrogen (E2) induced H19 expression in cholangiocytes (CCs), but not in hepatocytes (HCs). However, how CCs communicate with HCs in cholestatic liver injury remains unclear. The aim of this study to determine if Exs are involved in delivery of H19 from CCs to HCs to promote cholestatic liver injury. **Methods:** Age and gender-matched wild type (WT), Mdr2$^{-/-}$ and H19$^{-/-}$ mice were used for *in vivo* studies or for isolation of primary HCs and CCs. The Exs were isolated from CC culture media and mouse serum by ultracentrifugation and characterized by dynamic light scattering analysis, transmission electron microscopy and immunofluorescence staining. The expression of H19 and SHP were determined by real-time PCR and western blot analysis, respectively. Exs from aged female Mdr2$^{-/-}$ mice (100 days old) sera were injected into young female Mdr2$^{-/-}$ mice (50 days old) via tail vein twice a week. One week after the last injection, mice were sacrificed. Livers were processed for pathological and molecular analysis. **Results**: CC-derived Exs contained high levels of H19 and expressed common exosome markers, such as CD63, LAMP1 and LAMP2. In Mdr2$^{-/-}$ mice, H19 level in sera Exs was closely correlated with the severity of cholestatic liver injury as indicated. Exs from 30-day old Mdr2$^{-/-}$ mice contained minimal H19, but Exs from 100-day old mice contained significantly higher levels of H19, especially those from female mice. Treatment of 50-day old female Mdr2$^{-/-}$ mice with Exs from 100-day old female Mdr2$^{-/-}$ mice markedly increased serum bile acid levels and induced fibrotic liver injury, which was associated with downregulation of SHP. Overexpression of H19 significantly suppressed SHP expression in HCs. In contrast, hepatic SHP expression was upregulated in H19$^{-/-}$ mice. In addition, treatment of primary mouse HCs with Exs from TCA- and E2-treated WT primary mouse CCs suppressed SHP expression, but not with Exs from H19$^{-/-}$ primary mouse CC. **Conclusion:** H19-containing Exs from CCs play a critical role in cholestatic liver injury in Mdr2$^{-/-}$ mice. Our study suggests that serum H19-containing Exs can be used as a potential biomarker for cholangiopathies.

Disclosures:

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Activation of the HDC/histamine axis by reintroduction of mast cells or histamine treatment induces hepatobiliary damage, liver inflammation and fibrosis in Mdr2$^{-/-}$/HDC$^{-/-}$ double knockout mice

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Background: Primary sclerosing cholangitis (PSC) is characterized by biliary damage and fibrosis. Multidrug resistance-2 gene knockout (Mdr2$^{-/-}$) mice develop cholestatic liver disease and mimic some characteristics of human PSC. Mast cells (MCs) infiltrate the liver of Mdr2$^{-/-}$ mice and PSC patients increasing local histamine (HA) levels (synthesized by l-histidine decarboxylase (HDC)) and hepatic damage. We have shown that knockout of the HDC/HA axis in Mdr2$^{-/-}$ mice (Mdr2/HDC (DKO)) reduces biliary damage, liver inflammation and hepatic fibrosis. The **aim** of this study was to determine the effects of activation of the HDC/HA axis by reintroducing MCs or HA on PSC-induced damage in DKO mice. **Methods:** 12 week old homozygous DKO mice were injected with tagged MCs (or vehicle) via tail vein 3 days prior to euthanasia or implanted with osmotic minipumps to deliver vehicle or HA (10m/kg/BW) for 2 weeks. H&E and serum liver enzymes were analyzed to evaluate liver damage. We next evaluated: (i) HDC expression by qPCR, (ii) HA serum levels by EIA, and (iii) MC activation by qPCR for chymase, tryptase and c-Kit expression. Changes in biliary proliferation were evaluated by CK-19 and Ki67 immunohistochemistry. Liver fibrosis was evaluated by hydroxyproline assay, Sirius red staining in liver sections, and qPCR for a-SMA and collagen-type 1a. Hepatic stellate cell (HSC) activation was determined by immunofluorescence and qPCR for SYP-9. Liver inflammation was evaluated by staining for F4/80 and qPCR for TNF-a and CCL-2,-3, and -5. TGF-b1 secretion was determined by EIA and gene expression by qPCR. *In vitro,* cultured human HSCs (hHSCs) were stimulated with cholangiocyte supernatants collected from all groups of mice and activation was measured by qPCR for a-SMA and SYP-9. **Results:** Activation of the HDC/HA axis by reintroduction of MCs or HA treatment in DKO mice significantly increased (i) hepatic damage/serum liver enzymes; (ii) the HDC/HA axis and MC activation; (iii) biliary mass/prolifer-

ation; (iv) hepatic fibrosis/inflammation and (v) secretion and expression of TGF-b1 compared to DKO controls. *In vitro,* stimulation with Mdr2$^{-/-}$ mouse cholangiocyte supernatant increased hHSC activation that was reduced by treatment with DKO cholangiocyte supernatants. hHSCs treated with DKO + MC or HA treated cholangiocyte supernatants displayed increased activation compared to DKO controls. **Conclusion:** Our data demonstrates that activation of the HDC/HA axis by reintroduction of MCs or HA treatment promotes PSC-associated damage. Therefore, targeting MC-mediated HDC/HA axis may offer new therapeutic strategies for patients suffering from PSC.

Disclosures:

The following people have nothing to disclose: Laura Hargrove, Jennifer Demieville, Lindsey Kennedy, Joanne Thomson, Kristen Stephenson, Heather L. Francis

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Lysosomal nitrosative stress: A novel mechanism for obesity-associated hepatic insulin resistance

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Obesity greatly increases risk for a wide range of diseases, including diabetes and fatty liver disease. The lysosome is an acidic organelle that has central role in degrading recycling intracellular and extracellular components, cholesterol trafficking, and recycling of receptors including LDLR in the liver. Obesity results in a metabolically driven, low-grade, chronic inflammatory state in the liver, but how this affects lysosome dynamic and contributes to the disruption of glucose and lipid homeostasis are largely unknown. Here we show that obesity activates inducible nitric oxide synthase (iNOS) but disrupts S-nitrosoglutathione reductase (GSNOR) activity in the liver. Together, this results in increased S-nitrosylation of lysosomal enzymes, which consequently impairs the activities of lysosomal enzymes and lysosomal function, engendering lysosomal nitrosative stress. Accordingly, we find that in mice with liver-specific GSNOR deletion, diet-induced obesity (DIO) increases lysosomal nitrosative stress and impairs autophagy, worsening obesity-associated hepatic insulin resistance, steatosis and systemic glucose intolerance. Notably, expression of lysosomal components that are resistant to S-nitrosylation, transcription factor EB (TFEB, a lysosomal master transcriptional factor), or treatment with an autophagy enhancer all lead to improved insulin sensitivity in GSNOR-deficient primary hepatocytes. Conversely, both liver-specific overexpression of GSNOR and liver-specific suppression of iNOS markedly enhance lysosomal function and improve insulin action, steatosis and glucose homeostasis in DIO mice. These results indicate a direct role of the lysosomal nitrosative stress in defective autophagy and hepatic insulin resistance in obesity. Furthermore, we show that enhancing lysosome-specific denitrosylation capacity by overexpression of gamma-interferon-inducible lysosomal thiol reductase (GILT) and increasing GSNOR activity by nicotinamide riboside (NR) treatment both significantly improve autophagy and insulin sensitivity in primary hepatocytes from DIO mice. Finally, we observed that in human samples, NAFLD and diabetes are accompanied by decreases in hepatic GSNOR activity, indicating that this mechanism is conserved and relevant to human disease. Taken together, our study provides the first insight into the inflammatory mechanisms that compromise lysosome in obesity, and how this affects hepatic insulin sensitivity. This may lead to new strategies to develop therapeutics for many inflammatory diseases including fatty liver disease.

Disclosures:

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ZFP36L1 controls adiposity and steatosis via post-transcriptional regulation of bile acid metabolism

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Bile acids are detergents and important signaling molecules that activate the nuclear receptor FXR to control key metabolic processes, including feedback mechanisms to maintain bile acid homeostasis. Activation of FXR decreases the mRNA levels of several bile acid synthetic genes, including the expression of the gene encoding the rate-limiting enzyme of bile acid synthesis, *Cyp7a1*. In the present study, we show that *Cyp7a1* mRNA levels were very rapidly reduced (less than 30 minutes) following pharmacologic FXR activation in wild-type, but not *Fxr*$^{-/-}$ or liver-specific *Fxr* knockout mice (*Fxr*$^{L\text{-}KO}$). The rapid decrease in *Cyp7a1* mRNA was suggestive of a previously unidentified post-transcriptional mechanism. To identify the molecular mechanism, we used FXR ChIP-Seq and RNA-Seq to identify the RNA binding protein *Zfp36l1* as a novel FXR target gene. We showed that FXR activation using synthetic and endogenous agonists increases ZFP36L1 mRNA and protein levels in wild-type, but not *Fxr*$^{-/-}$ mice and the increased ZFP36L1 mRNA and protein was rapid, within 30 minutes after treatment with an FXR agonist. ZFP36L1 is a *bona-fide* RNA binding protein that promotes degradation of mRNA targets by binding to AU-rich elements (AREs) in the 3′ UTR. We generated *in vivo* and *in vitro* gain-of-function models and used reporter assays to show that ZFP36L1 targets the *Cyp7a1* UTR. We identified a 200bp region of the *Cyp7a1* 3′ UTR that contains tandem AREs that were required for the ZFP36L1 regulation of *Cyp7a1.* In mice, we show that hepatic overexpression of ZFP36L1 decreased *Cyp7a1* mRNA and decreased bile acid levels. To complement our gain-of-function studies, we generated liver-specific *Zfp36l1* knockout mice (*Zfp36l1*$^{L\text{-}KO}$) and we show that loss of *Zfp36l1* in hepatocytes resulted in elevated *Cyp7a1* mRNA and bile acid levels as well as reduced plasma cholesterol levels, consistent with increased bile acid synthesis. Given that bile acids are important signaling metabolites that also control lipid absorption, we investigated whether loss of hepatic *Zfp36l1* resulted in other metabolic disturbances. When littermate *Zfp36l1*$^{flox\text{-}flox}$ and *Zfp36l1*$^{L\text{-}KO}$ were fed a Western diet, we observed that *Zfp36l1*$^{L\text{-}KO}$ mice had reduced body weight gain, specifically in adipose tissue. In addition, *Zfp36l1*$^{L\text{-}KO}$ mice also had markedly reduced hepatic steatosis. The differences in adiposity and steatosis were attributed to reduced lipid absorption, which is consistent with altered bile acid metabolism. Thus, we have identified a novel pathway that controls *Cyp7a1* and bile acid metabolism but may also have wider implications in diseases such as obesity and hepatosteatosis.

Disclosures:

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Hepatocyte-derived mitochondrial DNA induces fibrogenic activation of stellate cells and promotes fibrosis in non-alcoholic steatohepatitis

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BACKGROUND/AIMS: The mechanisms of transition from hepatic steatosis to progressive non-alcoholic steatohepatitis (NASH) remain elusive. Mitochondria, abundant in the liver, share structural similarities with bacteria and its components can be recognized as "danger signals" if released from damaged cells. We hypothesized that mitochondrial DNA (mtDNA) species released from injured hepatocytes promote fibrosis in NASH. **METHODS:** Lipoapoptosis was induced by palmitic acid (PA) in primary hepatocytes in vitro. Liver steatosis and steatohepatitis were induced in C57Bl/6 mice fed with methionine-choline-deficient diet (MCD). Serum mtDNA levels were detected and quantified by real-time PCR of two mtDNA-specific sequences in 54 treatment-naïve NAFLD/NASH patients with different stages of the disease and 14 healthy controls and MCD-fed mice. Effects of crude mito-DAMPs and purified native mtDNA prepared from liver mitochondria on the fibrogenic activity of hepatic stellate cells (HSC) were studied in vivo and in vitro. **RESULTS**: PA-induced lipoapoptosis in primary hepatocytes in vitro lead to mtDNA release into the supernatant. Circulating mtDNA levels in serum were elevated 5-fold in NAFLD/NASH patients compared to healthy subjects ($p<0.01$). Serum mtDNA increased several-fold in mice with steatohepatitis starting from 4 and peaking at 8 weeks of MCD feeding ($p<0.05$), and correlated with the degree of fibrosis. Intravenous administration of mito-DAMPs or purified mtDNA at physiological doses (10μg/mouse) induced robust upregulation of α-SMA expression in HSC in mice primed with short-term MCD feeding, as determined by in situ immunostaining and immunoblotting of liver lysates 24h post-mtDNA ($p<0.05$). Activation of HSC was following by 2-3-fold increases in pro-fibrogenic gene expression (TGFβ1, procollagen α1(I) and TIMP-1) 48 hours after mtDNA administration ($p<0.05$). In vitro, the addition of both crude mito-DAMPs and purified mtDNA (1-5μg/ml) induced significant and dose-dependent upregulation of α-SMA expression and cell proliferation in primary HSC cultures (*$p<0.05$*). **CONCLUSIONS**: 1) mtDNA is elevated in serum of NAFLD/NASH patients 2) In mice with NASH, mtDNA is released from hepatocyte during lipoapoptosis, circulates in serum and correlates with fibrosis progression. 3) Administration of purified mtDNA is sufficient to directly activate hepatic stellate cells in vivo and in vitro. Our results suggest hepatocyte-derived mtDNA promotes fibrosis progression in NAFLD/NASH.

Disclosures:

Yury Popov - Consulting: Morphic Therapeutics, Inc; Grant/Research Support: Gilead Sciences, Inc, Pharmakea, Inc, Enanta Pharmaceutical, Inc, Biogen, Inc

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A Negative Feedback Circuit of miR-378, NRF1, AMPK, SIRT1, NF-κB and TNFα Controls the Development of Hepatosteatosis and NASH Progression

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Background: The progression of hepatosteatosis to liver injury is a critical step to hepatocarcinogenesis. However, the mechanistic pathways that link both disorders are essentially unknown. Our study was designed to determine the role of microRNA-378 (miR-378) in the pathogenesis of hepatosteatosis and its potential involvement in liver injury. **Methods:** Database mining, 5'RACE and Northern Blot were used to identify miR-378 primary transcript. Wild-type mice kept on a high fat diet (HFD) received tail-vein injections of miR-378-ASO (anti-sense oligonucleotide) or a mini-circle expression system of miR-378 for 8 weeks. Livers and blood were collected for pathological and molecular analysis. **Results**: MiR-378 expression was increased in livers of HFD-treated mice. Although miR-378 locates within the intron of *Ppargc1*β, it possesses its own transcription machinery which is independent of *Ppargc1*β and under the control of Nrf1. Both function and expression of *Ppargc1*β and miR-378 is inversely correlated in fatty livers. Further studies revealed that miR-378 directly targeted *Nrf1* and *Prkag2* which encodes nuclear respiratory factor 1 and protein kinase AMP-activated non-catalytic subunit gamma 2 (AMPKγ2) respectively. Activation of miR-378 expression in livers impaired the ability of hepatocytes to oxidize fatty acid, promoted hepatosteatosis, and induced hepatic inflammation and fibrosis. Antagonizing miR-378 in livers improved fatty acid oxidation of hepatocytes and alleviated hepatosteatosis, while additional knockdown of up-regulated *Nrf1* due to miR-378 deficiency offset the inhibitory effect of miR-378-ASO on hepatosteatosis. The results indicated that *Nrf1* mediated the role of miR-378 in promoting hepatosteatosis. Liver-specific expression of miR-378 also aggravated hepatic inflammation and fibrosis in HFD-treated mice by facilitating TNFα signaling. Mechanistically, miR-378 impaired AMPK signaling to activate SIRT1 that impairs the transactivation capacity of the NF-κB by deacylating p65. Meanwhile, *Nrf1* knockdown in the liver impaired biogenesis of miR-378 and simulated the role of miR-378 in inducing hepatosteatosis and liver injury. **Conclusion**: A negative feedback loop circuit consisting of miR-378, NRF1, AMPK, SIRT1, NF-κB and TNFα promoted hepatosteatosis and its progression to liver injury. Once this circuit is triggered, it maintains activation of miR-378 biogenesis, suppression of fatty acid oxidation, and activation of the AMPK-SIRT1-NF-κB-TNFα pathway. These results identify miR-378 as a molecular switch for hepatosteatosis and its progression to liver injury in addition to its potential as a therapeutic target for both conditions.

Disclosures:

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Novel Multiparametric Magnetic Resonance Elastography (MRE) Protocol Accurately Predicts Early NASH and Disease Activity

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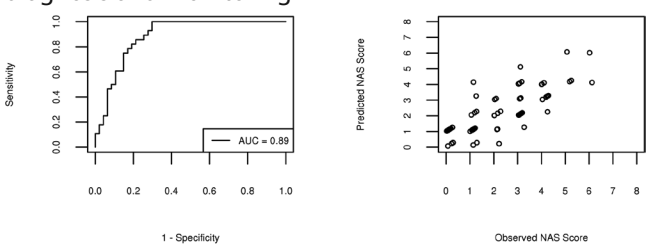
Background: The lack of a reliable, noninvasive method to diagnose nonalcoholic steatohepatitis (NASH) is a major unmet need. We examined the role of a multiparametric magnetic resonance elastography (MRE) protocol for the detection of NASH before onset of fibrosis. **Methods:** Obese subjects scheduled to undergo bariatric surgery were prospectively enrolled. Multifrequency 3D MRE (at 30, 40 and 60 Hz) was used prior to surgery to assess which viscoelastic imaging parameters correlated with early histologic changes in NASH, and MRI proton density fat fraction (MRI-PDFF) was used to quantify steatosis. NASH was diagnosed based on liver biopsy obtained during surgery, and the histologic NAFLD activity score (NAS) was calculated based on the NASH Clinical Research Network criteria. The imaging parameters that correlated with steatosis, lobular inflammation and ballooning were selected using logistic regression. These imaging parameters were included into a predictive regression model of NASH diagnosis. **Results:** A total of 83 subjects were included, of whom 37 had biopsy-proven NASH (45%). Only 3 subjects had advanced fibrosis (F3-4). From the complex shear modulus output generated by MRE at multiple mechanical frequencies, the damping ratio (loss modulus/storage modulus) at 30Hz and shear stiffness at 60Hz correlated with lobular inflammation and hepatocellular ballooning, respectively. The fat fraction obtained from MRI-PDFF best correlated with steatosis ($p<0.05$). These 3 parameters were fit into a generalized linear model which had high predictive performance of biopsy-proven NASH: AUROC=0.89 (Figure-left panel). When classified by predicted case probability ≥0.5, the performance parameters were the following: sensitivity=0.68, specificity=0.85, PPV=0.73, NPV=0.82. The relative agreement between the MRI-based predictive NAS and histologic NAS is illustrated in the right panel. **Conclusion:** Multifrequency 3D MRE allows identification of novel imaging parameters that predict early NASH and disease activity with high accuracy. This imaging biomarker represents a promising alternative to liver biopsy for NASH diagnosis and monitoring.



Disclosures:

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BMS-986036 (pegylated FGF21) in patients with non-alcoholic steatohepatitis: A phase 2 study

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Background: BMS-986036 is a pegylated analogue of human fibroblast growth factor 21, a key regulator of metabolism. In preclinical models of non-alcoholic steatohepatitis (NASH), BMS-986036 improved steatosis, inflammation, hepatocyte ballooning, and fibrosis. In a Phase 2 study in obese patients with type 2 diabetes, it improved insulin sensitivity, lipids, adiponectin, and fibrosis biomarkers. This Phase 2 study evaluated the safety, tolerability, and efficacy of BMS-986036 in NASH patients. **Methods**: This was a multicenter, randomized (1:1:1), double-blind, placebo-controlled study in adults with body mass index ≥25 kg/m^2, biopsy-confirmed NASH (F1-F3), and hepatic fat fraction ≥10%, assessed by magnetic resonance imaging – proton density fat fraction (MRI-PDFF). Randomization was stratified by diabetes status. Patients received subcutaneous injections of BMS-986036 10 mg daily (QD), BMS-986036 20 mg weekly (QW), or placebo QD for 16 weeks. The primary efficacy endpoint was absolute change in MRI-PDFF at Week 16; exploratory endpoints included serum pro-C3 (N-terminal type III collagen propeptide, a fibrosis biomarker), ALT, AST, and, in a subset of patients, liver stiffness, assessed by MR elastography (MRE). **Results**: 74 patients were treated (median age, 51.5 years; women, 65%; type 2 diabetes, 38%; mean hepatic fat fraction, 19.5%). Baseline characteristics, including histology, imaging, and biochemical parameters, were comparable among groups. 68 patients had MRI-PDFF data at both Baseline and Week 16. At Week 16, both BMS-986036 regimens significantly reduced MRI-PDFF versus placebo and improved pro-C3, MRE, adiponectin, ALT and AST (Table). The most frequent AEs in BMS-986036-treated patients were diarrhea (17% vs 8% [placebo]), nausea (15% vs 8%), and frequent bowel movements (10% vs 0%); most of these were mild, none was severe. Serious AEs occurred in 2 patients; none was considered treatment-related. There were no deaths and no discontinuations due to AEs. **Conclusions**: BMS-986036, QD and QW for 16 weeks, compared with placebo, significantly decreased hepatic fat fraction in patients with NASH (F1-F3). BMS-986036 also improved biomarkers of fibrosis (MRE and pro-C3), adiponectin, and markers of hepatic injury (ALT and AST). These results suggest that BMS-986036 has beneficial effects on steatosis, liver injury, and fibrosis in NASH.

Disclosures:

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Accuracy of vibration controlled transient elastography (VCTE) in grading steatosis and staging fibrosis in patients with nonalcoholic fatty liver disease (NAFLD): a prospective multicenter study

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Previous studies which evaluated the performance of VCTE in patients with NAFLD have used the M probe and found a higher failure rate in NAFLD compared to other liver diseases, probably due to obesity. Recent availability of an extra-large (XL) probe and automatic probe selection software, which chooses the probe based on skin to liver capsule distance, has reportedly decreased the failure rate of VCTE in NAFLD. **Aim:** To evaluate the performance of the newer Fibroscan 502 Touch, which offers a machine-derived choice between the M and XL probes, in grading steatosis and staging fibrosis in patients with histologically confirmed NAFLD. **Methods:** Adults with NAFLD who had liver biopsy within preceding 12 months were enrolled at 8 NASH CRN Clinical Centers. VCTE examinations were performed in a fasted state according to a standardized protocol. Liver histology was reviewed and scored centrally in a blinded fashion. Liver stiffness measurements (LSM) and the controlled attenuation parameter (CAP) were compared to the presence and histological severity of hepatic fibrosis and steatosis. **Results:** A total of 292 participants (32% male, 82% Caucasian, mean±SD age 52±11 years, BMI 34±6 kg/m^2) were eligible. The median time from liver biopsy to VCTE was 36 days. 58% had definite steatohepatitis and the mean NAFLD Activity Score was 4.3 (steatosis grade: 1.8, lobular inflammation: 1.6, ballooning: 1.0). Mean fibrosis stage was 1.7 and 20% had stage 2, 23% stage 3, and 9% stage 4 fibrosis (cirrhosis). CAP detected the presence of steatosis with cross-validated AUROC of 0.78 (95% CI: 0.67, 0.89). With sensitivity fixed at 90%, a CAP of 256, 279 and 283 dB/m distinguished steatosis grade 0 vs higher, 0-1 vs higher and 0-2 vs higher with a specificity of 0.33, 0.43 and 0.30 yielding a positive predictive value (PPV) of 0.96, 0.70 and 0.34 respectively. Using LSM cutoffs of 4.6, 5.5, 6.1 and 10.4 kPa, the specificity (PPV, NPV) of fibrosis stage with sensitivity fixed at 90% was 0.27 (0.79, 0.49), 0.46 (0.65, 0.82), 0.43 (0.43, 0.90) and 0.72 (0.25, 0.99) for detection of stage >0, >1, >2 and > 3. With cutoffs of 10.3, 11.6, 13.6, and 15.8 kPa and specificity fixed at 90%, the LSM sensitivity (PPV, NPV) were 0.41 (0.93, 0.34), 0.47 (0.84, 0.61), 0.44 (0.68, 0.77), and 0.59 (0.38, 0.96) for same fibrosis thresholds. The overall cross-validated AUROC for distinguishing cirrhosis from lesser stages was 0.91 (95% CI: 0.86, 0.96). **CONCLUSIONS:** In a multi-center setting, VCTE using Fibroscan 502 Touch accurately detects steatosis grade and fibrosis stage using histologic analysis of biopsies as a reference.

Disclosures:

Cynthia A. Behling - Consulting: ICON, Covance; Stock Shareholder: Pacific Rim Pathology Medical Group

Brent A. Neuschwander-Tetri - Advisory Committees or Review Panels: Nimbus Therapeutics, Bristol Myers Squibb, Janssen, Conatus, Allergan, Zafgen, Pfizer, Novartis, Galmed, Receptos, ConSynance, Afimmune, Arrowhead; Consulting: Medimmune, Karos, NuSirt

Danielle Brandman - Advisory Committees or Review Panels: Alnylam

Arun J. Sanyal - Advisory Committees or Review Panels: Bristol Myers, Gilead, Genfit, Abbott, Ikaria, Pfizer, Novartis, Virology Education, Intercept, Sanofi; Consulting: Salix, Immuron, Exhalenz, Nimbus, Genentech, Echosens, Takeda, Merck, Enanta, Zafgen, JD Pharma, Islet Sciences, ardelyx; Grant/Research Support: Salix, Genentech, Intercept, Ikaria, Takeda, GalMed, Novartis, Gilead, Tobira; Independent Contractor: UpToDate, Elsevier; Management Position: Sanyal Biotechnology; Stock Shareholder: Exhalenz, Hemoshear, Akarna, tiziana, Genfit

The following people have nothing to disclose: Mohammad Siddiqui, Raj Vuppalanchi, Mark L. Van Natta, Melissa J. Contos, James Tonascia

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Performance of liver stiffness by FibroScan in a Large Prospective Multicenter UK Study: Applicability, Reliability, Diagnostic Performance and Influence of The Probe Type And Of Steatosis on the Liver Stiffness Measurement

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Background & Aims: This prospective study evaluated the diagnostic performance of liver stiffness measurement (LSM) by FibroScan with either M or XL probe in a cohort of 450 patients with NAFLD. **Methods:** In the M118 study 450 patients underwent FibroScan examination within 2 weeks of a clinically indicated liver biopsy (LB) for suspected NAFLD. Recruitment took place (Mar 2014-Jan 2017) at seven UK centres. LB were scored in a blinded manner by two expert pathologists using the NASH CRN system. NASH was diagnosed using the FLIP algorithm. Diagnostic performance was reported in patients with reliable FibroScan examination (Boursier's criteria) using area under the ROC curves (AUC). Cutoffs were computed for high sensitivity (Se) >0.90, high specificity (Sp) >0.90 or for maximizing Se/Sp simultaneously. Univariate analysis was performed using Wilcoxon or Chi-square/Fisher-exact test. Influence of probe type and histological parameters on LSM were appraised using a backwards stepwise multiple linear regression. **Results:** 408 patients completed the study. Of the 380 patients with a LB of sufficient size that showed NAFLD, 43% were female, with a median age 55 [IQR 19] years and median BMI 33.8 [9.3] kg/m^2. Fibrosis distribution was: F0: 17%, F1: 23%, F2: 22%, F3: 28%, F4: 10%. 64% had NASH. 45% had a NAS score ≥5. 374/380 patients had a valid LSM and 331/374 had a reliable LSM giving 98% applicability and 89% reliability. Patients with unreliable LSM had a higher BMI: 39.0 *vs* 32.8, p<10^{-6}. 121 (37%) patients were measured using the M probe, 210 (63%) patients using the XL probe. Performance characteristics are shown in the Table. Univariate analysis showed that probe type (p=0.62) did not influence LSM, whereas steatosis (p=0.047), lobular inflammation (p<10^{-5}), ballooning (p<10^{-6}) and fibrosis (p<10^{-16}) all did. However, at multivariate analysis the only parameter significantly influencing LSM was fibrosis stage (p<10^{-16}), with no association seen for steatosis or probe type. **Conclusion:** LSM by FibroScan is a reliable technique to non-invasively assess liver fibrosis in NAFLD patients. Probe type and steatosis do not influence LSM.

Performance characteristics of FibroScan

	F≥2	F≥3	F=4
AUC [95% CI]	0.78 [0.72-0.86]	0.84 [0.77-0.90]	0.92 [0.85-0.98]
Cufoff max Se/Sp, all M probe XL probe	C=8.0 *(Pr=60%)* - PPV=78/NPV=61 C=8.3 *(Pr=56%)* - PPV=81/NPV=72 C=7.9 *(Pr=61%)* - PPV=77/NPV=57	C=9.1 *(Pr=36%)* - PPV=61/NPV=83 C=9.7 *(Pr=32%)* - PPV=67/NPV=90 C=8.8 *(Pr=39%)* - PPV=59/NPV=78	C=12.9 *(Pr=10%)* - PPV=33/NPV=98 C=12.8 *(Pr=10%)* - PPV=37/NPV=98 C=13.6 *(Pr=10%)* - PPV=33/NPV=98
Cutoffs Se≥0.9, all M probe XL probe	C=5.7 - PPV=68/NPV=74 C=6.1 - PPV=68/NPV=85 C=5.4 - PPV=68/NPV=69	C=6.6 - PPV=47/NPV=88 C=8.7 - PPV=59/NPV=95 C=6.1 - PPV=46/NPV=84	C=10.9 - PPV=28/NPV=99 C=10.9 - PPV=30/NPV=99 C=11.5 - PPV=28/NPV=99
Cutoffs Sp≥0.9, all M probe XL probe	C=10.3 - PPV=90/NPV=57 C=9.4 - PPV=90/NPV=68 C=10.5 - PPV=89/NPV=53	C=12.4 - PPV=76/NPV=78 C=12.8 - PPV=74/NPV=80 C=12.9 - PPV=79/NPV=76	C=18.4 - PPV=45/NPV=96 C=21.4 - PPV=62/NPV=96 C=19.8 - PPV=41/NPV=95

C: cutoff; Pr: prevalence; PPV: positive predictive value; NPV: negative predictive value.

Disclosures:

Quentin M. Anstee - Advisory Committees or Review Panels: NewGene; Consulting: Eli Lilly, Falk, Genfit, Intercept, Inventiva, Kenes, Janssen, Pfizer, Raptor Pharma, Tobira; Grant/Research Support: Abbvie, GSK, Tobira, Vertex

Philip N. Newsome - Advisory Committees or Review Panels: Novo Nordisk, Boehringer Ingelheim, Shire, DS BioPharma, Gilead; Consulting: AbbVie; Grant/Research Support: Pharmaxis

The following people have nothing to disclose: Peter J. Eddowes, Michael E. Allison, Emmanuel Tsochatzis, Indra Neil Guha, Pierre Bedossa

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*nor*Ursodeoxycholic acid (*nor*UDCA) improves non-alcoholic fatty liver disease (NAFLD): Results from a randomized placebo-controlled, double-blind phase IIa study

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*nor*UDCA is a side chain-shortened homologue of UDCA undergoing hepatic enrichment. **Aim:** To compare 500 mg/d or 1500 mg/d of oral *nor*UDCA with Placebo (PBO)

in the treatment of NAFLD. **METHODS:** Patients with a clinical diagnosis of NAFLD and serum ALT levels of > 0.8 ULN were randomized to a 12-week treatment period and a 4 week follow-up. Primary efficacy endpoint was the mean relative change (%) in ALT levels between the baseline and the end-of-treatment (EOT) visit. **RESULTS:** 198 patients were randomized and analysed. Baseline data were comparable between treatment groups (mean age 47 years, 62% males, BMI 30.2, and 10% T2D). Results for the primary endpoint are shown in Table 1. Similar results were found for other liver enzymes. Serum ALP, FGF-19, FGF-21and BMI remained unchanged. Serum triglycerides declined whereas LDL-C and HDL-C increased slightly. The number of patients with severe steatosis assessed by US declined; in line with data in a small subset of patients undergoing analysis of hepatic fat content by MRI or MR-spectroscopy (MRS). As such the mean value of hepatic fat fraction on MRI/MRS decreased under 1500mg/d (n=8) from 21.3% to 16.3% at the end of treatment, while remaining almost constant under 500mg/d (n=8: 14.6% to 15.5%) and PBO (n=5: 17.0% to 16.0%). About 50% of patients underwent Fibroscan (M, XL probe) or ARFI. The proportion of patients with low degrees of liver stiffness (stage 0/1) increased in the 1500 mg/d group (55.6% to 67.5%) while decreasing in the 500 mg/d- (62.5% to 58.3%) and the PBO group (72.7% to 61.1%). Serious adverse events occurred in 6 patients (2 in the 500 mg/d-, 1 in the 1500 mg/d-group, and 3 in the PBO group, respectively). Treatment Emergent Adverse Events were reported in a similar number of patients across groups. ADRs were reported for 25 patients in the 1500mg/d-, 15 patients in the 500mg/d- and 16 patients in the PBO-group. **CONCLUSION:** *nor*UDCA resulted in a significant reduction of ALT values within 12 weeks of treatment when compared to PBO. The highest effect occurred at 1.500 mg/d. The results are supported by improvement of steatosis and liver stiffness in the subsets analysed. *nor*UDCA was safe and well tolerated.

Table 1: Relative change (%) in ALT between baseline and EOT (ITT)

	NorUDCA 1500 (N = 67)	NorUDCA 500 (N = 67)	PBO (N = 64)
Mean ± SD	-17.4 ± 26.1	-4.2 ± 37.5	10.4 ± 51.0
Difference between means: norUDCA vs Placebo	-27.8	-14.6	
95%-RCI * for difference in means	[-34.7, -14.4]	[-20.7, 3.59]	
Testing of H0**, overall p-value (one-sided)	< 0.0001	0.0905	

* Repeated 95% CI

** Testing of H01: μN1500-μPlacebo ≥ 0 and H02: μ N500-μPlacebo ≥ 0 (inverse normal test statistic)

Disclosures:

Stefan Traussnigg - Grant/Research Support: Phenex Pharmaceuticals

Jörn M. Schattenberg - Advisory Committees or Review Panels: Intercept Pharmaceuticals, AbbVie, Gilead Sciences, Boehringer Ingelheim; Consulting: Genfit SA, Novartis; Grant/Research Support: Yakult Deutschland GmbH; Speaking and Teaching: MSD Sharp & Dohme

Andreas Geier - Advisory Committees or Review Panels: AbbVie, Gilead, Novartis, BMS, Sequana, Intercept, Alexion; Grant/Research Support: Novartis; Speaking and Teaching: Falk, Alexion, Novartis, Intercept, Gilead, AbbVie, BMS

Andreas E. Kremer - Advisory Committees or Review Panels: GSK, Intercept, MSD, Beiersdorf; Speaking and Teaching: Falk Pharma, BMS, Intercept, Janssen, MSD

Joerg Petersen - Advisory Committees or Review Panels: Bristol-Myers Squibb, Gilead, Novartis, Merck; Grant/Research Support: Roche, GlaxoSmithKline; Speaking and Teaching: Abbott, Tibotec, Merck

Roland Greinwald - Employment: Dr. Falk Pharma GmbH

Markus Proels - Employment: Dr. Falk Pharma GmbH

Michael P. Manns - Consulting: Falk, Novartis, Roche, Gilead, GlaxoSmithKline, AbbVie, Boehringer Ingelheim, Intercept, Bristol Myers Squibb, Janssen, Medgenics, Eiger, Enyo, Merck; Grant/Research Support: Merck, Falk, Novartis, Roche, Gilead, GlaxoSmithKline, Intercept, Boehringer Ingelheim, Janssen, Eiger, AbbVie; Speaking and Teaching: Falk, Gilead, Roche, AbbVie, Bristol Myers Squibb, Janssen, Merck

Peter Fickert - Consulting: Falk Foundation; Speaking and Teaching: MERCK Austria, Roche Austria, Gilead Austria, MSD Austria

Michael H. Trauner - Consulting: Albireo, Falk, Gilead, Novartis, MSD, Phenex; Grant/Research Support: Falk, Intercept, Gilead, Takeda, Albireo

The following people have nothing to disclose: Muenevver Demir, Johannes Wiegand, Gerlinde Teuber, Frank Spreda, Johannes Kluwe, Tobias Boettler

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4D Dynamic FDG-PET correlates with hepatic inflammation and steatosis in patients with non-alcoholic steatohepatitis

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Background: Non-alcoholic fatty liver disease (NAFLD) and its more severe form, non-alcoholic steatohepatitis (NASH), are major causes of chronic liver disease characterized by a progressive spectrum of steatosis, inflammation and fibrosis. Current noninvasive modalities remain limited for detection and quantitation of NASH, with liver biopsy providing an imperfect 'gold standard.' The lack of noninvasive tests has made management of NASH challenging. We propose to utilize four-dimensional (4D) dynamic positron emission tomography (PET) with the widely-used radiotracer ^{18}F-fludeoxyglucose (FDG) as a novel, clinically effective method for evaluating liver inflammation and steatosis and thus for determining NASH. **Methods:** The pilot cohort included 10 patients with a diagnosis of NAFLD/ NASH confirmed via biopsy within 6 months. Patients with concomitant liver diseases or prior bariatric surgery were excluded. Dynamic FDG-PET images were acquired over one hour using list-mode data acquisition. The time activity curves of 8 hepatic regions of interest corresponding to 8 segments, were extracted from the dynamic images. Kinetic parameters, representing various rate constants of FDG transport (K_1, k_2, k_3 and k_4), were estimated by tracer kinetic modeling accounting for liver's dual blood supply. The overall FDG influx rate $K_i=K_1*k_3/(k_2+k_3)$ and standardized uptake values (SUV) of FDG were calculated. Liver biopsy was scored per the NASH Clinical Research Network criteria for steatosis (0-3), inflammation (lobular inflammation + ballooning degeneration: 0-5) with total calculated as NAFLD activity score (NAS: 0-8). MR-PDFF was performed to measure hepatic steatosis. **Results:** The cohort (n=10) included patients aged 18-67 years, with 7 white, 3 Hispanic and 7 female patients. BMI range was 24.4-43.1 kg/m^2. ALT range was 13-121 U/L. The cohort had an equitable spread across steatosis (1-3), inflammation (1-5) and NAS scores (3-7), although the sample size was small. FDG-K_1 was significantly correlated with liver inflammation (r=0.805; p=0.005) and overall NAS (r= 0.67; p=0.031). SUV value was significantly correlated with biopsy steatosis grade (r= 0.74; p=0.01) and MR-PDFF (r=0.897, p=0.001), respectively. **Conclusions:** 4D dynamic FDG-PET has the potential to accurately determine liver inflammation and steatosis in NAFLD and NASH. This study

fills an essential gap in noninvasive evaluation of NAFLD and NASH patients and provides seed data for FDG kinetic parameters to understand physiologic changes in NAFLD patients especially with therapeutic interventions.

Disclosures:

Ramsey Badawi - Grant/Research Support: Philips Medical, Siemens Medical

The following people have nothing to disclose: Souvik Sarkar, Kristin Olson, Susan Stewart, Cole R. Johnson, Guobao Wang

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Deficiency of bile acid transporter Ostα alters plasma oxysterol mediated Lxrα/β signaling and lipid accumulation in mouse adipose

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BACKGROUND: Mice deficient in Organic solute transporter (OST) α/β, a bile acid (BA) and sterol/oxysterol transporter, have lower adipose mass and are protected from adiposity. While BAs activate Fxr/Tgr5, oxysterols activate Lxrα/β. We have previously demonstrated expression of Osta/β in mouse adipose, however mechanisms leading to lower adiposity in these mice remains unknown. **AIM:** To determine whether deficiency of Ostα alters differentiation and lipid accumulation in adipocytes via changes in substrate concentrations and activation of Fxr/Tgr5 and Lxrα/β pathways. **METHODS:** Sterol, oxysterol concentrations, Fxr, Lxrα/β and lipid metabolic gene expression were quantified in Ostα WT and KO adipose tissue (11 weeks, male) by qPCR. Mouse embryonic fibroblasts (MEFs) isolated from Ostα WT and KO mice were differentiated *in vitro*, treated with BA and oxysterol metabolites and gene expression quantified. **RESULTS:** There were no differences in Fxr and Tgr5 mRNA expression in Ostα KO adipose relative to WT. Further, Ostα WT and KO MEFs demonstrated similar changes in lipid accumulation upon treatment with Fxr (TCA, UDCA, GW4064) and Tgr5 (LCA, INT777) ligands. These observations indicated that differences between Ostα WT and KO adipose were not BA driven. Interestingly, there was a 40% decrease in adipose Lxrα, Lxrβ, Cyp27a1, and Cd36 mRNA expression in KO relative to WT. These decreases were even more pronounced when gene expression was quantified in WT and KO mature adipocytes only. Along with lower Lxrα/β, Ostα KO had significantly lower neutral sterol concentrations in plasma and adipose. Plasma concentration of 24,25-dihydrolanosterol was lower in Ostα KO indicating lower hepatic cholesterol synthesis relative to WT mice. Plasma concentrations of endogenous Lxrα/β ligands (27-hydroxyholesterol, 24(S),25-epoxycholesterol) were 1.5-4 fold higher in Ostα KO mice. While adipose levels of epoxy ligand remained high, those of 27-hydroxyholesterol were lower in Ostα KO. *In vitro*, the epoxy ligand induced Lxrα/β target genes in both Ostα WT and KO, but 27-hydroxycholesterol activated these genes only in the WT MEFs. **CONCLUSION:** Our observations indicate that deficiency in Ostα potentially leads to differential accumulation of Lxrα/β ligand in adipocytes. We speculate that lower Lxrα/β activity leads to altered regulation of genes involved in adipose differentiation resulting in lower adiposity in Ostα KO mice. Overall changes in adipose, liver and intestinal gene expression indicate the potential of Ostα/β as a target for therapy to reduce adiposity.

Disclosures:

James L. Boyer - Consulting: Allergan, Alnylam; Grant/Research Support: Gilead, Intercept pharma

The following people have nothing to disclose: Supriya Kulkarni, Carol J. Soroka

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Anti-inflammatory effects of miR-21 depletion in the macrophage response to cholestatic liver injury

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Background: MicroRNA-21 (miR-21) is a key microRNA in cholestatic liver injury. Although detailed functions of miR-21 in the liver are still unclear, our recent study has shown that miR-21$^{-/-}$ mice have ameliorated liver damage and fibrosis following bile duct ligation (BDL) surgery. We have also demonstrated that infiltration of macrophages and neutrophils in the liver is reduced in miR-21$^{-/-}$ mice during BDL. In this current study, we sought functional roles of miR-21 in cytokine production in liver macrophages and cholangiocytes during cholestatic liver inflammation. **Methods:** In the animal studies *in vivo*, wild-type (WT) and miR-21 knockout mice were subjected to sham or BDL surgery. After one week, mice were sacrificed and liver tissues were collected. Liver macrophages were isolated from frozen liver sections by laser capture microdissection using anti-F4/80 antibody and Leica LMD7 microscope. Virtually pure cholangiocytes were obtained by immunoaffinity separation by using a monoclonal antibody, rat IgG2a. RNAs were harvested from total liver, LCM-isolated macrophages, and isolated cholangiocytes. RT-PCR was performed for inflammatory cytokine expression (IL-1β, IL-6, TNFα and CCL2). Murine macrophage line RAW264.7 cells and human non-malignant cholangiocytes (H69) were transfected by lentivirus for negative control or miR-21 inhibitor *in vitro*. After two days of transfection, cells were stimulated by lipopolysaccharide (LPS, 100 ng/mL for RAW264.7, 200 ng/mL for H69) for 4 hours. RNAs were then harvested and RT-PCR for inflammatory cytokines was performed. **Results:** Inflammatory cytokine expression was elevated in total liver, LCM-isolated macrophages, and isolated cholangiocytes of WT BDL mice compared to WT sham mice. BDL also induced cytokine expression in miR-21$^{-/-}$ mouse liver, isolated macrophages, and cholangiocytes compared to sham controls. However, cytokine production levels were attenuated in miR-21$^{-/-}$ BDL mouse compared to WT BDL mice, suggesting that reduced cytokine production may be responsible for reduced liver damage in these knockout mice during BDL. Lentiviral miR-21 inhibitor also reduced inflammatory cytokine production in RAW264.7 cells and H69 cells induced by LPS stimulation, suggesting that miR-21 is associated with cytokine production both in macrophages and cholangiocytes. **Conclusion:** Our data suggest that miR-21 is associated with cytokine production in liver macrophages and cholangiocytes during BDL *in vivo*, and LPS stimulation *in vitro*. This study indicates that miR-21 may be a great therapeutic target to develop novel treatments of biliary inflammation and liver fibrosis during cholestatic liver injury.

Disclosures:

The following people have nothing to disclose: Keisaku Sato, Lindsey Kennedy, Thao Giang, Tianhao Zhou, Sugeily Ramos-Lorenzo, Heather L. Francis, Shannon S. Glaser, Gianfranco Alpini, Fanyin Meng

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Inhibition of hepatic senescence decreases biliary proliferation and liver fibrosis *in vitro* and in the Mdr2$^{-/-}$ mouse model of primary sclerosing cholangitis (PSC).

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Cellular senescence occurs in response to cellular stress and includes irreversible growth arrest, expression of p16/p21 and formation of senescence-associated secretory phenotypes (SASP) that secrete pro-inflammatory cytokines. We have shown enhanced biliary senescence but reduced senescence of hepatic stellate cells (HSCs) in Mdr2$^{-/-}$ mice and human PSC samples that contributes to the enhanced fibrotic reaction seen in PSC. We hypothesized that inhibition of cholangiocyte senescence reduces the activation of HSCs leading to reduced liver fibrosis. **Methods**: *In vitro,* siRNA against p21/p16 were used to downregulate the expression of p16/p21 in murine biliary lines (IMCLs) prior to evaluation of the expression of senescence (p21, p16, p15, p18, p53, and EGFR) and fibrosis genes (Fn-1, MMP9, TGF-β1 and TIMP1) by qPCR. The expression of the senescence markers p21/p16 was evaluated by immunofluorescence (IF), in IMCLs and human hepatic stellate cells (HHSTeCs). *In vivo,* cholangiocytes and liver samples were collected from male wild-type (WT) and Mdr2$^{-/-}$ mice (12 wk). HSCs from WT and Mdr2$^{-/-}$ liver sections were obtained by Laser Capture Microdissection (LCM). Senescence and fibrosis gene expression in cholangiocytes and HSCs was evaluated by qPCR. p21/p16 expression in bile ducts and HSCs was evaluated in liver sections co-stained with CK-19 (biliary marker) or synaptophysin-9 (HSCs marker) by IF. Intrahepatic biliary mass (IBDM) and liver fibrosis were evaluated in liver sections. Senescence and fibrosis gene expression was evaluated in HHSteCs treated with biliary supernatants (expected to express variable levels of SASP) from WT and Mdr2$^{-/-}$ mice and p16/p21-silenced IMCLs. **Results**: *In vitro,* p16 and p21 is expressed in IMCLs and HHSteCs by IF. Silencing of p16/p21 reduces cellular senescence and fibrosis of IMCLs. Incubation of HHSteCs with supernatants from p16/p21-silenced IMCLs increased senescence and reduced fibrosis gene expression of HHSteCs. In Mdr2$^{-/-}$ mice, there was: (i) enhanced IBDM, collagen deposition, fibrotic and senescence gene expression in cholangiocytes; and (ii) enhanced fibrotic marker expression, but reduced senescence in HSCs compared to WT mice. Incubation of HHSTeCs with Mdr2$^{-/-}$ biliary supernatant (displaying higher SASP phenotype) resulted in increased expression of senescence and decreased expression of fibrotic gene expression of HHSTeCs. **Conclusions**: Differential changes in cellular senescence of cholangiocytes and HSCs contributes to the pathogenesis of liver fibrosis in PSC model. Therapeutic modulation of cholangiocyte senescence may be a key approach for managing HSC activation and liver fibrosis in cholangiopathies.

Disclosures:

The following people have nothing to disclose: Tina Kyritsi, Tianhao Zhou, Fanyin Meng, Nan Wu, Julie Venter, Thao Giang, Gianfranco Alpini, Shannon S. Glaser

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β-catenin and IL-1β dependent CXCL10 production drives progression of disease in a mouse model of Congenital Hepatic Fibrosis

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Congenital Hepatic Fibrosis (CHF), a genetic disease caused by mutations in the PKHD1 gene, encoding for the protein fibrocystin (FPC), is characterized by biliary dysgenesis, progressive portal fibrosis, and by a PKA-mediated activating phosphorylation of β-Catenin at Ser675. Biliary structures of *Pkhd1*$^{del4/del4}$ mice, a mouse model of CHF, secrete CXCL10 a chemokine able to recruit macrophages. The aim of this study is to clarify whether CXCL10 secretion by cholangiocytes plays a pathogenetic role in disease progression in CHF/CD and to understand the mechanisms leading to increased CXCL10 secretion. We demonstrate that treatment of *Pkhd1*$^{del4/del4}$ mice for three-month with AMG-487, an inhibitor of CXCR3 the cognate receptor of CXCL10, reduces the peribiliary recruitment of M2 macrophages (CD45^{+}F4/80^{+} cells), spleen size, liver fibrosis (Sirius red), and cyst growth (K19^{+} area), consistent with a pathogenetic role of CXCL10. Furthermore, we show that in FPC-defective cholangiocytes, isolated from *Pkhd1*$^{del4/del4}$ mice, CXCL10 production is mediated by JAK/STAT3, in response to IL-1β and β-Catenin. Specifically, IL-1β promotes STAT3 phosphorylation whereas β-Catenin promotes its nuclear translocation. Increased pro-IL-1β was regulated by NF-kB and increased secretion of active IL-1β was mediated by the activation of NLRP3 inflammasome (increased expression of caspase 1 and NLRP33). ***Conclusions:*** In FPC-defective cholangiocytes, β-Catenin and IL-1β are responsible for STAT3-dependent secretion of CXCL10. *In vivo* experiments show that this mechanism is of pathophysiological relevance, as targeting the CXCL10/CXCR3 axis prevents the recruitment of macrophages, reduces inflammation and halts the progression of the disease. The increased production of IL-1β highlights the autoinflammatory nature of CHF and may open novel therapeutic avenues.

Disclosures:

The following people have nothing to disclose: Eleanna Kaffe, Romina Fiorotto, Valeria Mariotti, Massimiliano Cadamuro, Luca Fabris, Mario Strazzabosco, Carlo Spirli

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Inhibition of H2 histamine receptor by Vivo-Morpholino treatment decreases mast cell activation, large biliary proliferation, angiogenesis, fibrosis and inflammation in the Mdr2$^{-/-}$ mouse model of primary sclerosing cholangitis

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Background: Histamine (HA) (i) is a trophic factor that increases biliary damage, hepatic fibrosis and biliary vascular endothelial growth factor (VEGF) expression. HA binds to one of four G-protein coupled receptors, H1-H4 HRs, expressed by large cholangiocytes and mast cells (MCs). We have shown that HA increases large, but not small cholangiocyte proliferation, via H2HR. In Mdr2$^{-/-}$ mice (i) MC activation and HA levels are increased and (ii) the H2HR blocker, ranitidine, decreases liver damage. We hypothesized that targeting hepatic H2HR using Vivo-Morpholino decreases large biliary proliferation and liver damage in Mdr2$^{-/-}$ mice. **Methods:** Mdr2$^{-/-}$ mice were treated with mismatch or Vivo-Morpholinos to decrease H2HR expression by tail vein injection 2x/week for 1 week. Serum, liver and isolated cholangiocytes were obtained. Liver damage was assessed by H&E along with serum liver enzymes. MC activation was determined by staining for mouse MC protease-1 and by *q*PCR for chymase, tryptase and c-Kit in total liver (TL) and in laser micro-dissected (LMD) MCs. H2HR expression was measured in TL, isolated cholangiocytes and MCs by *q*PCR. Changes in large biliary mass and proliferation were detected in liver sections by CK-19 and Ki-67, as well as in large cholangiocytes by *q*PCR for PCNA. Angiogenesis was measured by *q*PCR and immunofluorescence (IF) for VEGF-A/C and von Willebrand factor in TL. We evaluated liver fibrosis using hydroxyproline assay and Sirius red staining. HSC activation was measured by IF and *q*PCR for SYP-9 in LMD-isolated HSCs. Inflammation was determined by staining for F4/80 and by *q*PCR for IL-6/10 and TGF-b1 expression in TL. Serum levels of HA, VEGF and TGFβ-1 were measured by EIA. *In vitro,* cultured MCs were treated with vehicle or ranitidine (10 μM, 48 hrs) prior to co-culture with large mouse cholangiocytes and human HSCs. Following treatment, biliary proliferation was detected by BrDU incorporation and HSC activation by IF for α-SMA. **Results:** Treatment with H2HR Vivo-Morpholino in Mdr2$^{-/-}$ mice decreased (i) hepatic damage/inflammation; (ii) large IBDM/proliferation; (iii) MC activation and H2HR expression; (iv) angiogenesis and fibrosis and (v) HA, VEGF and TGF-β1 secretion compared to mismatch. *In vitro,* co-culture with ranitidine-treated MCs decreased large biliary proliferation and HSC activation compared to vehicle. **Conclusion:** Inhibition of H2HR signaling via Vivo-Morpholino treatment decreases MC activation/HA secretion and large IBDM/proliferation, thereby ameliorating PSC-induced angiogenesis, fibrosis and inflammation. Therapeutics targeting the HA/H2HR axis may be beneficial in treating PSC.

Disclosures:

The following people have nothing to disclose: Lindsey Kennedy, Jennifer Demieville, Laura Hargrove, Fanyin Meng, Gianfranco Alpini, Steven Smith, Heather L. Francis

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Modelling Primary Sclerosing Cholangitis using Induced Pluripotent Stem Cell-Derived Cholangiocytes and 3-Dimensional Biliary Spheroids

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Introduction: Primary Sclerosing Cholangitis (PSC) is a chronic fibroinflammatory disease of the biliary tract characterized by cellular senescence and progressive periportal fibrogenesis. The etiology and pathophysiology of PSC remains unclear, due in part to a lack of patient-specific *in vitro* models. Recently, our group and others developed systems to create induced pluripotent stem cell (iPSC)-derived cholangiocytes (iDCs). The **purpose** of this study was to develop individualized iDCs and biliary spheroids (cholangioids) from subjects with PSC for disease modelling. **Methods and Results:** Fibroblasts from healthy controls and PSC subjects were isolated and reprogrammed to pluripotency via forced expression of KLF4, OCT4, SOX2, and c-MYC. iPSC clones underwent quality control including differentiation to the three primary germ layers (endoderm, mesoderm, and ectoderm), karyotype analysis, as well as staining and flow cytometry for Oct4, SSEA, Nanog, and TRA-1-60. Individualized iPSC were differentiated to cholangiocytes and subsequently grown in a 3-dimensional, matrigel-based culture to induce formation of cholangioids. Samples from PSC subjects differentiated as efficiently as those from healthy subjects, as assessed by the acquisition of the biliary cytokeratins, CK7 and CK19. PSC-derived iDCs showed increased secretion of the extracellular matrix (ECM) molecule, fibronectin (4.12±1.13 fold, $p<0.01$) as well as the inflammatory cytokines, IL-6 (10.3±3.7 fold, $p<0.05$) and CCl2 (22±6.5 fold, $p<0.05$). Moreover, PSC-derived cholangioids were smaller in size (18.3±2.5μm^2 vs 9.5±0.92μm^2, $p<0.005$), morphologically lacked a central lumen, and showed a 6-fold increase in cellular senescence by SA-βGal staining. Furthermore, the PSC-derived iDCs release approximately 3-fold more extracellular vesicles compared to the control iDCs, consistent with a senescence-associated secretory phenotype (SASP). In accordance with the SASP, conditioned media (CM) from PSC-derived iDCs more potently activated hepatic stellate cells compared to CM from control iDCs as assessed by alpha SMA staining. **Conclusion:** We have demonstrated efficient generation of iDCs and cholangioids from PSC patients. PSC samples showed disease specific features including ECM deposition, luminal narrowing, cellular senescence, and the SASP, along with an enhanced ability to activate HSCs. This approach thus represents a novel source of individualized biliary spheroids that can be analyzed as subject-specific disease models and serves as a platform for developing therapeutics for PSC and other cholangiopathies.

Disclosures:

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Comparison of Outcomes after DAA therapy among HCV-infected kidney transplant recipients who received grafts from either HCV-positive or negative donors.

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Direct acting antivirals (DAA) have transformed hepatitic C virus (HCV) treatment. In the kidney transplant (KT) setting, HCV-infected ESRD patients can now receive deceased donor KT (DDKT) from HCV positive donors (D+) and undergo treatment in the post-transplant period. However a few cases of rejection have been reported in this HCV D+R+ cohort. Therefore, we sought to compare outcomes among HCV infected DDKT recipients (R+) who received a graft from HCV negative donors (D-) and D+. This is a case series of 39 consecutive KT recipients of which 14 R+ have been transplanted with a kidney from D- and the rest from D+. All patients completed a full course of DAA therapy as per guidelines. Time to transplantation, efficacy of DAA therapy, number of rejection episodes, tacrolimus dose adjustments, and renal function were assessed in both groups. The average age of our cohort was 56.7±8.8 and 59.1±10.5 years with 64% and 77% male in D-R+ and D+R+, respectively. In both groups the predominant genotype was 1A. The median METAVIR fibrosis stage was 2.0 in D-R+ and 1.0 in D+R+. D-R+ had 100% SVR at 12 weeks whereas 96% of D+R+ did. The median waiting time to transplantation after signing consent was 802 days for D-R+ and 58 days in D+R+. Additionally, the median time between transplantation and initiation of DAA therapy was 405 days and 124 days in D-R+ and D+R+, respectively. There were 4 antibody mediated rejection episodes in D+R+ and 1 mixed rejection in D-R+. Tacrolimus dose adjustments were required in 64% of D-R+ and 52% of D+R+. When comparing kidney function before and after treatment with DAA, 42% of D-R+ and 28% of D+R+ had a significant change, which is defined as an increase or decrease in creatinine by ≥ 0.3mg/dL. Acceptance of a D+ kidney resulted in a significant decrease in transplant waiting time in R+ candidates without marked compromise. The response to DAA therapy was excellent and the SVR rates were similar to those reported in the general population. In both groups, tacrolimus dose adjustments were necessary. Our data suggests that KT recipients should be closely monitored during and immediately following HCV therapy with DAAs.

Comparing D-R+ to D+R+

	D-R+	D+R+
Age (years)	57.6 ± 8.8	59.1 ± 10.5
Gender (%)	64% male 36% female	77% male 23% female
HCV Genotype (1, 2, 3)	83%, 7%, 0%	92%, 4%, 4%
Time to DDKT (days)	806	58
Time from DDKT to start DAA (days)	405	124
SVR12 (%)	100%	96%
Rejection Episodes (%)	7%	16%
Tacrolimus Dose Changes	43% increase 22% decrease 36% no change	48% increase 28% decrease 4% no change
Changes in Creatinine (Δ 0.3mg/dL)	21% increase 21% decrease 58% no change	20% increase 8% decrease 72% no change

Disclosures:

Cynthia Levy - Advisory Committees or Review Panels: Target Pharmasolutions; Board Membership: Liver Transplantation; Consulting: Intercept, Novartis; Grant/Research Support: Intercept, Gilead, Novartis, Tobira, NGM, CymaBay; Speaking and Teaching: Medscape

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Efficacy and Safety of Sofosbuvir/Velpatasvir plus Ribavirin for 12 or 24 Weeks in Genotype 1 or 2 HCV-Infected Japanese Patients with Prior Treatment Failure to DAA-Based Regimens

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Background: There is a growing number of Japanese patients with HCV infection who have failed direct acting antiviral (DAA)-based regimens and currently have no salvage therapies available. This Phase 3 study evaluates the efficacy and safety of sofosbuvir/velpatasvir (SOF/VEL) plus ribavirin (RBV) for 12 or 24 weeks in Japanese patients with genotype (GT) 1 or 2 HCV infection who have been previously treated with DAAs. **Methods:** Approximately 110 subjects were randomized 1:1 to receive SOF/VEL+RBV for 12 or 24 weeks. Randomization was stratified by GT and presence of cirrhosis. All subjects must have been previously treated with a DAA for at least 4 weeks. Subjects with GT1 HCV infection must have previously been treated with an NS5A inhibitor. The primary efficacy analysis is comparison of the SVR12 rates for GT1 patients in each of the two treatment groups to a historical control SVR of 50%. **Results:** Of 117 patients enrolled, 45% were male, 81% had GT1 HCV infection, and 33% had cirrhosis. In terms of previous HCV treatment experience, 84% had previously been treated with at least 2 different DAAs and 71% had failed ≥2 regimens. 86% of GT1 patients had previously been treated with daclatasvir plus asunaprevir and 91% of GT2 patients with SOF. Virologic outcomes at post-treatment week 4 are presented in the table below. There were no on-treatment virologic failures. Complete SVR12 and virology data will be presented. Three (3%) patients discontinued study drugs due to adverse events

(AEs). One patient in the 12-week arm discontinued study drugs on Day 4 due to rash (related to study drugs). Two patients in the 24-week arm discontinued study drugs; one on Day 85 due to hepatic angiosarcoma (not related) and one on Day 57 due to depression (related). The two latter patients achieved SVR12. The most common AEs were nasopharyngitis, anemia, and headache. No Grade 3 or 4 AEs were considered related to study drugs. **Conclusions:** SOF/VEL+RBV has the potential to be a safe, well-tolerated, and effective treatment for Japanese patients with and without cirrhosis who have previously failed DAA-based regimens, a group without currently approved retreatment options. Baseline NS5A RASs did not affect treatment outcome.

Virologic Outcomes for DAA-Experienced Patients Treated with SOF/VEL+RBV for 12 or 24 Weeks

		12 Weeks			24 Weeks	
	Total	GT1	GT2	Total	GT1	GT2
SVR4, % (n/N)	86 (49/57)	89 (42/47)	70 (7/10)	98 (59/60)	98 (4748)	100 (12/12)
with BL NS5AVEL-specific RASs	87 (47/54)	89 (40/45)	78 (7/9)	100 (52/52)	100 (41/41)	100 (11/11)
without BL NS5A VEL-specific RASs	67 (2/3)	100 (2/2)	0 (0/1)	88 (7/8)	86 (6/7)	100 (1/1)
Relapse, % (n/N)	12 (7/57)	9 (4/47)	30 (3/10)	2 (1/60)	2 (1/48)	0

*RAS.resistance-associated substitution

Disclosures:

Tetsuo Takehara - Grant/Research Support: Janssen pharmaceutical Co., AbbVie, Gilead Sciences, Chugai Pharmceutical Co., MSD K.K., Bristol-Meyer Squibb, Mitsubishi Tanabe Pharma Corparation, Toray Industories Inc.; Speaking and Teaching: Bristol-Meyer Squibb, Gilead Sciences, AbbVie, MSD K.K.

Kazuaki Chayama - Grant/Research Support: Ajinomoto, Astellas, Asuka, MSD, Bayer, Daiichi Sankyo, Dainippon Sumitomo, Eisai, Nihon Kayaku, Nihon Shinyaku, Kowa, Mitsubishi Tanabe, Nippon Eli Lily, Roche, Otsuka, Takeda, Toray, Torii, Tsumura, Zeria; Speaking and Teaching: Bristol-Myers Squibb, Abbvie, Ajinomoto, Abbott, Astellas, AstraZeneca, Asuka, Chugai, Dainippon Sumitomo, Johnson and Johnson, Jimuro, Miyarisan, Nihon Kayaku, Nihon Shinyaku, Olympus, Tsumura, Otsuka, Takeda, Nippon Kayaku, MSD

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Hepatitis C Virus (HCV) Reinfection and Injecting Risk Behavior Following Elbasvir (EBR)/Grazoprevir (GZR) Treatment in Participants on Opiate Agonist Therapy (OAT): Co-STAR Part B

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Background: High efficacy was observed in Co-STAR Part A, a phase 3 trial of EBR/GZR for 12 weeks in participants on OAT. HCV viral recurrence consistent with reinfection was observed in 6/296 participants through follow-up week (FW)24, with a reinfection rate of 3.4 reinfections/100 person years (95% confidence interval [CI]: 1.3, 7.5). We provide further analysis of HCV reinfection and injection drug use risk behavior within the ongoing Co-STAR observational study. **Methods:** Co-STAR Part B is a 3-year observational study of participants who received at least one dose of EBR/GZR in Part A (n = 296). During Part B, follow-up occurs every 6 months (6M, 12M, 18M, 24M, 30M, and 36M); if HCV RNA is detected, viral genotype and sequencing are investigated. Participant-reported surveys are also administered at each visit to assess risk behavior. **Results:** Of 296 participants treated in Part A, 199 participants enrolled in Part B, and 194, 180, 172, and 43 participants have completed follow-up visits at 6M, 12M, 18M, and 24M, respectively thus far. Urine drug screen (UDS) results were similar in Parts A and B. Positive UDS results were obtained in 58% of participants at enrollment in Part A, compared with 60%, 59%, 61%, 60%, and 61% of participants at enrollment, 6M, 12M, 18M, and 24M, respectively, in Part B. Reported drug use, including injection drug use, also has remained stable in Part B (Table). In addition to the 6 viral recurrences observed from end-of-treatment through FW24 of Part A, a further 3 viral recurrences were identified in Part B: 1 at the enrollment visit, 1 at 6M, and 1 at 18M. Spontaneous clearance was seen in 3 of 5 reinfections detected through FW12 and in none of 4 reinfections detected after FW12. The incidence of reinfection from end of treatment through all completed visits in Part B is 2.3 reinfections/100 person-years (95% CI: 1.1, 4.4). Including only those with evidence of persistence of reinfection, the effective incidence of reinfection is 1.5 reinfections/100 person years (95% CI: 0.6, 3.3). **Conclusion:** HCV reinfection among participants on OAT following EBR/GZR therapy is uncommon, despite ongoing drug use. Additional follow-up data, including risk factors related to incidence of reinfection and specific risk behaviors, will be reported.

Reported Drug Use to Follow-up Month 24

	6-month follow-up	12-month follow-up	18-month follow-up	24-month follow-up
Reported drug use in the past month, n/N (%)	96/191 (50)	84/178 (47)	85/172 (49)	19/42 (45)
Reported injection drug use in the past month, n/M (%)	36/191 (19)	28/178 (16)	35/172 (20)	6/42 (14)
Heroin, n	29	20	23	4
Other opiates, n	1	1	4	--
Cocaine, n	4	3	3	2
Other, n	4	4	2	--

Disclosures:

Gregory J. Dore - Board Membership: Gilead, Merck, Abbvie, Bristol-Myers Squibb; Grant/Research Support: Gilead, Merck, Abbvie, Bristol-Myers Squibb; Speaking and Teaching: Gilead, Merck, Abbvie, Bristol-Myers Squibb

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100% SVR with 8 weeks of ledipasvir/sofosbuvir in HIV-infected Men with Acute HCV Infection: Results from the SWIFT-C Trial (Sofosbuvir-Containing Regimens Without Interferon For Treatment of Acute HCV in HIV-1 Infected Individuals)

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Background: The optimal duration of ledipasvir/sofosbuvir (LDV/SOF) for the treatment of acute hepatitis C virus (HCV) infection is unknown. The AASLD/IDSA HCV Treatment Guidance Panel recommends treating acute HCV like chronic infection, while the EASL Guideline Panel recommends 8-12 weeks of LDV/SOF. **Methods:** SWIFT-C is a multicenter, single arm trial of the AIDS Clinical Trials Group investigating the safety and efficacy of 8 weeks of LDV/SOF for the treatment of acute HCV infection in HIV-infected participants. Acute HCV was defined as the first 24 weeks of HCV infection after diagnosis, with enrollment in the first 12-24 weeks. Criteria for diagnosis were HCV antibody or RNA seroconversion in the preceding 6 months or new liver enzyme elevation (>5X ULN with documented normal baseline ALT or >10X ULN without baseline ALT) and detectable HCV RNA in those without recent HCV testing. Genotypes 1 and 4 were allowed and other liver diseases were excluded (e.g. active hepatitis B). The sample size for the cohort was determined by non-inferiority comparison to the study-defined historical sustained virologic response (SVR) rate of 60%, achieved with 24-48 weeks of pegylated interferon and weight based ribavirin. The primary endpoint was SVR12, defined as HCV RNA target not detected at 12-weeks post-treatment. **Results:** Of the 27 participants enrolled, all were male, median age was 46 years (IQR: 38 – 50), 9 (33%) were Hispanic, and 22 (81%) reported having never used IV drugs. The distribution of HCV genotype was: 26 participants (96%) genotype 1 and 1 (4%) genotype 4. Median baseline HCV RNA was 1,490,000 IU/mL (IQR: 32,500 – 3,560,000 IU/mL), with a high baseline RNA of >6 million IU/mL in 6 (22%) participants. Median baseline CD4+ T cell count was 561 cells/mm^3. All participants were on antiretrovirals with HIV-1 RNA <LLOQ, of which 10 were on a ritonavir- or cobicistat-boosted regimen with tenofovir disoproxil fumarate (TDF). All 27 participants took 8 weeks of study treatment and all (100%) achieved SVR12. The 90% CI was 90% to 100% showing superiority relative to the historical SVR of 60%. Ten (37%) participants had a Grade 2 or higher adverse event (AE); no nephrotoxicity AEs were reported. **Conclusion:** An 8-week course of LDV/SOF achieved 100% SVR12 in this trial of HIV-infected men with acute genotype 1 or 4 HCV infection, regardless of baseline HCV RNA. No renal toxicity was noted in participants on boosted TDF-inclusive HIV regimens. These data support an 8-week duration of LDV/SOF in the treatment of acute HCV infection in HIV-infected persons.

Disclosures:

Susanna Naggie - Advisory Committees or Review Panels: Merck; Consulting: IDSA; Grant/Research Support: Tacere, Gilead, AbbVie, BMS, Jenssen, Merck; Speaking and Teaching: IAS-USA, Platform Q, Practice Point, Clinical Care Options, Vindico

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Kristen M. Marks - Grant/Research Support: Merck, Gilead, BMS

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Impact of HCV Treatment before vs. after Renal Transplantation on Time from Listing to Transplantation: A Multi-Center Study

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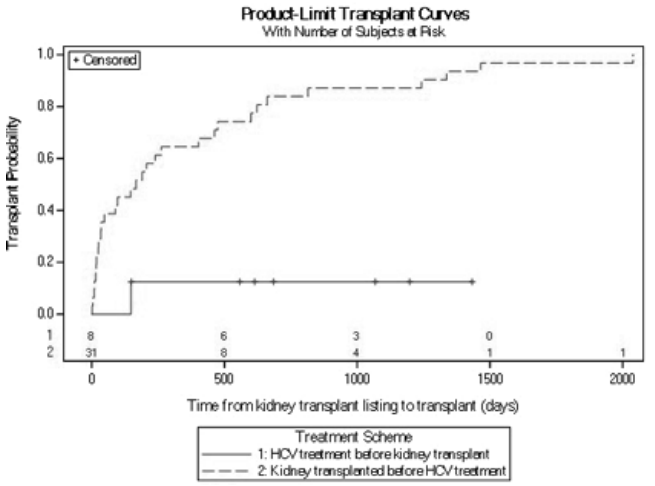
BACKGROUND: Wait list time (WLT) for renal transplantation (RT) is 3-5 years. Hepatitis C (HCV) pre-RT is associated with increased adverse events (AE) post-RT. Optimal timing of direct acting antivirals (DAA) to treat HCV is unknown. **AIM:** To compare the impact of HCV treatment pre- vs. post-RT on WLT. **METHODS:** We retrospectively reviewed the course of HCV+ patients listed for RT at two large volume liver & kidney transplant centers. All patients received DAA based interferon-free regimens between the years 2012 and 2017. Center A's practice (in UNOS region 3) was to treat HCV pre-RT, while HCV was treated post-RT at Center B (in UNOS region 5). Captured demographics included: age, gender, race, blood type, renal function, and RT quality and outcomes. WLT was evaluated. **RESULTS:** 54 patients were analyzed; 21 underwent HCV treatment pre-RT and 33 post-RT. There was no difference in age, gender, HCV genotype or cirrhosis. 81% of patients in both groups were male (p=0.87). 67% of those treated pre-RT were black compared to 79% white treated post-RT (p<0.0001). Of 21 pre-RT, 1 underwent RT;1 died awaiting RT. Median WLT in pre-RT group was 650 days (range 148-1433), compared to167 days (range 2-203) in post-RT group (p=0.03; figure 1). Median WLT nationally, in UNOS region 3 and region 5 are 701, 598, and 862 days respectively. 77% received brain dead donor allograft. 21 patients (63%) received HCV+ kidneys. All had functioning allografts at last follow up. Median time from RT to HCV treatment was 77 days (range 30-4433). There was no hepatic decompensation and all achieved SVR. **CONCLUSION:** A strategy of post-RT HCV treatment markedly reduced median WLT compared nationally, regionally, and with a center in the same health system. As all patients achieved SVR, delaying HCV treatment until after RT appeared to shorten WLT without adverse impact on recipients' liver condition or overall survival.



Disclosures:

Andrew P. Keaveny - Independent Contractor: UpToDate Inc

The following people have nothing to disclose: David M. Chascsa, Omar Y. Mousa, Surakit Pungpapong, Nan Zhang, Amy E. Chervenak, Sreecharita Nidamanuri, Eduardo Rodriguez, Diana Franco, Kristen Ryland, Janna Huskey, Hugo E. Vargas, Bashar A. Aqel

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Adherence to Pangenotypic Glecaprevir/ Pibrentasvir Treatment and SVR12 in HCV-infected Patients: An Integrated Analysis of the Phase 2/3 Clinical Trial Program

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Background: Previous studies of anti-HCV regimens suggest an association between lower treatment adherence and reduced SVR rates. This integrated analysis assessed the factors associated with non-adherence to all-oral, once-daily, interferon- and ribavirin-free, pan-genotypic glecaprevir/pibrentasvir regimen (G/P; glecaprevir identified by AbbVie and Enanta) and the impact of non-adherence on SVR12 rates (97% overall) in HCV patients enrolled in the G/P phase 2/3 clinical trial program. **Methods:** Data were pooled from 2,091 patients enrolled in 8-, 12-, or 16-week active-treatment arms from the studies ENDURANCE-1, ENDURANCE-2, ENDURANCE-3, ENDURANCE-4, EXPEDITION-1, EXPEDITION-4, SURVEYOR-II, and MAGELLAN-1. Adherence was measured via pill count and defined as compliance to the assigned G/P regimen of at least 80% at all on-treatment visits. Interaction of non-adherence with the treatment outcome was assessed using a multivariate linear regression model, controlling for baseline demographic and clinical characteristics. **Results:** Overall, 88.5% (1,851/2,091) patients were adherent to G/P at all consecutive between-visit intervals. Subgroups with the lowest adherence included patients with genotype 3 infec-

tion (80.0% [419/524]), severe renal impairment (79.8% [83/104]), and those on stable opioid substitution therapy (78.2%, [115/147]). Significant independent predictors of non-adherence were current alcohol use (vs non-use; odds ratio [95% confidence interval]: 1.96 [1.29, 2.98]), previous alcohol use (vs non-use; 1.87 [1.21, 2.90]), genotype 3 infection (vs genotype 1; 2.16 [1.54, 3.03]), and severe renal impairment (yes vs no; 2.79 [1.56, 4.97]). In the adherent subgroup, AEs were experienced by 66% (1,228/1,851) and serious AEs by 3% (55/1,851) of patients. In the non-adherent subgroup, those values were 72% (174/205) and 5% (13/240). The SVR12 rates were similar between adherent and non-adherent patients (table). **Conclusion:** In HCV-infected patients treated with the G/P regimen for 8, 12, or 16 weeks, overall adherence was high. Non-adherence was not associated with increased virologic failure rates, suggesting a good forgiveness of this once-daily regimen. These data provide further support for the high SVR rates seen in G/P clinical studies, regardless of patient characteristics.

	Adherent N=1,839	Non-adherent N=230	Overall N=2,069
SVR12 [95%CI], mITT population [a]	98.6 [98.0, 99.1]	98.7 [96.2, 99.6]	98.6 [98.1, 99.1]

[a] Includes all patients who received at least one dose of G/P, except those who did not have SVR12 data available due to reasons other than virologic failure.
CI, confidence interval; mITT, modified intent to treat; SVR12, sustained virologic response at post-treatment week 12.

Disclosures:

Ashley S. Brown - Advisory Committees or Review Panels: MSD, Bristol-Myers-Squibb, Gilead, Janssen, Abbvie; Grant/Research Support: Gilead; Speaking and Teaching: MSD, Bristol-Myers Squibb, Gilead, Janssen, Abbvie

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Heritability and shared gene effects of novel gut microbiome associated serum metabolite in NAFLD: A twin and family study

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Background: Studies have shown that nonalcoholic fatty liver disease (NAFLD) is heritable and has a shared gene-effect with metabolic risk factors. However, the heritability of the serum metabolites associated with NAFLD has not been assessed. The aim of this study was to investigate whether serum metabolites are heritable and if there are any shared gene-effects with NAFLD. **Methods:** This is a cross-sectional analysis of a well-characterized prospective cohort of community-dwelling Twins and Family from southern California (156 subjects: 100 twins; 37 monozygotic, 13 dizygotic twin pairs; 56 sibling-sibling or parents-offspring). Hepatic steatosis was assessed using magnetic resonance imaging proton density fat fraction (MRI-PDFF). Serum metabolite analysis was performed by Metabolon platform using UPLC-MS/MS and GC/MS. Serum metabolites associated with NAFLD were assessed using a Welch's t-test and a sampling permutation by running 1,000 random selections of the dataset for each serum metabolite was performed to assess the familial effect. The association of serum metabolites with NAFLD was confirmed using generalized estimating equations (PROC GENMOD) to account for intrapair correlation within twinship. A classic twin ACE model was used to estimate the heritability of the serum metabolites and the shared gene effect (R_G) between serum metabolites and NAFLD. **Results**: Mean (± sd) age and body mass index were 46.3 (± 19.8) years and 26.6 (± 6.0) kg/m2, respectively, and 36 subjects (23%) had NAFLD (MRI-PDFF≥5%). Among 713 serum metabolites analyzed, 156 were significantly associated with NAFLD in the whole cohort. We identified 30 serum metabolites that were heritable: belonging to Lipid, Amino Acid and Peptide super pathways. Four heritable serum metabolites were significantly associated with NAFLD after adjustment for age, sex and Hispanic ethnicity : phenyllactate, palmitic acid, gamma-glutamylisoleucine, 3-(4-hydroxyphenyl)lactate, with odd ratios of NAFLD (95% confidence interval, p-value) of 2.12 (1.09-4.10, 0.0258), 2.58 (1.31-5.17, 0.0065), 2.98 (1.36-6.51, 0.0062), 4.29 (1.87-9.81,0.0006), respectively. We identified a novel serum metabolite, Phenyllactate (derived from gut microbiome), which had a significant shared gene effect with NAFLD: R_G : 0.925 (95%CI, 0.031-1). **Conclusion:** This study provides evidence that several serum metabolites associated with NAFLD are heritable. We report the discovery of a single novel gut microbiome-liked serum metabolite, phenyllactate, that has a significant shared gene effect with NAFLD. These data suggest a potential link between genetics and gut-microbiome metabolites in the pathogenesis of NAFLD.

Disclosures:

Lisa M. Richards - Speaking and Teaching: Intercept, Merck, Gilead, Abbvie

Claude B. Sirlin - Grant/Research Support: GE, Pfizer, Bayer, Guerbet, Siemens

Rohit Loomba - Advisory Committees or Review Panels: Galmed Inc, Tobira Inc, Arrowhead Research Inc; Consulting: Gilead Inc, Corgenix Inc, Janssen and Janssen Inc, Zafgen Inc, Celgene Inc, Alnylam Inc, Inanta Inc, Deutrx Inc; Grant/Research Support: Daiichi Sankyo Inc, AGA, Merck Inc, Promedior Inc, Kinemed Inc, Immuron Inc, Adheron Inc

The following people have nothing to disclose: Cyrielle CAUSSY, Cynthia Hsu, Amy Liu, Ricki Bettencourt, Veeral H. Ajmera, Shirin Bassirian, Jonathan Hooker, Ethan Z. Sy, David A. Brenner, Chi-Hua Chen

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Dynamic adaptations of intestinal microbiota after liver transplantation

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Background: The effects of liver disease and transplantation (LT) on the intestinal microbiome are complex and incompletely understood. Changes in the microbiome post-LT may be due to the underlying cause of liver, immunosuppression, antibiotics, and the ischemia, reperfusion and allorecognition associated with LT. Moreover, LT recipients are disproportionally affected by colonization and infection with potentially fatal multi-drug-resistant organisms (MDRO). **Methods**: Consecutive adult LT candidates and recipients have been prospectively enrolled since 03/2014. Fecal samples were collected pre-LT, at weeks 1, 2, 3 and months 2, 3, 6, 9 and 12 post-LT. Fecal specimens were screened for MDR-O using ChromAgar. DNA was extracted from fecal samples and 16S rRNA (V3-V4 region) sequencing was performed (Illumina MiSeq). Alpha- and beta-diversity were compared between patient groups to evaluate the impact of severity and etiology of liver disease on microbiome composition, the natural history of microbiome diversity in serial samples with restoration of health, and the relationship between diversity and MDRO infection. **Results**: We enrolled 323 patients and 130 completed 1-year follow-up. Patient mean age was 60.4 years, 61% were male and median laboratory MELD at LT was 25. HCV (39%) and NAFLD (14%) were the most frequent indications for LT and 48.5% had HCC. MDRO colonization was detected at least once in 65% of patients during 1-year post-LT. *C. difficile* infections occurred in 24% of patients and MDRO infections in 15%. We collected 1098 fecal samples (median 7 per patient) and sequenced 384. In preliminary analysis of 129 samples alpha-diversity (Shannon index) differed significantly by liver disease severity measured by MELD (2.05 MELD >35 vs. 2.84 MELD <15), by post-LT period (p=0.023), and by underlying disease (1.76 alcohol vs. 2.59 in HBV). In linear discriminant analyses Actinomycetales were markers of early post-LT and Clostridiales of 1-year microbiota. During MDRO colonization (eg. carbapenem-resistant Enterobacteriaceae), patients had a decrease in *Lactobacillus* and Lachnospiraceae and differed in beta-diversity in weighted UniFrac analysis. In ongoing analyses we are further investigating how MELD, liver disease and post-LT period modulate interactions between MDROs and gut microbiota. **Conclusions**: Intestinal microbiota undergo dynamic adaptations in the peri- and post-transplant period. Our preliminary data suggest a critical role for modulation of gut microbiota through MDRO colonization and vice versa. Further studies are needed to characterize these interactions and their impact on transplant outcomes.

Disclosures:

Robert S. Brown - Consulting: Abbvie, Merck, Intercept, BMS, Gilead

The following people have nothing to disclose: Nenad Macesic, Medini Annavajhala, Marley Giddins, Stephania Stump, Angela Gomez-Simmonds, Anne-Catrin Uhlemann

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NLRP6 inflammasome-mediated dysbiosis augments acetaminophen induced acute liver injury

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Background & Aims: Acetaminophen (APAP) poisoning represents the leading cause of acute liver failure (ALF) in western countries. Whereas the link between intestinal dysbiosis and chronic liver disease is well established, insight into the role of gut-liver crosstalk for drug induced liver injury (DILI) remains scarce. Here, we hypothesized that the intestinal microbiota may affect the outcome of APAP overdose. **Methods:** Male 6-8 week old wildtype (WT) and *Nlrp6*$^{-/-}$ mice were injected with a sublethal dose of APAP to induce DILI. 12 hours after injection, a comprehensive analysis of liver injury was performed based on liver functions tests (LFTs), histology, flow cytometry immunophenotyping (FACS) and 16S rRNA-based microbiota profiling. Moreover, microbiota of WT and *Nlrp6*$^{-/-}$ mice was modulated by oral broad-spectrum antibiotics (ABx) or fecal microbiota transfer (FMT), respectively. **Results:** APAP administration induced significantly increased liver injury in *Nlrp6*$^{-/-}$ mice compared to WT controls as evidenced by LFTs and histological assessment which revealed necrosis as the predominant form of cell death. Enhanced DILI in *Nlrp6*$^{-/-}$ mice was associated with markedly increased infiltration of Ly6C^{hi} monocyte derived macrophages (MoMFs) as demonstrated by FACS analysis. Upon microbiota depletion by ABx liver injury in WT and *Nlrp6*$^{-/-}$ mice was indistinguishable, suggesting that previously reported dysbiosis in *Nlrp6*$^{-/-}$ mice contributes to disease exacerbation. Strikingly, WT mice gavaged with microbiota from *Nlrp6*$^{-/-}$ mice displayed significantly increased liver injury upon APAP treatment and resembled the inflammatory phenotype of *Nlrp6*$^{-/-}$ mice. Specifically, FMT skewed MoMF polarization in WT mice toward a Ly6C^{hi} inflammatory phenotype suggesting a strategic function of MoMF as sensors of gut-derived signals orchestrating the inflammatory response. **Conclusions:** Our data suggest an important, yet unknown function of the intestinal microbiota and gut-liver crosstalk during acute liver injury. Intestinal dysbiosis – as seen in *Nlrp6*$^{-/-}$ mice and transferrable to healthy WT controls via FMT - aggravated liver injury upon APAP administration by promoting pro-inflammatory Ly6C^{hi} macrophage polarization.

Disclosures:

Christian Trautwein - Advisory Committees or Review Panels: Abbvie, MSD, BMS, Gilead, Bayer, AstraZeneca; Speaking and Teaching: Falk, MSD, Abbvie, BMS, Gilead

The following people have nothing to disclose: Kai M. Schneider, Carsten Elfers, Antje Mohs, Eric J. Galvez, Till Strowig

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Maternal exposure to the bacterial-derived Butyrate protects neonatal mice against experimental biliary atresia

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Background: Little is known about the relationship between the intestinal microbiome and pathogenesis of biliary atresia (BA). Recently, we showed that the intestinal enrichment of butyrate-producing *Anaerococcus lactolyticus* in mice with impaired IL-8 signaling makes them resistant to rotavirus (RRV)-induced biliary atresia. To explore the mechanisms used by bacteria to regulate tissue injury, we investigated if treatment of mice with butyrate prevents experimental biliary atresia. Our hypothesis was that butyrate decreases epithelial injury and bile duct obstruction by suppressing neonatal immune cells. **Methods:** Adult Balb/c female mice were given *ad libitum* water with or without 200mM sodium butyrate throughout pregnancy and the first 3 weeks post partum. Their offspring were injected intraperitoneally with $1.5x10^6$ ffu of RRV within 24 hr of birth and monitored for symptoms. Histopathology, FACS, and mRNA for *Tnfa, Irf7, Tlr9, Cxcl10,* and *Il1b* were quantified at days 3-14. **Results:** Treatment of mothers with butyrate significantly attenuated jaundice in their newborn mice infected with RRV (51% vs 91% in controls, $P<0.0001$), decreased portal inflammation and extrahepatic cholangitis, prevented duct obstruction in 64% of mice (vs 30% in controls, $P<0.05$) and improve long-term survival (60% vs 20% in controls, N=65-72, $P<0.0001$). Investigating the immunological basis of this improvement, we performed FACS of hepatic mononuclear cells at 7 days (time of duct obstruction) and found a decrease in the number and activation of myeloid DCs (33±4.7% vs 44±7.8%, $P<0.02$) and CD3/CD4+ cells (24±2.4% vs 31±3.3%, $P<0.01$) and CD3/CD8+ cells (29±3.3% vs 37±2.7%, $P<0.01$), as well as their effector subpopulations of $CD4^+CD69^+$ (13±2.8% vs 19±3.9%, $P<0.02$) and $CD8^+CD69^+$ (12±1.4% vs 51±5.8%, $P<0.0001$). At the molecular level, butyrate decreased the hepatic expressions of *Tnfa, Irf7, Tlr9* and *Cxcl10* (2-3 fold, $P\leq0.03$-0.00001) at days 3-14 after RRV infection, but not *Il1b* mRNA. **Conclusion:** Maternal exposure to butyrate: 1) suppressed the activation of hepatic dendritic cells and effector lymphocytes in their newborn mice infected with RRV, and 2) decreased the susceptibility to RRV-induced experimental biliary atresia. These data suggest a regulatory role of bacterial products in pathogenesis of disease.

Disclosures:

Jorge A. Bezerra - Grant/Research Support: Molecular Genetics Laboratory, CHMC, Gilead, Shier

The following people have nothing to disclose: Yu Zhu, Junbae Jee, Sridevi Gutta, Reena Mourya, Pranavkumar Shivakumar

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Metagenomic Analysis of Bacteria-Derived Extracellular Vesicles as Biomarkers for Hepatocellular Carcinoma

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Background/Aims: Gut dysbiosis promotes the development and progression of various liver diseases through interactions with the host's immune systems. Bacteria-derived extracellular vesicles (EVs) have been suggested to be important in host-microbe communications. In this study, we evaluated whether disease-specific microbiome alterations are present in the bacteria-derived EVs from patients with cirrhosis and HCC, and their potential as diagnostic biomarkers for HCC. Methods: We performed cross-sectional metagenomic analyses of serum samples from 94 patients with HCC, 100 with cirrhosis and matching healthy controls. DNA was extracted from bacteria-derived EVs after EV isolation using the differential centrifugation method. Bacterial genomic DNA sequencing was performed using high-throughput pyrosequencing after amplification of the V3–V4 hypervariable regions of 16S rDNA. Results: There were specific differences in the proportion of several bacterial taxa in blood EVs that correlate with the presence of cirrhosis and HCC, thus defining a specific signature of the liver disease, compared with healthy controls. In addition, a significant reduction of within-individual microbial diversity was noted in HCC compared with cirrhosis (p=0.04). We identified 5 microbial gene markers distinguishing metagenomes of HCC from cirrhosis with a balanced accuracy of 84.4% and an area under the receiver-operating curve (AUC) of 0.905. Conclusion: Microbiome-based signatures may be potential biomarkers for the early diagnosis of cirrhosis or HCC.

Disclosures:

Yoon Jun Kim - Grant/Research Support: Bristol-Myers Squibb, Roche, JW Creagene, Bukwang Pharmaceuticals, Handok Pharmaceuticals, Hanmi Pharmaceuticals, Yuhan Pharmaceuticals; Speaking and Teaching: Bayer HealthCare Pharmaceuticals, Gilead Science, MSD Korea, Yuhan Pharmaceuticals, Samil Pharmaceuticals, CJ Pharmaceuticals, Bukwang Pharmaceuticals, Handok Pharmaceuticals

Jung-Hwan Yoon - Grant/Research Support: Bayer HealthCare Pharmaceuticals, Daewoong Pharmaceuticals, Bukwang Pharmaceuticals

The following people have nothing to disclose: Eun Ju Cho, Hyeki Cho, Young Youn Cho, Jeong-Hoon Lee, Su Jong Yu

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Intestinal Farnesoid X Receptor Deficiency Exacerbates Alcoholic Steatohepatitis

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Background: Alcoholic liver disease (ALD) is one of the major causes of liver morbidity and mortality worldwide. Bile acids (BA) and the BA-activated nuclear receptor, farnesoid X receptor (FXR), play important roles in the

development of ALD. In mice, fibroblast growth factor 15 (FGF15) is induced by intestinal FXR activation and subsequently FGF15 signaling to the liver inhibits BA synthesis in hepatocytes, creating a negative feedback loop. Thereby, the gut-liver FXR-FGF15 axis is critical for maintaining BA homeostasis. However, the contribution of intestine-specific FXR and FGF15 in the pathogenesis of ALD remains unknown. **Methods:** Intestine-specific FXR knockout ($FXR^{Int-/-}$), FGF15 whole-body knockout ($FGF15^{-/-}$) and wild-type (WT) mice were fed a Lieber-DeCarli ethanol-containing diet for 10 days, followed by a single "binge" dose of ethanol (5g/kg body weight) via gavage. All cohorts were sacrificed 9 hours after the final ethanol administration. Intestinal permeability was assessed by oral gavage of FITC-dextran (100 mg/ml in PBS, 440mg/kg body weight). Liver, intestine and serum samples were collected for analysis. **Results:** Serum alanine and aspartate aminotransferase activities and BA levels were higher in $FXR^{Int-/-}$ compared to WT and liver histology further confirmed $FXR^{Int-/-}$ and $FGF15^{-/-}$ were more susceptible to ethanol-induced liver steatosis and injury compared to WT. However, less steatosis and liver injury was observed in $FGF15^{-/-}$ compared to $FXR^{Int-/-}$ mice following ethanol feeding. Ethanol altered the expression of hepatic genes involved in lipid homeostasis. Specifically, CD36 levels were increased in $FXR^{Int-/-}$ mice potentially contributing to increased liver steatosis with greater fatty-acid uptake following ethanol feeding. Genes involved in fibrosis were all induced by ethanol feeding in WT mice and further induced in $FXR^{Int-/-}$ and $FGF15^{-/-}$ mice. Furthermore, Sirius Red staining revealed worsened fibrosis in $FXR^{Int-/-}$ and $FGF15^{-/-}$ mouse livers compared to those of WT mice. Ethanol feeding increased hepatic gene expression of pro-inflammatory cytokine TNF-α, but not IL-6, in all genotypes. Ethanol feeding induced gene expression of a LPS-binding protein, CD14, in all mice, with greater induction in $FXR^{Int-/-}$ and $FGF15^{-/-}$mice. $FXR^{Int-/-}$ mice had increased intestinal permeability. BA homeostasis was disturbed in $FXR^{Int-/-}$ and $FGF15^{-/-}$ mice by ethanol feeding. **Conclusion:** Intestinal FXR may protect mice from ethanol-induced liver injury and $FXR^{Int-/-}$ mice have increased gut leakage/permeability, increased serum LPS levels, and induced liver inflammation possibly due to decreased integrity of the intestine.

Disclosures:

The following people have nothing to disclose: Mingxing Huang, Bo Kong, Min Zhang, Laura Armstrong, Monica Chow, Justin D. Schumacher, Dan Rizzolo, Xin Li, Jinyu Xia, Yi-Horng Lee, Grace L. Guo

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The road to elimination of Hepatitis C: Analysis of SVR versus new HCV infections in 91 countries

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Background: Hepatitis C (HCV) can only be eradicated if annual rates of cure (SVR) are consistently and significantly higher than new HCV infections, across many countries. In 2016, the WHO called for a 90% reduction in new HCV infection by 2030. Direct acting antivirals (DAA) can cure over 90% of those treated, at potentially very low prices. We compared the net annual change in epidemic size across 91 countries using data on SVR, new HCV infections, and deaths. In a further 109 countries, we projected this figure using regional averages of epidemic size. **Methods**: Epidemiological data for 2016 were extracted from national reports, publications and the Polaris Observatory (http://polarisobservatory.org/polaris/hepC.htm): 91/210 countries and territories worldwide had data on SVR, HCV-related deaths and new infections available for analysis. 109 countries had net change in epidemic size projected from the regional prevalence of HCV, extrapolated to their population size. "Net Cure" was defined as the number of people with SVR, minus new HCV infections, plus HCV-related deaths in 2016. **Results**: Table 1 shows the number achieving SVR, new infections and HCV-related deaths by region. In the 91 countries analysed, there were 57.3 million with chronic HCV infection in 2016. In the remaining 109 countries and territories, the projected epidemic size was 12.2 million, giving a global epidemic size of 69.6 million. Across the 91 countries, there was a drop from 57.3 to 56.9 million people in 2017, a 0.7% reduction. The projected global net change was from 69.6 to 69.3 million, a 0.4% reduction. Ten countries had at least 5 times more people reaching SVR than new HCV infections, including Egypt and USA. In 47/91 countries, there were more HCV infections than SVR in 2016. **Conclusion**: Very few countries are on target to achieve elimination of HCV as a public health problem by 2030. While the North American, North African/Middle East and Western European regions have shown small declines in prevalence, the epidemic is growing in sub-Saharan Africa and Eastern Europe. Far higher rates of DAA treatment are required for worldwide elimination of HCV.

Table 1 - HCV Epidemiology

Region	Total viraemic (millions)	New HCV infections	Number cured	HCV-related deaths	Net cure (%)
North America (n = 3)	2.96	31,870	216,731	20,829	6.96% (205,690)
North Africa & Middle East (n = 18)	7.40	156,660	542,724	51,944	5.92% (438,008)
Western Europe (n = 20)	2.36	35,440	105,821	14,951	3.61% (85,332)
Central & South America (n = 11)	3.48	27,537	47,859	21,496	1.20% (40,548)
Asia & Pacific (n = 18)	29.6	574,330	456,552	179,810	0.21% (62,032)
Sub Saharan Africa (n = 8)	5.07	130,800	3,805	21,540	- 2.08% (- 105,455)
Central & Eastern Europe (n = 15)	6.51	322,800	26,110	15,505	- 4.32% (- 281,185)
Missing countries (n = 109)	12.2	318,375	113,157	57,923	- 1.21% (- 147,295)
Global estimate	**69.6**	**1.279,437**	**1,399,602**	**326,075**	**0.43% (298,945)**

Disclosures:

The following people have nothing to disclose: Andrew M. Hill, Sanjay Nath, Bryony Simmons

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Dynamics and Characterization of DAA Treatment Induced Serum AFP Reduction in HCV-infected Patients without Hepatocellular Carcinoma (HCC)

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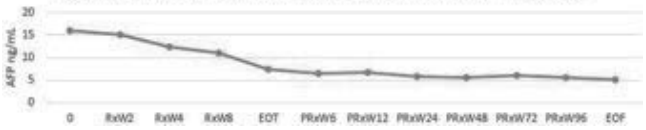
BACKGROUND. It was recently reported that DAA treatment (Rx) may result in reduction of elevated serum AFP, but larger and more detailed studies are needed to characterize how DAA Rx impacts elevated AFP in HCV-infected

patients. **AIMS.** To assess the frequency of baseline AFP elevations and their related factors, AFP dynamics during and after DAA Rx, and factors associated with AFP reduction. **METHODS.** A retrospective chart review and analysis on 149 HCV-infected patients without HCC, finished DAA Rx and minimum 12 wks post Rx follow-up (PRx F/U). 141 had SVR12, 8 relapsed; 4 were diagnosed for HCC during PRx F/U. 3 more cases with baseline HCV-cirrhosis, HCC, and elevated AFP were used for comparison. **RESULTS.** Mean PRx F/U was 54 (12-124) wks, mean age, 57.8 (20-85); 51%, males; 78% had GT 1; 47%, cirrhosis. 56.9% had baseline AFP elevation (> 5.5 ng/mL); 30%, > 10; 8.5% > 25; and 4.7%, > 50. Baseline AFP > 5.5 was associated with histologic G3-4 (p=0.016), F3-4 (p=0.008), NASH (p=0.018), cirrhosis (p<0.001), and end of follow-up (EOF) ALT > 30 IU/L (p=0.026). On multivariate analysis, baseline AFP > 5.5 was associated with NASH (p=0.035), cirrhosis (p<0.001), and GT1 (p=0.029). AFP normalization (norm.) was seen in 0%, RxW2; 19.0%, RxW4; 32.7%, end of treatment (EOT); 39.4%, PRxW12; 52.9%, PRxW24; 56.7%, PRxW48; and 57.1%, EOF. Figure 1 summarizes mean AFP dynamics in cases with baseline AFP > 5.5. Univariate analysis showed PRxW24 AFP norm. was associated with absence of cirrhosis (p=0.005), CPC < 6 (p=0.024), baseline AFP < 10 (p<0.001) and ALT and AST < 40 IU/L (p=0.029), and RxW4 AST < 30 (p=0.008). Multivariate analysis showed PRxW24 AFP norm. was associated with absence of cirrhosis (p=0.003), CPC < 6 (p=0.015), and baseline AFP < 10 (p=0.015). During PRx F/U, 4 developed HCC. All had baseline HCV cirrhosis and AFP elevation (7-51), 3 had AFP reduction, and none had AFP norm. at HCC diagnosis. 3 other cases with HCV-cirrhosis, uncontrolled HCC, and baseline elevated AFP (125-850) had continual AFP rise during Rx and PRx F/U. **CONCLUSIONS.** Baseline AFP elevation occurred in 56.9% of cases undergoing DAA Rx and is independently associated with NASH, cirrhosis, and GT1 infection. DAA Rx resulted in AFP norm. as early as RxW4 with progressive reduction through PRxW48, and by the EOF, 57.1% had AFP norm. PRxW24 AFP norm. is independently associated with absence of cirrhosis, CPC < 6, and baseline AFP <10 ng/mL.

Figure 1: Mean AFP Trend for Patients With Baseline AFP > 5.5 ng/mL



Disclosures:

Ke-Qin Hu - Speaking and Teaching: Gilead, Merck, Intercept, Abbvie

The following people have nothing to disclose: Johnathan Zhang, Tung Huynh, Mohit Mittal

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Significantly Improved Liver Transplant Waitlist Survival Among Chronic Hepatitis C Virus Patients After Introduction of Direct Acting Antiviral Therapies

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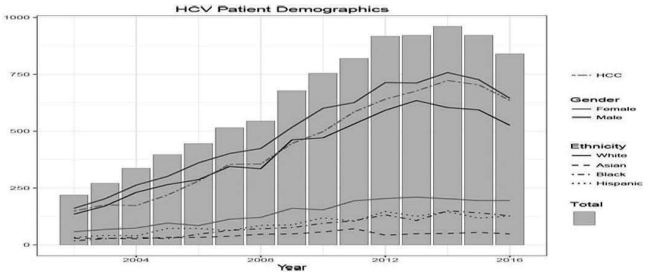
Background: Direct acting antivirals (DAA) for chronic HCV successfully reduce HCV-related disease progression. It is expected that rates of HCV-related liver transplantation (LT) and LT waitlist mortality have also declined. Aim: Evaluate trends in waitlist registrations, waitlist survival, and probability of receiving LT among U.S. adults with chronic HCV. Methods: We retrospectively evaluated adults (age ≥18) with chronic HCV listed for LT before and after the widespread use of sofosbuvir, allowing a 6-month period after its approval (Era 1: 1/1/2002-6/1/2014 vs Era 2: 6/1/2014-12/31/2016) using the United Network for Organ Sharing registry. LT waitlist survival and likelihood of receiving LT were evaluated with multivariate Cox regression models, stratified by Eras and adjusted for sex, age, ethnicity, MELD, and HCC. Results: Among 9546 HCV patients (7228 in Era 1, 2318 in Era 2), proportion of HCV patients with HCC increased by 1.2%/year (R^2 0.55, p=0.001 by linear regression), and over 75% of HCV patients listed for LT in Era 2 had concurrent HCC. There were no sex-specific differences in HCV-related LT listings in Era 1 vs. Era 2; proportion of black HCV patients increased from 12.0% to 16.0% and proportion of Asian HCV patients decreased from 7.3% to 5.8%, p<0.001. HCV patients in Era 2 had significantly lower waitlist mortality compared to Era 1 (HR 0.77, p=0.02). However, HCV patients in Era 2 were less likely to receive LT compared to Era 1 (HR 0.65, p<0.001). When stratified by ethnicity, Hispanics had significantly better waitlist survival in Era 1 compared to Era 2 (HR 0.69, p = 0.004), whereas no significant difference in waitlist survival between eras was seen among Asians. Blacks with HCV had lower likelihood of receiving LT in Era 2 compared to Era 1 (HR 0.76, p = 0.002). Compared to non-HCC, HCC patients were more likely to receive LT in both eras; HCC patients had higher waitlist mortality in both eras (Era 1: HR 1.27, p = 0.01; Era 2: HR 1.69, p = 0.03) Conclusion: While HCV and HCV-HCC as an indication for LT continue to rise, both appears to have peaked and is decreasing after the introduction of DAAs. Furthermore, LT waitlist survival among HCV patients is significantly higher after availability of DAAs.



Disclosures:

Robert G. Gish - Advisory Committees or Review Panels: Gilead, AbbVie, Arrowhead, Ionis, Merck; Consulting: Eiger, Genentech, Merck, Enyo, MedImmune, Avella, Quest; Speaking and Teaching: Gilead, AbbVie, Merck; Stock Shareholder: Arrowhead

Robert J. Wong - Advisory Committees or Review Panels: Gilead; Consulting: Gilead; Grant/Research Support: Gilead; Speaking and Teaching: Gilead, Salix, Bayer

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Hepatitis C virus-induced epigenetic changes associated with hepatocarcinogenesis persist following viral cure

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Background. Hepatitis C virus infection (HCV) is a major cause of hepatocellular carcinoma (HCC) worldwide. While direct-acting antivirals cure viral infection the large majority of infected patients, limited access to therapies and late stage diagnosis are challenges for prevention of HCC on a global level. Furthermore, there is accumulating evidence that cancer risk persists even after ten years of viral cure in particular in patients with advanced fibrosis. **Methods.** To study whether epigenetic and transcriptomic changes triggered by chronic HCV infection contribute to liver disease and HCC risk, we performed genome-wide ChIP-Seq defining active and repressed genomic regions (i.e., H3K4me3, H3K27ac, and H3K27me3) as well as RNA-Seq analyses in liver tissues from patients with chronic HCV infection, and in patients with viral cure and HCC. We then integrated the level of histone mark changes with differential expression of associated genes. Finally, we performed functional and mechanistic studies of epigenetic and transcriptional changes using a HCV cell culture model. **Results.** Chronic HCV infection induced genome-wide epigenetic changes in the liver of patients compared to liver tissue from non-infected controls. Interestingly, virus-induced epigenetic and transcriptional changes were only partially reversed following DAA-based treatment with subsequent viral cure. Gene set enrichment analysis of the virus-induced changes persisting following cure revealed dysregulated pathways associated with hepatocarcinogenesis. In an HCV cell culture model, chromatin remodeling inhibitors reversed the HCV-induced gene regulation associated with HCC risk, confirming a functional role of epigenetic modifications for virus-induced transcriptional reprogramming associated with carcinogenesis. **Conclusion.** Virus-induced epigenetic and transcriptional modifications are only partially reversed following DAA-based treatment, and therefore persist post cure. These findings suggest that HCV-induced epigenetic changes are an important driver for hepatocarcinogenesis and may contribute to persistent HCC risk post cure. These findings open novel opportunities to discover biomarkers that can identify patients at high remaining risk for HCC, and open a perspective for HCC chemoprevention.

Disclosures:

Patrick Pessaux - Board Membership: Merck; Speaking and Teaching: integra

Yujin Hoshida - Grant/Research Support: Allergan, Kyowa Hakko Kirin, AbbVie, Zafgen, Enanta Pharmaceuticals; Speaking and Teaching: Merck

The following people have nothing to disclose: Frank Jühling, Nourdine Hamdane, Christine Thumann, Simonetta Bandiera, Antonio Saviano, Christoph Bock, François Habersetzer, Stefan Wieland, Nabeel Bardeesy, Mirjam B. Zeisel, Thomas F. Baumert

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MicroRNA-130a Regulates HCV and HBV Replication through the PKLR/pyruvate Pathway

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Background & Aims: HBV and HCV infection have each been shown to positively regulate miR-130a expression. In this study, we sought to identify and characterize miR-130a target genes and to explore the mechanisms by which miR-130a regulates HCV or HBV replication. **Methods:** Bioinformatics software was used to predict potential miR-130a target genes. MiR-130a and its target genes were overexpressed, or knocked down by siRNA or by CRISPR/Cas9 gRNA, respectively. Selected gene mRNAs and their proteins, together with HCV replication in OR6 cells, JFH1 HCV-infected Huh7.5.1 cells and JFH1 HCV-infected human primary human hepatocytes (PHHs), and HBV replication in HepAD38 cells, HBV-infected NTCP-Huh7.5.1 cells and HBV-infected NTCP-PHHs, were monitored by qRT-PCR and Western blot, respectively. **Results**: We identified 2152 potential miR-130a target genes by bioinformatics analysis. Dual luciferase reporter assay specifically confirmed that miR-130a targets PKLR. We found that miR-130a overexpression down-regulated PKLR mRNA and protein levels in OR6 cells, Huh7.5.1 cells, HepAD38 cells, and PHHs. miR-130a inhibitor and gRNA increased PKLR expression. MiR-130a mimic and PKLR siRNA or gRNA knockdown inhibited HCV replication in JFH1-infected Huh7.5.1 cells and JFH1-infected PHHs, and HBV replication in HepAD38 cells, HBV infected NTCP-Huh7.5.1 cells and NTCP-PHHs, while miR-130a gRNA and PKLR overexpression increased HCV and HBV replication. Supplemental pyruvate increased HCV and HBV replication and rescued HCV and HBV replication inhibition by miR-130a mimic and PKLR knockdown. Moreover, miR-130a levels were not affected by PKLR overexpression, knock down or supplemental pyruvate. **Conclusion:** MiR-130a regulates HCV and HBV production through its targeting of PKLR and subsequent pyruvate production. Our data provide novel insights into miR-130a regulated metabolic pathway steps,

including PKLR and pyruvate synthesis, which are in turn subverted by HCV and HBV replication.

Disclosures:

Jacinta A. Holmes - Grant/Research Support: NIH, AASLD, AbbVie, ACTG

Raymond T. Chung - Advisory Committees or Review Panels: Alnylam; Grant/Research Support: Gilead, Abbvie, Merck, BMS, Boehringer Ingelheim, Janssen

The following people have nothing to disclose: Xiaoqiong Duan, Shilin Li, Zeng Tu, Yujia Li, Dachuan Cai, Xiao Liu, Wenting Li, Chunhui Yang, Baihai Jiao, Esperance A. Schaefer, Dahlene Fusco, Shadi Salloum, Limin Chen, Wenyu Lin

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Identification of Novel HCV Genotype and Subtypes in Patients Treated with Sofosbuvir Based Regimens

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Introduction: The hepatitis C virus (HCV) is classified into 7 genotypes and 67 subtypes. The determination of HCV genotype and subtype has historically been necessary to inform the optimal treatment regimen. Here we investigate the HCV genotype and subtype in patients with unresolved HCV genotyping results enrolled in clinical studies of sofosbuvir-based regimens. **Methods:** Assignment of HCV subtype was performed by BLAST analysis of NS3, NS5A and NS5B deep sequencing to GT1-7 reference strains (67 subtypes and 7 genotypes). HCV samples with unresolved subtype (<85% homology to references) or with different subtype assigned across the multiple sequencing targets were subsequently sequenced by full HCV genome sequencing. **Results:** Across Gilead clinical studies, 52 patients had <85% sequence homology and/or different subtype assigned across multiple targets. Random amplification and full genome sequencing was performed on 34/52 patients. Successful HCV sequencing data were obtained from 31/34 patients. Phylogenetic analysis revealed that out of 31 patients with full HCV genome sequencing data, 17 patients were infected with novel HCV subtypes and 2 patients were infected with a novel HCV genotype, distinct from GT1-7. Of the remaining patients, 9 patients were infected with one of the 67 known HCV subtypes and 3 were infected with multiple HCV strains. The novel subtypes identified were distinct from each other but assigned to the following genotypes; 9 to GT2, 4 to GT4, 2 GT6 and one each to GT5 and GT7. Twelve of 17 patients infected with novel subtypes were from Europe, particularly France. The 2 patients infected with a novel HCV genotype were both from Canada but originated from the Punjab state of India and they were epidemiologically unlinked. Across patients with novel HCV subtype/genotype the following substitutions were observed in multiple patients at previously identified amino acid positions associated with resistance to DAAs: 36L, 56F/L, 80G/K and 122A/H/K/R/N/T, 170V/H and 175M/I in NS3; 24E/S, 28C/L/F/T/V, 30A/H/K/S, 31M/P, 58P, 92C/L/S/T, 93S/V in NS5A; 159R/S, 237L/T and 289F/H/M in NS5B. Despite presence of polymorphism at RASs positions, 19/19 patients treated with sofosbuvir/velpatasvir±voxilaprevir achieved SVR12. **Conclusions:** Across Gilead clinical studies, 17 novel HCV subtypes and 1 novel HCV genotype were identified, unrelated to each other and to previously described HCV subtypes, suggesting an even greater genetic diversity of HCV subtypes than previously recognized. The pangenotypic regimens of sofosbuvir/velpatasvir±voxilaprevir were highly efficacious against previously uncharacterized subtypes and the novel genotype.

Disclosures:

Charlotte Hedskog - Employment: Gilead Sciences; Stock Shareholder: Gilead Sciences

Neeru Bhardwaj - Employment: Gilead Sciences

Bandita Parhy - Employment: Gilead; Stock Shareholder: Gilead

Ross Martin - Employment: Gilead Sciences

Stefan Zeuzem - Consulting: AbbVie, Bristol-Myers Squibb Co., Gilead, Merck/MSD, Janssen

Christophe Moreno - Consulting: Abbvie, Janssen, Gilead, BMS, MSD; Grant/Research Support: Janssen, Gilead, Roche, Astellas, Abbvie

Stephen Shafran - Advisory Committees or Review Panels: Bristol-Myers Squibb, Gilead Sciences, Merck; Grant/Research Support: AbbVie, Bristol-Myers Squibb, Gilead Sciences, Janssen, Merck; Speaking and Teaching: Pfizer

Sergio M. Borgia - Advisory Committees or Review Panels: Gilled Sciences, Merck Canada

Robert H. Hyland - Employment: Gilead Sciences, Inc; Stock Shareholder: Gilead Sciences, Inc

John McNally - Employment: Gilead Sciences; Stock Shareholder: Gilead Sciences

Hongmei Mo - Employment: Gilead Science Inc

Evguenia S. Svarovskaia - Employment: Gilead Sciences Inc.; Stock Shareholder: Gilead Sciences Inc.

The following people have nothing to disclose: Silvia Chang

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Proposal of GALADUS Score: Combining liver ultrasound with serum based biomarkers for Hepatocellular Carcinoma Surveillance

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Background: Early detection is essential to curb the rising mortality rate of Hepatocellular carcionoma (HCC). Screening with liver ultrasound is limited by its relatively low sensitivity and specificity. The GALAD score is a new serum biomarker based model incorporating three serum biomarkers, alpha-fetoprotein (AFP), AFP-L3%, and Des-gamma carboxyprothrombin (DCP), and patient characteristics (age, gender). GALAD has been found to be superior to individual biomarkers and validated in different patient populations. We aimed to incorporate ultrasound findings into this model and assess their combined performance in predicting the presence of HCC. **Methods**: Patients with cirrhosis or chronic hepatitis B seen at Mayo Clinic, Rochester, MN, between October 2013 and October 2016 who had AFP, AFP-L3%, and DCP measured in their clinical care or consented for their serum stored for measurement of these biomarkers, and also had ultrasound of the abdomen done were included. Cases were those who had these tests performed at diagnosis of HCC, excluding those who had prior HCC treatments. Controls were those without HCC as confirmed by cross sectional imaging and at least six months follow up. GALAD score was calculated using -10.08 + 1.67 x [Gender (1 for male, 0

for female)] + 0.09 x [Age] + 0.04 x [AFP-L3%] + 2.34 x log[AFP] + 1.33 x log[DCP]. GALAD-US score was GALADUS = GALAD + 1.84* (1 for positive and 0 for negative ultrasound). Identification of a solid lesion in the liver by ultrasound was considered a positive ultrasound. AUC was measured to compare performances of these models. **Results**: 116 patients with HCC and 179 controls with cirrhosis or chronic hepatitis B met inclusion and exclusion criteria. The AUC of the GALAD score for HCC detection was 0.95 [95% confidence interval (CI): 0.92-97], which was higher than the AUC of ultrasound (0.83, P<0.01). At a cut off of -0.76, the GALAD score had a sensitivity of 91% and a specificity of 85% for HCC detection. The AUC of the GALAD score for early stage HCC detection remained high at 0.91 [95%CI: 0.87-0.96] (cut off -1.18, sensitivity 91%, specificity 80%) in the test cohort. The combination of GALAD and ultrasound (GALADUS score) further improved the performance of the GALAD score, achieving an AUC of 0.98 [95%CI: 0.96-0.99] (cut off –0.27, sensitivity 95%, specificity 91%). **Conclusion**: The performance of the GALAD score was superior to that of ultrasound for HCC detection. Incorporation of the result of ultrasound to GALAD score, GALADUS score, resulted in even better performance. While further validation is necessary, the GALADUS score may reduce false positive and false negative ultrasound results.

Disclosures:

Gregory J. Gores - Advisory Committees or Review Panels: Conatus, Jecure

Lewis R. Roberts - Advisory Committees or Review Panels: Bayer, TAVEC; Grant/Research Support: Bristol Myers Squibb, ARIAD Pharmaceuticals, BTG, Wako Diagnostics, Inova Diagnostics, Gilead Sciences, Five Prime Therapeutics; Speaking and Teaching: OncLive, Medscape Education

The following people have nothing to disclose: Benyam D. Addissie, Ju Dong Yang, Kristin Mara, William S. Harmsen, Hawa Ali, Fatima Hassan, Nasra H. Giama, Alicia Algeciras-Schimnich, J Paul Theobald, Hiro Yamada, Melissa M. Ward, Denise M. Harnois

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Frequency, Evaluation and Subsequent Clinical Outcomes of Cirrhosis Patients with Abnormal Imaging Findings during Surveillance for Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) surveillance has been associated with early tumor detection and improved overall survival. We aimed to determine the effectiveness of an HCC surveillance program and the frequency and implications of the detection of liver nodules. **Methods:** Adults with cirrhosis enrolled in a HCC surveillance program were retrospectively analyzed. We used electronic reminders to prompt ultrasound (US) surveillance every 6 months. Outcomes included completion of imaging at recommended intervals; staging of HCC at diagnosis; and frequency and evaluation of indeterminate nodules (IN). **Results:** The cohort of 999 patients were predominantly white (91%), males (54%), with a median age of 58 years. Etiology of cirrhosis was diverse (35% HCV, 17% alcohol, 16% NASH) with median MELD 6. Median follow-up was 2.2 years (IQR 0.9-3.9). Surveillance imaging was completed every 6, 9, and 12 months in 46%, 51% and 68% of patients, respectively. 256 (26%) patients had at least one abnormal imaging test and downstream subsequent imaging is shown in the **Figure**. 40 patients were diagnosed with HCC on initial CT/MRI. Of the remaining 216 patients, 29 were later diagnosed with HCC after median 2 CTs/MRIs, 43 continued to have IN after median 3 CTs/MRIs, while 144 returned to US surveillance after median 2 CTs/MRIs. Of the 69 patients with HCC, 78% were within Milan criteria. Patients with an IN, diagnosed with HCC, had higher AFP, MELD, bilirubin, INR and alkaline phosphatase, and lower platelets and albumin than those with IN without HCC or no IN. **Conclusions:** HCC surveillance rates among patients with cirrhosis enrolled in a structured program were significantly higher than those reported in the literature with a high proportion of early stage HCC diagnosis. IN were identified in 21.6% patients undergoing surveillance, with 73% of those not resulting in an HCC diagnosis. Improved risk-stratification tools are needed to characterize IN in patients with cirrhosis undergoing HCC surveillance to reduce the psychological and physical harms associated with IN work-up.

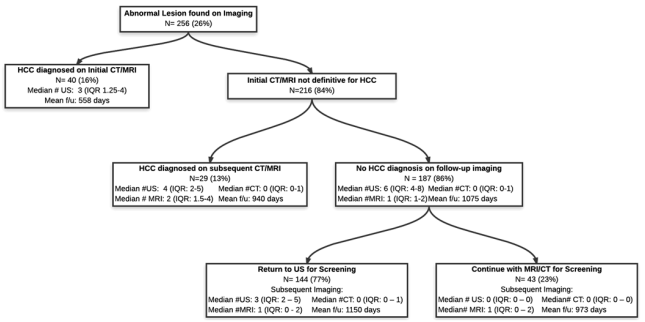

Figure. Patient schema

Disclosures:

Anna S. Lok - Grant/Research Support: Gilead, Target Solutions, BMS

Neehar D. Parikh - Advisory Committees or Review Panels: Eisai, Bayer; Consulting: Bristol Meyer Squibb

The following people have nothing to disclose: Aashesh Verma, Monica A. Konerman

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Routine Hepatocellular Carcinoma Surveillance for Hepatitis C Virus-Infected Alaska Native Persons Associated with Increased Likelihood of HCC Diagnosis at an Early Stage During 1991–2016.

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Background: Guidelines recommend hepatocellular carcinoma (HCC) surveillance for hepatitis C virus (HCV)-infected persons with cirrhosis because diagnosis at an earlier stage could potentially improve survival. The Alaska Tribal Health System (ATHS) maintains a clinical registry of HCV-infected Alaska Native (AN) persons that included approximately 1,300 patients in 2016. In 2006, ATHS initiated a routine HCC surveillance program that included sending semiannual reminders by mail to registry-persons with known cirrhosis to schedule an HCC screening ultrasound (US). We evaluated whether HCV-infected AN persons receiving care through the ATHS during 1991–

2016 were more likely to have HCC diagnosed at an early stage after implementation of the routine HCC surveillance program. Methods: We queried the ATHS electronic health records to identify and obtain data on HCV-infected persons diagnosed with HCC during 1991–2016. An early-stage HCC was defined as a single tumor ≤5 cm diameter or ≤3 tumors each ≤3 cm in diameter; all other tumors were considered late-stage. We calculated the adjusted odds ratio (aOR) and 95% confidence interval (CI) for early-stage HCC diagnosis by using a model that adjusted for sex, compliance with HCC surveillance (indicated by receipt of US <6 months before HCC diagnosis), and HCC diagnosis before (1991–2004) or after (2005–2016) implementation of routine HCC surveillance. Results: Of the 56 persons with HCC, 42 (75%) were male (median age at HCC diagnosis [years]: 57; Minimum–Maximum: 46–83) and 50 (89%) had cirrhosis; 15 (27%) tumors were diagnosed during 1991–2004, 21 (37%) during 2005–2011, and 20 (36%) during 2012–2016. Of the 51 participants with clinical data necessary to determine tumor stage, 28 (55%) were diagnosed at an early stage, including 3 of 12 (25%) diagnosed during 1991–2004, 12 of 20 (60%) diagnosed during 2005–2011, and 13 of 19 (68%) diagnosed during 2012–2016 (*P*-value for trend = .04). An US <6 months before HCC diagnosis was documented for 18 of 28 (64%) persons with an early-stage tumor and 4 of 23 (17%) persons with a late-stage tumor. After controlling for receipt of an US <6 months before HCC diagnosis (aOR: 10.5; CI: 1.9–82.7), the likelihood of early-stage HCC diagnosis during 2005–2016 compared with 1991–2004 was not significant (aOR: 6.3; CI: 0.8–81.7). Conclusions: The proportion of HCC tumors diagnosed at an early stage increased over time. That trend disappeared after controlling for receipt of an US <6 months, indicating that the routine HCC surveillance program implemented in 2006 contributed to the subsequent greater proportion of HCV-infected AN persons diagnosed with HCC at an early stage.

Disclosures:

Lisa J. Townshend-Bulson - Grant/Research Support: Gilead

Brian J. McMahon - Grant/Research Support: Gilead Sciences

The following people have nothing to disclose: Prabhu P. Gounder, Priyanka Pillai, Prishanya Pillai, Dana J. Bruden, Philip R. Spradling

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PyC3A: A Novel Manganese MRI Contrast Agent for the Evaluation of Hepatic Neoplasms

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Background: Contrast-enhanced MRI is routinely used to diagnose and characterize hepatic neoplasms. However, safety concerns limit the use of Gadolinium (Gd) based contrast agents in patients with renal insufficiency, which represents an increasing problem with an aging U.S. population. Manganese (Mn^{2+}) is a paramagnetic ion that is cleared via biliary excretion, and provides an ideal alternative to Gd. We present a novel Mn-based contrast agent, $[Mn(PyC3A)(H2O)]^-$ (referred to as PyC3A), and compare its efficacy to gadoxetic acid (Eovist) by imaging orthotopically implanted colorectal cancer hepatic metastases in mice. **Methods:** Male BALB/c mice (n = 8) were injected in the left lobe of the liver with $5x10^4$ MC26/MCA-26 murine colon carcinoma cells via a midline laparotomy incision. Tumors were allowed to grow for 10 days prior to MR imaging. The imaging protocol included two-dimensional rapid acquisition with refocused echo (RARE) imaging to delineate anatomy and a 3D fast low-angle shot (FLASH) sequence on a 4.7T scanner. Mice were imaged twice, once with PyC3A followed by gadoxetic acid, each session separated by 24 hours for complete probe clearance. MR contrast-to-noise ratio (CNR) was calculated for normal liver relative to tumor, and compared between probes for each animal. **Results**: Relaxivity of PyC3A in blood plasma was shown to be comparable to commercial Gd contrast agents. Biodistribution analysis confirmed that PyC3A clears via a mixed renal/hepatobiliary pathway with greater than 99% clearance within 24 hours. For imaging hepatic metastases, all mice survived to the study endpoint and developed radiographically discernible tumors (5 ± 2mm diameter) without evidence of carcinomatosis. Calculated CNRs for liver relative to tumor for all recorded times are shown in **Table 1.** By CNR, there was no statistical difference between gadoxetic acid and PyC3A for all time points, suggesting near-equivalent enhancement of normal liver and tumor by both probes. **Conclusion:** PyC3A provides resolution of metastatic liver metastases that is comparable to gadoxetic acid. PyC3A may provide a safe and effective alternative to Gd constrast agents for imaging hepatic neoplasms in patients with renal impairment.

Table 1. Contrast-to-Noise Ratio (CNR) of Normal Liver Relative to Tumor

Time (minutes post injection)	Gadoxetic Acid	PyC3A	p-value
0	2.61 ± 1.08	2.60 ± 1.64	0.92
3	7.72 ± 4.67	7.53 ± 3.02	0.93
8	7.85 ± 3.89	8.85 ± 4.43	0.66
13	8.02 ± 3.98	7.62 ± 5.49	0.88
18	9.79 ± 4.17	5.60 ± 3.08	0.06
23	8.72 ± 4.16	7.03 ± 2.06	0.36

Disclosures:

Kenneth K. Tanabe - Patent Held/Filed: EGF SNP and risk for HCC, EGFR inhibition and HCC, gene signature for prognosis in cirrhosis

Peter Caravan - Consulting: Guerbet, UCB Biopharma; Grant/Research Support: Pfizer, Biogen, Agilent; Stock Shareholder: Collagen Medical, Factor 1A LLC

Bryan C. Fuchs - Advisory Committees or Review Panels: Pharmakea Therapeutics; Consulting: Collagen Medical, Enanta Pharmaceuticals, Gilead Sciences; Grant/Research Support: Nimbus Therapeutics, Collagen Medical, Enanta Pharmaceuticals, Gilead Sciences

Eric Gale - Stock Shareholder: Reveal Pharmaceuticals

The following people have nothing to disclose: Derek J. Erstad, Christian T. Farrar, Ian Ramsay, Nicholas Rotile

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Less Than Half of Hepatocellular Carcinoma Patients Among the 1945-1965 Birth Cohort in the U.S. Were Within Milan Criteria at Time of Diagnosis

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Background: Individuals from the 1945-1965 birth cohort account for 75% of HCV infections in the U.S. Understanding HCC trends among the 1945-1965 birth cohort is particularly important given the increasing burden of chronic liver disease among this group **Aim:** To evaluate

HCC trends in the U.S. among the 1945-1965 birth cohort with a focus on disparities in tumor stage at diagnosis. **Methods:** Adults born from 1945-1965 who were diagnosed with HCC from 2004 to 2014 were retrospectively evaluated using data from the Surveillance, Epidemiology, and End Results registry. Tumor stage at diagnosis was assessed using Milan Criteria. Multivariate logistic regression models, which adjusted for age, sex, race/ethnicity, year of diagnosis, insurance status, and marital status, evaluated for predictors of having HCC within Milan Criteria at diagnosis. **Results:** Among 38,045 HCC patients (81.6% male, 50.1% non-Hispanic white, 16.2% African American, 12.6% Asian, 19.8% Hispanic), 66.2% had Medicare or commercial insurance, 27.2% had Medicaid, 6.6% were uninsured. From 2004-2006 to 2013-2014, number of HCC patients from the 1945-1965 birth cohort increased by 58%, and in 2013-2014, the 1945-1965 birth cohort represented 26.4% of all HCC patients. The proportion of HCC patients within Milan Criteria increased from 36.4% in 2003-2006 to 46.3% in 2013-2014, p<0.01. On multivariate regression, men with HCC in the 1945-1965 birth cohort were less likely to have HCC within Milan compared to women (OR 0.88, 95% CI 0.83-0.93, p<0.01). Compared to non-Hispanic whites, African Americans were less likely to have HCC with Milan (OR 0.73, 95% CI 0.68-0.78, p<0.01). Compared to patients with HCC diagnosed in 2004-2006, those diagnosed in 2013-2014 were more likely to have HCC within Milan (OR 1.12, 95% CI 1.05-1.20, p<0.01). Patients with Medicaid or no insurance were also significantly less likely to have HCC within Milan Criteria. **Conclusions:** Rates of HCC among the 1945-1965 birth cohort increased by 58% over the past decade. However, less than half of these HCC patients met Milan Criteria at diagnosis in the most recent era. Sex-specific, race/ethnicity-specific, and payer-specific disparities in tumor stage at diagnosis were observed.

	Odds Ratio	95% CI	P-Value
Male (vs. Female)	0.88	0.83-0.93	<0.001
Non-Hispanic White	1.00	Reference	-
African American	0.73	0.68-0.78	<0.001
Asian	0.85	0.79-0.92	<0.001
Hispanic	1.02	0.96-1.09	0.48
Medicare or Commercial Insurance	1.00	Reference	-
Medicaid	0.86	0.81-0.90	<0.001
No Insurance	0.46	0.41-0.51	<0.001

Disclosures:

Robert J. Wong - Advisory Committees or Review Panels: Gilead; Consulting: Gilead; Grant/Research Support: Gilead; Speaking and Teaching: Gilead, Salix, Bayer

The following people have nothing to disclose: Ann Robinson, Hesam Tavakoli, Benny Liu, Taft Bhuket

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Combination of Killer Immunoglobulin-like Receptor and HLA Genes is Associated with Hepatitis C Virus-Related Hepatocellular Carcinoma

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Background & Aims: Natural killer (NK) cell responses have been shown to play a crucial role in the innate immune response against infection with viruses. Since the killer immunoglobulin-like receptor (KIR), in combination with its cognate human leukocyte antigen (HLA) ligand, is associated with treatment-induced and spontaneous clearance of hepatitis C virus (HCV) infection, we examined the impact of *KIR*, and *HLA* on the development of HCV-related hepatocellular carcinoma (HCC) in a Japanese population. **Methods:** We investigated 16 KIR genotypes along with *HLA-B* and *-C* ligands in 787 HCV-positive patients with chronic hepatitis C (n = 613), HCC (n = 174), and 325 volunteer controls. **Results:** The frequency of *KIR3DS1* in all HCV-infected patients was significantly lower than controls ($P < 0.0001$; odds ratio [OR] 0.57). Combination of *KIR3DL1/HLA-Bw4* was significantly higher in HCV-infected patients ($P < 0.001$; OR 1.65). In contrast, the frequency of *KIR2DL1/HLA-C2* was lower in patients with chronic hepatitis C ($P <0.0001$; OR 0.57). No statistically significant differences were found for HLA and KIR between patients with HCC and those without. When HCV-related HCC patients were stratified according to 65 years, the frequency of *KIR2DL2*, *KIR2DS2*, and *HLA-C1C1* homozygote were significantly higher in younger patients (<65 years) than in older patients (>65 years) ($P = 0.021$; OR 2.50, $P = 0.037$; OR 2.40, $P = 0.045$; OR 3.40, respectively). Moreover, younger patients with *KIR2DL2/HLA-C1* and *KIR2DS2/HLA-C1* had significantly higher HCC development compared with those without ($P = 0.014$; OR 2.69 and $P = 0.023$; OR 2.61, respectively). Conversely, *KIR2DL1/HLA-C2* was more frequently found in older patients with HCC than in those without ($P = 0.045$; OR 0.29). Multivariate logistic regression analysis subsequently identified *KIR2DS2/HLA-C1* ($P = 0.026$; OR 2.61), female ($P = 0.01$; OR 0.28), serum albumin ($P < 0.001$; OR 0.13), and platelet count ($P < 0.001$; OR 0.86) as independent predictive factors of development of HCC in younger patients. **Conclusions:** These results indicate a central role of NK cells in the innate-system for developing chronic HCV infection and HCC development. In particular, combination of *KIR2DS2/HLA-C1* is associated with HCV-related HCC in younger patients.

Disclosures:

The following people have nothing to disclose: Takeji Umemura, Hiromi Saito, Satoru Joshita, Tomoo Yamazaki, Eiji Tanaka, Masao Ota

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Superiority of Avatrombopag (AVA) to Placebo (PBO) for the Treatment of Chronic Liver Disease (CLD)-Associated Thrombocytopenia (TCP) in Patients Undergoing Scheduled Procedures: Results of 2 Randomized, PBO-Controlled Phase 3 Studies

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Background: Patients with CLD and TCP may require platelet (PLT) transfusions before routine procedures,

increasing the risk of PLT refractoriness. AVA is an oral, 2nd generation, small molecule, thrombopoietin receptor agonist in development as an alternative to PLT transfusions. Two randomized, double-blind, PBO-controlled Phase 3 studies evaluated the potential for AVA to reduce the need for PLT transfusions in patients with CLD undergoing scheduled procedures. **Methods**: ADAPT1 (NCT01972529) and ADAPT2 (NCT01976104) enrolled adults with CLD and severe TCP (mean baseline [BL] PLT <50x10^9/L), scheduled to undergo invasive procedures. Cohorts were defined based on BL PLT count (**Cohort 1**, <40x10^9/L or **Cohort 2**, 40 to <50x10^9/L), and patients were randomized 2:1 to once-daily oral AVA (60 mg for **Cohort 1**, 40 mg for **Cohort 2**) or PBO for 5 days with the procedure scheduled 5-8 days after their last dose. The primary efficacy endpoint was the proportion of patients not requiring PLT transfusion or any bleeding rescue procedure up to 7 days post-procedure. Secondary endpoints assessed the proportion of patients achieving the target PLT count (≥50x10^9/L), change in PLT count from BL to Procedure Day, and safety. **Results:** ADAPT1 randomized 231 patients (median age 57 y; 68% male; BL median PLT 38x10^9/L; CLD etiology: alcohol 14%, viral hepatitis 62%, other 23%) to **Cohort 1**, 90 AVA /48 PBO or **Cohort 2**, 59 AVA /34 PBO. ADAPT2 randomized 204 (median age 59 y; 62% male; BL median PLT 39x10^9/L; CLD etiology: alcohol 15%, viral hepatitis 53%, other 33%) to **Cohort 1**, 70 AVA /43 PBO or **Cohort 2**, 58 AVA/33 PBO. Significantly greater proportions of AVA-treated patients across all cohorts did not require PLT transfusion or bleeding rescue procedures compared with PBO--ADAPT1: **Cohort 1**, 66% vs 23%; **Cohort 2**, 88% vs 38%; each p<0.0001; ADAPT2: **Cohort 1**, 69% vs 35%, p=0.0006; **Cohort 2**, 89% vs 33%, p<0.0001. AVA was also superior to PBO for both secondary endpoints, increasing mean PLT counts on Procedure Day to 64x10^9/L in **Cohort 1** and 85x10^9/L in **Cohort 2**. The most common treatment-emergent adverse events (TEAE) were pyrexia, abdominal pain, nausea, and headache, which were similar for PBO and AVA arms in both studies. Most were mild to moderate in severity; one thrombotic TEAE occurred with 40 mg AVA in **Cohort 2** in ADAPT2. **Conclusions:** AVA given over 5 days significantly reduced the need for PLT transfusions or rescue procedures for bleeding. It was well tolerated with a safety profile similar to PBO. The availability of an alternative to platelet transfusions for patients with CLD undergoing procedures would reduce their risk of developing platelet refractoriness.

Disclosures:

Norah Terrault - Advisory Committees or Review Panels: Dynavax, Gilead; Consulting: Conatus, BMS, Novartis, Merck, Intercept; Grant/Research Support: Biotest, Vertex, Gilead, AbbVie, Merck, BMS, Eisai

Francesco Bibbiani - Employment: Eisai Inc

Tarek I. Hassanein - Advisory Committees or Review Panels: AbbVie Pharmaceuticals, Bristol-Myers Squibb, Trek Therapeutics; Grant/Research Support: AbbVie Pharmaceuticals, Obalon, Bristol-Myers Squibb, Eiasi Pharmaceuticals, Gilead Sciences, Merck Sharp & Dohme, NGM BioPharmaceuticals, Ocera Therapeutics, Salix Pharmaceuticals, Sundise, TaiGen Biotechnology, Vital Therapies, Tobria, Shinoghi & Co. Ltd, La Jolla Pharmaceuticals, Trek Therapeutics, Novo Nordisk, Intercept, GenFit, Norvartis, Shire; Speaking and Teaching: Baxter, Bristol-Myers Squibb, Gilead Sciences, Salix Pharmaceuticals, AbbVie Pharmaceuticals

The following people have nothing to disclose: Yi-Cheng Chen, Namiki Izumi, Zeid Kayali, Paul Mitrut, Won Young Tak

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Impact of SVR in the development of all complications and fibrosis regression in a cohort of patients treated with interferon-base Triple Therapy and Direct Acting Antiviral

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Background: Antiviral treatment with interferon-based Triple Therapy (TT) and Direct-Acting Antiviral (DAA) has achieved a very high sustained virological response (SVR). The aim of this study was to evaluate the improvement of liver fibrosis in the entire cohort and the development of liver decompensation (LD) in cirrhotic patients **Methods:** We prospectively collected data on liver fibrosis and clinical events in a hepatitis C cohort of patients treated with TT and DAA from 3 University Hospital in Spain **Results:** A total of 538 consecutive patients were included. A total of 64.5% (n=196/304) of patients in TT cohort and a 97.8% (n=229/234) of patients in DAA cohort achieved an SVR. Patients in TT group with SVR showed greater decreased of kilopascals (Kpa) than non-SVR patients (20.0KPa SVR vs 9.9KPa non-SVR)(*p<0,001).* In DAA group the decreased was (7 KPa SVR *vs* 1 KPa non-SVR) (p= 0.3). Overall, a 51.5% (n= 277) were classified as F4 and of these a 12.6%(n=35/277) had a LD; 24/136(17.6%) in TT cohort by a mean follow up of 24.6 months and 11/141 (7.8%) in DAA cohort by a mean follow up of 7.7 months. The most frequent complications in TT cohort were ascites observed in 11% (n=15/136) and Hepatocellular carcinoma (HCC) in 6.6% (n=9/136) of the patients; 6 (4.4%) required liver transplant and 4 (2.9%) died. LD was significantly lower in SVR patients (4.5%SVR vs 14.3% Non-SVR)(*p*=0,006) and one year cumulative probability of developing LD in the SVR was (4,3% SVR vs 16,3% Non-SVR)(p=0,02). In DAA cohort the most frequent complication was HCC observed in 7% (n=10/141) of the patients, 1 (0.7%) required liver transplant and 3 (2.1%) died. LD was significantly lower in SVR patients vs Non-SVR (4.8% vs 20%)(*p*=0.2). One year cumulative probability of developing LD in the SVR DAA cohort was (1.6% SVR vs 33.7% Non-SVR) p=<0.05. Multivariate analysis in DAA group shows bilirrubin levels related to LD (p= 0.04) (OR 1,026-0,5471). Concerning HCC, in order to estimate the effect of the treatments on the incidence of HCC a propensity score matching was performed by adjusting it to the HCC risk variables in the 2 cohorts; we observed that the probability of incidence in both cohorts were similar (7% in TT vs 7.1% DAA) p= 0.9. **Conclusions:** SVR in patients with advanced disease was associated with an improvement in fibrosis stage, lower rate of decompensation and liver transplantation. Although with DAA there was a subtle increase in the incidence of HCC in a shorter time, the propensity score showed that in both cohorts the probability was the same without significant differences.

Disclosures:

Jose L. Calleja - Advisory Committees or Review Panels: Gilead, Abbvie; Speaking and Teaching: Abbvie, Gilead, Janssen, BMS

The following people have nothing to disclose: Christie Perelló, Joaquin Cabezas, José A. Carrión, Susana Llerena, Marta Hernández-Conde, Javier Crespo

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OCR-002 (Ornithine Phenylacetate) is a Potent Ammonia Scavenger as Demonstrated in Phase 2b STOP-HE Study

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Background: In serious liver disease, elevated ammonia levels contribute to the neurocognitive effects that characterize hepatic encephalopathy (HE). Ornithine phenylacetate (OCR-002) is an ammonia scavenger that acts, in part, by combining with excess glutamine to form phenylacetylglutamine, which is then excreted via the kidneys. OCR-002 is in clinical development for treating HE in patients with cirrhosis. A Phase 2b study, STOP-HE, in hospitalized patients with HE demonstrated that OCR-002 was a potent ammonia scavenger, shortened the time to clinical improvement, and was safe and well tolerated. **Methods**: This was a double-blind, randomized, placebo-controlled study (clinicaltrials.gov NCT01966419) of OCR-002 in patients hospitalized with cirrhosis, patients with persistent Stage 2 or higher HE, and Screening local plasma ammonia level >ULN. After a minimum 12-h prescreening phase on standard of care (SOC), patients with HE were screened and if they met entry criteria were randomized to 5 days treatment with SOC plus OCR-002 10, 15 or 20 g/day (continuous IV infusion) vs. placebo. The pre-determined OCR-002 dose was based on the severity of underlying liver disease. Study outcomes included time to normal plasma ammonia levels, reduction in plasma ammonia, and correlation of ammonia levels with clinical improvement. **Results**: 231 patients were randomized to OCR-002 (n=116) or placebo (n=115). Ammonia at screening correlated with HE severity (p=0.032). Time to clinical improvement in patients with a confirmed baseline plasma ammonia level >ULN was significantly (p=0.034) improved with OCR-002 vs. placebo. The time to achieve normal plasma ammonia levels was significantly (p=0.028) reduced with OCR-002 vs. placebo Hazard Ratio 1.692 (1.137, 2.514). A dose-related reduction in plasma ammonia levels was observed (-11.8, -19.3, -28.4, and -38.9 mmol/L with placebo and OCR-002 10, 15, and 20 g/day), although rigorous dose-response assessment was complicated by the assignment of dose based on disease severity. The difference between OCR-002 and placebo was statistically significant (p=0.014). A reduction in plasma ammonia levels (-9.0 mmol/L no improvement vs. -28.2 mmol/L improvement) correlated with clinical improvement from Stage 2/4 to Stage 0/1 HE (p=0.0006). **Conclusion**: Findings from STOP-HE support the benefits of OCR-002 as an ammonia scavenger in cirrhotic patients hospitalized with HE. OCR-002 + SOC normalizes and reduces plasma ammonia levels faster than placebo + SOC and leads to faster clinical improvement. A phase 3 study of OCR-002 is planned to confirm these results.

Disclosures:

Robert S. Rahimi - Grant/Research Support: Ocera

Charles E. DiLiberti - Consulting: Ocera Therapeutics, Inc.

Laurene Wang - Independent Contractor: Ocera Therapeutics, Inc.

Nikolaos T. Pyrsopoulos - Advisory Committees or Review Panels: GILEAD, MERCK, VITAL THERAPIES, BMS, INTERCEPT, BAYER, ABBVIE; Grant/Research Support: HOLOGIC, VITAL THERAPIES, MERCK, ABBVIE, INTERCEPT, OCERA, CONATUS, SALIX, Hologic

Amy Potthoff - Management Position: Ocera Therapeutics, Inc; Stock Shareholder: Ocera Therapeutics, Inc

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Increased myocardial extracellular volume define patients with more advanced cirrhosis and at higher risk of mortality: Results from a cardiac MRI and Echo study

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Background: Cirrhotic cardiomyopathy is an established complication in cirrhosis. Cardiac dysfunction has been linked to disease severity, development of hepatorenal syndrome, and increased mortality. However, the pathogenesis and precise definition of cirrhotic cardiomyopathy remain a matter of debate. It has been speculated that structural changes in the myocardium are involved. Cardiac MRI (CMR) with T1-mapping and quantification of extracellular volume (ECV) is a new non-invasive method to assess diffuse myocardial fibrosis in conditions such as amyloidosis. This technique has, to our knowledge, never been applied in cirrhosis. Our aim was therefore to perform a detailed characterization of cardiac structure and function using CMR and echocardiography in cirrhotic patients to determine the relationship between myocardial fibrosis and cardiac function, liver disease severity, and adverse events. **Methods:** 63 cirrhotic patients and 12 healthy controls were included. All patients underwent contrast-enhanced CMR (including ECV determination), echocardiography with Tissue Doppler Imaging (TDI), and clinical and biochemical assessments. Follow-up with registration of death and liver transplantation was ascertained. In the controls, contrast-enhanced CMR alone was performed. **Results:** ECV was higher in the patients compared with healthy controls (31.1±6 vs. 27.4±3 %, *p*=0.04). Moreover, ECV increased across Child Pugh A/B/C classes (26.9±4/ 31.5±5/ 34.4±6%, *p*=0.02). During a median follow-up of 22 months (1-31)16 patients had either died (12) or been transplanted (4) and the ECV was significantly higher than in patients without events (33.4±5 vs. 30.5±6 %, *p*=0.043). ECV correlated with MELD (r=0.31, *p*=0.028), cardiac index (r=0.35, *p*=0.015), CRP (r=0.47, *p*=0.001), proANP (r=0.4, *p*<0.001), and proBNP (r=0.5, *p*=0.005). No association was found with age, etiology or HVPG (*p*=NS). **Conclusion:** Cirrhotic patients have an increased ECV compared to healthy controls. ECV is related to disease severity with the highest values in the patients with the most advanced disease and in those who die or are transplanted during follow-up. We suggest that these changes reflect diffuse myocardial fibrosis and are of pathophysiological relevance, defining cirrhotic patients who may

develop cirrhotic cardiomyopathy and increased mortality. Further studies on the potential of ECV in the definition of cirrhotic cardiomyopathy are warranted.

Disclosures:

The following people have nothing to disclose: Signe Wiese, Jens D. Hove, Rajeshwar Mookerjee, Flemming Bendtsen, Søren Møller

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L-Ornithine L-Aspartate In Acute Overt Hepatic Encephalopathy

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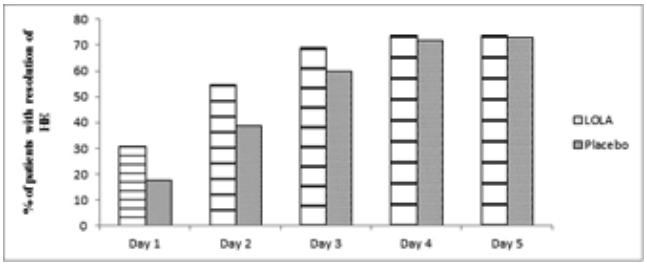
Background & Aims: Good quality data on effectiveness of L-Ornithine L-Aspartate (LOLA) in cirrhotic patients with acute overt hepatic encephalopathy (HE) is absent. We aimed to evaluate efficacy of intravenous LOLA in reversal of acute overt HE in cirrhotic patients. *Methods:* A prospective double-blind, randomized placebo controlled trial was conducted at Gastroenterology department of two tertiary care institutes in India. Three hundred and seventy cirrhotic patients with acute overt HE were screened. After exclusion, 196 (52.97%) patients were randomized to receive either intravenous infusions of LOLA (n=98), 30 grams daily or Placebo (n=98) for 5 days. Standard of care treatment (including Lactulose) was given in both groups. Fasting venous ammonia levels were estimated daily from 0–5 days. Serum concentration of, Interleukin 1β, Interleukin 6, Interleukin 10, Tumor Necrosis Factor α, Hemogram, liver, renal function tests were performed at day 0 and 5. Primary outcome was mental state grade at day 5 of treatment. *Results*: On completion of 5 days of treatment, 75.5% patients in LOLA group and 74.5% patients in placebo group had resolution of overt HE. Mean time taken for recovery was 1.92±0.93 days in LOLA group & 2.45±1.06 days in placebo group, p=0.002(95% CI -0.852 to - 0.202). Length of hospital stay and Venous ammonia at day 5 were significantly lower in LOLA group. No significant change was seen in Interleukins and Tumor Necrosis Factor α in both the groups. *Conclusion*: In patients suffering from acute overt HE, intravenous LOLA decreases the time of recovery from HE, shortens the length of hospital stay and decreases the venous ammonia.



Disclosures:

The following people have nothing to disclose: Sandeep S. Sidhu, Barjesh C. Sharma, Harsh Kishore, Navpreet Kaur

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Sarcopenia and Opiate Use May Be Modifiable Risk Factors for Incident Overt Hepatic Encephalopathy: A Prospective Study

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Background: Hepatic encephalopathy (HE) is associated with increased mortality, hospitalization, motor vehicle accidents, and falls. Given that muscle metabolizes ammonia and sedating medications alter neurotransmission, both sarcopenia and medications may be associated with the development of incident overt HE. Prospective data, however, are lacking. **Aims**: We assessed baseline cognitive performance and predictors of overt HE and falls in a longitudinal cohort of patients with compensated cirrhosis with no prior history of HE. **Methods:** Adults with Child A-B cirrhosis and portal hypertension without a history of HE were enrolled. At baseline, patients had clinical evaluation and a point-of-care test for covert HE – the Inhibitory Control Test (ICT; performance is defined in part by number of 'lures' with higher number indicating worse performance). We also evaluated patients for frailty (30-second chair stands, hand grip), muscle bulk (midarm muscle area – MAMA), and medication burden. Primary outcome was development of overt HE and secondary outcome was falls. Penalized regression models for rare outcomes were employed. **Results**: 136 patients were enrolled, 51% men, age 58.1±11.3 years, 74% Child A 74%, mean MELD 10.0±3.9. Most common etiologies of liver disease were HCV 31%, NAFLD 26% and alcohol 19%. At enrollment, 19% of patients were taking benzodiazepines and 19% opiates. The median number of lures/ICT was 11 IQR (7-21). In stepwise regression models, only hand-grip was associated with ICT performance: for every 10 kg increase in hand grip, there was 1.8 (95%CI, 0.4-3.2) fewer lures/ICT – meaning that muscle strength was the only clinical variable linked to cognitive performance. Within 6 months of follow-up, 12 patients developed overt HE and 11 experienced a fall. Child class B (OR 3.39, 95%CI 1.70-7.85), Opiate use (OR 2.88, 95%CI 1.42-6.36), and MAMA (OR for every 10 mm^2 1.37, 95%CI 1.08-1.86) but not ICT performance predicted new overt HE. Coversely, only ICT performance was predictive of falls: for each lure followed, the OR of a fall was 1.07 (95%CI 1.14-1.44). **Conclusion**: These data suggest a need for proactive screening of cognitive function and sarcopenia in patients with cirrhosis and portal hypertension in order to prevent adverse events. Simple, bedside markers of sarcopenia are associated with cognitive performance while both sarcopenia measures and opiates predicted the development of overt HE. Further, we show that cognitive assessments for covert HE such as ICT are predictive of falls. These risk factors are potentially modifiable and should be addressed in future intervention studies.

Disclosures:

Anna S. Lok - Grant/Research Support: Gilead, Target Solutions, BMS

The following people have nothing to disclose: Elliot B. Tapper

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Association of Aspirin Therapy with Reduced Risk of Hepatocellular carcinoma in Patients with Chronic Hepatitis B

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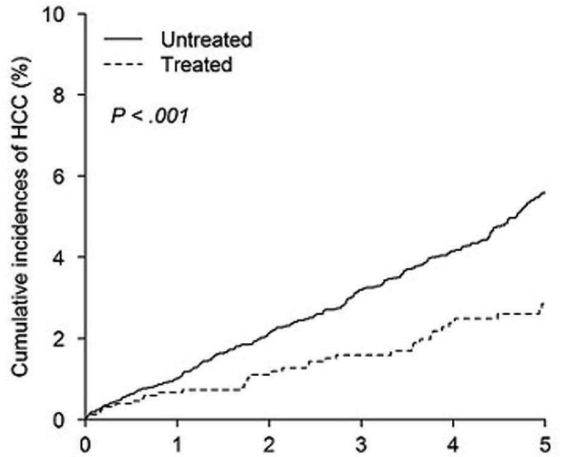
Background: Aspirin may prevent cancer development, but clinical evidences in HBV-related HCC remain lacking. We aimed to investigate the association of aspirin therapy with HBV-related HCC risk. Methods: In this nationwide cohort study, medical records were retrieved from the National Health Insurance Research Database between years 1998 and 2012. We screened 204,507 patients with chronic hepatitis B, and patients with other infectious hepatitis were excluded. After excluding patients with HCC before the follow-up index dates, 1,553 patients who continuously received daily aspirin ≥ 90 days were randomly matched 1: 4 with 6,212 patients who never received anti-platelet therapy by means of propensity scores, consisted of baseline characteristics, the index date, and nucelos(t)ide analogue (NA) use during follow-up. Both cumulative incidences of and hazard ratios (HRs) for HCC development were analyzed after adjusting for competing mortality. Results: The cumulative incidence of HCC in the treated group was significantly lower than that in the untreated group in 5 years (2.86%, 95% CI: 1.89-3.83 vs. 5.59%, 95% CI: 4.91-6.27; P< 0.001). In the multivariable regression analysis, aspirin therapy was independently associated with a reduced HCC risk (HR 0.63, 95% CI: 0.47-0.85; P= 0.002). Sensitivity subgroup analyses also verified this association. In addition, older age (HR 1.03 per year), male gender (HR 2.65), cirrhosis (HR 1.89), and diabetes mellitus (HR 1.51) were independently associated with an increased HCC risk, but NA (HR 0.57) or statin (HR 0.57) use was with a decreased HCC risk. Conclusion: Aspirin therapy is significantly associated with a reduced risk of HBV-related HCC.



Number at risk						
Untreated	6212	5259	4516	3794	3030	2373
Treated	1553	1378	1212	1044	836	671

Disclosures:

Yao-Chun Hsu - Speaking and Teaching: AbbVie, BMS, Gilead

The following people have nothing to disclose: Teng-Yu Lee, Shi-Hang Yu, Jaw-Town Lin, Ming-Shiang Wu, Chun-Ying Wu

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HNF4α Gene Signature Can Accurately Predict Stage Specific Disease Progression In Cirrhosis And Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy, which has extremely limited treatment options. Tools to determine the disease progression from the pre-malignant stages to cancer and further disease progression from early stage HCC to later stage HCC are limited. Our recent studies have identified the role of Hepatocyte Nuclear Factor 4α (HNF4α) in inhibition of hepatocyte proliferation and HCC pathogenesis. Here we report that HNF4α activity as measured by using a specific HNF4α target gene signature can be used as an excellent predictive tool in HCC disease progression. **Methods:** We identified a specific HNF4α gene expression signature using RNAseq-ChIPseq data obtained from hepatocyte specific HNF4α knockout (HNF4αKO) mice, which was further refined for use in human samples. Validation studies were performed using HNF4a KO mice, mouse HCC samples and a set of 23 human HCC samples. Next, we performed extensive *in silico* analysis to determine the use of this gene signature as a prognostic tool. We analyzed a total of 23 independent with global gene expression datasets comprising of total 2200 individual samples of increasing disease progression including normal livers, cirrhosis with and without HCC, early, and late stage HCC, paired tumor-normal tissues, paracarcinoma, HCC with portal vein thrombosis, HCC-cholangiocarcinoma mixed tissues and metastatic HCC. Survival analysis was performed on a dataset of 365 samples. **Results:** HNF4α gene signature was successful in distinguishing regenerative nodules from cirrhosis, cirrhosis without HCC from cirrhosis with HCC with >80% specificity; differentiate normal, cirrhotic non-HCC, dysplastic nodules, early stage HCC and advanced HCC with >85% specificity and distinguish HCC from paired non-tumor tissues with 100% specificity. In the survival analysis the HNF4α target gene signature was able to clearly distinguish cases with shorter survival, which had significantly lower HNF4α activity. In all cases, lower HNF4a target gene expression was associated with highly progressive metastatic disease. HNF4a gene signature clearly segregated highest grade of disease from the controls in each case. **Conclusions:** The novel HNF4a target gene signature has outstanding prognostic power to distinguish stage dependent HCC pathogenesis and survival. Furthermore, these data indicate that loss of HNF4α is a critical factor in HCC pathogenesis. Supported by 5 P20 GM103549 and R01 DK 098414

Disclosures:

The following people have nothing to disclose: Sumedha S. Gunewardena, Ian Huck, Steven A. Weinman, Anusha Vittal, Josiah Cox, Udayan Apte

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Phenotypic diversity spanning the spectrum of hepatocyte differentiation impacts the outcome of patients with β-catenin-mutated hepatocellular carcinomas.

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Background: Hepatocellular carcinomas (HCCs) are heterogeneous tumors that display a spectrum of molecular phenotypes. Non-proliferative, well-differentiated HCCs have favorable outcomes, preserve metabolic liver zonation programs and include two mutually exclusive subclasses: *Periportal-type* (wild-type β-catenin) and *Perivenous-type* (mutant β-catenin). *Periportal-type HCCs* have the lowest potential for early (< 2 year) recurrence. At the opposite, proliferative HCCs include *Extracellular Matrix*- and *STEM*-type HCCs with bad outcomes. *Periportal-, Extracellular Matrix*- and *STEM-type HCCs* distribute across a spectrum which ranges from hepatocyte-like to stem/progenitor-like phenotypes. In contrast, *Perivenous-type HCCs* deviate from that spectrum because they express β-catenin *(CTNNB1)* target genes (Hepatology 2017, doi:10.1002/hep.29254). Thus, it is not clear how phenotypic diversity contributes to cancer aggressiveness in *Perivenous-type* HCCs. **Aim:** To explore the impact of phenotypic diversity in the outcome of *Perivenous-type* HCCs. **Methods**: First, to confirm the specificity of our previous 5-gene score used to predict *CTNNB1* mutations (Hepatology 2017, doi:10.1002/hep.29254), we retrovirally transduced mutant (T41A) *CTNNB1* to well-differentiated, hepatocyte-like human HCC cells (HepaRG cell line). Then, we predicted *CTNNB1* mutations in a 242-HCC transcriptomic dataset (GSE14520) and analyzed 12509 genes by Cox-PLS combined to genetic algorithms to allow feature selection. Cell proliferation was assessed in an independent series of 72 *CTNNB1*-Sanger-sequenced HCCs by immunohistochemistry for MKI67. **Results:** HepaRG cells expressing mutated *CTNNB1* confirmed the specificity of the mutation markers *GLUL, LGR5, HAL, VNN1* and *ODAM.* Analysis of 72 HCCs showed that cell proliferation rates were low in tumors with mutated *CTNNB1* (Sanger-sequenced or predicted) or with high GLUL staining. As expected, high cell proliferation was associated with HCC recurrence (p = 0.007). However, neither GLUL staining nor *CTNNB1* mutation rates were associated with recurrence. In the 242-HCC dataset, *CTNNB1* was predicted to be mutated in 63 and wild-type in 179 tumors. Discriminant analyses revealed the phenotypic diversity of HCCs with mutated *CTNNB1*, which ranged from well-differentiated tumors with hepatocyte-like features to HCCs expressing a stem/progenitor-like cell program. Thus, HCCs with mutated *CTNNB1* could develop substantial cancer stem/progenitor cell subpopulations overtime. **Conclusions:** Albeit non-proliferative, HCCs with mutant *CTNNB1* may evolve toward an undifferentiated phenotype with bad outcome, which justifies early HCC detection.

Disclosures:

The following people have nothing to disclose: Romain Desert, Christelle Reynès, Robert Sabatier, Damien Grégoire, Florian Rohart, Frédéric Canal, Marie Sicard, Mireille Desille, Stephanie Renaud, Bruno Turlin, Laurent Sulpice, Damien Bergeat, Bruno Clement, Kim-Anh Lê Cao, Orlando Musso

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The incidence of hepatocellular carcinoma is not increased in individuals with chronic hepatitis C after SVR with interferon-free regimens compared with interferon-containing regimens: an ERCHIVES study

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Background: Sustained virologic response (SVR) after interferon-based treatment for chronic hepatitis C virus (HCV) infection has been strongly associated with decreased incidence of hepatocellular carcinoma (HCC). Surprisingly, several recent studies have reported higher rates of HCC in individuals treated with direct-acting antivirals (DAAs). However, making definitive conclusions has been challenging due to the heterogeneous populations and methodologies of these reports. We sought to determine whether DAA use is associated with increased rates of incident HCC in our large, well-characterized cohort. Methods: Using the Electronically Retrieved Cohort of HCV Infected Veterans (ERCHIVES) database, we identified 17,836 patients with chronic HCV and did not have a prior diagnosis of HCC. Patients were divided into 3 groups based on treatment: (a) pegylated interferon + ribavirin (IFN) (n=3,534); (b) DAA-only (n=5,829); and (c) no treatment (n=8,643). Additional analyses were performed on subgroups of DAA-only treatment, including sofosbuvir/simeprevir (SOF/SMV) and sofosbuvir/ledipasvir (SOF/LDV). Predictors of HCC were identified using multivariate Cox proportional hazards analysis. HCC-free survival in cirrhotics was assessed by Kaplan-Meier analysis. Results: SVR was achieved by 66.6% and 96.2% of the IFN and DAA groups, respectively. Among all treated patients, the risk of HCC was not higher in the DAA group compared to the IFN group (HR 1.16; [95% CI: 0.79, 1.71]). Among cirrhotics who achieved SVR, neither the HCC incidence rate nor HCC-free survival were significantly different in the DAA group compared to the IFN group (21.2 vs. 22.8 per 1000 patient years; p=0.78; and log-rank p=0.17, respectively). Untreated cirrhotics had a significantly higher HCC incidence rate (45.3 per 1000 patient years) compared to those treated with IFN or DAAs (p = 0.03). Further, both groups of treated patients had significantly longer HCC-free survival compared to untreated

patients (log-rank p = 0.006). Analyzing the DAA treatment subgroups separately, among SVR cirrhotics, the HCC incidence rate was higher in the SOF/SMV subgroup compared to both the SOF/LDV subgroup and the IFN group (33.3, 15.5, and 21.2 per 1000 patient years, respectively). Notably, the SOF/SMV subgroup had a higher incidence Child-Pugh B/C disease. Conclusions: DAA treatment is not associated with an increased risk of HCC. Previously reported increases in HCC associated with DAA treatment may be explained by the presence of pre-existing HCC risk factors as well as selection bias from treatment with DAA regimens historically used to treat patients at higher inherent risk for developing HCC.

Disclosures:

Daniel S. Fierer - Grant/Research Support: Gilead; Stock Shareholder: Gilead

Vincent Lo Re - Advisory Committees or Review Panels: FDA Antimicrobial Advisory Committee, AASLD/IDSA Hepatitis C Guidance Panel; Board Membership: International Society for Pharmacoepidemiology; Grant/Research Support: AstraZeneca

Raymond T. Chung - Advisory Committees or Review Panels: Alnylam; Grant/Research Support: Gilead, Abbvie, Merck, BMS, Boehringer Ingelheim, Janssen

Adeel A. Butt - Grant/Research Support: Gilead, Merck

The following people have nothing to disclose: Darrick K. Li, Stephanie Rutledge, Obaid S. Shaikh, Tracey G. Simon, Abdul-Badi Abou-Samra

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Neoangiogenic transcriptomic signature identifies HCCs with worse therapeutic outcome

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Abstract Body: Background: Clinical and radiological features are used for prognostication in patients with hepatocellular carcinoma (HCC). The present study aimed to prospectively evaluate the impact of HCC treatment in patients with HCC bearing a transcriptomic signature (TS) (Gut 2016.doi: 10.1136/gutjnl-2014-308483) associated with aggressive tumour behaviour and worse survival. Methods: Candidates for HCC treatment were prospectively subjected to histological HCC evaluation, both for diagnosis and for performance of the TS (qRT/PCR). Physicians deciding and performing treatment were blinded to the presence of TS. Outcome results were matched with TS presence only after follow up finished. Results: 237 patients were enrolled, 81% were male, median age 65 years; 39.7% of them were alive at the end of follow-up in March 2017. Overall median survival was 31 months; 26.6% of patients bearing the TS, had significantly worst survival (median survival: 12 vs. 42 months; p <0.001). The 80% of entire population underwent at least one treatment for HCC. The cohort with TS showed a significant lower survival independently from having a therapeutic option (HCC with TS vs. no-signature: median survival: 20 vs. 48 months; p<0.001) or undergoing supportive therapy only (median survival: 5 vs. 13 months; p<0.001). The presence of TS was always associated with worse survival independently from undergoing surgical resection (33 vs. 68 months; p<0.001), multiple loco-regional treatments (33 vs. 69 months; p<0.001) or systemic drug therapy alone (8 vs. 19 months; p<0.001). Twenty patients (11.8%) undergoing liver transplant (LT) had the best survival of the entire cohort (98 vs. 36 months; p< 0.001), however, the 3 patients with TS undergoing LT had a significantly lower survival within the LT group (75±19 vs. 101±58 months, p<0.001). One out of 3 (33.3%) HCCs with TS recurred vs. 1 out of 17 (5.8%) without TS. At Cox Regression analysis, the presence of transcriptomic signature (HR 2.636,95% CI 1.676-4.145), no treatment vs. performance of any treatment (HR .361, 95% CI .217-.601), and liver function (Child-Pugh score: HR:1.263, 95% CI 1.063- 1.490)) were independently related with worse survival. Conclusion: HCCs bearing the transcriptomic signature have an extremely aggressive clinical course that ultimately impacts on survival despite the application of all the available treatment for HCC. Liver transplant could be the only real therapeutic option but this should be prospectively assessed, as in HCC with transcriptomic signature, a high rate of recurrence is biologically extremely plausible.

Disclosures:

Erica Villa - Advisory Committees or Review Panels: MSD, Abbvie, GSK, Gilead; Speaking and Teaching: Novartis

The following people have nothing to disclose: Alberto Borghi, Luca Marzi, Federica Morellini, Rosina M. Critelli, Cristian Caporali

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Long non-coding RNA DANCR correlated to prognosis of Hepatitis C Virus-related hepatocellular carcinoma

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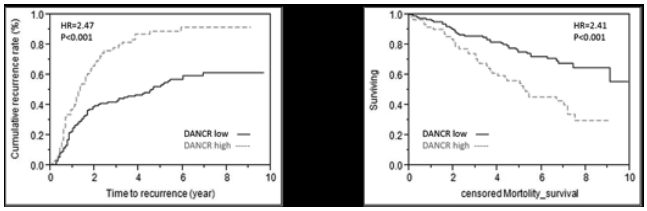
Long non-coding RNAs (lncRNAs), non-protein coding transcripts with size greater than 200 nucleotides, have been associated with different types of neoplasms. Hepatitis C virus (HCV) is one of major etiologies of hepatocellular carcinoma (HCC) worldwide. HCC is a highly prevalent and deadly cancer because of high risks of recurrence and/or metastasis. Identification of potential prognostic markers and/or target molecules could help in the preventing and managing HCV-related HCC. Herein, we characterized and compared lncRNA expression of tumor part and adjacent non-tumor part (ANT) between patients with non-virus-related HCC and HCV-related HCC by RNA-seq to identify candidate lncRNA relevant to HCV-related HCC. In screening panel, 10 lncRNAs significantly differentially expressed between ANT and tumor lesions among HCV-related HCC (p < 0.05, fold change > ±3.0), but not among non-virus-related HCC, were characterized as specific to HCV-related HCC. Of the 10 candidate lncRNAs, 4 were validated by quantitative real-time PCR analysis in confirmation panel and were selected for further analysis in HCV subgenomic replicon system in vitro. We found that lncRNA-DANCR is overexpressed in the HCV infectious replicon cells. In confirmation panel of 55 HCC patients (22 HCV-related), DANCR was also overexpressed in human tumor than in ANT lesions of HCV-related HCC. In another longitudinal cohort of 200 patients of HCV-related HCC post-surgical resection with 10-year followup, higher tumor expression of DANCR (> -6.2 dCt [lncDANCR-U6]) was significantly associated with higher vascular tumor invasion (HR/CI: 1.97/0.97-4.12), recurrence (HR/CI: 2.69/1.31-5.71) and mortality (HR/CI: 3.29/2.22-4.93)

by Cox regression model. The 5-y cumulative incidence of recurrence and survival was 89% and 54.6%, respectively, among patients with higher tumor DANCR, compared to 53.3% and 75.3%, respectively, among those of lower tumor DANCR (Fig 1). In conclusion, DANCR, a lncRNA highly linked to HCV infection and disease progression of HCV-HCC, could serve as a prognostic biomarker and potential target of HCV-related HCC.



Disclosures:

Ming-Lung Yu - Advisory Committees or Review Panels: ABBOTT, MSD, ABBVIE, GILEAD, J&J, ROCHE, BMS; Consulting: MSD, ABBVIE, GILEAD, J&J, ROCHE, BMS; Grant/Research Support: ABBOTT, ROCHE, MSD, ABBVIE, GILEAD, ABBVIE, GILEAD, ROCHE, BMS; Speaking and Teaching: ABBOTT, ROCHE, MSD, GILEAD, BMS, GSK

Wan-Long Chuang - Advisory Committees or Review Panels: Gilead, BMS, Abbvie, MSD, PharmaEssentia; Speaking and Teaching: BMS, Gilead, MSD, Abbvie, PharmaEssentia, Roche

The following people have nothing to disclose: Chung-Feng Huang, Ming-lun Yeh, Jee-Fu Huang, Chia-Yen Dai

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Sphingosine-1 Phosphate Receptor 2 plays a critical role in regulating macrophage recruitment and polarization in cholestatic liver injury in Mdr2$^{-/-}$ mice

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Background: Hepatic macrophages are central players in the pathogenesis of cholestatic liver diseases. Our previous studies demonstrated that conjugated bile acid-induced activation of sphingosine-1 phosphate receptor 2 (S1PR2) plays a critical role in cholestatic liver injury in the bile duct ligation (BDL) mouse model. The multi-drug resistance 2 knockout (Mdr2$^{-/-}$) mouse is a well-established model of cholestatic cholangiopathies and a higher proportion of taurocholate (TCA) in the bile is associated with more severe hepatobiliary damage. We recently show that S1PR2 level is correlated to the progression of fibrosis in Mdr2$^{-/-}$ mouse. However, the role of TCA-induced activation of S1PR2 in regulating hepatic macrophages remains unclear and is the focus of this study. **Methods:** Age and gender-matched wild type (WT), Mdr2$^{-/-}$ and S1PR2$^{-/-}$ mice were used. WT and S1PR2$^{-/-}$ mice were subjected to 2-week BDL. The specific chemical antagonist of S1PR2, JTE-013, was used to treat Mdr2$^{-/-}$ mice via intraperitoneal injection every other day for 8 weeks. At the end of the experiment, the mice were sacrificed. Blood was collected for measuring serum total bile acids and ALP, ALT and AST. Liver samples were processed for HE and Masson's trichrome staining. Primary hepatocytes and macrophages were isolated from WT and Mdr2$^{-/-}$ mice for quantitative real-time RT-PCR, and fluorescence-activated cell-sorting. **Results:** S1PR2 is highly expressed in both hepatocytes and macrophages, but only induced in Mdr2$^{-/-}$ macrophages during disease progression. Serum ALP and total bile acid levels were increased in Mdr2$^{-/-}$ mice. Compared to WT mice, Mdr2$^{-/-}$ mice also had an increased hepatic macrophage infiltration and significant induction of M2 marker genes and fibrotic genes, such as CD206, CD68, CD11b, TGF-β1, CCL17, CCL2, CK-19, Ki67 and collagen 1. BDL-induced upregulation of CD206, CCL17, CCL22, TGF-β1 and CD11b and liver fibrosis were attenuated in S1PR2$^{-/-}$ mice. Flow cytometric analysis further showed that both F4/80^{m}CD11b^{hi} and F4/80^{hi}CD11b^{m} subset macrophages were significantly increased in Mdr2$^{-/-}$ mice but was markedly inhibited by JTE-013 treatment. **Conclusion:** S1PR2 is significantly induced in hepatic macrophages during cholestatic liver injury. Activation of S1PR2 not only induces macrophage infiltration into the liver, but also promotes M2 macrophage polarization, which results in fibrotic liver injury. Our recent studies show that S1PR2$^{-/-}$ mice are protected from BDL-induced liver injury. These results suggest that S1PR2 is a key player in bile acid-induced cholestatic liver injury and represents a potential therapeutic target for cholestatic liver disease.

Disclosures:

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Differential impact of MT1 and MT2 melatonin receptor deletion on biliary proliferation, senescence, and liver fibrosis during cholestatic liver injury

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Senescent cholangiocytes (a key feature in primary sclerosing cholangitis, PSC) contribute to liver fibrosis through activation of hepatic stellate cells (HSCs) by autocrine/paracrine pathways. Melatonin decreases biliary hyperplasia and liver fibrosis in bile duct ligated (BDL) rats. No data exists on the role of MT1/MT2 melatonin receptors on biliary homeostasis and liver fibrosis. We studied the role of melatonin MT1/MT2 on biliary senescence, proliferation and liver fibrosis during BDL. **Methods:** Wild-type (WT), MT1$^{-/-}$ or MT2$^{-/-}$ mice with/without BDL for 1 wk were used. BDL WT mice were treated with Vivo-Morpholinos to silence MT1 or MT2. MT1/MT2 levels were evaluated in liver sections by IHC and in normal and PSC human liver samples by IHC and *q*PCR. Intrahepatic biliary mass (IBDM) and proliferation were evaluated by IHC for CK-19 and Ki67 in liver sections and *q*PCR/immunoblots for PCNA in cholangiocytes. SA-β-gal staining and *q*PCR for p18/p21 was used to evaluate biliary senescence. Liver fibrosis was measured by Sirius red staining and *q*PCR for fibrosis genes (TGF-β1, α-SMA, fibronectin and collagen alpha 1) in total liver, cholangiocytes and Laser Capture Microdis-

section (LCM)-isolated HSCs. *In vitro*, human HSCs (hHSCs) were treated with biliary supernatants from all groups of mice before measuring the expression of fibrosis genes by *q*PCR. **Results:** MT1/MT2 in bile ducts was increased in BDL compared to WT mice. There was increased MT1/MT2 expression in human PSC samples compared to controls Biliary senescence, proliferation and liver fibrosis were increased in BDL compared to WT mice. Biliary senescence, proliferation and liver fibrosis were increased in MT2$^{-/-}$ mice during BDL, but there was reduced biliary senescence, proliferation and liver fibrosis in MT1$^{-/-}$ mice during BDL. A similar profile was observed in BDL WT mice treated with Vivo-Morpholinos for MT1 or MT2. In LCM-HSCs from BDL mice, fibrotic gene expression was increased in MT2$^{-/-}$ but decreased in MT1$^{-/-}$ mice. In hHSCs treated with biliary supernatants from BDL WT mice, there was increased expression of fibrosis genes compared to controls. Fibrotic gene expression was reduced in hHSC treated with biliary supernatants from BDL MT1$^{-/-}$ mice and enhanced by supernatants from BDL MT2$^{-/-}$ compared to hHSC treated with supernatants from BDL WT mice. Increased hHSC fibrotic gene expression is due to senescence of MT2$^{-/-}$ cholangiocytes. **Conclusion:** Biliary senescence, proliferation and liver fibrosis are differentially regulated in MT1$^{-/-}$ and MT2$^{-/-}$ mice. Specific targeting of MT1 receptor may be a novel approach for ameliorating biliary damage and liver fibrosis during chronic liver diseases.

Disclosures:

The following people have nothing to disclose: Nan Wu, Tina Kyritsi, Pietro Invernizzi, Julie Venter, Francesca Bernuzzi, Fanyin Meng, Keisaku Sato, Heather L. Francis, Tianhao Zhou, Paolo Onori, Antonio Franchitto, Gianfranco Alpini, Shannon S. Glaser

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Systems genetics of cholestatic liver disease in ABCB4 deficiency: identification of genetic modifiers that are involved in hepatic cholesterol homeostasis

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Background: Mutations in the ABCB4 transporter gene cause progressive cholestatic liver disease. Modifier genes of familial cholestasis have yet to be identified systematically. In this study we generated congenic *Abcb4*$^{-/-}$ knockout mice, in which deficiency of the hepatobiliary phosphatidylcholine translocator causes variable cholangiopathy in different inbred mouse strains. We applied a systems genetics approach to elucidate the genetic control of liver fibrosis in an experimental cross of congenic *Abcb4*$^{-/-}$ lines. Methods: The *Abcb4* knockout was transferred from the fibrosis-resistant FVB-*Abcb4*$^{-/-}$ strain to the susceptible BALB/cJ background by repeated backcrossing. To identify genetic modifiers that contribute to the fibrosis susceptibility linked to ABCB4 deficiency, we crossed the two congenic strains to generate an intercross (F2) population. By quantitative trait locus (QTL) analysis phenotypic differences among the 217 F2 progeny were mapped to polymorphic regions across the whole genome. Single and two-dimensional QTL scans were applied to identify modifiers and pairwise gene interactions. For translation, the effect of genetic variation in the orthologous *PCSK9* locus was tested a cohort of 193 patients with primary sclerosing cholangitis (PSC). Results: Compared to FVB-*Abcb4*$^{-/-}$ mice, BALB-*Abcb4*$^{-/-}$ mice progress to higher fibrosis stages. The heterogenic F2 population shows marked phenotypic variation. Whereas single modifiers demonstrate minor effects, gene-gene interaction scans identified a significant interaction of two QTLs on chromosomes 4 and 17. Underlying these loci we identified the genes *Lrp8* (LDL receptor related protein 8), *Pcsk9* (proprotein convertase subtilin-kexin type 9) and *Scp2* (sterol carrier protein 2) on chromosome 4, as well as *Abcg5/g8* and the trigenic region *Rhoq-Pigf-Cript* on chromosome 17. The specific genes are functionally related to hepatobiliary cholesterol homeostasis and resemble creedal modifier genes. The analysis of the PSC cohort revealed that carriers of a variant *PCSK9* allele presented with significantly higher serum alkaline phosphatase activities. Conclusions: The congenic *Abcb4*$^{-/-}$ lines allow the systematic genomic exploration of chronic cholestasis in inbred mice. The experimental cross of ABCB4-deficient strains with distinct differences in fibrosis susceptibility resulted in the identification of ABCB4-dependent modifiers of cholestatic liver injury. Several candidate genes identified in mice, such as *Pcsk9*, are involved in the regulation of hepatic cholesterol metabolism and accordingly, the human *PCSK9* gene was also associated with serum surrogate markers in PSC patients.

Disclosures:

The following people have nothing to disclose: Rabea A. Hall, Katrin Hochrath, Marcin Krawczyk, Malgorzata Milkiewicz, Piotr Milkiewicz, Frank Grünhage, Frank Lammert

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Vitamin D receptor (VDR) deficiency in *Abcb4*$^{-/-}$ mice causes an aggravation of primary sclerosing cholangitis features that can be prevented by VDR-independent therapeutic interventions

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Background: Primary sclerosing cholangitis (PSC) pathophysiology could involve the impairment of functions controlled by the vitamin D receptor (VDR), such as mucosal immunity or barrier integrity in the bile ducts and in the intestine. In this study, we aimed to address the protective functions of the VDR axis in the *Abcb4*$^{-/-}$ mouse model of PSC. Methods: To evaluate the impact of VDR deficiency on the development of PSC, we compared the survival and liver phenotype of *Vdr*$^{+/+}$-*Abcb4*$^{-/-}$ mice and *Vdr*$^{-/-}$-*Abcb4*$^{-/-}$ mice, a mouse line that was developed in our group. We also tested the potential therapeutic effects of Vitamin D by treating both simple and double knockout mice with either a rescue diet supplemented in Vitamin D that normalizes mineral ion levels (2% calcium, 1.25% phosphorus, 20% lactose, 2.2 IU vitamin D/g) or calcipotriol, a vitamin D analog. Liver injury, inflammation and fibrosis were assessed by serum tests, RT-qPCR, histo-

pathology and collagen quantification. Results: Mortality rate was markedly higher in *Vdr*$^{-/-}$-*Abcb4*$^{-/-}$ mice compared to *Vdr*$^{+/+}$-*Abcb4*$^{-/-}$ mice from 8 months on (47% *vs.* 27%). *Vdr*$^{-/-}$-*Abcb4*$^{-/-}$ mice displayed significantly higher serum alanine amino transferase levels from the age of 4 weeks when compared to *Vdr*$^{+/+}$-*Abcb4*$^{-/-}$ mice. In 8 weeks old *Vdr*$^{-/-}$-*Abcb4*$^{-/-}$, 1.8-fold higher levels of phosphatase alkaline and serum bile acids were also detected, indicating more severe cholestasis. An increase in the expression of the pro-inflammatory genes *Mcp1*, *Vcam-1 and Tnf*α and in the ductular reaction, ascertained by a 1.7-fold increase in CK19-positive area, was also observed in *Vdr*$^{-/-}$-*Abcb4*$^{-/-}$ mice compared to *Vdr*$^{+/+}$-*Abcb4*$^{-/-}$ mice. Consistently, *Vdr*$^{-/-}$-*Abcb4*$^{-/-}$ mice at 8 weeks had more severe fibrosis as ascertained by a 1.6 times higher collagen content. Noteworthy, *Vdr*$^{-/-}$-*Abcb4*$^{-/-}$ mice fed with the rescue diet developed less severe cholestasis and fibrosis than those with the standard chow. By contrast, no such effect of the rescue diet was observed in the *Vdr*$^{+/+}$-*Abcb4*$^{-/-}$ mice. In the double knockout mice, calcipotriol treatment was able to reduce the expression of pro-inflammatory genes (*Mcp1*, *Vcam-1*) to levels comparable to those observed in the simpe knockout. Calcipotriol also caused a decrease both in fibrosis and in the intensity of ductular reaction in the double knockout mice, whereas no significant effect was observed in the simple knockout. Conclusion: VDR deficiency aggravates PSC features in *Abcb4*$^{-/-}$ mice. Both a Vitamin D/phospho-calcic supplementation and a vitamin D analog improved the exacerbated PSC features observed in VDR deficient mice, suggesting yet uncovered VDR-independent mechanisms of action of Vitamin D.

Disclosures:

Nicolas Chignard - Employment: Novartis Pharma

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Hepatic X-box Binding Protein 1 (XBP1) Plays a Protective Role in Cholestatic Liver Injury.

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Background: Endoplasmic reticulum (ER) stress occurs in cholestatic liver diseases and the X-box binding protein 1 (XBP1) pathway of the unfolded protein response (UPR) is a protective response to ER stress. However, the role of the hepatic XBP1 in cholestatic liver injury remains unexplored. We demonstrate a protective role of the hepatic XBP1 during cholestasis using: 1) a bile duct ligation (BDL) model and 2) in FXR/XBP1 double knockout mice. Methods: BDL was performed on C57BL/6J, liver specific XBP1 knockout (LS-XBP1$^{-/-}$) or XBP1$^{fl/fl}$ control mice for up to 10 days. Serum liver chemistries, hepatic gene expression and hepatic histology (H&E and Sirius Red staining quantified by Image J) were measured. We also developed and characterized FXR$^{-/-}$/LS-XBP1$^{-/-}$ double knockout (DKO) and FXR$^{-/-}$/XBP1$^{fl/fl}$ single knockout (SKO) mice as a new model of cholestatic liver injury. Results: 48 hrs after BDL, C57BL/6J mice had a 4-fold increase of hepatic XBP1s gene expression (P<0.001) and its downstream target gene *ERdj4* (P<0.01); nuclear XBP1s protein expression also significantly increased. LS-XBP1$^{-/-}$ mice subjected to BDL for 72 hours had greater serum alkaline phosphatase (ALP) levels (1475±465 vs 80±27 U/L, P<0.05) and hepatic *Col1a1* gene expression (1.8±0.3 vs 1.0±0.2, P<0.05) compared to XBP1$^{fl/fl}$ mice. Ten days after BDL, serum ALP remained higher in LS-XBP1$^{-/-}$ mice compared to XBP1$^{fl/fl}$ mice (997±41 vs 591±33 U/L, P<0.001). LS-XBP1$^{-/-}$ mice also had greater hepatic gene expression of *Col1a1* (3.1±0.8 vs 1.0±0.1; P<0.05), *Timp1* (3.7±0.7 vs 1.0±0.3; P<0.01), *Chop* (3.6±1.0 vs 1.0±0.1; P<0.05) and *Dr5* (1.6±0.1 vs 1.0±0.1; P<0.01). H&E staining demonstrated a greater bile infarct area (20.6±1.4% vs 5.5±2.8%; P<0.01), and Sirius Red staining revealed more fibrosis (2.8±0.8% vs 0.8±0.1%; P<0.05) in LS-XBP1$^{-/-}$ mice compared to XBP1$^{fl/fl}$ mice. FXR$^{-/-}$ mice are a frequently utilized model of cholestasis. The FXR/XBP1 DKO mice had greater serum ALT levels (209±17 vs 81±14 U/L; P< 0.001) compared to the SKO mice. DKO mice also had higher hepatic gene expression of *Timp1* (6.6±1.1 vs 1.0±0.3, P<0.01), *Chop* (2.4±0.4 vs 1.0±0.1; P<0.05) and *Dr5* (6.2±1.2 vs 1.0±0.02; P<0.01) compared to the SKO mice. Sirius Red staining demonstrated that DKO mice had more fibrosis than SKO mice (1.1± 0.3% vs 0.2±0.02%, P<0.05). Conclusion: BDL activates the XBP1 pathway of the UPR. Loss of hepatic XBP1 results in more liver injury and fibrosis in both BDL and FXR$^{-/-}$/LS-XBP1$^{-/-}$ DKO models of cholestatic liver injury. We speculate that hepatic XBP1 has an important role in lessening liver injury in cholestatic liver diseases.

Disclosures:

The following people have nothing to disclose: Xiaoying Liu, Susan C. Hubchak, Brian E. LeCuyer, Xin Y. Yeap, Richard M. Green

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Bile acids induce release of pro-inflammatory extracellular vesicles from mouse hepatocytes

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BACKGROUND and AIMS: Recent studies demonstrate that cholestatic liver injury may be largely caused by sterile inflammatory response induced by overload of bile acids. Extracellular vesicles (EVs) are nanometer-sized, membrane-bound vesicles loaded with proteins, lipids, RNAs and DNAs that can be transported and exchanged between cells. EVs play an important role in inflammatory liver diseases such as nonalcoholic steatohepatitis and alcoholic hepatitis. Here we examine the role of EVs in cholestatic liver injury where pro-inflammatory mediators such as chemokines have been shown to participate in the inflammatory response. **METHODS:** Wild-type primary mouse hepatocytes (PMH) were treated with bile acids and EVs released to the culture medium were isolated at different time points by differential centrifugation. EVs were also isolated from plasma of sham-operated and 2-day bile duct-ligated (BDL) mice by size exclusion chromatography column. The obtained EVs were characterized with nanoparticle tracking analysis and immunoblotting. **RESULTS:** Increased number of EVs were released from PMH treated with 100 μM glycocholic acid (GCA) compared with no treat control or 100 μM ursodeoxycholic acid (UDCA) treatment. GCA induced approximately 5- and 40-fold increase in EV release at 16 hr and 22 hr, respectively, without significantly increased cell death compared with no treat control. The size distribution of the EVs derived from PMH was 40-400 nm with a mode sizes of ~90 nm. Immunoblotting demonstrated that these EVs contain the EV markers CD 63 and Cyp2E1, a marker for hepatocyte-derived EV. CXCL10, a potent chemokine for

the innate immune system, was induced with 100μM GCA, but not with 100μM UDCA in PMH. Whereas monomer CXCL10 was the major form on the EVs from PMH treated with 100 μM GCA for 6 hr, both monomer and dimer CXCL10, which has been reported being necessary for its in vivo activity, were detected on EVs from 16 hr and 22 hr treatment. However, only monomer CXCL10 that was secreted in a soluble form was detected in the culture medium at these time points. EVs isolated from the plasma of BDL mice showed significantly increased concentration as well as size distribution compared with sham-operated mice. **CONCLUSION:** Bile acids induce release of CXCL10-laden extracellular vesicles from mouse hepatocytes. The concentration and size distribution of circulating EVs are also increased in cholestasis induced by bile duct ligation, suggesting that EVs induced by bile acid overload participate in the inflammatory response in cholestasis.

Disclosures:

Gregory J. Gores - Advisory Committees or Review Panels: Conatus, Jecure

James L. Boyer - Consulting: Allergan, Alnylam; Grant/Research Support: Gilead, Intercept pharma

The following people have nothing to disclose: Man Li, Petra Hirsova, Albert Mennone

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Hepatic Stellate Cell-Derived Hyaluronan Synthase 2 Mediates Hyaluronic Acid Production That Promotes Liver Fibrosis

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Backgrounds: Hyaluronic acids (HA), a major extracellular matrix, have been used as a biomarker for cirrhosis. However, the molecular mechanism of HA synthesis and the role of endogenous HA in liver fibrosis are poorly understood. This study investigated the role of HA synthase (HAS) 2 in HA production, hepatic stellate cell (HSC) activation, and fibrosis. **Methods**: 65 patient samples were used to determine HAS2 expression in human liver fibrosis (F0-F4). HSC-specific *Has2* knockout mice (*Has2*$^{\Delta HSC}$) were generated by crossing *Has2 flox* mice with *Lrat-Cre* mice. αSMA promoter-driven HAS2 Tg mice were also used. Matrigel invasion assay was used to examine the invasive behavior of HSCs. **Results**: Hepatic HAS2 expression was significantly increased in patients with cirrhosis (F4). The immunohistochemical analysis demonstrated high levels of HAS2 in activated HSCs in cirrhotic livers. In murine liver fibrosis models, HA production was dramatically increased. *Has2*$^{\Delta HSC}$ mice showed reduced HA production and liver fibrosis induced by bile duct ligation (BDL), CCl_4, or CD-HFD. In contrast, ASMA-HAS2 Tg mice had aggravated BDL-induced liver fibrosis. Treatment of 4-methylumbelliferone, an inhibitor of HA synthesis, attenuated BDL-induced liver fibrosis. Col1a1 and αSMA mRNA levels were reduced in *Has2*$^{-/-}$ HSCs but increased in HSCs derived from ASMA-HAS2 Tg mice compared with WT HSCs. Matrigel invasion assay showed that *Has2*$^{-/-}$ HSCs had less invasive phenotype that is critical for fibrosis progression, whereas HAS2-overexpressing HSCs acquired more invasive behavior than WT HSCs. Consistently, HSC proliferation was suppressed in *Has2*$^{-/-}$ HSCs but was enhanced in HAS2 Tg HSCs. To characterize the signaling pathway of two major HA receptors, CD44 and TLR4, RNA-seq was performed for culture activated WT, CD44$^{-/-}$, and TLR4$^{-/-}$ HSCs. Fibrogenic genes (Col1a1, Col4a3, Acta2, Tgfb1, Tgfb2) were suppressed in both CD44$^{-/-}$ and TLR4$^{-/-}$ HSCs, but inflammatory genes (Ccl2, Ccl5, Cxcl1, Cxcl2) were suppressed only in TLR4$^{-/-}$ HSCs. Finally, we examined the regulatory mechanisms of HAS2 expression in HSCs. We found that Wilm's tumor 1 (WT1) regulates Has2 expression by binding Has2 promoter region and miR-200c inhibited Has2 mRNA expression by binding 3'-UTR of Has2 mRNA. **Conclusion**: Hepatic HAS2 expression was associated with the degree of fibrosis in patients. HSC-derived HAS2 mediates HA production in the liver, which promotes HSC activation and liver fibrosis. Inhibition of HA synthesis inhibits liver fibrosis. HAS2 contributes to HSC activation, proliferation, and invasive property. This is the first study showing the pathogenic role of HAS2 and endogenous HA in liver fibrosis.

Disclosures:

Robert F. Schwabe - Consulting: Merck, Dicerna

Ekihiro Seki - Consulting: SUNTORY; Grant/Research Support: Nippon Zoki

The following people have nothing to disclose: Yoon Mee Yang, Cheng Liu, Koichiro Ohashi, Yoon Seok Roh, Hiroshi Matsushita, Dianhua Jiang, Paul W. Noble

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SHP2 promotes liver fibrosis by inducing secretion of hepatic stellate cell-derived PDGFRα-enriched extracellular vesicles

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Background: Platelet-derived growth factor (PDGF)/PDGF receptor (PDGFR) signaling plays a crucial role in liver fibrosis by promoting hepatic stellate cell (HSC) activation and migration. Upon PDGF binding, PDGFRs are phosphorylated and recruit molecules such as SHP2 to activate downstream signaling. PDGFRs are later transferred to the endosomal compartment and then degraded. The endosomal compartment also generates extracellular vesicles (EVs), which impact cell-to-cell communication. Based on this understanding, the **aim** of this study is to investigate the mechanism and the role of PDGFRα signaling by EVs in liver fibrosis. **Methods/Results**: Patients with liver fibrosis had 2-fold increase of PDGFRα levels in liver lysates and 3-fold increase of PDGFRα in serum EVs ($p<0.05$) compared to healthy individuals, as assessed by western blot. *In-vitro* PDGF treatment of human HSCs induced 2-fold enrichment of PDGFRα in EVs ($n=5$ $p<0.05$). Inhibition of autophagic degradation with BafilomycinA1 or Atg5 siRNA enhanced PDGFRα enrichment in EVs by 3-fold ($n=3$) or 1.5-fold ($n=5$), respectively ($p<0.05$) suggesting that inhibition of PDGFRα degradation leads to targeting of the protein to EV. To examine the mechanism of PDGFRα phosphorylation in this process we mutated the tyrosine residue 720 to phenylalanine (Y720F) and overexpression of this dominant negative mutant abolished PDGFRα enrichment in EVs ($n=5$ $p<0.05$). A constitutive active PDGFRα phospho-mimetic mutant (Y720E) promoted a 2-fold increase of PDGFRα enrichment of EVs ($n=3$ $p<0.05$). SHP2 is a regulatory binding partner of phosphorylated Y720. Y720F mutant showed 8-fold lower association with SHP2 ($n=3$ $p<0.05$), as assessed by co-IP. SHP2 inhibition using SHP099 (10μM $n=6$), SHP2 siRNA ($n=3$) or SHP2 dominant negative construct ($n=3$) abolished PDGFRα enrichment in EVs

(p<0.05). *In-vitro* wound-healing assay demonstrated that EVs derived from PDGFRα overexpressing HSCs induced 2-fold increase of cell migration compared to control cells (n=3 p<0.05) indicating that HSC derived EV can act on adjacent HSC to promote migration. Finally, administration of SHP099 (5mg/kg) to CCl_4-chronically injected mice inhibited PDGFRα enrichment in serum EVs and promoted a 40% reduction of Sirius red staining and 50% decrease of αSMA protein levels in CCl_4 livers (p<0.05). **Conclusion**: These results demonstrate a novel mechanism of action of PDGFRα through EVs, promoted by SHP2 binding to phosphorylated Y720. Therapeutically targeting SHP2 reduces PDGFRα enrichment of EVs, which correlates with decrease of liver fibrosis.

Disclosures:

Vijay Shah - Consulting: Merck Research Laboratories, Durect Corporation, Novartis Pharma

The following people have nothing to disclose: Enis Kostallari, Petra Hirsova, Alena Prasnicka

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EZH2 Histone Methyltransferase Promotes TGFβ Dependent Fibrogenic Genes *In Vitro* and Liver Fibrosis *In Vivo*

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Background/aims: Transdifferentiation of hepatic stellate cells (HSCs) into myofibroblasts is regulated in part through epigenetic modifications of histone proteins that control gene transcription programs. Trimethylation of lysine 27 on histone H3 (H3K27Me3) is a transcription-suppressing epigenetic mark mediated by the histone methyltransferase, enhancer of zeste homolog 2 (EZH2). The goal of this study was to characterize the consequences of EZH2 inhibition using molecular and pharmacologic strategies. **Methods:** We conducted *in vitro* and *in vivo* experiments with human HSCs and mice chronically treated with CCl4. siRNA-mediated and pharmacological inhibition of EZH2 (GSK-503) were used to analyze the role of EZH2 in fibrogenesis. **Results:** Blockade of EZH2 with siRNA significantly attenuated the TGF-β induced increase in fibronectin protein expression and mRNA (p<0.05), collagen 1α1 mRNA (p<0.05) and αSMA mRNA (p<0.05). Conversely, adenoviral overexpression of EZH2 enhanced TGF-β dependent stimulation of fibronectin protein and mRNA levels (p<0.05). Concordantly, pharmacologic inhibition of EZH2 using GSK-503 (10 mM), also significantly attenuated the TGF-β induced increase in fibronectin protein expression and mRNA (p<0.05), α-SMA mRNA (p<0.05) and collagen 1α1 mRNA (p<0.05). As expected, this effect correlated with the decrease in H3K27me3 in HSCs. *In vivo*, administration of GSK-503 to mice receiving chronic CCl4 led to attenuated CCl4 induced fibrosis. This effect was assessed by trichrome and Sirius red stains (p<0.05) from fixed liver tissue, as well as by hydroxyproline assays from liver lysates (p<0.05). The H3K27me3 mark was globally attenuated in response to administration of GSK-503 as assessed by Western blot and immunohistochemistry (p<0.05). **Conclusion:** Inhibition of EZH2 attenuates fibrogenic gene transcription in TGF-β treated HSCs and reduces liver fibrosis in mice administered chronic CCl4. A better understanding of these fibrogenic pathways in liver fibrosis and the effects of epigenetic pharmacology may lead to new approaches to treat liver fibrosis.

Disclosures:

Vijay Shah - Consulting: Merck Research Laboratories, Durect Corporation, Novartis Pharma

The following people have nothing to disclose: Rosa M. Martin Mateos, Juan Pablo Arab, Thiago Milech de Assuncao, Vikas K. Verma, Nidhi Jalan-Sakrikar, Guang Shi, Usman Yaqoob, Angela Mathison, Robert C. Huebert

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Modeling compound-induced hepatic fibrosis *in vitro* using three-dimensional liver tissue constructs

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Background. Hepatic fibrosis develops from a series of complex and cumulative interactions among resident and recruited cells making it a challenge to replicate using standard *in vitro* models. Previous work demonstrated the utility of bioprinted liver tissue (ExVive™ Human Liver, Organovo) to model fundamental aspects of fibrotic injury as a result of physiologically relevant methotrexate (MTX) exposure. The present studies were undertaken to 1) further optimize the exposure regimen to understand the injury/fibrotic response and 2) evaluate the role of Kupffer cells (KCs) in modulating these outcomes over an extended timeframe. ***Methods.*** Tissues comprised of primary human hepatocytes (HCs), endothelial (ECs), and hepatic stellate cells (HSCs) with or without KCs were fabricated according to standard protocol (Organovo, Inc.), allowed to mature for 7 days, and exposed to MTX for 14-28 days. Culture medium was sampled on alternate days to assess markers of tissue and HC-specific injury (*e.g.*, LDH and ALT, respectively), function (*e.g.*, urea and albumin), and fibrogenic activity. At the conclusion of the study, tissues were harvested for gene expression and histologic assessment. ***Results.*** While baseline LDH, IL-8, and IL-1β levels decline steadily post fabrication, treatment with MTX after tissue maturation resulted in a dose-dependent elevation in LDH, peaking at 2.5-fold at the highest concentration tested (10 μM). In contrast, a rapid and dose-dependent increase in ALT was observed, peaking at 15-fold on treatment day (Tx) 3 for 10 μM MTX. Patterns of LDH and ALT release were consistent across 2 donor tissue lots with early HC injury preceding general, sustained tissue injury. These trends were accompanied by progressive, dose-dependent increases in collagen deposition suggesting the extent of injury impacts the magnitude of fibrogenic outcome. The temporal expression of fibrogenic genes *ACTA2* and *COL1A1* at Tx14 further support the histological outcome. Expansion to a continuous 28-day exposure at the LC20 and LC50 resulted in sustained LDH release over time (Tx9-Tx19). However, incorporation of KCs attenuated this response resulting in a narrowed injury window (~Tx13-Tx15). Preliminary evaluation of collagen deposition and cytokine release suggest KCs may limit fibrogenic activity early on, while persistent MTX exposure in the presence of KCs resulted in decreased cellularity and an increase in collagen deposition at late time points (Tx28). ***Conclusion.*** These data demonstrate the utility of this model to mimic the onset and progression of injury and suggest KCs may play an important bimodal role during early versus later phases of the response.

Disclosures:

Leah M. Norona - Grant/Research Support: Organovo Inc.

Deborah G. Nguyen - Stock Shareholder: Organovo

David A. Gerber - Consulting: Organova

Sharon C. Presnell - Employment: Organovo, Inc.

Paul B. Watkins - Consulting: Abbott, Actelion, Boerringer-Ingelheim, Cempra, Alecra, Roche, GSK, Merck, Reservlogix, Astra Zeneca, Intercept, Janssen, Novartis, Otsuka, Pfizer, Sanolfi, Takeda, Bristol-Myers Squibb, UCB

The following people have nothing to disclose: Merrie W. Mosedale

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Heat Shock Transcription Factor 1 (HSF1), a key cellular proteostasis regulator, is crucial for resolution of liver fibrosis in mice

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Background/Aims: Liver fibrosis is wound healing adaptive response to hepatocellular injury caused by various etiologies such as chronic viral hepatitis, NAFLD and alcoholic liver disease. These etiologies are known to generate reactive oxygen species, which activates heat shock factor 1 (HSF1), the key transcription factor for regulation of the proteostasis network. Previously, our group demonstrated that HSF1 can regulate macrophage inflammatory responses. Inflammation plays an important role in mediating liver fibrosis. We hypothesized that HSF1 plays a protective role in liver fibrosis by decreasing hepatic stellate cell (HSC) activation and inflammation. Methods: HSF1 expression and function was analyzed in human and murine fibrotic liver tissues by RT-PCR and EMSA. HSF1 deficient (HSF1-/-) and corresponding wild type littermates (WT) were injected with CCl_4 for 6 weeks followed by 4 weeks without CCl_4 (recovery phase) and extent of liver injury was assessed. *In vitro* mechanistic studies were performed using dominant negative-HSF1 (dnHSF1) stably transfected to HSC cell line, LX-2. Functional significance of HSF1 in TGFβ mediated HSC activation was analyzed by 1) assessing effect on dnHSF1 cells and 2) heat shocked LX2 cells, by evaluating the expression of pro-fibrogenic genes. Results: HSF1 was elevated in human cirrhotic livers (p=0.0094). Murine fibrotic livers induced by CCl_4, MCD diet and ethanol (7 weeks plus multiple binges) demonstrated increased HSF1 expression. However, functionally nuclear localization of HSF1 was impaired in CCl_4 induced fibrotic livers. HSF1-/- mice demonstrated higher pro-fibrogenic gene expression such as α-SMA (p=0.0129), α1-(I)-collagen (p=0.0229), TIMP1 (p=0.0036) and MMP-8 (p=0.0048) whereas delayed resolution was identified by lack of reduction of pro-fibrogenic genes in HSF1-/- mice. Human LX-2 cells treated with TGFβ, exhibited loss of HSF1 DNA binding by EMSA. Concomitantly, dnHSF1 cells demonstrated higher expression of α-SMA (p=0.0057) and α1-(I)-collagen (p=0.0011) compared to the control cells. On the other hand heat shock mediated activation of HSF1 significantly decreased the expression of α-SMA and α1-(I)-collagen. Conclusion: We conclude that HSF1 DNA binding activity is impaired during liver fibrosis with a likely compensatory HSF1 expression. Absence of HSF1 significantly exacerbated liver fibrosis through increasing HSC activation and delayed resolution of liver fibrosis, whereas activation of HSF1 reduced pro-fibrogenic gene induction. Our study highlights HSF1 activation, an important proteostasis regulator, as a promising molecular candidate for resolution of liver fibrosis and HSC activation.

Disclosures:

The following people have nothing to disclose: Asmita Choudhury, Arlene Lim, Christopher L. Moore, Matthew Shoulders, Pranoti Mandrekar

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Tyrosine kinase SYK is a potential therapeutic target for liver fibrosis

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Background/Aims: Spleen tyrosine kinase (SYK) has been reported as a novel biomarker for human hepatocellular carcinoma (HCC) and plays a critical role in immune cell signaling pathways. We previously demonstrated that SYK regulated tumor invasion and metastasis through the differential expression of two SYK isoforms in HCC (Hong J. et al, Cancer Res 2014, JCI 2012). The SYK inhibitor entospletinib is currently in Phase II/III clinical trials for treatment of hematological malignancies and rheumatoid *arthritis*. In this study, we sought to evaluate the mechanism by which SYK induces liver fibrosis and by which entospletinib inhibits SYK-mediated liver fibrosis/cirrhosis and hepatocarcinogenesis. **Methods:** We evaluated the association between SYK expression and liver fibrogenesis in HBV, HCV infected or alcohol-related liver tissues with progressive fibrosis/cirrhosis stages. The effects of HBV or HCV infection on SYK-induced fibrosis were also monitored in HBV replicon HepG2215 and JFH1 HCV-infected Huh7.5.1 cells models. We used a transwell co-culture system to evaluate SYK's role in crosstalk between Huh7.5.1 and hepatic stellate cells (HSCs) LX2. We tested SYK inhibitor (entospletinib) effects on gene transcription factors (TFs) in primary human HSCs. We also examined the effects of a SYK inhibitor (entospletinib) on liver fibrogenesis in three animal models including diethylnitrosamine (DEN) induced rat model, bile duct ligation rat model, or carbon tetrachloride (CCl_4) induced mouse model. **Results:** We found that increased SYK protein staining mainly in HSCs and hepatocytes correlated positively with liver fibrosis/cirrhosis stages in HBV or HCV infection, or alcohol induced liver disease in human liver tissue. HBV or HCV infection significantly increased SYK mRNA levels compared to uninfected hepatocytes. Moreover, coculture of HCV or HBV infected hepatocytes with LX2 cells increased fibrogenesis related gene expression compared to uninfected Huh7.5.1 cells. SYK knockdown or entospletinib treatments effectively suppressed the expressions of fibrogenesis-related genes including TGF-β1, PAI-1, COL1A1 and CTGF in both hepatocytes and HSCs. SYK overexpression promoted HSCs activation via up-regulation of transcription factors including c-Myb, CREB-binding protein, and c-Myc. In animal models, entospletinib treatment significantly reduced HSC activation, prevented cirrhosis progression, regressed fibrosis, and reduced HCC development. **Conclusions:** SYK promotes

liver fibrosis across etiologies via activation of HSCs. SYK inhibition represents an attractive potential therapeutic target to reduce liver fibrosis and HCC development.

Disclosures:

Raymond T. Chung - Advisory Committees or Review Panels: Alnylam; Grant/ Research Support: Gilead, Abbvie, Merck, BMS, Boehringer Ingelheim, Janssen

The following people have nothing to disclose: Chen Qu, Dandan Zheng, Sai Li, Anna Lidofsky, Yuchuan Jiang, Lu He, Hui Yuan, Xijun Chen, Qiaoting Hu, Xiaojun Luo, Yuchen Zhou, Wenyu Lin, Jian Hong

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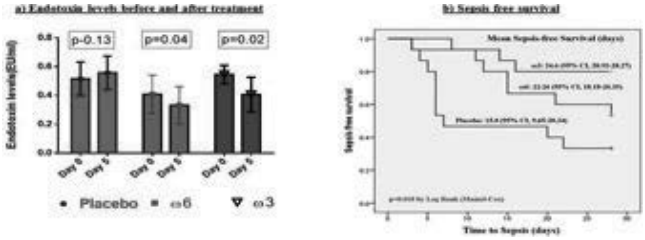
ω3 polyunsaturated fatty acids(PUFA) lipid emulsions are safe and effective to reduce endotoxemia and sepsis in patients with Acute on Chronic Liver Failure -A randomised controlled trial.(NCT 02691533)

Anand V. Kulkarni, Lovkesh Anand, Ashish K. Vyas, Nirupma Trehanpati, Aakaksha Kakran, Happy Chauhan, Jaya Benjamin, Guresh Kumar, Y.K. Joshi, Shiv K. Sarin; Hepatology, Institute of Liver and Biliary Sciences, New Delhi, India

Background- Acute on chronic liver failure (ACLF) is often a progressive disease with high 28day mortality. Providing adequate nutrition & reducing incidence of sepsis are main challenges.ω3 PUFA have been shown to be efficacious in reducing inflammation, decreasing mortality & morbidity in postoperative patients. There is limited data on the use of PUFA in ACLF. **Methods-**Fifty-one ACLF patients(APASL criteria) without sepsis, shock, AKI(creatinine>1.5mg/dl), immunosuppressive therapy were randomised to 3 groups-Gr.I-Controls, Gr.II-ω6(20% intralipid 50ml~100kcal)& Gr.III-ω3(10% omegavan 100ml~100kcal) for 5days. Primary aim was to assess immunomodulation(change in levels of Interleukins, TNF α, endotoxin) & secondary were occurrence of sepsis at day 7 & survival at day 28. **Results**– Forty-five ACLF patients(41 male, age 42.96±10.4yr., alcohol 78%)completed the study;15 in each Gr. Baseline parameters were comparable. After 5 days of infusion, expression of TLR 2/4 on monocyte, macrophage & neutrophil significantly increased in Gr.III.Serum endotoxin levels decreased in patients on lipid emulsions;20% in Gr.II,27% in Gr.III but increased by 8% in Gr.I.At day 5, TNFα levels increased in Gr I by24.6%(p=0.13),6.8% in Gr. II(p=0.05) but decreased by 22.3% in Gr.III(p=0.03). A decrease in IL-1β (p=0.03), increase in IL-6 (p=0.04) & IL-10 (p=0.02) was seen after 5 days in Gr.III.CRP levels decreased in Gr.III(I-2.36%, II-13.83%, III-42.56%;p<0.001) at day5. Twenty ACLF patients developed sepsis by day 28;Gr. III>II>I{I-66.7%, II-46.7% & III-20%; p=0.03}, though without improved survival (p=0.8). The time to development of sepsis was shorter in Gr. I than II & III {I-6(3,22), II-15(8,28), III-14(3,16)days;p=0.09}.The most common source of sepsis was pneumonia(50%). On multivariate analysis INR{2.03(1-4.17,0.05)} & HDL {0.06(0.39-0.93),0.02} were significant predictors of sepsis. No adverse effects of lipid emulsion infusion were noted, except flushing in 20% in Gr.II & 13.4% in Gr.III. **Conclusions**-ω3 PUFA are well tolerated & are effective in reducing immune mediated injury, controlling inflammation, reducing endotoxemia & incidence of sepsis in ACLF patients. Longer duration & higher doses of PUFA should be evaluated in ACLF.



Disclosures:

The following people have nothing to disclose: Anand V. Kulkarni, Lovkesh Anand, Ashish K. Vyas, Nirupma Trehanpati, Aakaksha Kakran, Happy Chauhan, Jaya Benjamin, Guresh Kumar, Y.K. Joshi, Shiv K. Sarin

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Prediction of Fungal Infection Development and Their Impact on Survival using the NACSELD Cohort

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Infections impair outcomes in cirrhotic patients. With rampant antibiotic use, fungal infections are being increasingly recognized but have not been studied systematically. Aim: Define the determinants of fungal infection development & their impact on 30-day survival in the NACSELD cohort. Methods: Cirrhotic inpatients recruited into a large multi-center prospective inpatient study (NACSELD) were evaluated for bacterial and fungal infections. Demographics, cirrhosis details, ICU utilization, organ failures/NACSELD-ACLF and 30-day survival were evaluated. Patients with fungal infections (associated/ not with bacterial infections) were compared to pts with isolated bacterial infections & those without infections. Variables associated with fungal infection were defined using multi-variable regression. An ordinal variable (0=none, 1=bacterial only, 2=fungal infection) was utilized in a model studying 30-day survival in all pts. Results: 2743 pts [1743 no-infection, 866 bacterial & 134 fungal infections (110 developed after an initial bacterial infection)] were included. Fungal infections were more often nosocomial, more often occurred in pts with diabetes, more advanced liver disease admitted with bacterial infections & on SBP prophylaxis. Fungal infections were associated with higher ACLF, longer length-of-stay (LOS), ICU admission and poor 30-day survival. On multi-variable analysis, age, AKI, MELD, NACSELD-ACLF, ICU admission & source of infection variable were negatively associated with survival (p<0.0001) with an AUC of 0.83 (0.80-0.86). Of the 134 fungal infections, only 12 were diagnosed on admission. After excluding these pts, multi-variable analysis of fungal infection development showed male sex to be protective

while diabetes, greater LOS, ICU admission, NACSELD-ACLF & admission bacterial infection were associated with a higher risk of fungal infection development (AUC = 0.81). Conclusions: Fungal infections impair 30-day survival in hospitalized patients with cirrhosis. Patients with diabetes, NACSELD-ACLF and those admitted with a bacterial infection are at the highest risk for fungal infection.

**p<0.01, ***p<0.001	No Infection (n = 1743)	Bacterial Infection (n = 866)	Fungal Infection (n = 134)
Age (years)	57.38 (10.61)	57.23 (11.18)	55.66 (11.80)
Gender (Male)	64%	59%	50%**
Diabetes	33%	34%	47%**
Admitted with an infection	-	78%	49%***
Episodes of AKI	35%	55%	73%***
MELD score on admission	18.68 (7.45)	20.65 (7.83)	23.25 (8.09)***
Length of hospital stay (days)	9.59 (10.89)	14.48 (14.70)	36.38 (106.58)***
ICU admission	18%	29%	60%***
NACSELD-ACLF	6%	14%	43%***
30-day survival	93%	86%	66%***

Disclosures:

Jasmohan S. Bajaj - Consulting: Abbott, Norgine, Valeant; Grant/Research Support: Salix, Grifols

K. Rajender Reddy - Advisory Committees or Review Panels: Merck, Janssen, BMS, Abbvie, Gilead; Grant/Research Support: Merck, BMS, Gilead, Janssen, Abbvie

Florence Wong - Consulting: Gore Inc, Sequana; Grant/Research Support: Mallinckrodt

Patrick S. Kamath - Advisory Committees or Review Panels: Sequana Medical, Gilead

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Jennifer C. Lai - Consulting: Third Rock Ventures

Paul J. Thuluvath - Advisory Committees or Review Panels: Abbvie, Gilead; Grant/Research Support: Gilead, AbbVie, BMS, Isai, Salix; Speaking and Teaching: AbbVie, Bayer/Onyx, Gilead

Jacqueline G. O'Leary - Advisory Committees or Review Panels: Gilead, Intercept, Grifols; Consulting: Abbvie; Speaking and Teaching: Gilead, Abbvie, Merck; Stock Shareholder: Medfusion

The following people have nothing to disclose: Puneeta Tandon, Scott W. Biggins, Ram Subramanian, Hugo E. Vargas, Leroy Thacker

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Early introduction of a combination of low dose terlipressin and noradrenaline as vasopressors is superior to high dose noradrenaline alone in patients of cirrhosis with septic shock(NCT02468063).

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Background and Aims: Sepsis in cirrhosis causes rapid deterioration in haemodynamics in an already vasodilated system, with significant down regulation of vasoconstrictive receptors. While timely institution of vasopressors could restore microcirculation, the choice and dosage of vasoconstrictor(s) is not yet clear. We compared early introduction of a combination of terlipressin and noradrenaline versus noradrenaline alone as vasopressors in patients of cirrhosis with septic shock. **Methods:** Cirrhotics with septic shock admitted from April 2015 to January 2017 were screenedand randomized in an open label manner to receive either a combination (Gr. A) of terlipressin (2 mg/24 hours fixed dose infusion) plus Noradrenaline (3.75 mcg/min to 30 mcg/min, n=91) or noradrenaline alone (Gr. B, 7.5 mcg/min to 60mcg/min, n=93) to achieve a target Mean Arterial Pressure (MAP) of >65mm Hg and were followed till death or 48 hrs. **Results:** 184 cirrhotics with septic shock were randomized to Gr A (N= 91) or B (N= 93), having comparable baseline demographic, clinical and laboratory parameters, MELD (28 vs 30, p=0.17) and SOFA scores (12 vs 13, p=0.33). Forty six (25%) patients had 3 organ failures at start of study. At 6 hours, target MAP was achieved more often in Gr A than B (97.8% vs. 86%, p=0.005). Patients in Gr A had lower incidence of fall in MAP once target MAP was achieved (1.5±3.3) than B(2.7±4.7)(p <0.006). The frequency (2 vs 3 vs >=4) of fall in MAP in Gr A (19 vs 5 vs 7) was lower than GrB (26 vs 7 vs 8) (p <0.006). Adverse effects occured more frequently in Gr. A (18%) than B (4%). Most common adverse effect in Gr A was peripheral cyanosis while arrhythmia in Gr B. Overall survival at day 30 was 50%, higher in Gr A than B (59% vs, 39%, p =0.01). On Multivariate analysis, combination treatment and presence of pneumonia significantly predicted mortality. **Conclusions**: Early institution of a combination of low dose terlipressin with noradrenaline achieves greater hemodynamic stability with fewer fluctuations in MAP and a reduced mortality than noradrenaline alone in cirrhotics with septic shock.

Disclosures:

The following people have nothing to disclose: Vikash Prakash, Ashok k. Choudhury, Shiv K. Sarin

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Early addition of prokinetics reverses gut paralysis and improves survival in critically ill cirrhotics- An open label placebo controlled RCT (Feed Intolerance and Treatment-FIT protocol). NCT02528760

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Background and aims- Gut-paralysis causing feed intolerance (FI) is common in critically ill cirrhotics (CIC), requiring discontinuation of enteral nutrition. FI is associated with longer ICU stay and higher mortality. Prokinetics, such as Metoclopramide (Met) and Erythromycin (Ery) are first line treatment for FI but their efficacy and safety in CIC is unknown. We evaluated the incidence, role of prokinetics and predictors of 28 day mortality in CIC who developed FI. **Patients and Methods-** Consecutive CIC patients, admitted in Liver ICU, who developed new onset FI without hypokalemia, tense ascites, mechanical cause, uncontrolled sepsis, high vasopressors or DIC were randomized to either IV Met (Gr. A, n=24), IV Ery (Gr. B, n=25) or Placebo (Gr. C, n=23). FI was defined with presence of 3 of 5 variables- absence of bowel sounds, vomiting, diarrhoea, bowel distension and gastric residual volume (GRV)≥500ml and a FI score (positive variables out of 5) was calculated. Primary end-point was resolution (atleast 3 of 5 variables resolved) of FI at 24 hours. **Results-** In this ongoing study, 186 of 956 CIC patients (19.5%, 95% CI:17.1-22.1) had FI and 72 patients fulfilling criteria were randomized to 3 groups. Baseline parameters and length of ICU stay prior to FI were comparable. Median FI score was 3 (Range- 3-5). Resolution of FI was significantly better at 24 hr (p=0.026) in Gr A and B compared to C with a trend to better resolution in Gr B than A (24% vs 8.7%,

p=0.16). Partial response (2 of 5 variables resolved) at 24 hr was better in Gr A, B than C (p<0.001) and in Gr B than A (p=0.018). Decrease in GRV beyond 24 hr was significantly more in Gr A and B than C and the time to restart enteral nutrition was shorter (Gr A, B, C= 2.61±0.72, 2.20±0.91, 3.47±1.29 days) with Gr B>A (p=0.03). The 7 day survival was better in Gr A, B compared to C (p=0.07) with significantly higher 28-day all cause mortality in Gr. C (p=0.007). On univariate analysis, resolution of FI at 72 hr (OR:3.45: 95% CI 1.19-9.99, p=0.02), presence and persistence of organ failures at 72 hours (OR:2.59; 95% CI 0.91-7.38, p=0.07) and new sepsis after development of FI (OR: 0.18; 95% CI 0.02-1.53, p=0.08) were predictors of 7 and 28-day survival, though on multivariate analysis only resolution of FI at 72 hours was significant (OR:3.45: 95% CI 1.19-9.99, p=0.02). **Conclusions-** Early detection and addition of prokinetics helps in resolution of FI in critically ill cirrhotics. Erythromycin is safe and superior to Metoclopramide for early resolution of gut paralysis in CIC. Gut paralysis manifesting as FI should be regarded as an independent organ failure requiring early treatment.

Disclosures:

The following people have nothing to disclose: V Rajan, Rakhi Maiwall, Ashok k. Choudhury, Kapil D. Jamwal, Jaya Benjamin, Guresh Kumar, Y.K. Joshi, Shiv K. Sarin

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Prevalence, spectrum, predictors and outcome of infections in patients with Acute on Chronic Liver Failure

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Background and Aims: In Acute on Chronic Liver Failure (ACLF), continued injury and persistent inflammation and subsequent immunoparalysis predisposes to a perpetuating cascade and high incidence of infections. Therefore, the spectrum and source of infection and sepsis is likely to be different in ACLF patients. We investigated the prevalence, microbiological spectrum, predictors and outcome of infections in a large cohort of ACLF patients from Asia. **Methods:** 2,825 ACLF patients recruited prospectively between 2012-2017 upto 90 days for onset of sepsis or death or liver transplant. Standard terminologies were used to define Pneumonia, Urinary Tract Infection(UTI), Spontaneous Bacterial Peritonitis(SBP), Spontaneous Bacterial Empyema(SBE), Spontaneous bacteremia(SB), Fungemia, skin and soft tissue infections(SSTI). **Results:** Overall, 845(29.9%) patients had sepsis, of which 183(21.7%, Gr.A) had infections only at presentation, 454(53.7%, Gr.B) nosocomial and 208(24.6%, Gr.C) had second-hit sepsis. UTI(35.9%), pneumonia(26.3%), SBP(19.2%) and SB(13.4%) were common infections with small contribution by SSTI(1.4%) and SBE(1.05%). 30.7% of pneumonia and 71.4% of SBP and SBE were culture negative. Common bacteria identified for UTI were E.Coli(44.9%) and enterococcus(25.1%); for pneumonia, Klebsiella(15.8%) and methicillin resistant staphylococcus(MRSA;5.7%); for SBP and SBE, E.coli (10.9%,21.4%) and MDR klebsiella (7.3%,14.2%) and for SB, E.coli(25.9%), Klebsiella(20.3%), MRSA(16.1%) and enterococcus(15.4%). Fungal pneumonia (aspergillus) was seen in 27.2%, candidia in UTI in 14.2%, fungemia in 2.5% and fungal SBP in 3% patients; 89.7% fungal infections had associated bacterial infections. Predictors of mortality included bacteremia[2.6 (1.81-3.74)], fungal infections [2.33(1.63-3.32)] and pneumonia [2.6(1.93-3.50)]. Survival was lowest in Group C <B< A (median survival A:B:C – 49:45:18 days, p=0.003). Alcoholic hepatitis (AH)[2.01(1.49-2.71), p<0.001] and DILI [1.86(1.26-2.74), p=0.002] were independent predictors of sepsis compared to other etiologies. Low albumin [0.58(0.49-0.68), p<0.001] and high MELD score [1.4(1.3-1.5), p<0.001] also significantly predicted sepsis in MV analysis. **Conclusions:** The risk of infection is significantly increased with low serum albumin, high MELD and with AH and DILI as acute insult in ACLF patients. Fungal infections alone or with bacterial infections and second hit sepsis result in poorer clinical outcome. Maintaining near normal serum albumin along with emperical antibiotics (for Klebsiella and E.coli) and low threshold for antifungals (in select cases) is recommended to prevent sepsis and improve survival in ACLF patients.

Disclosures:

Diana A. Payawal - Advisory Committees or Review Panels: Inogen Pharmaceutical, SV More Pharmaceutical; Consulting: United Laboratory; Speaking and Teaching: Gilead

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Treatment of patients with decompensated cirrhosis with IFN-free regimens is associated with high SVR and early improvement in liver function but increased frequency of decompensation and bacterial infections. Results of prospective cohort.

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Background: even though DAAs made treatment of patients with decompensated HCV-cirrhosis possible the effect of SVR on liver function complications of cirrhosis is not entirely established. Frequency of complications of cirrhosis and adverse effects of treatment, is also largely unknown, as well as their effect on treatment and patient's outcome. Objective: evaluate changes in liver function and portal hypertension (PH) parameters, frequency of decompensations of cirrhosis and bacterial infection during treatment and their possible effect on patients outcomes. Methods: prospective study with consecutive patients with decompensated cirrhosis (previous or current ascites, hepatic encephalopathy (HE), varicose GI bleeding and/or a Child-Pugh B/C) treated with IFN-free regimens. Patients with G1 were treated for 24weeks the remaining for 12W of treated with SOF/DCV combination (+RBV in 76%). Results: 103 patients (61±9 years, 37% male, G1 82%, MELD 12±4, CSPH 88%) were evaluated. Patients were on treatment for ascites and HE in 42 and 25% and were classified as Child A, B and C in 18%, 75% and 7% of cases. SVR at 3 months was achieved in 48 out 49 patients. Treatment was interrupted in 4 patients due to adverse event. Six patients died during treatment, (bacterial infections in 4) and 1 was transplanted. At EOT significant increases in bilirubin (1.6±0.8 vs 1.3±0.8) albumin (29±5 vs. 32±4) and platelet count (85±35 vs. 92±35) were observed (p<0.05 for all). Resolution of ascites and HE was observed in in 39% and 36% of patients and 41% of Child B/C patients were classified as child A, while 25% of Child A patients progressed to Child B. Decompensation was observed in 19 patients, most frequently ascites and/or increase in diuretic dosage (14%), kidney dysfunction and HE (12 and 5%). Serum AST(xULN) was the only predictive of decompensation and the best cut-off point was 3.8 (55%vs17% for lower values, p=0.007). Bacterial infections were observed in 20 patients (skin and urinary tract infections in 7 and 5 patients). MELD score (HR1.14, 95%CI 1.03-1.27, p=0.012) and CSPH (HR5.1, 95%CI 1.7-15.1, p=0.003) were independent predictors of infection. The frequency of decompensation was similar in patients with and without infections (30 vs 21%, p=0.43). Development of infection (80vs.96%, p=0.02), but not decompensation, was associated with lower survival. Conclusion: Treatment of hepatitis C virus in patients with decompensated cirrhosis is associated early improvement in liver function and PH related parameters. Nevertheless, development of decompensations of cirrhosis and bacterial infections are common and associated with lower survival.

Disclosures:

The following people have nothing to disclose: Livia B. Victor, Caroline B. Souza, Lívia Guimarães, Juliana Piedade, Joana Duarte, Zulane d. Veiga, Renata M. Perez, Flavia F. Fernandes, Cristiane Villela-Nogueira, Gustavo Pereira

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Degree of Hepatic and Systemic Hemodynamic Alterations Predicts Development of AKI and Mortality in Patients With Cirrhosis - A Prospective Cohort Study

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Background and Aim: There is limited data in cirrhotics on the correlation of altered portal, systemic and pulmonary hemodynamics and development, progression and severity of AKI and the impact of hemodynamic response to beta-blockers (BB). **Methods**: Consecutive cohort of cirrhotics (n=3493) without AKI, who underwent a hemodynamic assessment at baseline as part of clinical evaluation, were prospectively followed for the development/ resolution of AKI. **Results:** Cirrhotics aged 50±12 years, 79% male, mean hepatic venous pressure gradient (HVPG) 15.6±6.1mm Hg were followed for a median of 369 (61-790) days. A total of 1105 episodes of AKI occurred in 604 patients during follow-up of 321(40-726) days. Of these, 53% developed >1 episode of AKI, peak-AKI stage {(3:2:1- 55%:19%:26%)} and 67% had resolution after the first episode. Patients who developed AKI than those who did not, were significantly more vasodilated; higher median cardiac index [4.3(3.2-5.1)*vs* 3.3(2.9-3.9) L/min/m^2; P<0.001)], lower systemic vascular resistance index (SVRI) [869 (1324-2232)*vs*2094 (1570-2689) dynes/cm/m^2; P<0.001], pulmonary vascular resistance index [89 (53-141)*vs*94(58-147) dynes/cm/m^2; P=0.001], higher HVPG [17(13-21)*vs*15(11-19); P=0.001]. In addition, they had lower eGFR [61(38-96)*vs*87(67-114) ml/min/m^2; P<0.001], higher MELD [17.2±9.1*vs* 12.6±5.6; P<0.001] and CTP scores [9.5±1.7*vs*8±1.9; P<0.001]. Higher HVPG (P=0.03) and lower eGFR (P=0.013) were noted in patients who developed one versus >1episode of AKI. On multivariate analysis (HR,95%CI), higher HVPG (3.1,1.1-9.3), lower SVRI (0.23, 0.1-0.5) and eGFR (0.08,0.05-0.16) and higher CTP score (1.5,1.4-1.6) predicted AKI development and consequential increased mortality [HR 6.7, 95%CI3.2-9.1]. Of the 1344 patients who received BB (Carvedilol-79%, propranolol 21%, second HVPG in 781), 43.4% were responders (>20% reduction or ≤12 mm Hg) showing greater resolution (P<0.001) and lower degree of AKI (P<0.001). High HVPG (HR 4.2) and MELD score (HR 1.1) independently predicted mortality. A significant increase (P<0.05) was noted for both AKI development (HR) [1 *vs* 1.4 *vs* 1.8 *vs* 3.4] and mortality [1 *vs* 1.4 *vs* 1.9 *vs* 2.7] on stratification of patients based on baseline HVPG (<12 *vs* 12.1-16 *vs* 16.1-22 *vs* >22mm of Hg) respectively. **Conclusions:** High portal pressure, severe systemic and pulmonary vasodilatation, lower renal reserve and severity of liver disease determine the risk of AKI development and mortality in cirrhosis. BB therapy, especially in responders, reduces degree of AKI and helps in its resolution. Stratifying patients with high portal pressure for additional pharmacologic intervention could enable prevention of AKI.

Disclosures:

The following people have nothing to disclose: Rakhi Maiwall, Guresh Kumar, Ankit Bhardwaj, Priyanka Jain, Kapil D. Jamwal, Manoj Kumar, Barjesh C. Sharma, Shiv K. Sarin

★ Denotes AASLD Presidential Poster of Distinction

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Neutrophil-Gelatinase-Associated Lipocalin and Cystatin C Predict Acute Kidney Injury Progression and Transition to Chronic Kidney Disease in Critically Ill Cirrhotics

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Background and Aim: Bacterial infections and systemic inflammation(SIRS) are the major precipitants of organ failure including acute kidney injury (AKI) in critically ill(CIC) and predispose to increased progression to Chronic Kidney Disease(CKD). Timely identification of AKI is therefore an unmet need. Biomarkers can help in differential diagnosis and prediction of the trajectory of AKI course. We evaluated Cystatin C(CysC) and urinary neutrophil-gelatinase-associated lipocalin (NGAL) as biomarkers of glomerular and tubular injury respectively in predicting a progressive AKI and transition to CKD in CIC. **Methods:** CIC admitted to the Liver ICU were prospectively followed up. Baseline serum creatinine, history of prior AKI (past 3 months) and precipitant for AKI was recorded at admission. Progressive AKI was defined at day 7 as an increase of serum creatinine by 0.3 mg/dl from baseline or percentage increase of more than 50% or an increase in AKI stage or requirement of dialysis. **Results:** A total of 519 CIC, mean age 48.4±11.4 years, 86% males were followed for 26(8-97) days. The median serum NGAL and CysC was 759(223-1230) ng/ml and 2.1(1.5-2.7)mg/l respectively. SIRS was seen in 84%, sepsis in 58%, prior AKI in 18%. AKI at admission was present in 60% (Stage 1:2:3 43%vs 14%vs 43%) of which 48% recovered and 52% had progressive AKI. Sepsis was the commonest (46%) cause of AKI. At day 7, 317(61%) patients had progressive AKI of which 51% had new onset AKI. Of these, 56% had HRS, 10% prerenal AKI and remaining had acute tubular necrosis (ATN). The levels of NGAL and CysC were higher in patients in sepsis vs non-sepsis AKI (<0.001), correlated with the severity of AKI stage (p<0.001), response to terlipressin (p<0.001), and recovery of AKI (p<0.001) but could not differentiate HRS from ATN. On multivariate analysis (OR,95% CI), u-NGAL (2.53,1.6-4), Cyst C (1.9, 1.1-3.4) and prior AKI (3.15,1.36-7.3) significantly predicted progressive AKI which in-turn was associated with higher mortality (HR 2.32,95%CI 1.8-3.1). Further, of all patients with progressive AKI, 144(45%) had transition to chronic kidney disease. The levels of NGAL(OR 1.9,1.3-2.9) and Cyst C(OR 1.9,1.3-2.5) could also predict the transition of AKI to CKD in CIC. **Conclusion:** Almost two-thirds of CIC have AKI at presentation, which in majority is a result of sepsis, is progressive and is associated with worse outcome. u-NGAL and CysC can accurately predict progression of AKI, transition to CKD and can help in stratifying patients for early therapeutic intervention. Prior AKI itself predisposes to AKI progression in CIC and should be carefully assessed in readmissions.

Disclosures:

The following people have nothing to disclose: Rakhi Maiwall, Priyanka Jain, Naveen Yadav, Lalita G. Mitra, Sherin Thomas, Kapil D. Jamwal, Manoj Kumar, Barjesh C. Sharma, Shiv K. Sarin

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Gene therapy with endothelial-specific liposomal short interfering siRNA targeting kinase insert domain receptor inhibits portosystemic collateralization in portal hypertension

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Background: Development of portosystemic collateral vessels and gastroesophageal varices is responsible for the most serious clinical consequences of portal hypertension and chronic liver disease. Although much has been learned about the pathophysiology of portosystemic collateral growth, this knowledge has not been accompanied by parallel advances in therapies. Here we investigated the therapeutic potential of a novel and advanced short-interfering RNA (siRNA) technology for downregulating the pathological overexpression of the kinase insert domain receptor (KDR), also known as VEGF receptor-2. KDR is an endothelial cell surface receptor that is abundantly expressed in portal hypertension but not in normal tissues, and has a central role in portosystemic collateralization, pathological angiogenesis, and disease progression and aggravation, making it an ideal therapeutic target. **Methods:** We developed liposomally-formulated siRNAs with clinically suitable delivery materials that were optimized for precise and specific therapeutic targeting of KDR in vascular endothelial cells. We first validated these siRNA^{KDR}-lipoplexes, and control siRNA^{Luc}-lipoplexes, in vitro in human umbilical vein endothelial HUVEC cells and murine endothelioma H5V cells. Then, we tested their therapeutic potential on portosystemic collateralization and pathological angiogenesis in vivo in mouse models of portal hypertension (portal vein-ligation). **Results:** siRNA^{KDR}-lipoplexes efficiently transported the siRNA^{KDR} cargo to vascular endothelial cells in mesenteric microvenules and portal vein of portal hypertensive mice, where collaterogenesis and angiogenesis take place. This systemic treatment significantly downregulated pathological KDR overexpression, without causing complete KDR knockout, preserving homeostatic baseline KDR levels and thus limiting adverse effects. siRNA^{KDR}-lipoplex-induced endothelial-specific KDR knockdown drastically reduced portosystemic collateralization by 73%, and impaired the pathologic angiogenic potential of endothelial cells at different levels (cell proliferation, sprouting and remodeling). **Conclusions:** Targeting endothelial KDR with therapeutic siRNA^{KDR}-lipoplexes could be a promising and plausible treatment modality for attenuating the formation of portosystemic collaterals in a clinical setting. Of interest, a related formulation that we have also developed is currently being evaluated in clinical trials for treatment of patients with advanced solid tumors, further supporting the translational relevance and therapeutic potential of this approach for clinical portal hypertension and chronic liver disease.

Disclosures:

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Rho-kinase inhibitor coupled with peptide-modified albumin carrier reduces fibrogenesis and portal pressure in cirrhotic rats without systemic effects

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Background & Aim. Rho-kinase (ROCK) activation in hepatic stellate cells (HSC) is a key mechanism for liver fibrosis and portal hypertension. Untargeted ROCK-inhibitors decrease portal pressure but have major systemic effects, while HSC-specific ROCK inhibitors targeted to mannose-6-phosphate-receptors blunted portal pressure and fibrogenesis with a drop in cardiac contractility as a minor systemic effect. To improve targeting, this study coupled the ROCK inhibitor Y-27632 (Y-27) to human serum albumin (HSA) carrier modified with platelet-derived growth factor receptor beta (PDGFRβ) recognizing peptides (pPB) and investigated dose-dependent effect of Y27-pPBHSA on fibrogenesis and portal hypertension in cirrhotic rats. ***Methods.*** MALDI-TOF mass spectrometry and HPLC characterized the compound. *In vitro* collagen contraction assays tested biological activity on LX2 cells. Fibrogenesis, systemic and portal hemodynamics were analyzed in ascitic BDL and CCl_4-intoxicated rats using i.v. administration of Y27-pPBHSA (0.5 and 1 mg/kg b.w) after 3, 6 and 24h. Phosphorylation of the myosin light chain (MLC) and cytoskeletal linker protein moesin assessed Rho-kinase inhibition in liver, femoral muscle, mesenteric artery and heart. ***Results.*** Three Y-27 molecules were coupled to pPBHSA as confirmed by HPLC/MS, which was sufficient to relax LX2 cells *in vitro* (contraction index 100±0.0% control; 43.5±5.3% Y27-unconjugated; 60.7±7.4% Y27-pPBHSA). *In vivo,* BDL- and CCl_4-induced cirrhotic rats treated with Y27-pPBHSA had lower portal pressure (29-33% reduction) and hepatic vascular resistance (57-60% reduction) without any systemic or cardiac changes. In addition, hepatic MLC and moesin phosphorylation were significantly reduced in Y27-pPBHSA-treated cirrhotic rats. No ROCK inhibition was observed in femoral muscle, mesenteric artery and heart. Likewise, hepatic decreases in alpha smooth muscle actin (αSMA) IHC and *acta2* mRNA expression suggested less HSC activation. Decreased c*ol1a1*, *rock2, pdgfr* mRNA expression in the liver after 24h of inhibition of Rho-kinase, suggested Y27-pPBHSA affects fibrogenesis. ***Conclusions.*** Targeting the ROCK inhibitor to PDGFRβ decreases fibrogenesis, portal pressure, hepatic vascular resistance, and HSC activation without any systemic and cardiac off-target effects.

Disclosures:

Klaas Poelstra - Consulting: BiOrion Technologies BV; Grant/Research Support: BiOrion Technologies BV; Stock Shareholder: BiOrion Technologies BV

The following people have nothing to disclose: Fernando Magdaleno, Sabine Klein, Franziska Frohn, Catharina Reker-Smit, Frank E. Uschner, Robert Schierwagen, Leonie Beljaars, Jonel Trebicka

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Inflammation provokes endothelial nitric oxide synthase trafficking inducer expression and decreases hepatic nitric oxide biosynthesis and associated endothelial dysfunction in cirrhosis

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Background: A previous study showed in cirrhotic patients that increased hepatic endothelial nitric oxide synthase (eNOS) trafficking inducer (NOSTRIN) was associated with the severity of portal hypertension (PHT), however, the precise underlying molecular mechanism is not well understood. Our aims were to investigate whether superimposed inflammation increases NOSTRIN overexpression and decreases hepatic nitric oxide (NO) synthesis and associated endothelial dysfunction (ED) in two different models of cirrhosis and also to study the effect of Candesartan Cilexetil (CC, an angiotensin II type 1 receptor blocker) on the regulation of hepatic eNOS-NOSTRIN pathway in cirrhosis. **Methods:** Four weeks after Bile duct ligation (BDL) or a sham operation, BDL rats were randomized 3 h prior to sacrifice, to receive either lipopolysaccharide (LPS; 1 mg/kg, i.p.) or saline. In addition, CD-1 mice received carbon tetrachloride injections (CCl_4 15% v/v corn oil, 0.5 ml/kg, twice weekly i.p.) for 13 weeks to induce cirrhosis. After three months, mice were randomized to two weeks oral administration of CC (8mg/kg) or DMSO; with or without LPS. At the time of sacrifice, plasma (biochemical, cytokines and angiotensin II measurements) and liver tissues (histopathology and sirius red stains) were collected for various analysis. **Results:** When compared to control, BDL and CCl_4 animals showed markedly elevated ($p<0.0001$) hepatic gene and protein expressions of NOSTRIN whilst hepatic eNOS activity was significantly ($p<0.05$) decreased. LPS challenge to cirrhotic rodents showed further significant ($p<0.05$) increase of NOSTRIN whereas eNOS activity was reduced further. Portal pressure changes were not observed between BDL and BDL+ LPS groups (BDL, 14 ± 1.2 vs. sham, 5.8 ± 0.9 mmHg; $p<0.0001$). Confocal microscopy findings showed NOSTRIN and eNOS were expressed within the hepatic vascular endothelial cells. CC treatment to CCl_4 and that received LPS showed significantly ($p<0.05$) reduced NOSTRIN and significantly ($p<0.01$) increased hepatic NO synthesis. eNOS, iNOS and caveolin-1 protein expressions were significantly increased in CCl_4 mice compared to control. CC treatment lowered eNOS and iNOS protein expressions towards sham values but caveolin-1 protein expression was unaltered. **Conclusion:** Our study is the first indication of evidence for an association between increased NOSTRIN and decreased hepatic eNOS, thus severe ED and associated PHT in cirrhosis with superimposed inflammation. CC treatment attenuated NOSTRIN overexpression and improved hepatic NO synthesis, thereby attenuating ED and associated PHT in experimental cirrhosis.

Disclosures:

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The mechanism of splenic fibrosis in portal hypertension: The role of miR133 and connective tissue growth factor (CTGF)

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Background: Portal hypertension is accompanied with splenomegaly and spleen stiffness, which is likely related to splenic fibrosis. The molecular and cellular mechanism of splenomegaly, spleen stiffness and splenic fibrosis is poorly understood. Connective tissue growth factor (CTGF) is a potent pro-fibrotic factor. The aim of this study was to elucidate the mechanism of splenic fibrosis in portal hypertension. **Methods:** Spleens and splenic fibroblastic reticular cells (FRCs, stromal cells in the spleen and a major contributor to splenic fibrosis) were collected from rats given sham or partial portal vein ligation (PPVL) surgery for 10 days to assess splenic fibrosis. Gene chip analyses were performed for spleens and exosomes released from splenic lymphocytes of these rats. Human spleen specimens from normal, non-cirrhotic and cirrhotic patients with portal hypertension were assessed for fibrosis. A human monoclonal antibody to CTGF (FG-3019, 100 mg/kg, FibroGen, Inc., San Francisco, CA) was injected intraperitoneally every other day for 10 days to PPVL rats to evaluate CTGF as a potential therapeutic target for splenic fibrosis in portal hypertension. **Results:** Splenic fibrosis was significantly increased in rats with PPVL for 10 days (2.5-fold, $p<0.01$), accompanied with splenomegaly (30% increase in weight, $p<0.0001$). Gene chip analyses identified a significant reduction of miR133 in exosomes from lymphocytes and a significant increase in its target gene CTGF in the spleens of PPVL rats. Splenic FRCs from PPVL rats showed a significant increase in CTGF mRNA (4.5-fold, $p<0.0001$), α-SMA protein (2-fold, $p<0.05$), collagen type1α mRNA (4-fold, $p<0.01$) and Ki67 level (2.5-fold, $p<0.001$). Increased levels of fibrosis and CTGF were also observed in the spleens of portal hypertensive patients. *In vitro*, low levels of CTGF in splenic FRCs isolated from normal rats were significantly increased by TGFβ treatment (10 ng/ml, 24 hrs). miR133 mimic suppressed TGFβ-induced CTGF levels in FRCs. miR133 was negative in FRCs, but abundant in splenic lymphocytes. Treating PPVL rats with a neutralizing antibody to CTGF (FG-3019) significantly reduced splenomegaly (20%, $p=0.004$) and splenic fibrosis indicated by Sirius red staining (36%, $p<0.001$) and hydroxyproline (18%, $p=0.02$), compared to PPVL rats treated with mIgG (control). **Conclusion:** Increased CTGF levels in splenic FRCs likely caused by decreased miR133 released from lymphocytes via exosomes contribute to splenic fibrosis and splenomegaly in portal hypertension. CTGF can be a potential therapeutic target for these pathological conditions of the spleen in portal hypertension.

Disclosures:

Hisashi Hidaka - Consulting: Nihon Pharmaceutical Co., Ltd.

Kenneth E. Lipson - Employment: FibroGen, Inc.; Stock Shareholder: FibroGen, Inc.

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Application of human NASH-based transcriptome and metabolome profiles in preclinical models for the translational study of drug effects on liver fibrosis

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Background: NASH patients are characterized by complex metabolic disturbances in liver resulting in chronic inflammation that drives disease progression towards fibrosis. Recent human studies have comprehensively profiled the metabolic and inflammatory perturbances in patient livers and plasma and have identified molecular patterns that characterize NASH patients and can differentiate between mild and severe pathology. Concerns have been raised whether preclinical models sufficiently mimic these molecular disease profiles, bringing into question their translational value in studies of therapeutic interventions in the process of NASH/fibrosis. Here, we applied molecular patterns derived from human NASH in an experimental disease model, to improve the translational value of pre-clinical efficacy studies. **Methods:** Genome-wide liver transcriptome (Tx) and multi-platform plasma metabolomic (Mx) profiles representing healthy and NASH subjects were compared to corresponding profiles from high-fat-diet (HFD) treated LDLr-/-.Leiden mice, a preclinical model with translational characteristics. Mice were profiled at the stage of mild (24 weeks HFD) and severe (34 weeks HFD) fibrosis, as well as after OCA intervention to attenuate or resolve fibrosis (treatment from 24 to 34 weeks of HFD; 10 mg/kg). Effects of OCA were analyzed histologically, biochemically, by IHC, deuterated water technology (for de novo collagen formation) and the human-based Tx/Mx profiles. **Results:** After 24 weeks of HFD treatment the Tx/Mx profile of LDLr-/-.Leiden mice largely reflected the molecular signature of NASH patients, and this representation of human molecular patterns was even more pronounced at 34 weeks. OCA counter-regulated the expression of these molecular human NASH profiles, quenched specific inflammatory-profibrotic pathways and reverted the human-based molecular profile towards the healthy situation. Consistent with Tx and Mx profiling, OCA attenuated specific facets of cellular inflammation, affected bile acid and energy metabolism, reduced de novo collagen formation and attenuated further progression of liver fibrosis, but did not reduce fibrosis below the level at start of the intervention. **Conclusion:** Ldlr-/-.Leiden mice recapitulate specific molecular Tx/Mx signatures of NASH patients. Intervention with OCA in developing fibrosis counter-regulates the effects of HFD and normalizes Tx/Mx profiles. OCA reduces collagen deposition and de novo synthesis but does not resolve already manifest fibrosis in the period studied (10 weeks). These data show that human

molecular signatures may be used to increase the translational value of preclinical models for NASH.

Disclosures:

Marta Iruarrizaga-Lejarreta - Employment: One Way Liver S.L.

José M. Mato - Advisory Committees or Review Panels: MIDATECH, MITOTHERAPEUTICS; Consulting: ABBOTT, GALMED; Stock Shareholder: OWL Metabolomics

Cristina Alonso - Employment: OWL Metabolomics

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Down-regulation of hepatic MBOAT7 by hyperinsulinemia favors steatosis development

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Background and aims: We have recently shown that the rs641738 C>T variant in Membrane bound O-acyltransferase domain-containing 7 gene (MBOAT7), involved in phosphatidylinositol acyl-chain remodeling, increases the risk of nonalcoholic fatty liver disease (NAFLD), inflammation and fibrosis due to lower protein expression. Aim was to evaluate the regulation of hepatic MBOAT7 and the impact on hepatic fat accumulation. ***Methods:*** We examined hepatic MBOAT7 in 119 obese patients and in experimental models. We silenced hepatic MBOAT7 by i.v. administration of antisense oligonucleotides modified by morpholinos (MPO) for 4 consecutive days in C57Bl/6 male mice (n=5). ***Results:*** In obese patients, hepatic mRNA levels of MBOAT7 progressively decreased from normal liver to simple steatosis and NASH ($p<0.05$). At multivariate analysis, type 2 diabetes ($p<0.05$), necroinflammation ($p<0.01$) and MBOAT7 genotype ($p<0.01$) were independently associated with MBOAT7 downregulation (Table1). The mRNA and protein levels of MBOAT7 were reduced in experimental models of NAFLD, such as the methionine-choline deficient diet, but more so in genetically obese *ob/ob* mice and in insulin resistant mice with Insulin receptor haplo-insufficiency, characterized by hyperinsulinemia ($p<0.05$). Furthermore, in wild-type male mice MBOAT7 was downregulated by refeeding concomitantly with the rise of insulin levels and activation of hepatic insulin signaling through PI3K and AKT. Insulin downregulated MBOAT7 in primary mouse hepatocytes in a PI3K-depedent manner. Finally, MPO induced a 45% silencing of hepatic MBOAT7 comparable to that associated with the genetic risk variant, resulting in a 80% increase in hepatic triglyceride content ($p<0.05$ *vs* scramble) and with the development of microvesicular steatosis. ***Conclusion:*** These data suggest that hyperinsulinemia causes downregulation of hepatic MBOAT7, which both genetic and experimental data indicate is causally implicated in steatosis development. Further studies are needed to investigate the mechanisms linking reduced phosphatidyl-inositol desaturation by MBOAT7 with the development of steatosis and hepatic inflammation.

Variables associated with hepatic MBOAT7 gene expression in 119 severely obese patients

	Estimate SE	P value
Age, years	-0.00±0.01	0.98
Sex, F	-0.07±0.09	0.43
BMI, Kg/m2	+0.00±0.01	0.87
T2DM, yes	-0.16±0.08	0.04*
Steatosis	-0.02±0.07	0.84
Necroinflammation	-0.31±0.11	0.005*
PNPLA3, I148M alleles	-0.04±0.09	0.66
MBOAT7, T alleles	-0.25±0.08	0.004*

Disclosures:

The following people have nothing to disclose: Marica Meroni, Paola Dongiovanni, Raffaela Rametta, Sara Badiali, Silvia Fargion, Stefano Romeo, Stefano Gatti, Luca Valenti

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Responding to metabolic demand: Key role for Thioesterase superfamily member 2 in the hepatic partitioning of fatty acids between oxidative and synthetic pathways

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Background: Thioesterase superfamily member 2 (Them2) is a mitochondria-associated long-chain fatty acyl-CoA thioesterase that is abundant in the liver and oxidative tissues. Evidence from whole-body knockout mice and primary cultured hepatocytes suggests that Them2 controls the partitioning of fatty acids into β-oxidation versus glycerolipid synthetic pathways, depending upon nutritional status. During prolonged (16 h) fasting, Them2 promotes hepatic fatty acid oxidation, which in turn sustains gluconeogenesis. However, because it also suppresses thermogenesis in brown adipose tissue by limiting fatty acid oxidation, the true contribution to fatty acid metabolism of Them2 in the liver remains unclear. Aim: This study was designed to evaluate the contribution of Them2 in liver to fatty acid metabolism during a physiologically-relevant period of moderate (6 h) fasting. Methods: Them2 liver-specific knockout (*L-Them2*$^{-/-}$) mice were created by breeding *Them2* floxed mice with albumin-Cre transgenic mice. Floxed littermates were used as controls. Body composition was determined by magnetic resonance spectroscopy. Fatty acid and triglyceride concentrations were determined in plasma and liver by enzymatic assays. Rates of fatty acid oxidation and glycerolipid synthesis were measured by radiolabel-based assays using liver homogenates and membrane fractions, respectively. Hepatic VLDL-triglyceride secretion rates were determined following IV injection of tyloxapol. Immunoblot analysis was performed using standard techniques. Results: *L-Them2*$^{-/-}$ mice exhibited similar body weights and compositions as controls. In the absence of changes in plasma or hepatic concentrations of free fatty acids, a 30% increase in rates of fatty acid oxidation was accompanied by enhanced activation of AMP-activated protein kinase (AMPK). Whereas steady state concentrations of hepatic triglycerides and glycerolipid synthetic rates were unchanged in *L-Them2*$^{-/-}$ mice, plasma triglyceride concentrations were reduced by 38% and apoB100 concentrations by 41%. This was accompanied by a marked (70%) reduction in hepatic VLDL-triglyceride secretion rates. Conclusion: Under conditions of moderate fasting, hepatic

Them2 functions to direct fatty acids towards triglyceride incorporation into VLDL particles for secretion and away from mitochondrial β-oxidation, thereby sustaining the export of lipids from the liver to the plasma and peripheral tissues. Collectively, these findings demonstrate that Them2 in the liver plays a key role in determining the metabolic fates of fatty acids in response to nutritional status and metabolic demands.

Disclosures:

David E. Cohen - Advisory Committees or Review Panels: Gemphire, Amarin, Ardelyx, Aegerion, Esperion, Merck, Immuron; Consulting: Intercept, Amgen, Janssen

The following people have nothing to disclose: Michele Alves-Bezerra, Yingxia Li

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The role of X-box binding protein 1 in the hepatic response to fasting and refeeding in mice

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Background: The inositol requiring enzyme 1α (IRE1α) branch of the unfolded protein response has been shown to regulate the metabolic adaptive response to fasting. It has been speculated that this function of IRE1α is mediated by the major IRE1α target, X-box binding protein 1 (XBP1). In the present study we examine the role of hepatic *Xbp1* in mediating the hepatic metabolic adaptive program in mice. **Methods:** Mice bearing a hepatocyte-specific deletion of *Xbp1* (*Xbp1*^{LKO}) and littermate floxed controls (*Xbp1*$^{fl/fl}$) were subjected to overnight fasting (18hrs) followed by refeeding for 6 hours. **Results:** The hepatic expression of activated *Xbp1* was suppressed in the fasted state followed by induction upon refeeding in *Xbp1*$^{fl/fl}$ control mice. As expected, *Xbp1*$^{fl/fl}$ controls showed suppression of hepatic lipogenesis genes in the fasted state followed by induction upon refeeding. Despite the known function of *Xbp1* in the regulation of hepatic *de novo* lipogenesis, deletion of hepatic *Xbp1* allowed for normal postprandial induction of hepatic lipogenesis genes including sterol regulatory element binding protein 1c, acyl-CoA carboxylase, and fatty acid synthase. Fasted *Xbp1*^{LKO} mice also demonstrated normal induction of PPARα targets controlling fatty acid β-oxidation and an equivalent increase in plasma β-hydroxybutyrate relative to fasted *Xbp1*$^{fl/fl}$ controls. *Xbp1*^{LKO} mice showed normal induction fibroblast growth factor 21, a critical mediator of the metabolic response to fasting, followed by normal suppression upon refeeding. *Xbp1*^{LKO} mice demonstrated normal physiologic hepatic steatosis in response to fasting. The postprandial state is characterized by an increased hepatic protein load and, accordingly, we found that refeeding *Xbp1*$^{fl/fl}$ control mice resulted in marked induction of hepatic UPR genes involved in protein folding and processing including *Slc33a1*, *Edem*, *ERdj5*, *Sec61α*, and *ERo1α*. Deletion of hepatic *Xbp1* prevented postprandial induction of hepatic genes involved in protein homeostasis. On the contrary, refeeding *Xbp1*^{LKO} mice resulted in preferential activation of proapoptotic elements of the unfolded protein response including *Chop*, *Bim* and *Trb3.* **Conclusions:** Although hepatic *Xbp1* has clearly been shown to regulate hepatic lipid metabolism in response to lipogenic diets, our data strongly suggest that hepatic *Xbp1* does not regulate the hepatic lipogenic program in response to fasting or refeeding in mice. Instead, we find that hepatic *Xbp1* is required for postprandial induction of genes controlling protein homeostasis. We speculate that activation of *Xbp1* in the postprandial state serves to accommodate the increased protein load caused by refeeding.

Disclosures:

The following people have nothing to disclose: Shantel Olivares, Anne Henkel

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Autophagic changes of both adipose tissues and the liver induced by high fat diet feeding contribute to hepatic lipid accumulation

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Background: NAFLD (Non-alcoholic fatty liver disease) is known to be associated with autophagy but its mechanism is not investigated in detail. Especially, the role of adipose tissue autophagy in NAFLD has not been studied. We investigated the changes of autophagy in hepatocytes and adipocytes in high fat diet (HFD) fed mice and their effect on the liver. Method: C57BL/6J mice, hepatocyte-specific Rubicon knockout (KO) mice (Alb-Cre Rubicon fl/fl), or adipocyte-specific Atg7 KO mice (Adiponectin-Cre Atg7 fl/fl mice) were fed either HFD containing 32% fat (HFD32®) or control diet. Results: We previously reported that HFD feeding suppresses autophagy in wild type mice livers via the increase of Rubicon, which suppresses autophagy at the autophagosome-lysosome fusion step, and that hepatocyte-specific Rubicon KO mice improved their liver steatosis and injury (Tanaka, et al. Hepatology 2016). In the present study, we found that hepatocyte-specific Rubicon KO significantly increased the volume of visceral fat pads in HFD-fed mice, while it reduced the liver volume, suggesting that autophagy of the liver affect adipose tissue. In adipose tissues of wild type mice, HFD feeding increased the protein expression levels of LC3-II but decreased those of p62 without change in p62 mRNA levels. To investigate autophagic flux in vivo, HFD-fed mice were injected with 20 mg/kg leupeptin, a lysosome inhibitor, and sacrificed after 2 hours. LC3-II step up by leupeptin injection was clearly observed in adipose tissues, while that was not observed in livers. HFD feeding did not affect the levels of expression of Atg5, Atg7, Beclin-1, and UVRAG but (significantly) suppressed Rubicon expression in adipose tissues. These data suggested that HFD feeding promotes autophagy with decrease of Rubicon in adipose tissues. To investigate the effect of adipocyte autophagy on hepatic lipid accumulation, we generated adipocyte-specific Atg7 KO mice. Atg7 KO mice showed significant increase of inguinal fat pads, an indicator of subcutaneous fat, compared to wild type littermates after 2 months HFD feeding. HFD-fed Atg7 KO mice showed significantly lower levels of serum non-esterified fatty acids and ameliorated liver steatosis compared with than HFD-fed wild type littermates. These data suggested that adipocyte autophagy regulates the release of fatty acids from adipose tissues and affects hepatic lipid accumulation. Conclusion: HFD feeding suppressed autophagy with increase of Rubicon in the liver and promoted autophagy

with decrease of Rubicon in adipose tissues. These autophagic changes contribute to hepatic lipid accumulation.

Disclosures:

Hayato Hikita - Grant/Research Support: Bristol-Myers Squibb; Speaking and Teaching: Bristol-Myers Squibb, EA Pharma Co., Ltd

Ryotaro Sakamori - Grant/Research Support: MSD; Speaking and Teaching: Gilead, SYSMEX

Tetsuo Takehara - Grant/Research Support: Janssen pharmaceutical Co., AbbVie, Gilead Sciences, Chugai Pharmceutical Co., MSD K.K., Bristol-Meyer Squibb, Mitsubishi Tanabe Pharma Corparation, Toray Industories Inc.; Speaking and Teaching: Bristol-Meyer Squibb, Gilead Sciences, AbbVie, MSD K.K.

The following people have nothing to disclose: Sadatsugu Sakane, Satoshi Tanaka, Kumiko Shirai, Yuta Myojin, Yuto Shiode, Yasutoshi Nozaki, Yoshinobu Saito, Tasuku Nakabori, Takahiro Kodama, Tomohide Tatsumi

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Level of a Prototypical Splicing Factor is Severely Diminished in Hepatocytes of Non-alcoholic Steatohepatitis Patients

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Background: Non-alcoholic steatohepatitis (NASH) is emerging as one of the most common liver diseases in the western world. However, the mechanisms underlying the progression and pathology of this disease are still not completely understood. A recent study by Chen, et al (PLosOne 2013), examining the single nucleotide polymorphisms (SNPs) in patients with NASH revealed a significant association for pathways involved in mRNA splicing, an essential step in gene expression. Specifically, it is a post-transcriptional event in which introns within pre-mRNA are removed and exons are ligated together, resulting in a mature mRNA. Additionally, certain exons can also be differentially included in the mature transcript and is referred to as alternative splicing. This essential step is regulated by a class of proteins known as splicing factors. **Methods:** We performed a screen measuring the levels of various RNA splicing factors in fatty liver mice models. This screen revealed a specific decrease in levels for a highly conserved pre-mRNA splicing factor known as SRSF1. To further corroborate this finding in humans, we measured the levels of SRSF1 using a semi-quantitative immunofluorescence (IF) based assay using biopsy sections. We began with a preliminary retrospective study using biopsy tissue from patients with advanced stages of fatty liver disease. Levels of SRSF1 were measured in the following groups of patients; Stage 1 NASH (5 patients), Stage 4 NASH (4), ASH (4) and healthy controls (4). In addition to the human studies, we also created a hepatocyte-specific SRSF1 knockout mice model (SRSF1 HKO) using Cre-loxP technology to study the function of SRSF1 in maintaining normal liver homeostasis. **Results:** The IF-based assay on patient samples revealed that SRSF1 levels are significantly diminished (> 4-fold decrease, $p < 0.001$) in hepatocytes in all forms of advanced fatty liver disease with respect to healthy controls. This finding was further supported by phenotypic analysis of the SRSF1 HKO mice model which revealed spontaneous and progressive liver injury including steatosis, inflammation, and fibrosis. **Conclusion:** In this study, we have identified a previously unknown role for SRSF1 in protecting the liver from fatty liver disease. We have also shown, to our knowledge, one of the first genetic mice model which results in spontaneous NASH-like pathology further strengthening the importance of SRSF1. We conclude that SRSF1 is essential for maintaining transcriptome integrity in hepatocytes and impairment of its activity promotes NASH pathology.

Disclosures:

The following people have nothing to disclose: Raiya Sarwar, Waqar Arif, Auinash Kalsotra, Andrew Batey, Ike Uzoaru

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Mayo Spheroid Reservoir Bio-Artificial Liver Improves Survival and Promotes Liver Regeneration in Post Hepatectomy ALF Pigs

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Background: Acute Liver Failure (ALF) is a catastrophic condition that can occur after major liver resection. The aim of our study was to determine the survival benefit of a novel spheroid reservoir bio-artificial liver (SRBAL) in the setting of post-hepatectomy ALF and assess its impact on liver regeneration. **Methods:** Wild-type large white female pigs (20-30kg) underwent placement of an ambulatory intracranial pressure (ICP) probe followed by 85% hepatectomy one week later. Volumetric CT scans were performed immediately pre- and post-resection to measure the extent of hepatectomy, and again 48 hours post-hepatectomy to assess regeneration of the remnant liver. Animals were randomized into three groups based on treatment delivered 24 to 48 hour post-hepatectomy: Group1 - Standard therapy (ST, n=6); Group2 - ST plus BAL treatment using no hepatocytes (0gBAL, n=6); Group3 – ST plus BAL treatment using 200 grams of primary porcine hepatocyte spheroids (200gSRBAL, n=5). Primary end point was survival 90 hours post-hepatectomy. Death equivalent was defined as unresponsive grade IV hepatic encephalopathy or ICP >20 mmHg with clinical evidence of brain herniation. **Results:** All animals in both (ST, 0gBAL) control groups met death equivalent prior to 50 hours post-hepatectomy. In contrast, 4 out of 5 animals in 200gSRBAL group survived to 90 hours ($p<0.03$). Mean peak levels of ammonia and peak intracranial pressure were significantly lower ($p<0.01$) in animals treated with 200g SRBAL (Table 1). Volume index (mean ± SD), defined as the ratio of liver volume at 48-hour to post-hepatectomy, was greatest in the 200gSRBAL group (2.2±0.3) compared to ST group (1.8±0.1, $p<0.01$) and 0gBAL group (1.9±0.2, $p<0.01$). **Conclusions:** SRBAL improved survival and accelerated liver regeneration after major hepatectomy. Improved survival was associated with a neuroprotective benefit of SRBAL therapy. These favorable results warrant further clinical testing of SRBAL.

Table1. Peak ammonia and ICP between groups (mean ± SD, ** $p<0.01$ vs SRBAL)

Group	Mean Peak NH3 (μM)	Mean Peak ICP (mmHg)
ST (n=6)	407±66 **	26.2±6.5 **
0gBAL (n=6)	590±231 **	21.3±1.0 **
200gSRBAL (n=5)	252±68	14.0±6.3

Disclosures:

Scott L. Nyberg - Board Membership: Liver Cell Therapies, Liver Cell Technologies

The following people have nothing to disclose: Harvey S. Chen, DongJin Joo, Mohammed F. Shaheen

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Chemical and Toxicology Analysis of Ayurvedic and Herbal Drugs Causing Severe Liver Injury

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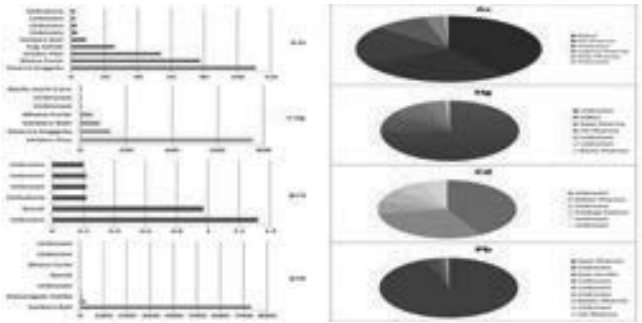
Introduction: Ayurvedic and herbal drugs (AHD) are known to cause varying degrees of liver injury. We present chemical analysis on 45 samples of AHD (33solids, 14 liquids) retrieved from 33 patients with AHD-liver injury. **Methods:**94 patients diagnosed with drug induced liver injury - 33 AHD related. Heavy metal concentration (mg/kg) determined by Inductively Coupled Plasma Atomic Emission Spectrometer (Thermo Electron, Germany). Analyses for hepatotoxic volatile organic compounds (hVOC) performed using Gas Chromatography-Mass Spectrometry (Agilant Technologies, USA). **Results:** Males (63.6%, n=21/33), mean age 45.9±15.1yr;AHD related liver injury probable in 57.5% (n=19), possible in 42.4%(n=14). 72.7%(n=24) survived-median follow up 102(6-255) d. 33.3%(n=11) consumed AHP prescribed by unregistered traditional healers- associated with mortality [p=0.031; OR 7.6(1.4-41.6)]. Arsenic(As), cadmium(Cd), mercury(Hg), lead(Pb), antimony(Sb) found in 19(57.6%), 12(36.4%), 21(63.6%), 24(72.7%)&3(9%) of 33 solid samples-range 110.6mg/kg(min-max, 0.4-111); 1.29(0.30-1.32); 751.3(0.2-751.5);7317.2(0.25-7318.1);6.9(0.46-37.4). Heavy metals in liquids(n=14)- As(1,7.1%); Cd(4,28.5%); Hg(35.7%), Pb(35.7%):mean 0.37mg/L; range 0.1mg/L(0.01-0.11); 3(0.1-3.1); 0.54(0.02-0.56) resp. **Highest As content** in *Swarna Guggullu* (111 mg/kg;Dabur), *Bitana Forte*(77.6mg/kg;HV-Pharma),*Hriden-Plus*(54mg/kg;unknown);**Hg–***Hriden Plus*(751.5mg/kg, unknown), *Swarna Guggullu*(128.4;Dabur),*Sankara Bati*(83.7;Vyas-Pharma); **Pb-***Sanakara Bati*(7318.1mg/kg, Vyas-Pharma), *Hriden Plus*(229.8;unknown); *Kaisoragulu-Vatika*(95.9; Raja-Health) [Figure]. ***Arsenic was significantly associated with mortality*** (p=0.12). **hVOC-analysis:pentane, cyclopentane, cyclobutane, dimethylamine** in 70.6%(n=12/17),58.8%,35.3%,23.5%&**heptane, ethylamine, cyclobutylamine, phenol and propanol** in 6% resp. **Conclusion:** Approved&traditional healer AHD's contain high heavy metals and hVOCs. Arsenic content was associated with higher mortality and consumption of AHP from unregulated, unregistered traditional healers significantly associated with mortality. Stringent policies are warranted from authoritative bodies concerned with AHP regulation.



Disclosures:

The following people have nothing to disclose: Cyriac A. Philips, Adarsh Joy, K L. Antony, Rosmin Francis, Rajeev Tharath

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Evaluation of spheroid reservoir bioartificial liver with porcine hepatocytes in rhesus monkey model of acute liver failure

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Background & aims Bioartificial liver devices are under development for supportive therapy of liver failure. The Spheroid Reservoir Bioartificial Liver (SRBAL) is a novel extracorporeal liver assist device composed of functional porcine hepatocyte spheroids. This study aims to evaluate the effectiveness and safety of the system in a preclinical primate model of acute liver failure (ALF). **Methods** Thirty healthy rhesus monkeys were infused with hepatotoxin α-amanitin and lipopolysaccharide (LPS) and randomly distributed into five groups (ALF control group, n=6; No cell SRBAL (sham SRBAL treatment) group, n=6; group A, namely starting SRBAL treatment at 12 h after ALF induction, n=6; group B, starting SRBAL treatment at 24 h after ALF induction, n=6; group C, starting SRBAL treatment at 36 h after ALF induction, n=6). Animals were continuously treated with SRBAL device for 6 h and followed for the biochemical, immunological, histological and inflammatory changes up to 336 h. **Results** The SRBAL improved survival rate and prolonged median survival time (MST) in a rhesus monkey model of drug induced ALF. Blood ammonia and total bilirubin were lower, and albumin levels were higher in all hepatocyte SRBAL treatment groups. There was no evidence of porcine endogenous retrovirus infection in treated animals. IgM levels rose initially after initiation of SRBAL therapy but returned to normal by the end of SRBAL treatment; IgG levels showed no response to cell therapy. The livers of SRBAL treated animals showed less liver injury and accelerated regeneration compared to controls. Levels of pro-inflammatory cytokines (TNF-α, IL-6, IL-12, IL-1β, IL-8, IFN-γ and IL-2) were ameliorated after SRBAL treatment. Other cytokines associated with liver regeneration (M-CSF; HGF, EGF and VEGF; IL-1RA, MIF showed a transient rise in associated with SRBAL treatment. **Conclusions** Improved survival of ALF monkeys during SRBAL therapy was multifactorial and included a reduction in the hepatotoxic effect, inhibition of the pro-inflammatory reaction, and creation of a microenvironment more suitable for regeneration of the injured liver. The porcine SRBAL did not elicit a xenogeneic immune response in ALF monkeys.

Disclosures:

Scott L. Nyberg - Board Membership: Liver Cell Therapies, Liver Cell Technologies

The following people have nothing to disclose: Yi Li, Bruce Amiot

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Development of a new inhibitory antibody for prevention of HCV infection

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Background: Direct-acting antivirals (DAAs) have been introduced as new Hepatitis C virus (HCV) therapeutic agents, but DAA-resistant HCV variants have emerged. Also, prevention of HCV infection has yet to be established. Host factors involved in HCV entry are potential targets for prevention or therapy. Based on this background, we focused on occludin, a tight-junction protein that is essential for establishment of HCV infection, and designed a new anti-human occludin monoclonal antibody. Here, we describe an in vitro study in which we evaluated this antibody for prevention of HCV infection and examined its mechanism of inhibition of infection. **Methods**: Mouse anti-human occludin monoclonal antibody was fabricated with amino acid sequence 214-230 as the antigen derived from the ECL2 domain of occludin responsible for human-specific HCV sensitivity. Binding of the antibody to the antigenic peptide was screened by ELISA. Inhibition of HCV infection by the anti-occludin antibody was evaluated in a monolayer culture system, double chamber culture system, and Matrigel-embedded 3D culture system of a well-differentiated human hepatic carcinoma-derived cell line, Huh7.5.1. HCVpv (VSV covered with HCV envelope proteins) and HCVcc (a laboratory HCV) were used for HCV infection. Cell damage due to the antibody was examined using a XTT cell growth assay. The structures and mechanism of action of the antibody were clarified by isotype and epitope analyses. Localization of endogenous occludin after administration of the anti-occludin antibody was determined by immunofluorescence staining. **Results**: All 15 subclones of the anti-occludin antibody had antigen-binding capacity, but there was no HCV infection inhibition in the monolayer culture. However, the anti-occludin antibody significantly inhibited HCV infection in the double chamber culture and Matrigel-embedded 3D culture, which better reproduce the liver cell environment *in vivo* and the HCV infection route. The antibody had no cytotoxicity. The isotype was IgG1/λ and the epitope may be ALCN (214-217), which contains two human-specific amino acids (A214, L215). The antibody also changed the localization of occludin from the tight junction region to inside the cytoplasm in the presence or absence of HCV. These findings suggest that the antibody endocytosed occludin, and thus prevented binding with HCV or other host factors, which may then prevent HCV infection. **Conclusion**: An anti-human occludin antibody was shown to have HCV infection inhibitory effects *in vitro*. The presumed mechanism of action of the antibody indicates that it could act independently of the genetic background of the virus.

Disclosures:

The following people have nothing to disclose: Ken Okai, Hiromasa Ohira

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Human stem cell-derived Encapsulated Mature Liver Tissue as an effective, consistent, and durable *in vitro* tool for drug testing and development

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Aim: to develop an innovative stem cell-based product performing mature liver functions *in vitro* for drug development and testing. **Methods:** We used human pluripotent stem cells (iPSCs) to generate posterior foregut cells and endothelial and mesenchymal progenitor cells. The 3 cell types were seeded at a fixed ratio and cultured for 5/10 days in suspension in custom-made, 500μm-diameter microcavities, in a defined medium. At day 10 the obtained organoids were characterized for liver-specific markers and functions, and then encapsulated in a PEG-based hydrogel. The resulting Encapsulated Mature Liver Tissue (EMLT), containing 10 or 100 organoids, was characterized and compared to human hepatocytes. **Results:** Over the first 72h from seeding the co-cultured 3 iPSC-derived cell types undergo condensation to form organoids measuring 194.3±41.5 mm in diameter. At day 10 they show a complex 3D structure consisting in a core composed of endothelial and mesenchymal cells and an outer layer of hepatocyte-like cells. Maturation of the 3 cell types was confirmed at single-cell mRNA-Seq (Drop-Seq). Liver cells express markers typical of human fetal hepatocytes. Nevertheless, such liver organoids start secreting albumin and urea at day 4 post-seeding, and at day 10 show a synthetic function comparable to thawed primary human hepatocytes (19.6±4.6 mg albumin/24h/10^6 liver cells and 30.9±4 mg urea/24h/10^6 liver cells). The activity of cytochrome P450 3A4 was detected in our liver organoids at day 10 (as compared to day 25 of standard iPSC-derived hepatocyte-like cells). Organoids were encapsulated within 5% photopolymerized PEG-vinyl sulfone (20/100 organoids/gel). The resulting EMLT showed significantly higher Cyp3A4 activity and urea production capabilities than non-encapsulated organoids after only 10 days. Albumin synthesis increased over the first days within the hydrogel, and reached levels comparable to freshly-isolated primary human hepatocytes (1.2±0.2 mg/24h/10^6 liver cells). Such a function was stable for at least 28 days. The EMLT expresses phase I, II and III drug-metabolizing enzyme, metabolizes ammonia and tacrolimus, can be generated in a 96-well format and is resistant to cryopreservation. **Conclusions:** To our knowledge, these are the first complex liver organoids generated using 3 cell types all derived from a single iPSC population. The EMLT is the first stem cell-derived product to show *in vitro* functions comparable to primary human hepatocytes, with the added advantages of unlimited supply, consistent and durable efficacy, and resistance to cryopreservation. All this features make the EMLT a promising product for *in vitro* drug testing and development.

Disclosures:

Massimiliano Paganelli - Grant/Research Support: Gilead Sciences

The following people have nothing to disclose: Claudia Raggi, Marie-Agnès M'Callum, Chenicka-Lyn Mangahas, Zackary Cohen, Ariella Shikanov