Distinguishing the metabolic signal of liver tumors from surrounding liver cells using hyperpolarized $^{13}$C MRI and gadoxetate


Hyperpolarized $^{13}$C MRI is a valuable technique for evaluating liver metabolism in vivo, but the signal is not selective for different cell types. We propose to separate the metabolic signals arising from tumor cells and normal hepatocytes by using gadoxetate, a targeted liver-specific gadolinium-based contrast agent that is selectively transported into hepatocytes but not tumors. Gadoxetate within hepatocytes supresses the hyperpolarized MR signals from these cells, leaving the signals from tumor cells unaffected. To test this, a WAG-Rij rat bearing CC531 rat-derived colon cancer in the liver were imaged using a 3 Tesla clinical MRI scanner and HyperSense DNP polarizer. We analyzed the conversion of [1-$^{13}$C]pyruvate into [1-$^{13}$C]lactate and performed an initial analysis by considering the total lactate as a fraction of total pyruvate before and after gadoxetate administration. The lactate/pyruvate ratio in normal liver changed from 0.7 to 0.4 (43% decrease), while the lactate to pyruvate ratio in the tumor changed from 0.78 to 0.57 (27% decrease). The 43% signal suppression in the unaffected liver was similar to that in prior studies. Persistent signal suppression in tumor was likely due to the small tumor size and “partial volume” effects, with a significant amount of “normal” liver contained in the slice. Future studies will test these effects in larger tumors. We successfully demonstrated the ability of gadoxetate to distinguish metabolic signals arising from tumors and hepatocytes. We expect these results to be rapidly translatable to future human studies in patients with liver tumors.
ABSTRACT #2

Hyperglycemia enhances pro-inflammatory properties of macrophage-derived exosomes to drive hematopoiesis in apolipoprotein E-deficient mouse

Bouchareychas L, Chung A, Wong D, Duong P, Raffai RL

Background and Purpose: Diabetes is strongly associated with a spectrum of liver diseases including non-alcoholic fatty liver disease (NAFLD). Recent studies have shown that hyperglycemia is associated with enhanced hematopoiesis, increased hepatic monocytes/macrophages numbers and an inflammatory macrophage phenotype. We explored whether hyperglycemia can enhance intercellular communication between mature macrophages and hematopoietic progenitors via exosomes to promote liver inflammation.

Methods: Bone marrow derived macrophages (BMDM) from C57BL/6 mice were cultured with normal (5mM) or high glucose concentrations (25mM). Exosomes were isolated by our cushioned-density gradient ultracentrifugation method and characterized by nanoparticle tracking and western blot analysis. Pro-inflammatory properties of high glucose exosomes (HGexo) were tested in vitro by exposing BMDM cultured in normal glucose. The capacity for BMDM-derived exosomes to alter systemic and hepatic inflammation were next tested by infusing 25-30 week-old ApoE−/− mice with 3 x 10^{10} exosomes three times a week, for four weeks.

Results: Our data demonstrated that HGexo can stimulate the expression of inflammatory cytokines (IL-6, IL-1β) as well as NADPH oxidases (Nox-1 and Nox-4) in cultured BMDM. Our in vivo experiments showed that intraperitoneally injected exosomes distribute primarily to the liver and the spleen. Furthermore, HGexo promote the expansion of multipotent and lineage committed hematopoietic progenitors. Lastly, HGexo elevated levels of triglycerides and esterified cholesterol in the liver of ApoE−/− mice.

Conclusions: We identified that exosomes derived from cultured BMDM exposed to high glucose have the ability to exert intercellular communication both in vitro, and in vivo. Our findings suggest that exosomes produced by macrophages exposed to hyperglycemia could be an unsuspected source of inflammation and in turn, accelerate liver injury.
New SLK allocation policy may impact transplant outcomes in women

Cullaro G, Hirose R, Lai JC

Background: We aimed to understand the impact of an SLK policy change on liver transplant (LT) outcomes in Women (W) and Men (M).

Methods: Included were non-Status 1 adults listed for LT from 5/07-7/14. Excluded were those with exceptions. We defined patients (pts) who met the new SLK policy as having an eGFR<60ml/min (by CKD-EPI) for 90d, with a final eGFR<30 ml/min. Cox regression associated LTAv.SLK with post-LT survival in women.

Results: Of 40,979 LT candidates, 4,330 were listed for SLK; 37% were W. 3,123 pts would have met new SLK criteria: 46% would have been W. Of the 3,123 pts who would have met new SLK criteria, 47% W v. 62% M were actually listed for SLK (p<0.01).

Of 1449 W who would have met new SLK criteria, 248(17%) W received SLK and 302 W(21%) received LTA. 1% of the 302 W who received LTA vs. 12% of the 248 who received SLK died. In these 550 W who would have met new SLK criteria, W who had LTA experienced better adjusted survival than W who had SLK (HR 0.06, p<0.01), even when accounting for Final MELD (HR 1.01, p=0.67) and Final Albumin (HR 0.48, p=0.007).

Conclusion: Under the new SLK policy, W will likely represent a substantially larger % of pts listed for SLK than before. Our data suggest that W meeting the new SLK criteria will have a lower adjusted survival if they receive a SLK vs. LTA, highlighting the need for monitoring of SLK outcomes, after implementation of the new policy.
ABSTRACT #4

Ceramide inhibits YAP/TAZ to promote HSC inactivation and reduce fibrosis

Chen JY, Ghoshal S, Christenson S, Wei L, Pondick J, Fuchs B, Mullen A, Sheppard D

Background: Hepatic stellate cells (HSCs) are the primary cell type responsible for hepatic fibrosis, the final common pathway leading to cirrhosis and liver failure for nearly every cause of chronic liver disease. Activation of HSCs in response to injury represents the key step in hepatic fibrogenesis. Through a small molecule screen, we identified that accumulation of the sphingolipid ceramide can profoundly inhibit the activated effector phenotype of HSCs. It remains unknown how ceramide inhibits HSC activation and whether ceramide reduces fibrosis in vivo.

Methods: We performed RNA sequencing and Ingenuity Pathway Analysis on human HSCs treated with vehicle or ceramide-C6. Immunofluorescence and immunoblotting were also performed to investigate the effect of ceramide on YAP/TAZ, the key effectors of the Hippo signaling pathway. In our mouse model of fibrosis, mice were treated with carbon tetrachloride (CCl₄) and B13, an acid ceramidase inhibitor, or CCl₄ and vehicle for 4 weeks. Fibrosis was assessed by quantifying hydroxyproline and collagen proportional area, immunohistochemical staining, mRNA expression of profibrotic genes including type 1 collagen, and histology.

Results: RNA seq analysis revealed that ceramide significantly regulates the Hippo signaling pathway, and qPCR confirmed that ceramide suppresses YAP/TAZ target genes in HSCs. Ceramide inhibits nuclear localization of YAP/TAZ by promoting their degradation. Furthermore, in a CCl₄ mouse model, increasing ceramide levels by treatment with B13, an aCDase inhibitor, promoted YAP degradation and reduced fibrosis.

Conclusion: Ceramide inactivates HSCs and reduces fibrosis development in vivo by inhibiting YAP/TAZ.
ABSTRACT #5

Tricyclic antidepressant use and the risk of fibrosis progression in hepatitis C-infected persons: results from ERCHIVES

Chen JY, Ren Y, Yan P, Belina ME, Chung RT, Butt AA

Background: Recent preclinical studies have suggested an antifibrotic role for tricyclic antidepressants (TCAs). Using the Electronically Retrieved Cohort of HCV Infected Veterans, we aimed to evaluate the impact of TCA use on fibrosis progression and development of hepatocellular carcinoma (HCC) among HCV-infected persons.

Methods: Subjects were categorized according to use of TCAs, selective serotonin reuptake inhibitors (SSRIs), or no antidepressants. TCA or SSRI use was defined according to cumulative defined daily dose, and categories were mutually exclusive. Subjects with HIV coinfection, HBsAg positivity, cirrhosis, or HCC at baseline were excluded. Outcomes were liver fibrosis progression measured by APRI scores and incident HCC (iHCC). We utilized Cox proportional hazards regression to determine predictors of cirrhosis, defined as APRI > 2, and iHCC.

Results: Among 128,201 eligible HCV+ persons, 4% received TCAs, 43% received SSRIs, and 53% received no antidepressants. Fewer TCA users had drug abuse (34% and 43%) and alcohol abuse (32% vs 42%) compared to SSRI users. After adjusting for age, baseline APRI score, diabetes, hypertension, alcohol use, drug abuse, and HCV RNA levels, TCA use was associated with decreased risk of cirrhosis (hazard ratio [HR] = 0.77, 95% CI = 0.60, 0.99) and delayed time to development of cirrhosis, but not with decreased iHCC.

Conclusion: Among a large cohort of HCV-positive Veterans, TCA use was associated with decreased fibrosis progression and lower risk of developing cirrhosis. These data provide supportive evidence for the beneficial effects of TCAs on progression of liver fibrosis in patients with chronic HCV infection.
Hepatocyte stem cells as cell replacement therapy for liver disease

Karnam G, Patkar R, Wang B

Cellular therapy in the form of hepatocyte transplantation is a potential alternative to liver transplant. However, its usage is limited by the shortage of healthy human hepatocytes. A longstanding scientific bottleneck in the field has been the inability to expand hepatocytes in vitro. We recently identified a replication-competent subpopulation of hepatocytes in vivo, important for homeostatic maintenance of the adult liver. In normal mouse liver tissue, these Axin2+ hepatocytes self-renew and continually generate fully differentiated hepatocytes that populate the liver lobule to maintain hepatocyte homeostasis. Axin2+ hepatocytes respond to and depend on a local Wnt signaling source from endothelial cells. We have found that a similar cell population exists in the normal human liver. These cells can be identified by antibody staining for the cell surface marker GLT1, which we have shown in mouse to enrich for Axin2+ hepatocytes. Significantly, using the data from the in vivo characterization of Axin2+ hepatocytes and their niche, we have been able to rapidly expand human and mouse hepatocytes in vitro, with retention of essential hepatocyte stem cell properties, including expression of the progenitor marker TBX3. In cell culture, these cells can be induced to differentiate into mature hepatocytes. Transplantation of in vitro expanded mouse hepatocytes show that they generate mature hepatocyte in vivo and can rescue a mouse model of liver failure. Our data suggest that Axin2+ hepatocytes may have significant potential as cell transplantation therapy for human liver disease.
The Y-encoded TSPY enhances the expression of cancer-related genes in liver cancer cells

Kido T, Lau YC

Liver cancer is an extraordinarily heterogeneous disease. Hence, it is crucial to identify the cancer subgroups and their biological features for the improvement of clinical outcomes. We previously reported that TSPY, a Y-located gene that is predominantly expressed in the mitotically proliferating male germ cells, is also expressed in approximately 30% of male hepatocellular carcinoma (HCC) cases, associating with the global DNA hypomethylation. Although various studies demonstrated that TSPY enhances proliferation of cancer cell lines, its downstream molecules and the characteristics of the TSPY-positive HCC still remain to be elucidated. In the present study, we investigated the TSPY-driven differential gene expression in a hepatocellular carcinoma cell line Huh7 by RNAseq transcriptome analysis. The results showed that TSPY enhanced the expression changes of various cancer-related genes in Huh7 cells, such as upregulation of CCND1 and FOXM1, and downregulation of CDKN1A/p21. Noteworthy, a combination analysis with the HCC gene expression data of The Cancer Genome Atlas (TCGA) revealed that the expression level of TSPY positively correlated with that of ADGRD1, a member of the adhesion G protein-coupled receptor family, in both Huh7 cells and the HCC samples of TCGA. Analyses of the TCGA clinical data demonstrated that the HCC patients in the high ADGRD1 expression group had significantly poorer prognosis than those in the low expression group. Similarly, the TSPY-positive HCC group had poorer prognosis than the TSPY-negative HCC group. Our results suggest that TSPY could enhance the expression of cancer-related genes in a subgroup of HCC, and lead to worse prognosis.
Defining signatures of impaired tolerance in autoimmune hepatitis

Klepper A, Whitaker E, Feng S, Tana MM

Introduction: The pathogenesis of autoimmune hepatitis (AIH) remains unknown, extrapolated from general theories of autoimmune disease. Our group is working on and RNA-Seq study comparing AIH patients to controls. We sought to perform a systematic review of the literature to identify primary studies assessing AIH pathogenesis in order to define signatures of impaired tolerance in AIH.

Methods: Search terms were defined for PubMed and Embase targeting three categories, (1) Disease (i.e. autoimmune hepatitis), (2) Technique used (e.g. PCR, Flow Cytometry), (3) Study result type (e.g. cytokine profile, microarray). Inclusion criteria: Type 1/2/3 AIH, humans, all ages (both pediatric and adult), peripheral blood or liver tissue source. Exclusion criteria: non-human data, non-English studies, tissues other than liver or peripheral blood, studies on PBC or PSC, or other hepatitis without AIH data, post-transplant AIH.

Results: Results of the search, prior to the application of inclusion and exclusion criteria, yielded over 2800 results. First pass assessment of results prior to study selection are notable for 68/100 papers describing cell types or chemokines or cytokines, with 10/100 addressing cell signaling on environmental triggers in AIH. Less than 10/100 studies addressed mRNA or other transcription profiling. Further analysis is pending.

Discussion: Preliminary conclusions highlight that most AIH studies look at cell types, cytokines or chemokines. An unbiased, molecular approach would be novel and may help elucidate AIH pathogenesis. This data can be applied as important background for further unbiased molecular studies.
ABSTRACT #9

Influence of liver-specific ablation of gp78 E3-ligase on hepatic cytochrome P450-dependent drug metabolism: clinical implications

Kwon D, Kim S, Correia MA

The autocrine motility factor receptor (AMFR)/gp78 is a membrane-anchored E3 ligase involved in ubiquitin-dependent proteasomal degradation (UPD). We have previously documented that gp78 is involved in the UPD of two clinically relevant hepatic cytochromes P450 CYP2E1 and CYP3A4 engaged in therapeutic drug and/or xenobiotic metabolism. To determine the role of gp78 in the UPD of hepatic P450s, we generated a liver specific gp78 knockout (KO) mouse model. Cultured primary hepatocytes from KO and wild type (WT) mice were treated with P450 inducer β-naphthoflavone (Cyp1a2), phenobarbital (Cyps2a, 2b and 2c), isoniazid (Cyp2e1) or dexamethasone (Cyp3a). Immunoblotting and selective functional marker analyses revealed that the functional content of Cyps1a2, 2a, 2c, 2e1 and 3a was increased, but that of Cyp2b or Cyp2d was decreased/unchanged in KO relative to WT. To determine the potential clinical relevance of these findings, the in culture metabolism of tamoxifen, a breast cancer chemotherapeutic prodrug, to its active metabolite endoxifen was monitored by LC/MS. Upon 8 h of tamoxifen incubation, the levels of Cyp3a-generated metabolites N-desmethyltamoxifen and endoxifen were significantly increased in KO over WT hepatocytes, but the Cyp2d-generated metabolite 4-hydroxytamoxifen was unchanged, consistent with the corresponding functional levels of hepatic Cyps3a and 2d. Collectively, hepatic gp78 deficiency significantly increased several drug-metabolizing P450s most likely by impairing their UPD. These increases in turn enhanced the hepatic metabolic activation of a clinically relevant anti-cancer drug. Our findings suggest that gp78 can be considered a significant modulator of P450-mediated metabolism of clinically relevant/chemotherapeutic drugs and associated drug-drug interactions.
Race/ethnicity is an independent risk factor for autoimmune hepatitis


Although autoimmune hepatitis (AIH) is more common in women and affects people of all races/ethnicities, there is currently limited information regarding the relationship between race/ethnicity and AIH, especially in the context of underserved populations. We aim to evaluate the relationship between race/ethnicity and AIH and better characterize its clinical features among different racial groups. We conducted a 15-year retrospective analysis, from January 2002 to June 2017, of patients seen at Zuckerberg San Francisco General Hospital (ZSFG). Sixty-three AIH patients and 2049 non-AIH controls were eligible for the study. The main predictor of interest was race/ethnicity, and the main outcome of interest was AIH diagnosis; other secondary measures recorded include clinical features such as ALT, bilirubin, and biopsy fibrosis at presentation. In a multivariable model adjusting for age and sex, we found that black (OR 9.6, 95% CI 1.8 to 178), Latino (OR 25.0, 95% CI 5.3 to 448), and Asian/Pacific Islander (API) (OR 10.8, 95% CI 2.2 to 196) race/ethnicity were associated with increased odds of an AIH diagnosis compared to the white reference group. Among people of color with AIH, there were no significant differences in baseline ALT (p = .45), total bilirubin at presentation (p = .06), fibrosis at presentation (p = .74), and hospitalization (p = .27).

Race/ethnicity is an independent risk factor for AIH. The clinical features of AIH did not differ significantly among black, Latino, and API patients.
Induced pluripotent stem cells (iPSCs) provide an important tool for the generation of hepatocyte-like cells for the characterization of liver diseases and metabolic pathways. iPSCs are typically differentiated to hepatocyte-like cells via developmental cues through an endoderm intermediate. However, most iPSCs fail to differentiate into endoderm, with induction resulting in apoptosis. Thus, studies of hepatocyte-like cells have been limited to the iPSCs that differentiate efficiently. We developed an improved protocol that dramatically enhances the iPSC to endoderm induction efficiency and allows culturing in 96-well plates. Our method incorporates Activin A as a primary inducer of Nodal pathways towards definitive endoderm, as well as compound ZD005. We tested this new protocol in 32 iPSC lines from 16 donors and found consistent formation of endoderm in 29 lines (90%) within 3 to 7 days. Endoderm generated by our method achieves near identical transcriptomic profiles, immunohistochemical staining for FOXA2, HNF1β, CXCR4, and SOX17, and ability to be further differentiated into hepatocyte-like cells. GO term analysis of genes differentially expressed in endoderm derived from iPSCs treated with or without ZD005 found that addition of ZD005 decreased expression of genes involved in intrinsic apoptosis while increasing gene expression of regulation of cellular metabolic processes, suggesting inhibition of apoptosis and altered metabolism. Thus, here we report generation of a simple, efficient and scalable protocol for the creation hepatocyte-like cells from nearly any iPSCs, which will enable future studies to characterize hepatocyte diseases in vitro and generate hepatocyte-like cells for transplantation studies.
The epigenomic signature of Wilson Disease


Introduction: Wilson Disease (WD) is an autosomal recessive disease caused by hepatic and brain copper accumulation. WD is due to mutations affecting the ATP7B copper transporter with consequent lack of adequate biliary copper excretion. The phenotypic presentation is characterized by hepatic, neurological, and psychiatric symptoms. There is evidence from animal studies that both genetic and epigenetic factors may contribute to WD clinical presentation and progression.

Aim: The goal of the present study was to characterize the epigenomic signature of WD in patients with hepatic or neurological manifestations, compared to healthy subjects and subjects with other liver diseases.

Methods: We conducted whole-genome bisulfite sequencing on DNA samples from whole blood (n=20 with prevalent hepatic manifestations; n=20 with prevalent neurologic manifestations) and liver (n=10) from patients with WD compared to healthy subjects, PSC, and NASH patients to identify WD-specific differentially-methylated regions (DMRs).

Results: In the liver, 1840 DMRs were identified specifically differentiating WD from both healthy control and subjects with other conditions. These DMRs were enriched for liver-specific transcriptional regulatory elements including enhancers and promoters. Associated genes were enriched for functions in inflammatory response, as well as lipid and folic acid metabolism. In blood samples, 262 DMRs were identified distinguishing WD from both healthy subjects and other diseases. DMR regions in blood could distinguish hepatic from neurologic phenotype and genes near hypomethylated regions were enriched for functions in mental deterioration.

Conclusions: We have characterized a mechanism-relevant epigenomic signature of WD providing new insights into potential biomarkers and treatments for this genetic disease.
Single cell transcriptomic analysis of fetal liver macrophages

Patkar R, Wang B, Nijagal A

In addition to their essential role in the initiation and resolution of infection, inflammation, and injury, macrophages also contribute to the development of tissues. We performed a single cell transcriptomic analysis to characterize the maturation and function of macrophage populations during fetal liver development. Single cell suspensions from C57BL/6 mouse livers were created at six time points: E12.5, E13.5, E15.5, E17.5, P0, and P49, and analyzed on a microfluidic droplet-based platform. The expression of genes vital for critical macrophage functions were analyzed in the 2,830 cells expressing F4/80 (Adgre1), a marker for macrophages. The results confirmed previously known findings such as the downregulation of the Kupffer cell chemokine receptor Cx3cr1, and the upregulation of the Kupffer cell specification marker Id3 in adult cells. Our analysis also suggests that fetal macrophages may be impaired in apoptotic cell clearance based on expression patterns of Axl, Timd4, and Mertk, and that self-renewal properties in macrophages are established over time, based on expression pattern of Mafb. Patterns of Igf1 and Tgfb1 expression indicate that key differences may exist between fetal and adult macrophage function during wound healing. The ongoing analysis of unbiased dimensional reduction by tSNE will provide insight into the heterogeneity of fetal macrophage populations and how their functions change during liver development.
Human and insect-produced AAV vectors differentially transduce liver tissue in vitro and in vivo


Background: Despite encouraging outcomes in pre-clinical and early clinical trials, AAV vector efficacies in several recent liver gene therapy trials were discordant. Mechanisms underlying this remain unknown. Transitions to new manufacturing platforms using baculovirus-infected insect cells appeared to correlate with low-efficacy trials. We hypothesized that the two leading recombinant AAV manufacturing platforms, baculovirus-infected insect and transiently-transfected human cell systems, result in different capsid post-translational modifications (PTMs), affecting function.

Methods: We utilized multiple analytical approaches including deep proteomic profiling with high-resolution and high-mass-accuracy mass spectrometry (nLC-ESI-MS/MS), two-dimensional isoelectric focusing, transmission EM, cryo-EM, and comparative functional liver transduction assessments in vitro and in vivo.

Results: Our data show that rAAV capsids are post-translationally modified, and differentially so in human and baculo-insect production schemes. Additionally, host cell protein (HCP) contaminant levels and composition were different between methods. Together, rAAV and HCP vector lot modifications included O-linked glycosylation, acetylation, phosphorylation, methylation and disulphide bonds. Capsid PTMs were seen on every serotype of AAV tested to date (AAVs 1, 2, 4, 5, 6, 8, 9), including those serotypes (AAVs 1 and 5) which were used in recent discordant human trials. Highly controlled vector lots produced using a custom dual-use transfer vector plasmid--employable in both human and insect systems--compared head-to-head for functional liver transduction in vivo, saw significant differences in expression from insect and human-produced vectors in 72 mice. Significant sexually dimorphic functional transduction differences were also seen where male mice experienced greater liver expression than females.

Conclusions: Our results demonstrate that AAV vector lots are differentially post-translationally modified when produced by human and insect manufacturing platforms. These findings were reproducible across numerous rAAV vendors including commercial producers, leading academic core facilities, and individual lab preparations. Collectively, differences in capsid PTMs and HCP contaminants may have profound implications for capsid folding, hepatocyte receptor binding, intracellular trafficking, expression kinetics, functional activity, stability, half-life regulation, immunogenicity, and more. Our findings may inform future directions and priorities for resource investments in GMP manufacturing facilities, quality control requirements, and may explain numerous discordant findings between research groups.
Increasing split liver transplantation in the U.S. could decrease pediatric deaths on the liver transplant waiting list

Perito ER, Roll G, Dodge JL, Rhee S, Roberts JP

Background: In the U.S., 1 in 10 infants and 1 in 20 older children die on the liver transplant waiting list. Increasing split liver transplantation could increase organ availability for these children, without decreasing transplants in adults.

Methods: Using UNOS STAR data, we identified livers transplanted 2010-2015 that could potentially have been used for split transplant, based on strict criteria. Livers not suitable for pediatric patients or allocated to high-risk recipients were excluded. Number and distribution of potentially “split-able” livers were compared to pediatric waitlist deaths in each region.

Results: Of 37,333 deceased donor livers transplanted, 6.3% met our strict criteria for utilization in split liver transplant. Only 3.8% of this select group were actually utilized for split liver transplantation. 96% were utilized for a single adult recipient. Among the 2,253 transplanted as whole livers, 82% of their recipients were listed as willing to accept a segmental liver, and only 3% as requiring a cold ischemia time <6 hours. Over the same 5 years, 299 children died on the waitlist. In every UNOS region, there were more potentially “split-able” livers than pediatric waitlist deaths. 37% of pediatric waitlist deaths occurred at transplant centers that averaged ≤1 pediatric split liver transplant annually during the study period.

Conclusion: This comparison suggests that we might be missing opportunities to reduce pediatric waitlist mortality without decreasing access for adults—using split liver transplant. Barriers are significant, but further work on strategies to increase split liver transplant is warranted.
ABSTRACT #16

Loss of Axin1 induced hepatocarcinogenesis requires intact β-catenin in mice

Qiao Y, Tao J, Che L, Calvisi DF, Monga SP, Chen X

Inactivation mutation of Axin1 is one of the frequent genetic events in human hepatocellular carcinoma (HCC). Axin1 is known to negatively regulate Wnt/β-catenin cascade in cells. However, in human HCC, whether inactivation mutation of Axin1 activates Wnt/β-catenin signaling remains controversial. We found that deletion of Axin1 in human HCC cell lines induced very low levels of canonical β-catenin pathway activation. We generate a murine HCC model via CRISPR/Cas9 based gene deletion of Axin1 (sgAxin1) in combination of transposon based expression of c-Met in the mouse liver (sgAxin1/c-Met). sgAxin1/c-Met HCC does not show the evidence of β-catenin activation. Gene expression analysis also supports that HCCs induced by activated β-catenin and c-Met (β-catenin/c-Met) and sgAxin1/c-Met have distinct gene expression profiles. To study whether endogenous β-catenin is required for sgAxin1/c-Met driven HCC development, we expressed sgAxin1/c-Met in Ctnnb1 null genetic background. We found that genetic ablation of Ctnnb1 completely prevents sgAxin1/c-Met induced HCC development in mice, but not β-catenin/c-Met HCC formation. Consistently, in Axin1 mutant human HCC cell lines, silencing of β-catenin strongly inhibits cell proliferation. Wnt/β-catenin functions promoting loss of Axin1 induced hepatic carcinogenesis via TCF4 mediated transcriptional regulation as the dominant negative form of TCF4 (dnTCF4) effectively inhibited sgAxin1/c-Met mouse HCC formation and human HCC cell growth. In conclusion, we demonstrate that loss of Axin1 induces HCC and it requires intact β-catenin signaling.
Cushioned–density gradient ultracentrifugation (C-DGUC) improves the isolation efficiency of exosomes for their use in liver research

Duong P, Chung A, Wong DK, Li K, Hong KY, Bouchareychas L, Raffai RL

Background and Purpose: Ultracentrifugation (UC) is recognized as a robust approach for the isolation of exosomes especially when combined with a second step that involves density gradient ultracentrifugation (DGUC). However, recent studies have highlighted limitations associated with the use of UC including low recovery efficiencies and possible aggregation of exosomes. Such effects could subsequently impact on downstream assessments of exosome function in biological systems.

Methods: We tested the benefit of using a liquid cushion of iodixanol during the first UC step to improve the yield of exosomes that are concentrated from the conditioned media (CM) of J774.1 murine macrophages in a method we recently termed Cushioned(C)-DGUC. We also compared the yield and purity of exosomes isolated by C-DGUC with those isolated by first subjecting CM to two other forms of concentration that included: ultrafiltration (UF) and polyethylene glycol (PEG) sedimentation prior to DGUC.

Results: Our data show that the concentration step largely determines the yield and purity of exosomes isolated following the second DGUC step. The use of a high-density iodixanol cushion in cushioned-UC (C-UC) led to a threefold improvement in exosome yield over conventional UC. Although subjecting the CM to UF resulted in a similar exosome recovery efficiency, it retained eight-fold more soluble proteins than C-UC method. Strikingly, PEG precipitation of the CM generated a substantial number of non-exosomal nanoparticles, which could not be efficiently eliminated by the DGUC step. Western blot analysis reproducibly detected exosome markers CD-81, TSG101 and Alix in fractions 6 and 7. Finally, an in vitro assay of exosome-mediated microRNA delivery confirmed that C-DGUC provided the highest yield of functional exosomes.

Conclusions: Collectively, our data demonstrate that the use of a high-density liquid cushion of iodixanol during the concentration step of C-DGUC substantially improves the yield and purity of exosomes derived from cell culture media. This approach can therefore facilitate functional studies of cell-derived exosomes in liver diseases.
ABSTRACT #18

Differential lipid uptake and metabolism in intrahepatic cholangiocarcinoma

Shihadih D, Park HM, Chen X, Stahl A

In recent years there has been an increase in the incidence of liver cancers, specifically intrahepatic cholangiocarcinomas (ICCs), which are highly malignant adenocarcinomas of the biliary tree. Metabolic reprogramming is a common feature of cancers with many showing specific alterations in lipid metabolism, particularly, an up regulation of de novo lipogenesis concomitant with a decreased dependence on exogenous fatty acids. Increased expression and activity of fatty acid synthase (FASN), the rate-limiting enzyme involved in de novo lipogenesis, is required for the survival and proliferation of many tumor cells, including hepatocellular carcinoma (HCC). Our investigator team has demonstrated in a recent study that ICC development is insensitive to FASN deprivation. Moreover, we found that ICC maintains robust fatty acid uptake rates suggesting a role of exogenous fatty acids for the growth of ICC. Using genetic manipulation and in vivo bio-imaging techniques, we were able to show that ICC tumor growth is dependent on the uptake of these fatty acids. We have also begun to use metabolomics to determine the fate and function of these fatty acids in comparison to HCC and non-tumorous liver. Preliminary data shows differing lipidomics profiles when comparing HCC and ICC suggesting a difference in lipid handling.
ABSTRACT #19

Oleate- but not palmitate-enriched diets induce iNKT cell activation in adipose tissue, resulting in hepatic steatosis

Amin AM, Duwaerts CC, Siao K, Goodsell A, Baron JL, Maher JJ

Background: Non-alcoholic fatty liver disease is driven by adipose tissue inflammation and dysfunction. Reports indicate that adipose tissue inflammation can be initiated by local activation of invariant natural killer T cells (iNKT cells) by lipid antigens. We investigated whether individual dietary fats have a unique capacity to activate adipose iNKT cells and induce hepatic steatosis.

Methods: Mice were fed either chow or one of 4 isocaloric high-energy diets with in a 40% carbohydrate:40% fat ratio containing palmitate or oleate as fat. After 9 weeks, liver and adipose tissue were collected and processed for histology and biochemical analyses, and immunophenotyping by flow cytometry.

Results: Mice fed all high-energy diets gained more body weight and adipose weight than chow controls ($P<0.0001$). Despite this, palmitate-fed mice developed mild steatosis, whereas oleate-fed mice developed more marked steatosis (13-41 mg/g triglyceride, $P<0.0001$). Adipose tissue from oleate-fed mice contained larger numbers of immune cells than palmitate-fed mice (6-20 x 10^5 cells/g adipose tissue, $P<0.0001$). Oleate-fed adipose tissue also contained more activated iNKT cells than palmitate-fed tissue (300-1800 cells/g, $P<0.0001$). This increase was accompanied by significant expansion of pro-inflammatory M1 macrophages.

Conclusion: Diets enriched in oleate, but not palmitate, provoke marked expansion of iNKT cells in visceral adipose tissue. iNKT cell expansion coincides with accumulation of M1 pro-inflammatory macrophages in adipose tissue and leads to hepatic steatosis. The data suggest that excess dietary oleate prompts the generation of an important lipid antigen in obesity that triggers iNKT cell activation and leads to adipose tissue and liver dysfunction.
The utility of MR elastography in hepatology practice: perspectives from a safety-net hospital

Patel R, Oberoi R, Ohliger M, Khalili M, Tana MM

This study aimed to assess the clinical scenarios in which MRE was ordered and assess the impact of MRE findings on clinical management. As we gain experience utilizing MRE in the clinic, it is informative to reflect on its utility and impact. A list of MREs ordered from July 2016 to August 2017 was obtained from the Radiology Department. A total of 96 MRE examinations were performed during the study period. MRE was ordered most frequently in patients with either HBV (25 patients, 26.0%), suspected NAFLD (30 patients, 31.3%), or a number of other etiologies (41 patients, 42.7%). MRE was most commonly ordered when patients declined liver biopsy (24 patients, 25.0%), or when providers sought cross sectional imaging for additional information (21 patients, 21.9%). MRE was obtained in 7 instances as follow-up to a prior imaging study suggesting advanced fibrosis, and in 5 instances for monitoring of progression or regression of fibrosis on therapy. 30 studies were obtained for more than one scenario. Median stiffness was 2.3 kPa (range 1.5-10.0). 73.0% of patients (65) had no significant fibrosis, 20.2% (18) had mild-moderate fibrosis, and 6.7% (6) had advanced fibrosis. The MRE result led to ongoing biochemical monitoring in 54.8% of cases, treatment initiation in 10.8%, and liver biopsy in 11.8%. Our experience with MRE demonstrates that it is widely applicable, feasible to obtain even in health system for vulnerable populations, and technically successful in nearly all cases.
CT texture analysis quantitatively distinguishes patients with acute alcoholic hepatitis from controls

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This study aims to determine if computed tomography (CT) texture features can distinguish patients with acute alcoholic hepatitis (AAH) from controls. This was a retrospective study of inpatients for whom gastroenterology consultations were obtained based on suspected AAH from 2013-2016. Patients who had contrast enhanced CT within 30 days of initial consultation were included. Control images were selected from CT scans of trauma patients with no intra-abdominal abnormalities. All scans were acquired using 64-slice CT scanners in the portal venous phase of contrast. For each patient, a single 1.25-mm axial slice was chosen at the bifurcation of the right portal vein. The liver was manually segmented from each slice, excluding major vessels. Using software written in R, 176 texture features were calculated for each segmented liver slice. Random forest elimination (RFE) was used to detect the most significant features distinguishing AAH patients from controls. A bootstrapped random forest algorithm was used to determine median accuracy of predicting AAH based on texture features of the liver ROI. 34 AAH patients and 35 controls were analyzed. Median accuracy for determining alcoholic hepatitis for test sets was 90%. The RFE algorithm determined the best features used in distinguishing AAH from controls were metrics from the grey level run length matrix, specifically grey-level non-uniformity and run-length non-uniformity. CT texture analysis can be used to differentiate AAH cases from controls, with a small number of significant texture features. Future studies will determine whether these features can predict clinical outcomes.
Prevalence of chronic subacute symptoms in carriers of mutation for autosomal dominant acute porphyria: a prospective, blinded study


In carriers of a mutation for acute porphyria (AP), severe attacks are uncommon, affecting less than 5% of the at-risk population. However, the prevalence of subacute recurring symptoms is debated. We have studied families with an index case genetically confirmed AP, with a focus on relatives who had never undergone screening for the family AP mutation. We anticipated a study population with similar numbers of mutation carriers and non-carriers, the latter serving as controls for symptoms that are non-specific and frequent in the general population. Candidates completed a health history questionnaire and provided samples (DNA and urine). In all cases, the questionnaire was administered before the lab results were known. For the analysis, subjects with an AP mutation were compared to those without a mutation. To date 99 subjects have been enrolled: 35 males; 64 females; 40 without an AP mutation; 59 with a mutation. A urine PBG, which was available for 69 subjects, is elevated in 33% of those with an AP mutation. In the no-mutation group, the values for females are significantly higher than for males. Porphyria-like symptoms are reported by both carriers and non-carriers but in the carrier group are more numerous, of longer duration and accompanied by PBG elevation. We conclude that approximately 20% of carriers of an AP mutation suffer from subacute symptoms. An unexpected result is the difference in urinary PBG between males and females in the non-carrier group, which is consistent with the marked predilection of young women to acute attacks of porphyria.
MEK inhibitor suppresses K-Ras wildtype cholangiocarcinoma growth via inhibiting cell proliferation and modulating tumor microenvironment


Intrahepatic cholangiocarcinoma (ICC) is an aggressive malignancy with limited therapeutic options. PD901 and U0126 are MEK inhibitors, which have been reported to reduce cell proliferation and induce apoptosis in ICC with activated mutant form of K-Ras oncogene. In this study, we investigated the therapeutic potential of MEK inhibitor, alone or in combination of pan-mTOR inhibitor, MLN0128 for the treatment of K-Ras wildtype ICC in vitro and in vivo using human ICC cell lines and AKT/YapS127A ICC mouse model, respectively. In vitro, treatment with MEK inhibitor or mTOR inhibitor alone suppressed ICC cell growth, whereas combined MEK and mTOR inhibitors led to increased growth inhibition. In AKT/YapS127A murine ICC model, we found treatment of PD901 treatment alone resulted in tumor regression whereas combined PD901 and MLN0128 treatment did not lead to additional tumor burden reduction. Mechanistically, PD901 efficiently inhibited ERK activation both in vitro and in vivo, leading to strong inhibiting of tumor cell cycle progression. Intriguingly, we found that PD901 but not MLN0126 treatment modulated tumor microenvironment of AKT/YapS127A ICC. In summary, our studies demonstrate the MEK inhibitors could be effective for the treatment of K-Ras wildtype ICC via inhibiting cell proliferation and modulating tumor microenvironment.
mTORC2 is required for hepatocyte proliferation during liver regeneration


Liver development and regeneration is important for normal liver function and restoring liver function after injury. Emerging evidence has pointed out cytokines, growth factors and various signaling pathways contributes to the process of liver development and regeneration. Mammalian target of rapamycin complex 2 (mTORC2) is one of the two TOR complexes. mTORC2 has been linked to modulate cell survival and metabolism by regulating multiple AGC kinases, especially AKT kinases. In the current study, we investigated the functional role of mTORC2 during liver regeneration. We performed 2/3 partial hepatectomy (PHx) in liver specific Rictor (the unique component of mTORC2) knock out (KO) mice (Rictor$^{LO}$) as well as widetype (Rictor$^{f/f}$) mice. We observed increased mortality in Rictor$^{LO}$ cohort after PHx. Mechanistically, we discovered that hepatocyte proliferation was delayed in Rictor$^{LO}$ mice; and this was accompanied with delayed lipid droplet formation. At the biochemical levels, we found that loss of Rictor strongly inhibited p-AKT(S473) activation and had abnormal expression patterns of cell cycle regulator cyclin D1 during liver regeneration. Together, our studies support that mTORC2/AKT cascade regulates cell cycle progression during liver regeneration.
ABSTRACT #25

Prevalence of liver fibrosis in a sample of young adult injectors with newly identified hepatitis C


Background: HCV is endemic among people who inject drugs (PWID), and is the most common blood borne disease in the United States affecting an estimated 4-6 million people. Incidence is highest among those early stages of injection drug use. In San Francisco, HCV incidence is 23%, and has the highest rate of hepatitis-related liver cancer.

Objective: To measure the prevalence of fibrosis in a sample of young adult injectors with newly identified hepatitis C virus (HCV) using non-invasive FIB4 and to examine associations between fibrosis and alcohol use among this sample population.

Methods: Observational study with cross-sectional design of young adult PWID with newly identified HCV living in the San Francisco Bay Area.

Results: Between March 2015 and March 2018, 108 HCV positive participants with FIB-4 scores were identified and included for this analysis. The overall median FIB-4 score was 0.49 (IQR 0.38-0.73). Median age was 25 years (IQR 23-27.5), and those who were 25 years or older had significantly higher median FIB-4 scores than those aged 24 and under (p=.02). Participants with acute HCV infection had the lowest median FIB-4 score and those with chronic infection the highest. We observed significant differences in median FIB-4 scores among acute and chronically infected participants as well as those who have resolved infection.

Discussion: Chronic hepatitis C infection is a slow progressing disease. Given that our participants are young (<30 years of age) and either resolved or in the early stages of disease, we found FIB-4 scores indicative of moderate to severe fibrosis.
ABSTRACT #26

Rate and predictors of phase transition among North American adults with HBeAg-negative chronic hepatitis B and low level viremia: a hepatitis B research network (HBRN) study


Background: Data on the natural history of inactive chronic hepatitis B (CHB) outside Asia and Europe are limited. We examined rates and predictors of phase transition among a North American population of HBeAg-negative CHB persons with low level viremia.

Methods: Adults within the HBRN were included if HBeAg-negative, HBV DNA≤10,000 IU/mL at entry and without treatment for 6 months. The primary predictor was HBV DNA≤2000 vs >2000-10,000 IU/mL. Outcome was phase transition defined as first measurement of HBV DNA>10,000 IU/mL and ALT>2xULN, or treatment initiation. Factors associated with this outcome were examined using Cox regression.

Results: Of 2003 HBRN adults, 962 met inclusion criteria. 52% of participants were female with median age 43 years (35-54) and predominantly Asian (64%) or Black (20%) race. Median follow-up was 3.7 years (1.4-4.8) and median number of follow-up measures per participant was 8 (4-11). 77% had baseline HBV DNA≤2000 IU/mL (28% HBV DNA≤100 IU/mL). ALT was normal (males ≤30 U/L; females ≤19 U/L) in 43%, >1-2xULN in 45% and >2xULN in 12%. Overall, 88% remained phase transition- and treatment-free at 4 years: 91% in those with HBV DNA≤2000 IU/mL and 80% with HBV DNA>2000 IU/mL (p=0.0001). In the fully-adjusted model, factors independently associated with higher risk of outcome included genotype, higher ALT, HBV DNA>2000 IU/mL, APRI≥0.5, and qHBsAg>1000 IU/mL.

Conclusion: Most patients in this North American cohort with HBeAg-negative CHB and low level viremia remained inactive and off treatment over 4 years. Viral rather than host factors were most strongly associated with phase transition.
ABSTRACT #27

PI(4,5)P$_2$ regulates plasma cholesterol through hepatic LDLR lysosomal decay


Hepatic low-density lipoprotein receptor (LDLR) plays an important role in cholesterol metabolism. We previously reported that transmembrane protein 55B (TMEM55B), annotated as a phosphatidylinositol 4,5-bisphosphate [PI(4,5)P$_2$] phosphatase, is a novel regulator of cholesterol metabolism. In human hepatoma cell lines, TMEM55B knockdown reduced cell surface LDLR, impaired LDL uptake, and increased LDLR decay. To test Tmem55b effects in vivo, we treated western diet fed C57BL/6J mice with antisense oligonucleotides against either Tmem55b or a non-targeting control for 3-4 weeks. Hepatic Tmem55b transcript and protein levels were both reduced by ~70% with Tmem55b knockdown (KD), resulting in increased plasma total (~1.5-fold, p<0.0001) and non-HDL cholesterol (~1.8-fold, p<0.0001). Analysis of FPLC fractions by immunoblot revealed apoE-enriched particles in the size range of small LDLs. Notably, Tmem55b KD had no effect on plasma cholesterol levels in Ldlr$^{-/-}$ mice. TMEM55B KD in HepG2 cells significantly increased PI(4,5)P$_2$, decreased LDLR, increased lysosome staining, and reduced LDLR-lysosome colocalization. Impairment of lysosome function by incubation with NH$_4$Cl or knockdown of the lysosomal proteins LAMP1 or RAB7 abolished the effect of TMEM55B KD on LDLR. Although there was no change in RAB11, a recycling endosome marker, LDLR-RAB11 colocalization was reduced by 50% upon TMEM55B KD. Incubation of HepG2 cells with PI(4,5)P$_2$ increased LDL uptake and reversed the inhibitory effect of TMEM55B OE. These findings suggest that hepatic TMEM55B regulates plasma cholesterol by mediating LDLR lysosomal degradation and LDLR recycling through PI(4,5)P$_2$. To our knowledge, this is the first demonstration of a phosphatidylinositol metabolizing enzyme regulating plasma cholesterol levels.
Deceased pediatric donor livers: how current policy drives allocation and transplantation

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Background: The current liver allocation algorithm for livers from deceased pediatric (<18y; "pedi") donors prioritizes adults listed locally/regionally over children listed nationally.

Methods: We examined U.S. pedi-liver allocation from 2010-2014 using national registry data. DSAs with ≤25 pediatric LTs during the 5y period were classified as “Pediatric Deserts” (n=37/58).

Results: 3,318 livers from pedi-donors were transplanted into 3,482 recipients. Pedi-donors whose livers were transplanted in children <12y were younger [3y (IQR 1-9) vs 15y (IQR 13-17)], more likely to be split (12% vs 8%), and less likely allocated within the Organ Procurement Organization (OPO, 25% vs 78%).

47% (1,569/3,482) of all LT recipients of pedi-livers were adults: 25% (390) were transplanted with a pedi-liver that was never offered to a child. Of 390 pedi-livers never offered to children, 52% (204) originated in a Pediatric Desert. Compared to adults, children<12y and 12-17y received a greater % of regionally (43% and 32% vs. 20%) and nationally (32% and 5% vs. 2%) shared livers.

A greater % of adults in Pediatric Deserts underwent LT with a pedi-liver (48% vs. 42%) compared to those in Donor Service Areas (DSAs) with >25 pedi-LTs. 278 children died/were delisted during the 5y study period, with a higher % occurring in Pediatric Deserts v DSAs with >25 pedi-LTs (10% vs. 6%).

Conclusions: 390 (12%) of all pedi-livers in 5 years were transplanted into adults before being offered to a child while 278 children died on the LT waitlist during this period. Prioritizing national sharing of pedi-livers to children may address this inequity.
Waitlist candidates who travel for liver transplantation and the donor organs they receive

Ge J, Lai JC

Background: Geographic disparities in access to liver transplantation (LT) has led to candidates seeking LT outside their home UNOS region (“Travelers”).

Methods: We analyzed all 2010-2014 US non-status 1 adult LT candidates. Travelers were defined as those listed ≥2 regions away from their home. We explored migration patterns, used linear regression to associate travel with donor quality, and Cox regression to evaluate outcomes.

Results: Of 83,352 candidates, 2,036 (2.4%) Travelers listed ≥2 regions from home. Travelers were more likely to be older (56 vs 58y), White (77 vs 69%), male (71 vs 64%), privately (62 vs 57%) or VA insured (15 vs 1%). They had higher median listing aMELD (17 vs 15), received more HCC exceptions (22 vs 20%), more likely to receive LT (55 vs 41%), less likely to have died (16 vs 22%).

Of the 2,036 Travelers, 1,040 (51%) traveled to a region with a median allocation MELD ≥5 less than home. 62% and 66% of Traveler listings and LTs were at 6 centers. Travelers received a greater % of DCDD (9 vs 5%) or nationally-shared livers (8 vs 3%), and had shorter cold ischemic times (5.9 vs 6.0h).

Nationwide, Traveler status was associated with DRI 0.02 [p=0.05] points higher than non-Travelers. Travelers had 20% decreased mortality post-transplant (HR 0.80, 95%CI 0.67-0.95, p=0.01).

Conclusions: The benefits of traveling were largely utilized by candidates who were older, White, male, and privately insured. Traveling for LT is associated with higher rates of LT at lower allocation MELDs at a small cost in donor quality.
De novo formation of the biliary system by TGFβ-mediated hepatocyte transdifferentiation


Transdifferentiation represents a form of cellular plasticity whereby one mature cell type converts into another mature cell type. In the context of injury, mature cells, as opposed to stem cells, may undergo transdifferentiation in order to facilitate organ repair. However, instances of transdifferentiation in the organs of adult mammals have previously been limited to cellular environments in which a scaffold and niche have been established during development. Here we show that in the liver, hepatocytes can undergo transdifferentiation to become cholangiocytes, which proceed to form an entire functional biliary network in a mouse model of intrahepatic bile duct paucity. Unlike previous studies in mice with a fully developed biliary system, these hepatocyte-derived cholangiocytes persist for life, and are equivalent to primary cholangiocytes. In contrast to development, NOTCH signaling is not required for de novo regeneration of the biliary network, and in its absence, proliferation of cholangiocytes is suppressed. In response to the strong environmental pressure caused by severe cholestasis, we identify TGFβ signaling as the driver of hepatocyte transdifferentiation and bile duct maturation.
Patient and graft survival among liver transplant recipients with alcoholic hepatitis versus alcoholic cirrhosis


The American Consortium of Early Liver Transplantation for Alcoholic Hepatitis (ACCELERATE-AH) is a multicenter consortium studying early liver transplantation (LT) for alcoholic hepatitis (AH). We evaluated early and late post-LT mortality in AH vs. alcoholic cirrhosis (ALC) using all alcohol-related LTs since first LT for AH at 12 ACCELERATE-AH sites. AH included clinically-diagnosed severe AH, no prior diagnosis of liver disease or AH, and LT without specific period of abstinence. ALC was a UNOS listing diagnosis of alcoholic cirrhosis and not within the AH group. Site-specific and UNOS data were utilized. We included 822 LT recipients from 2006-2016: 123 AH and 699 ALC. Median follow-up was 2.0 years. Only 28% of AH patients had AH as listing diagnosis in UNOS. AH patients vs. ALC were younger (42 vs. 54, p<0.001) and higher MELD (38 vs. 30, p<0.001). Cumulative unadjusted 3-year patient survival (85% vs. 86%, p=0.47) was similar for AH vs. ALC. With adjustment for covariates, AH as LT indication was associated with higher mortality (aHR 1.42, p=0.02), but not graft failure (aHR 1.24, p=0.08). The differential rate of death in AH vs. ALC was present ≤90 days post-LT but not beyond 90 days and age was an effect modifier; in older patients (age≥50), AH (vs. ALC) had a fourfold higher risk of early death post-LT (HR 4.07, p=0.003) but not in those <50 years (HR 1.09, p=0.92).

Conclusion: AH as indication for LT achieves 3-year survival (~85%) similar to ALC, but in older patients, the indication is associated with significantly higher risk of early post-LT death. Future studies of LT for alcoholic hepatitis need to elucidate the factors contributing to this early mortality risk.