

Caring Ambassadors Program

Hepatitis C Newsletter

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CLINICAL TRIALS, COHORT STUDIES, PILOT STUDIES

Multiple ascending dose study of BMS-790052, an NS5A replication complex inhibitor, in patients infected with hepatitis C virus genotype 1.

Nettles RE, Gao M, Bifano M, et al. Hepatology. 2011 Aug 11. doi: 10.1002/hep.24609. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21837752>

The antiviral activity, resistance profile, pharmacokinetics (PK), safety and tolerability of BMS-790052, an NS5A replication complex inhibitor, were evaluated in a double-blind, placebo-controlled, sequential panel, multiple ascending dose study. Thirty patients with chronic hepatitis C virus (HCV) genotype 1 infection were randomized to receive a 14-day course of BMS-790052 (1, 10, 30, 60 or 100 mg once daily or 30 mg twice daily) or placebo in a ratio of 4:1.

RESULTS: The mean maximum decline from baseline in HCV RNA ranged from 2.8 to 4.1 log(10) IU/mL; the placebo group showed no evidence of antiviral activity. Most patients experienced viral rebound on or before day 7 of treatment with BMS-790052 monotherapy; viral rebound was associated with viral variants that had been previously implicated in resistance development in the in vitro replicon system. The PK profile was supportive of once-daily dosing with median peak plasma concentrations at 1-2 hours postdose and mean terminal half-life of 12-15 hours. Steady state was achieved following 3-4 days of daily dosing. BMS-790052 was well tolerated in all dose groups with adverse events occurring with a similar frequency in BMS-790052- and placebo-treated groups. There were no clinically relevant changes in vital signs, laboratory, or electrocardiogram parameters. **CONCLUSION:** BMS-7590052 is the first NS5A replication complex inhibitor with multiple dose proof-of-concept in clinic. At doses of 1-100 mg daily, BMS-790052 was well-tolerated, had a PK profile supportive of once-daily dosing, and produced a rapid and substantial decrease in HCV-RNA levels in patients chronically infected with HCV genotype 1.

Efficacy of pegylated interferon plus ribavirin combination therapy for hepatitis C patients with normal ALT levels: a matched case-control study.

Hiramatsu N, Inoue Y, Oze T, et al. J Gastroenterol. 2011 Aug 20. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21858637>

BACKGROUND: The antiviral effect of pegylated interferon (Peg-IFN) plus ribavirin combination therapy in chronic hepatitis C (CHC) patients with normal alanine aminotransferase (ALT) levels (N-ALT) has been reported to be equivalent to that for patients with elevated ALT

levels (E-ALT). However, the actual antiviral effect in N-ALT patients remains obscure because efficacy can be overestimated in patients with an advantageous background. **METHODS:** In this study, 386 patients were extracted, for a matched case-control study, from 1320 CHC patients treated with Peg-IFN alpha-2b plus ribavirin combination therapy; 193 N-ALT patients [116 with hepatitis C virus genotype 1 (HCV-1), 77 with HCV genotype 2 (HCV-2)] were matched with 193 E-ALT patients by a propensity score method using the variables of age, sex, IFN treatment history, body mass index, and platelet counts. **RESULTS:** On multivariate analysis for sustained virological response (SVR) in N-ALT patients, younger age, low HCV RNA level at baseline, and HCV-2 were significant factors. The matched case-control study showed that the SVR rates of N-ALT patients were equivalent to those of E-ALT patients; at 49 and 40% in the HCV-1 group ($P = 0.146$), and 78 and 81% in the HCV-2 group ($P = 0.691$). However, in N-ALT patients with non-SVR, approximately 40% showed ALT elevation at 24 weeks post-treatment. **CONCLUSION:** Our findings indicate that the antiviral effect of Peg-IFN plus ribavirin therapy in N-ALT patients is comparable to that for E-ALT patients irrespective of their advantageous background; however, the application of this therapy for N-ALT patients, especially for those with HCV-1, should be considered carefully.

Pegylated interferon-Alfa-2a monotherapy in patients infected with HCV genotype 2 and importance of rapid virological response. Etoh R, Imazeki F, Kurihara T, et al. BMC Res Notes. 2011 Aug 31;4(1):316. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21884572>

BACKGROUND: Pegylated (PEG)-interferon (IFN)-alfa-2a plus ribavirin (RBV) therapy for 24 weeks is now a standard treatment protocol for patients with hepatitis C virus (HCV) genotype 2. As RBV cannot be used in certain situations, we examined whether PEG-IFN-alfa-2a monotherapy for 24 weeks or less would be sufficient to obtain a sustained virological response (SVR) in patients infected with HCV genotype 2. **METHODS:** Forty-nine consecutive patients with HCV genotype 2 received PEG-IFN-alfa-2a (180 mcg/week) subcutaneously without oral RBV for 8-64 weeks. HCV RNA level was determined by COBAS AMPLICOR HCV Test, v2.0. **RESULTS:** HCV RNA was equal to or less than 100 KIU/mL (defined as low viral load) in 15 of 49 patients, and the remaining 34 had HCV RNA above 100 KIU/mL (defined as high viral load). All 15 patients with low viral load achieved rapid virological response (RVR; HCV RNA negative at week 4), and also achieved SVR with an average treatment duration of 17.1 weeks. The 34 patients with high viral load were treated for 33.7 weeks on average, and 19 of them (55.9%) achieved RVR. The SVR rates of these patients were significantly higher in those with RVR than without RVR (16/19 vs. 6/15 $p=0.0074$). **CONCLUSION:** PEG-IFN-alfa-2a monotherapy for 24 weeks or less might be sufficient to treat selected patients with HCV genotype 2, especially those with low viral load and becoming negative for HCV RNA by week 4 of treatment.

Development rate of chronic kidney disease in hepatitis C virus patients with advanced fibrosis after interferon therapy. Arase Y, Suzuki F, Kawamura Y, et al. Hepatol Res. 2011 Aug 24. doi: 10.1111/j.1872-034X.2011.00845.x. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21883737>

AIM: The aim of this retrospective cohort study is to assess the development incidence and predictive factors for chronic kidney disease (CKD) after the termination of interferon therapy in hepatitis C virus (HCV) positive Japanese patients with liver cirrhosis. **METHODS:** A total of 650 HCV positive, liver cirrhotic patients who were treated with interferon and showed an estimated glomerular filtration rate (eGFR) of ≥ 60 mL/min per 1.73 m² after the termination of interferon therapy were enrolled. CKD was defined as an eGFR of < 60 mL/min per 1.73 m².

End-stage-CKD was defined as an eGFR of <15 mL/min/1.73 m². The primary goal is the new development of CKD and end-stage-CKD. **RESULTS:** Eighty-five patients developed CKD, and six patients progressed to end-stage-CKD. The development rate of CKD was 5.2% at the 5th year, 14.5% at the 10th year and 30.6% at the 15th year. Multivariate Cox proportional hazards analysis showed that CKD occurred when patients had age increments of 10 years (hazard ratio: 2.32; 95% confidence interval [CI] 1.61-3.35; P < 0.001), eGFR decrements of 10 mL/min per 1.73 m² (hazard ratio: 1.66; 95% CI 1.27-2.16; P < 0.001), hypertension (hazard ratio: 2.00; 95% CI 1.13-3.53; P = 0.017), diabetes (hazard ratio: 1.79; 95% CI 1.02-3.14; P = 0.042), and non-clearance of HCV (hazard ratio: 2.67; 95% CI 1.34-5.32; P = 0.005). The development rate of end-stage-CKD was 0.4% at the 5th year, 1.6% at the 10th year and 2.8% at the 15th year. **CONCLUSIONS:** The annual incidence for CKD among cirrhotic patients with HCV was determined to be about 1.0-1.5%. In addition, the annual incidence for end-stage-CKD is one order of magnitude lower than that of CKD.

Antiviral Activity of Danoprevir (ITMN-191/RG7227) in Combination With Pegylated Interferon {alpha}-2a and Ribavirin in Patients With Hepatitis C. Forestier N, Larrey D, Marcellin P, Guyader D, et al. J Infect Dis. 2011 Aug;204(4):601-8.
<http://www.ncbi.nlm.nih.gov/pubmed/21791662>

BACKGROUND: Current therapy options for patients with chronic hepatitis C virus (HCV) infection genotype 1 are effective in <50%. Danoprevir (ITMN-191/RG7227) is a potent, selective, and orally active inhibitor of the HCV NS3/4A serine protease. **METHODS:** The safety and antiviral efficacy of danoprevir was examined over 14 days in combination with pegylated interferon α -2a (180 μ g once weekly) and ribavirin (1000-1200 mg/day) in a double-blind, placebo-controlled, phase 1b, multiple ascending dose study consisting of 6 dose cohorts (400 mg, 600 mg, and 900 mg twice daily and 100 mg, 200 mg, and 300 mg 3 times daily). **RESULTS:** Danoprevir in combination with pegylated interferon α -2a and ribavirin was safe and generally well tolerated. The median change in HCV RNA level from baseline to the end of treatment with danoprevir at 400 mg, 600 mg, and 900 mg twice daily was -4.7 log(10) IU/mL, -5.4 log(10) IU/mL, and -5.3 log(10) IU/mL, respectively, and at 100 mg, 200 mg, and 300 mg 3 times daily was -5.5 log(10) IU/mL, -5.7 log(10) IU/mL, and -5.6 log(10) IU/mL, respectively. Placebo administered in combination with standard of care resulted in median decrease in HCV RNA level of -2.6 log(10) IU/mL (with twice daily regimen) and -2.0 log(10) IU/mL (with 3 times daily regimen). **CONCLUSIONS:** Our study showed substantial antiviral efficacy of danoprevir in combination with pegylated interferon α -2a and ribavirin. Exploration of the safety and antiviral efficacy of danoprevir in longer clinical studies is warranted.

Retreatment of patients with chronic hepatitis C relapsers to a previous antiviral treatment. Floreani A, Cazzagon N, Furlan P, et al. Eur J Gastroenterol Hepatol. 2011 Aug;23(8):711-5.

<http://www.ncbi.nlm.nih.gov/pubmed/21654322>

BACKGROUND: The efficacy of retreatment with pegylated interferon (PEG-IFN) plus ribavirin for patients relapsing after a previous treatment remains to be fully elucidated, although extended treatment seems to be the best option in such cases. **AIM:** To evaluate the efficacy of two extended protocols in patients with genotypes 1 or 4, or those with genotypes 2 or 3. **METHODS:** A total of 181 patients who had relapsed after a previous antiviral treatment with PEG-IFN α 2a plus weight-based ribavirin were offered retreatment with the same dose of both PEG-IFN plus ribavirin, to be continued for 48 weeks in those with genotypes 2 or 3 (group 1), and for 72 weeks in those with genotypes 1 or 4 (group 2). **RESULTS:** A total of 59 patients (32.5%) refused the retreatment, while 122 (78 men, 44 women) patients were enrolled in the

study: 41 were allocated in group 1 and 81 in group 2. Cirrhosis at baseline (staging 5/6 according to Ishak's score was recorded in 11 patients, six in group 1 and five in group 2). Nine patients (7.3%) in group 2 discontinued the treatment (due to lack of response). The remaining patients completed the treatment and were followed-up for at least 12 months after the treatment. Sustained virological response (SVR) rate was 82.9% in group 1 and 50.6% in group 2.

CONCLUSION: Patients with chronic hepatitis C with 'easy genotypes' relapsers to a previous antiviral treatment have more than 80% probability of achieving a SVR with a 48-week retreatment. Patients with 'difficult genotypes' have more than 50% chance of a SVR after a 72-week extended treatment.

Effects of mutation number in interferon sensitivity determining region on peripheral blood CD4(+) T cell subsets (Th1, Th2) in chronic hepatitis C patients with hepatitis C virus genotype 1b and high viral load. Ishii K, Shinohara M, Kogame M, et al. *Hepatol Int.* 2011 Aug 6. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21818686>

BACKGROUND/AIM: The number of amino acid (AA) mutations in the interferon sensitivity determining region (ISDR) of NS5A is reported to affect the response to interferon (IFN) therapy in patients with chronic hepatitis C (CHC). The aim of this study was to clarify whether host immunity is influenced by the number of AA mutations in the ISDR. **PATIENTS AND**

METHODS: Subjects included 44 patients with CHC infected with genotype 1b and high viral load. The number of AA mutations in the ISDR was retrospectively determined using stored serum samples taken immediately before starting therapy. All patients received IFN-alpha 2b or pegylated-IFN (PEG-IFN)-alpha 2b and ribavirin. When serum hepatitis C virus-ribonucleic acid (HCV-RNA) was negative at 4 or 12 weeks after starting therapy, the patient was defined as having rapid viral response (RVR) or early viral response (EVR), respectively. CD4(+) T cell (Th1 or Th2) in peripheral blood (PB) before and until day 56 of treatment was analyzed.

RESULTS: Rates of RVR and EVR were 0 (0/21) and 14% (3/21), respectively, in patients with one or fewer AA mutations in the ISDR (ISDR0-1), and 30 (7/23), and 74% (17/23), respectively, with two or more AA mutations in the ISDR (ISDR > 2). Although the percentage of PB Th1 cells did not differ between the two groups during the study period, the percentage of PB Th2 cells was significantly lower in the ISDR0-1 group than in the ISDR > 2 group at baseline and on days 3, 7, 14, and 28 of treatment. **CONCLUSION:** The number of AA mutations in the ISDR influenced PB Th2 cells before and until day 28, and was associated with higher RVR and EVR rates.

Higher serum testosterone is associated with increased risk of advanced hepatitis C-related liver disease in males. White DL, Tavakoli-Tabasi S, Kuzniarek J, Pascua R, Ramsey DJ, El-Serag HB. *Hepatology.* 2011 Aug 19. doi: 10.1002/hep.24618. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21858849>

BACKGROUND: Males have strikingly increased risk of advanced liver disease. However, the association between testosterone and risk of hepatitis C virus (HCV)-related advanced liver disease is unknown. **METHODS:** We performed a cross-sectional study in male veterans with chronic HCV. Blood samples were obtained to measure total serum testosterone and perform the FibroSURE-ActiTest. Other risk factor data were obtained through systematic questionnaires (e.g., alcohol), physical measurements (e.g., BMI) and serological tests (e.g., viral load). The association between total testosterone and risk of advanced hepatic fibrosis (F3 and F3/F4) and inflammatory activity (A3 and A2/3) measured by FibroSURE-ActiTest was evaluated with logistic regression. **RESULTS:** A total of 308 eligible study participants were prospectively recruited (mean age 57, 52% African-American). There were 105 cases with advanced fibrosis

and 203 mild fibrosis controls; and 88 cases with advanced inflammatory activity and 220 mild activity controls. Mean total serum testosterone was significantly higher in advanced fibrosis cases as well as advanced inflammatory activity cases compared to mild disease controls (6.0 ng/ml vs. 5.3 ng/ml and 5.9 ng/ml vs. 5.4 ng/ml, respectively). We observed a significant 27% increase in advanced fibrosis risk and 16% increase in advanced inflammatory activity risk for each 1 ng/ml increase in total serum testosterone. Total testosterone in the upper tertile was associated with an even greater excess risk of advanced fibrosis than advanced inflammatory activity (OR(adjusted advanced fibrosis) =3.78, 95% CI 1.88-7.61 vs. OR(adjusted advanced inflammatory activity) =2.64, 95% CI 1.29-5.45, respectively). **CONCLUSIONS:** Total serum testosterone is associated with an increased risk of both advanced hepatic fibrosis and advanced hepatic inflammatory activity in HCV-infected men. Testosterone may be important in the pathogenesis of HCV-related advanced liver disease in males.

Changes in Depressive Symptoms and Impact on Treatment Course Among Hepatitis C Patients Undergoing Interferon- α and Ribavirin Therapy: A Prospective Evaluation.

Chapman J, Oser M, Hockemeyer J, Weitlauf J, Jones S, Cheung R. Am J Gastroenterol. 2011 Aug 9. doi: 10.1038/ajg.2011.252. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21826113>

OBJECTIVES: Accounting for severity of depressive symptoms at baseline (pretreatment), this study describes (i) depressive symptom change over the course of antiviral treatment among patients with hepatitis C virus (HCV), and (ii) the relationship of such symptom change to treatment duration and response. **METHODS:** Depressive symptoms, measured with the Beck Depression Inventory (BDI), were examined prospectively among 129 HCV patients (95% male) who endorsed minimal (n=91), mild (n=28), or moderate depressive symptoms (n=10) prior to commencement of antiviral therapy. Assessments were obtained at baseline, 2 weeks, 4 weeks, and thereafter at 4-week intervals until treatment was discontinued or completed. **RESULTS:** The average depression score of the participants prior to commencing treatment was 7.4 (minimal depression). Depressive symptoms increased over the course of treatment, with average scores of 12.6 (mild depression) at the final assessment at the end of treatment. Patients with mild depressive symptoms at baseline demonstrated the greatest increase (M(increase)=12.7) and the greatest change (M(Δ)=5.8) in depressive symptoms from baseline to treatment completion. Patients who were minimally depressed at baseline completed the least amount of treatment (74%). Likewise, minimally depressed patients were less likely than mildly and moderately depressed patients to attain an antiviral treatment response. **CONCLUSIONS:** Depressive symptoms may worsen during antiviral therapy among patients with HCV. Notable changes in patients with subclinical depressive symptoms at baseline may be of significant concern, as the present work suggests that their depressive symptom changes are the most unstable. Thus, findings suggest that the degree of within treatment symptom change may be a more useful predictor (compared with baseline depression status) of ability to tolerate treatment. As the findings of the present study are preliminary, we urge further research and replication before drawing firm conclusions.

BASIC AND APPLIED SCIENCE, PRE-CLINICAL STUDIES

Selection of hepatitis C virus resistant to ribavirin. Feigelstock DA, Mihalik KB, Feinstone SM. Virol J. 2011 Aug 15;8(1):402. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21843317>

BACKGROUND: Given the side effects associated with intravenous injections of interferon, an interferon-free regimen for the treatment of HCV infections is highly desirable. Recently

published clinical studies show that interferon-free combination therapies containing ribavirin are efficacious, suggesting that an interferon-free therapy could be adopted in the near future. Therefore, understanding HCV resistance to ribavirin could be of major importance. In an approach to understand the effect of ribavirin on HCV replication and HCV resistance, we have selected a ribavirin resistant mutant of HCV in vitro. **METHODS:** We serially passed the J6/JFH1 strain of HCV in Huh7D cells (a Huh7 cell derivative more permissive to HCV replication) in the presence of different concentrations of ribavirin. Virus replication was assessed by detection of HCV antigens by immunofluorescence of infected cells and titration of recovered virus present in the supernatant. cDNAs from virus RNA grown in 0 or 250 uM concentrations of ribavirin were synthesized by RT-PCR, and sequenced. **RESULTS:** A concentration of 125 uM of ribavirin did not have a dramatic effect on HCV replication, while 500 uM of ribavirin lead to viral extinction. Concentrations of 250 uM of ribavirin dramatically reduced virus replication which was sustained over six passages. At passage seven viral resurgence began and over two passages the level of virus reached that of the wild type virus grown without ribavirin. Virus recovered from these cultures were more resistant to 250 uM ribavirin than wild type virus, and showed no difference in replication relative to wild type virus when grown in the absence of ribavirin. The ribavirin resistant virus accumulated multiple synonymous and non-synonymous mutations that are presently being analyzed for their relationship to ribavirin resistance. **CONCLUSIONS:** It is possible to select a ribavirin resistant mutant of HCV that can replicate to levels similar to wild type virus grown without ribavirin. Analysis of the mutations responsible for the ribavirin resistance may aid in understanding the mechanism of action of ribavirin.

Effects of hypolipidemic agent nordihydroguaiaretic acid on lipid droplets and hepatitis C virus. Syed GH, Siddiqui A. *Hepatology*. 2011 Aug 19. doi: 10.1002/hep.24619. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21858850>

Hepatitis C virus (HCV) relies on host lipid metabolic pathways for its replication, assembly, secretion and entry. HCV induces de novo lipogenesis, inhibits β -oxidation and lipoprotein export resulting in a lipid enriched cellular environment critical for its proliferation. We investigated the effects of a hypolipidemic agent, nordihydroguaiaretic acid (NDGA) on host lipid/fatty acid synthesis and HCV life cycle. NDGA negated the HCV induced alteration of host lipid homeostasis. NDGA decreased sterol regulatory element binding protein (SREBP) activation and enhanced expression of genes involved in β -oxidation. NDGA inhibited very low-density lipoprotein (VLDL) secretion by affecting mediators of VLDL biosynthesis. Lipid droplets (LDs), the neutral lipid storage organelles, play a key role in HCV morphogenesis. HCV induces accumulation and perinuclear distribution of LDs whereas NDGA most notably reduced the overall number and increased the average size of LDs. The antiviral effects of NDGA resulted in reduced HCV replication and secretion. **CONCLUSIONS:** NDGA-mediated alterations of host lipid metabolism, LD morphology and VLDL transport appear to negatively influence HCV proliferation.

The effect of cytokine profiles on the viral response to re-treatment in antiviral-experienced patients with chronic hepatitis C virus infection. Zhang Y, Guo D, Zhao Y, et al. *Antiviral Res*. 2011 Aug 26. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21889543>

BACKGROUND: There have been few studies on the potential immunological factors associated with viral controls in antiviral-experienced patients on a second round of combination therapy. In this study, we evaluated the level of systemic cytokines and potential impact on

combination therapy in both antiviral-naïve and -experienced patients chronically infected with hepatitis C virus. **METHODS:** Longitudinal analysis of 27 cytokines and chemokines was performed using the multiplex Biorad 27 plex assay in 37 antiviral-naïve and 24 experienced chronically HCV-1b-infected patients during combination therapy with peginterferon-alfa and ribavirin. A group of healthy donors was included as the control (n=11). **RESULTS:** Fifty percent of antiviral-experienced chronically HCV-patients could achieve a delayed and slow virologic response after 48weeks combination therapy, comparing with an early and fast virologic response in antiviral-naïve patients. A distinction of immune mediators profiling before and during antiviral therapy between antiviral-naïve and -experienced patients was identified, IL-4, IFN- γ and CCL-3 (MIP-1a) were significantly higher in naïve patients than those in experienced patients (P=0.005, 0.047 and 0.017, respectively) while G-CSF in naïve was lower than in experienced patients (P<0.05). Notably, higher Th1 type cytokine IFN- γ and lower Th2 type cytokine IL-4 at baseline and week 4 were associated with HCV clearance in naïve patients, and a similar trend appeared at week 12 in experienced patients. **CONCLUSIONS:** We found a successful second round therapy in antiviral-experienced patients appears to be associated with the host immune response. Dominant Th1-polar cytokines, especially IFN- γ , is a potential predictor of viral responsiveness.

The Hepatitis C Virus E1 Glycoprotein Undergoes Productive Folding but Accelerated Degradation When Expressed as an Individual Subunit in CHO Cells. Botti V, Bianchi A, Fong SK, Merola M. PLoS One. 2011;6(8):e23838. Epub 2011 Aug 17.

<http://www.ncbi.nlm.nih.gov/pubmed/21858229>

Hepatitis C Virus E1E2 heterodimers are components of the viral spike. Although there is a general agreement on the necessity of the co-expression of both E1 and E2 on a single coding unit for their productive folding and assembly, in a previous study using an in vitro system we obtained strong indications that E1 can achieve folding in absence of E2. Here, we have studied the folding pathway of unescorted E1 from stably expressing CHO cells, compared to the folding observed in presence of the E2 protein. A DTT-resistant conformation is achieved by E1 in both situations, consistent with the presence of an E2-independent oxidative pathway. However, while the E1E2 heterodimer is stable inside cells, E1 expressed alone is degraded within a few hours. On the other hand, the oxidation and stability of individually expressed E2 subunits is dependent on E1 co-expression. These data are consistent with E1 and E2 assisting each other for correct folding via different mechanisms: E2 assists E1 by stabilizing a semi-native conformation meanwhile E1 drives E2 towards a productive folding pathway.

Mutations in the E2 and NS5A regions in patients infected with hepatitis C virus genotype 1a and their correlation with response to treatment. Yahoo N, Sabahi F, Shahzamani K, et al. J Med Virol. 2011 Aug;83(8):1332-7. doi: 10.1002/jmv.22144.

<http://www.ncbi.nlm.nih.gov/pubmed/21678437>

Heterogeneity of subgenomic regions of hepatitis C virus (HCV) may be associated with response to interferon (IFN) therapy. The amino acid sequences of the PKR/eIF-2 α phosphorylation homology domain (pePHD), IFN sensitivity determining region (ISDR), PKR binding domain (PKRBD), and variable region 3 (V3) were studied in 19 patients before and after 4 weeks of treatment. All patients were infected with HCV genotype 1a and were treated with pegylated-IFN and ribavirin. Thirteen patients achieved sustained viral response (responders) and six failed to clear viral RNA (nonresponders). The amino acid sequences in the pePHD and ISDR were identical in responders and nonresponders. However, amino acid substitution at position 2252 of PKRBD was significantly different between responders and nonresponders (P = 0.044). A larger number of mutations were observed in the V3 region of

responders ($P < 0.001$). In this region, the amino acid in position 2364 differed between responders and nonresponders (responders: aspartic acid and serine, nonresponders: asparagine, $P = 0.018$). The amino acid sequences in the regions which were studied did not change after 4 weeks of treatment. **It is concluded** that the presence of specific amino acids in position 2252 of PKRBD and position 2364 of V3 might be associated with clinical response to IFN.

Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. Horner SM, Liu HM, Park HS, Briley J, Gale M Jr. Proc Natl Acad Sci U S A. 2011 Aug 15. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21844353>

RIG-I is a cytosolic pathogen recognition receptor that engages viral RNA in infected cells to trigger innate immune defenses through its adaptor protein MAVS. MAVS resides on mitochondria and peroxisomes, but how its signaling is coordinated among these organelles has not been defined. Here we show that a major site of MAVS signaling is the mitochondrial-associated membrane (MAM), a distinct membrane compartment that links the endoplasmic reticulum to mitochondria. During RNA virus infection, RIG-I is recruited to the MAM to bind MAVS. Dynamic MAM tethering to mitochondria and peroxisomes then coordinates MAVS localization to form a signaling synapse between membranes. Importantly, the hepatitis C virus NS3/4A protease, which cleaves MAVS to support persistent infection, targets this synapse for MAVS proteolysis from the MAM, but not from mitochondria, to ablate RIG-I signaling of immune defenses. Thus, the MAM mediates an intracellular immune synapse that directs antiviral innate immunity.

A polymorphism that delays fibrosis in hepatitis C promotes alternative splicing of AZIN1, reducing fibrogenesis. Paris AJ, Snapir Z, Christopherson CD, et al. Hepatology. 2011 Aug 11. doi: 10.1002/hep.24608. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21837750>

Among several single nucleotide polymorphisms (SNPs) that correlate with fibrosis progression in chronic HCV, a SNP in the antizyme inhibitor (AzI) gene is most strongly associated with slow fibrosis progression. **Our aim** was to identify the mechanism(s) underlying this observation by exploring the impact of the AzI SNP on hepatic stellate cell (HSC) activity. Seven novel AZIN1 splice variants ("SV2-8") were PCR-cloned from the LX2 human HSC line. Expression of a minigene in LX2 containing the AZIN1 slow-fibrosis SNP yielded a 1.67 fold increase in AZIN1 splice variant 2 (AZIN1 SV2) mRNA ($p = 0.05$). In healthy human leukocytes, the SNP variant also correlated with significantly increased SV2 mRNA. Cells (293T) transfected with shRNA complementary to the exonic splicing chaperone SRp40 expressed 30% less SRp40 ($p = 0.044$) and 43% more AzI SV2 ($p = 0.021$) than control shRNA-expressing cells, mimicking the effect of the sequence variant. LX2 cells transfected with AZIN1 full-length cDNA expressed 35% less collagen I mRNA ($p = 0.09$) and 18% less SMA mRNA ($p = 0.09$). Transient transfection of AZIN1 SV2 cDNA into LX2 cells reduced collagen I gene expression by 64% ($p = 0.001$) and α SMA by 43% ($p = 0.005$) compared to vector-transfected controls, paralleling changes in protein expression. Both AZIN1 and AZIN-SV2 mRNAs are detectable in normal human liver and reduced in HCV cirrhotic livers. The AZIN1-SV2 acts via a polyamine-independent pathway, as it neither interacts with antizyme nor affects the ability of AZIN1 lacking this variant to neutralize antizyme. **CONCLUSIONS:** A SNP variant in the AZIN1 gene leads to enhanced generation of a novel alternative splice form that modifies the fibrogenic potential of HSCs.

Zebrafish as a potential model organism for drug test against hepatitis C virus. Ding CB, Zhang JP, Zhao Y, Peng ZG, Song DQ, Jiang JD. PLoS One. 2011;6(8):e22921. Epub 2011 Aug 8.

<http://www.ncbi.nlm.nih.gov/pubmed/21857967>

Screening and evaluating anti-hepatitis C virus (HCV) drugs in vivo is difficult worldwide, mainly because of the lack of suitable small animal models. We investigate whether zebrafish could be a model organism for HCV replication. To achieve NS5B-dependent replication an HCV sub-replicon was designed and created with two vectors, one with HCV ns5b and fluorescent rfp genes, and the other containing HCV's 5'UTR, core, 3'UTR and fluorescent gfp genes. The vectors containing sub-replicons were co-injected into zebrafish zygotes. The sub-replicon amplified in liver showing a significant expression of HCV core RNA and protein. The sub-replicon amplification caused no abnormality in development and growth of zebrafish larvae, but induced gene expression change similar to that in human hepatocytes. As the amplified core fluorescence in live zebrafish was detectable microscopically, it rendered us an advantage to select those with replicating sub-replicon for drug experiments. Ribavirin and oxymatrine, two known anti-HCV drugs, inhibited sub-replicon amplification in this model showing reduced levels of HCV core RNA and protein. Technically, this method had a good reproducibility and is easy to operate. Thus, zebrafish might be a model organism to host HCV, and this zebrafish/HCV (sub-replicon) system could be an animal model for anti-HCV drug screening and evaluation.

Ceestatin, a novel small molecule inhibitor of hepatitis C virus replication, inhibits 3-hydroxy-3-methylglutaryl-coenzyme a synthase. Peng LF, Schaefer EA, Maloof N, et al. J Infect Dis. 2011 Aug;204(4):609-16.

<http://www.ncbi.nlm.nih.gov/pubmed/21791663>

BACKGROUND: Hepatitis C virus (HCV) chronically infects >170 million persons worldwide and is a leading cause of cirrhosis and hepatocellular carcinoma. The identification of more effective and better- **METHODS:** tolerated agents for treating HCV is a high priority. We have reported elsewhere the discovery of the anti-HCV compound ceestatin using a high-throughput screen of a small molecule library. To identify host or viral protein targets in an unbiased fashion, we performed affinity chromatography, using tandem liquid chromatography/mass spectrometry to identify specific potential targets. **RESULTS:** Ceestatin binds to 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase and irreversibly inhibits HMG-CoA synthase in a dose-dependent manner. Ceestatin's anti-HCV effects are reversed by addition of HMG-CoA, mevalonic acid, or geranylgeraniol. Treatment with small interfering RNA against HMG-CoA synthase led to a substantial reduction in HCV replication, further validating HMG-CoA synthase as an enzyme essential for HCV replication. **CONCLUSIONS:** Ceestatin therefore exerts its anti-HCV effects through inhibition of HMG-CoA synthase. It may prove useful as an antiviral agent, as a probe to study HCV replication, and as a cholesterol-lowering agent. The logical stepwise process employed to discover the mechanism of action of ceestatin can serve as a general experimental strategy to uncover the targets on which novel uncharacterized anti-HCV compounds act.

HIV/HCV COINFECTION

Patient and Provider Characteristics Associated with the Decision of HIV Coinfected Patients to Start Hepatitis C Treatment. Osilla KC, Wagner G, Garnett J, Ghosh-Dastidar B, Witt M, Bhatti L, Goetz MB. AIDS Patient Care STDS. 2011 Sep;25(9):533-8. Epub 2011 Aug <http://www.ncbi.nlm.nih.gov/pubmed/21823907>

Hepatitis C (HCV) and HIV coinfection is common and liver disease is a leading cause of morbidity and mortality among coinfecting patients. Despite advances in HCV treatment, few HIV coinfecting patients actually initiate treatment. We examined patient and provider characteristics associated with a patient's decision to accept or refuse HCV treatment once offered. We conducted patient chart abstraction and surveys with 127 HIV coinfecting patients who were offered HCV treatment by their provider and surveys of their HCV care providers at three HIV clinics. Participants were mostly male (87%), minority (66%), and had a history of injection drug use (60%). Most had been diagnosed with HIV for several years ($X=13.7$ years) and reported HIV transmission through unprotected sex (47%). Of the 127 patients, 79 accepted treatment. In multivariate analysis, patients who had a CD4 greater than 200 cells/mm³ and a provider with more confidence about HCV treatment were more likely to accept the recommendation to start treatment; younger age was marginally associated with treatment acceptance. In bivariate analysis, added correlates of treatment acceptance included male gender, no recent drug use, and several provider attitudes regarding treatment and philosophy about determination of patient treatment readiness. Patient and provider characteristics are important when understanding a patient's decision to start or defer HCV treatment. Further research is needed to better understand barriers to treatment uptake as new and more effective HCV treatments will soon be available.

Association of Vpu with hepatitis C virus NS3/4A stimulates transcription of type 1 human immunodeficiency virus. Kang L, Luo Z, Li Y, et al. *Virus Res.* 2011 Aug 25. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21889553>

Type 1 human immunodeficiency virus (HIV-1) and hepatitis C virus (HCV) are deadly bloodborne-transmitting pathogens. Due to sharing the routes of transmission, co-infection of HIV-1 and HCV is common with a high rate. Co-infection of HCV affects morbidity and mortality of patients with AIDS and impairs their tolerance to antiretroviral therapy. In this study, the roles of HCV proteins in the regulation of HIV-1 replication and the molecular mechanism involved in such regulation were investigated. We demonstrated that HCV NS3 protein stimulated HIV-1 LTR transcription and that HIV-1 Vpu protein was required for the activation of HIV-1 transcription regulated by HCV NS3/4A complex. Further study revealed that Vpu mediated ubiquitination-associated degradation of NS4A, detached NS3/4A complex and release NS3 for nuclear translocation. Since both degradation of NS4A and activation of HIV-1 LTR were closely correlated and mediated by Vpu, we proposed that Vpu impairs the stability of NS4A and releases NS3 from NS3/4A complex for the stimulation of HIV-1 transcription. This study enriched our understanding on HIV-1/HCV co-infection and provided new insights in molecular mechanism involved in the co-infection of the two viruses.

A Higher Correlation of HCV Core Antigen with CD4+ T Cell Counts Compared with HCV RNA in HCV/HIV-1 Coinfecting Patients. Shen T, Chen X, Zhang W, et al. *PLoS One.* 2011;6(8):e23550. Epub 2011 Aug 12.

<http://www.ncbi.nlm.nih.gov/pubmed/21858166>

Development of HCV infection is typically followed by chronic hepatitis C (CHC) in most patients, while spontaneous HCV viral clearance (SVC) occurs in only a minority of subjects. Compared with the widespread application of HCV RNA testing by quantitative RT-PCR technique, HCV core antigen detection may be an alternative indicator in the diagnosis of hepatitis C virus infections and in monitoring the status of infectious individuals. However, the correlation and differences between these two indicators in HCV infection need more investigation, especially in patients coinfecting by HIV-1. In this study, a total of 354 anti-HCV

and/or anti-HIV serum positive residents from a village of central China were enrolled. Besides HCV-related hepatopathic variables including clinical status, ALT, AST, anti-HCV Abs, as well as the altered CD4+/CD8+ T cell counts, HCV core antigen and HCV viral load were also measured. The concentration of serum HCV core antigen was highly correlated with level of HCV RNA in CHC patients with or without HIV-1 coinfection. Of note, HCV core antigen concentration was negatively correlated with CD4+ T cell count, while no correlation was found between HCV RNA level and CD4+ T cell count. Our findings suggested that quantitative detection of plasma HCV core antigen may be an alternative indicator of HCV RNA qPCR assay when evaluating the association between HCV replication and host immune status in HCV/HIV-1 coinfecting patients.

Treatment of chronic hepatitis C in HIV-infected patients with compensated liver cirrhosis.

Martín-Carbonero L, Tuma P, Vispo E, et al. J Viral Hepat. 2011 Aug;18(8):542-8. doi: 10.1111/j.1365-2893.2010.01334.x. Epub 2010 Aug 31.

<http://www.ncbi.nlm.nih.gov/pubmed/20819149>

The greatest benefit of hepatitis C virus (HCV) therapy is seen in cirrhotics attaining sustained virological response (SVR). However, concerns about toxicity and poorer responses often discourage treatment of cirrhotics. This may be particularly relevant in HIV-HCV-coinfecting patients, in whom progression of liver fibrosis is faster and treatment responses lower. This is a retrospective analysis of HIV-HCV-coinfecting patients who had received peginterferon-ribavirin therapy at our institution. Individuals naïve for interferon in whom liver fibrosis had been assessed using elastometry within the year before being treated were chosen. Response rates and toxicities were compared in cirrhotics (>14.5 KPa) and noncirrhotics. Patients with previous liver decompensation were excluded. Overall, 41 cirrhotics and 190 noncirrhotics entered the study. Groups were similar in age, gender, HCV genotypes and baseline serum HCV-RNA. SVR occurred at similar rates in cirrhotic and noncirrhotics, either considered by intention-to-treat (39% vs 45%; P=0.4) or as treated (50% vs 52%, P=0.8). In multivariate analysis (odds ratio, 95% CI, P), SVR was associated with HCV genotypes 2-3 (5, 2.9-11, <0.01) and lower serum HCV-RNA (2, 1.4-3.03 for every log decrease, <0.01) but not with cirrhosis (1.2, 0.4-3.6, 0.6). Treatment discontinuations because of adverse events tended to be more common in cirrhotics than in noncirrhotics (17% vs 12%; P=0.2), but only severe thrombocytopenia was more frequent in cirrhotics than in non-cirrhotics (20% vs 3% at week 24; P<0.01). Response to peginterferon-ribavirin therapy is similar in HIV-HCV coinfecting patients with and without liver cirrhosis. Therefore, treatment must be encouraged in all compensated cirrhotic patients, although closer monitoring and management of side effects, mainly thrombocytopenia, may be warranted.

Positive impact of HCV treatment on ART adherence in HIV-HCV coinfecting patients: one more argument for expanded access to HCV treatment for IDUs.

Roux P, Fugon L, Winnock M, et al. Addiction. 2011 Aug 5. doi: 10.1111/j.1360-0443.2011.03608.x. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21819472>

AIMS: Treatment for the hepatitis C virus (HCV) may be delayed significantly in HIV/HCV coinfecting patients on antiretroviral treatment (ART) for fear that its burden could compromise ART adherence. However, the effect such treatment has on ART adherence in observational settings remains largely unknown. Longitudinal data were used to investigate the relationship between initiating HCV treatment and adherence to ART in HIV-HCV coinfecting patients.

DESIGN: ANRS-CO-13-HEPAVIH is a French multi-center cohort. **SETTING:** Seventeen outpatient hospital services delivering HIV and HCV care in France. **PARTICIPANTS:** HIV/HCV coinfecting patients on ART (n = 593 patients, 976 visits). **MEASUREMENTS:** Self-administered questionnaires and medical records. A mixed logistic regression model based on

GEE to identify factors associated with non-adherence to ART. **FINDINGS:** Among the 593 patients, 36% were classified as non-adherent to ART at the enrolment visit and 12% started HCV treatment during follow-up. ART adherence was not statistically associated with HCV treatment initiation. The proportion of patients maintaining adherence or becoming adherent to ART for those starting HCV treatment was higher than in the rest of the sample ($p = 0.07$). After multiple adjustment for known correlates such as poor housing conditions, binge drinking, recent drug use and depressive symptoms, patients who initiated HCV treatment were less likely to be non-adherent to ART (OR[95%CI]= 0.41[0.24-0.71]). **CONCLUSIONS:** Engaging HIV/HCV co-infected individuals in HCV treatment is associated with high adherence to anti-retroviral treatment. Physicians should prioritise HCV treatment as part of a multidisciplinary approach.

Challenges Facing Providers Caring for HIV/HCV-Coinfected Patients. Lekas HM, Siegel K, Leider J. Qual Health Res. 2011 Aug 8. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21825278>

Despite the high prevalence of hepatitis C virus (HCV) infection among injection drug users also infected with human immunodeficiency virus (HIV), and the synergistic adverse effect of the two diseases on patients' health and survival, research on the clinical management of these patients and particularly the low uptake of HCV therapy is limited. We conducted qualitative interviews with 17 HIV providers from two urban public hospitals. We discovered that the limitations of the current state of medical knowledge, the severe side effects of HIV and HCV therapies, and the psychosocial vulnerability of HIV/HCV-coinfected patients combined with their resistance to becoming informed about HCV posed significant challenges for providers. To contend with these challenges, providers incorporated key dimensions of patient-centered medicine in their practice, such as considering their patients' psychosocial profiles and the meaning patients assign to being coinfected, and finding ways to engage their patients in a therapeutic alliance.

Reliability and predictive validity of a hepatitis-related symptom inventory in HIV-infected individuals referred for Hepatitis C treatment. Cachay ER, Wyles DL, Goicoechea M, et al. AIDS Res Ther. 2011 Aug 10;8(1):29. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21831314>

BACKGROUND: We aimed to determine the reliability and validity of a hepatitis symptom inventory and to identify predictors of hepatitis C (HCV) treatment initiation in a cohort of HIV-infected patients. **METHODS:** Prospective clinic based study that enrolled patients referred for HCV therapy consideration. A hepatitis symptom inventory and the Center for Epidemiologic Studies Depression Scale (CES-D) were administered to HIV/HCV individuals. The symptom inventory was factor analyzed and subscale reliability estimated with Cronbach's alpha. Predictive validity was evaluated using generalized estimating equations (GEE). Predictors of HCV treatment were identified using logistic regression. **RESULTS:** Between April 2008 to July 2010, 126 HIV/HCV co-infected patients were enrolled in the study. Factor analysis using data from 126 patients yielded a three-factor structure explaining 60% of the variance for the inventory. Factor 1 (neuropsychiatric symptoms) had 14 items, factor 2 (somatic symptoms) had eleven items, and factor 3 (sleep symptoms) had two items, explaining 28%, 22% and 11% of the variance, respectively. The three factor subscales demonstrated high intrinsic consistency reliability. GEE modeling of the 32 patients who initiated HCV therapy showed that patients developed worsening neuropsychiatric and somatic symptoms following HCV therapy with stable sleep symptoms. Bivariate analyses identified the following as predictors of HCV therapy initiation: lower HIV log₁₀ RNA, lower scores for neuropsychiatric, somatic and sleep symptoms, lower CES-D scores and white ethnicity. In stepwise multiple logistic regression

analysis, low neuropsychiatric symptom score was the strongest independent predictor of HCV therapy initiation and HIV log₁₀ RNA was inversely associated with a decision to initiate HCV treatment. **CONCLUSIONS:** A 41-item hepatitis-related symptom inventory was found to have a clinically meaningful 3-factor structure with excellent internal consistency reliability and predictive validity. In adjusted analysis, low neuropsychiatric symptom scores and controlled HIV infection were independent predictors of HCV treatment initiation. The usefulness of the HCV symptom inventory in monitoring HCV treatment should be evaluated prospectively.

The Impact of Hepatitis C Coinfection on Kidney Disease Related to Human Immunodeficiency Virus (HIV): A Biopsy Study. George E, Nadkarni GN, Estrella MM, Lucas GM, Sperati CJ, Atta MG, Fine DM. *Medicine (Baltimore)*. 2011 Aug 18. [Epub ahead of print] <http://www.ncbi.nlm.nih.gov/pubmed/21857365>

Approximately 1 in 4 individuals infected with the human immunodeficiency virus (HIV) in the United States is coinfecting with the hepatitis C virus. Both conditions increase the risk for the development and progression of kidney disease. The effect, however, of coexisting HIV and hepatitis C infection on the spectrum and progression of kidney disease is not well known. To compare the clinical features, histopathologic kidney diagnoses, and proportion of individuals progressing to end-stage kidney disease (ESKD), we reviewed the clinical records of HIV-infected individuals with and without hepatitis C coinfection who underwent ultrasound-guided percutaneous kidney biopsies between February 7, 1995, and March 30, 2009. Of the 249 HIV-infected individuals included in this study, 58% were coinfecting with hepatitis C. Coinfecting individuals were older (mean age, 46 ± 7 vs. 44 ± 10 yr, respectively; $p < 0.01$) and more likely to have used illicit drugs (85% vs. 14%, respectively; $p < 0.01$) compared to HIV-infected individuals without hepatitis C. HIV-associated nephropathy was the most common histopathologic diagnosis in both groups. Immune-complex glomerulonephritides (ICGNs), including lupus-like nephritis, postinfectious glomerulonephritis, membranous glomerulopathy, membranoproliferative glomerulonephritis, IgA nephropathy, and nonspecific ICGNs, occurred more frequently in individuals coinfecting with hepatitis C than in those not coinfecting (22% vs. 11%, respectively; $p = 0.02$). Although the proportion of those who died was similar between the 2 groups, hepatitis C coinfection was independently associated with a greater risk of progression to ESKD (hazard ratio, 1.81; 95% confidence interval, 1.09-2.99; $p = 0.02$). **The current study demonstrates** that coinfection with hepatitis C in individuals infected with HIV predisposes these individuals to immune-complex glomerulonephritides and is associated with increased risk of ESKD in the biopsied population.

Assessment of liver disease (noninvasive methods). Mehta SH, Buckle GC. *Curr Opin HIV AIDS*. 2011 Aug 18. [Epub ahead of print] <http://www.ncbi.nlm.nih.gov/pubmed/21857222>

PURPOSE OF REVIEW: The purpose of this review is to highlight new findings published in 2010-2011 related to noninvasive fibrosis assessment in HIV/hepatitis C virus (HCV) co-infected patients. Overall, in 2010-2011, 15 studies were published, of which two were excluded because they were published in languages other than English. **RECENT FINDINGS:** Eleven studies focused on serum marker panels. Studies sought to validate established panels in HIV/HCV co-infected patients often by comparing multiple serum marker panels in the same population; establish new marker panels using combinations of markers used in previously validated panels; and develop new marker panels using novel methodology. Overall, all panels performed within similar ranges of diagnostic accuracy as measured by the area under the receiver operating characteristic curve (AUROC) but the FibroMeter panel and its derivations achieved the highest performance. Four studies focused on transient elastography. Two studies

confirmed its accuracy for identifying fibrosis and cirrhosis and two studies confirmed that misclassification rates are higher in the presence of elevated triglycerides and steatosis.

SUMMARY: Overall, performance of transient elastography appeared superior to the majority of serum marker panels for the detection of significant fibrosis and cirrhosis in HIV/HCV co-infected patients. Challenges of widespread application of transient elastography remain high misclassification in some subgroups, lack of standardized cut-points and lack of widespread availability. Panels that were newly developed in 2010-2011 specifically for HIV/HCV appeared to perform better than existing panels such as APRI and FIB-4; however, additional external validation will be needed to confirm their accuracy.

European mitochondrial DNA haplogroups and liver fibrosis in HIV and hepatitis C virus coinfecting patients. García-Álvarez M, Guzmán-Fulgencio M, Berenguer J, et al. AIDS. 2011 Aug 24;25(13):1619-1926.

<http://www.ncbi.nlm.nih.gov/pubmed/21673559>

BACKGROUND: HIV infection, hepatitis C virus (HCV) liver disease, and mitochondrial DNA (mtDNA) polymorphisms are three possibly interrelated factors that might be associated with progression of liver disease. The aim of this study was to investigate whether mtDNA haplogroups had any influence on liver fibrosis progression in HIV/HCV coinfecting patients.

METHODS: We carried out a cross-sectional study in 231 patients who were genotyped via Sequenom's MassARRAY platform (San Diego, California, USA). Liver fibrosis was estimated based on the METAVIR score. In each patient, fibrosis progression rate (FPR) was calculated by dividing the fibrosis stage (0-4) by the estimated duration of HCV infection in years.

RESULTS: The cluster or major haplogroup HV was significantly associated with reduced odds ratios (OR) for advanced fibrosis [OR 0.35, 95% confidence interval (CI) 0.16-0.77, P=0.009], cirrhosis (OR 0.16, 95% CI 0.04-0.60, P=0.007), or high FPR (OR 0.43, 95% CI 0.21-0.84, P=0.015). Within the major haplogroup HV, haplogroup H was significantly associated with an absence of advanced fibrosis (OR 0.40, 95% CI 0.18-0.91, P=0.029), cirrhosis (OR 0.14, 95% CI 0.03-0.67, P=0.014), or high FPR (OR 0.47, 95% CI 0.23-0.95, P=0.035). We also found a significant association with increased odds of cirrhosis (OR 5.25, 95% CI 1.76-15.64, P=0.003) in the closely related major haplogroup U. **CONCLUSION:** The mtDNA haplogroups HV and H were associated with slower fibrosis progression, and the haplogroup U was associated with faster fibrosis progression in HIV/HCV coinfecting patients. These data suggest that mtDNA haplogroup may play a significant role in liver fibrogenesis during HCV infection.

Acute Hepatitis C Virus in an HIV Clinic: A Screening Strategy, Risk Factors, and Perception of Risk. Taylor LE, Delong AK, Maynard MA, et al. AIDS Patient Care STDS. 2011 Aug 22. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21859307>

Acute hepatitis C virus (HCV) infection is being acquired undetected among HIV-infected individuals. A practical way to regularly screen HIV-infected patients for acute HCV irrespective of perceived risk or symptoms is needed. We piloted implementation of an acute HCV screening strategy using routine HIV clinical care schedules and the least costly blood tests, in a Rhode Island HIV care center. Study participants had ongoing HCV risk, completed questionnaires encompassing risk behaviors and perception of risk, and were screened with quarterly alanine aminotransferase (ALT). ALT rise triggered HCV RNA testing, with pooled rather than individual specimen HCV RNA testing for underinsured participants. Participants were primarily older, college-educated men who have sex with men (MSM) with history of sexually transmitted infection other than HIV. One of 58 participants developed acute HCV in 50 person-years of observation for an annual incidence of 2.0% per year (95% confidence interval [CI] 0.05-11.1%).

The majority (54%) of MSM did not perceive that traumatic sexual and drug practices they were engaging in put them at risk for HCV. Unprotected sex often occurred under the influence of drugs or alcohol. Self-reported HCV risk and participation in several risk behaviors declined during the study. It was possible to collect frequent ALTs in a busy HIV clinic with 71% of total projected ALTs obtained and 88% of participants having at least one ALT during the 9-month follow-up period. All instances of ALT rise led to reflexive HCV RNA testing. Tracking quarterly ALT for elevation to systematically prompt HCV RNA testing before seroconversion is a promising approach to screen for acute HCV in a real-world HIV clinical setting.

Gender Differences in liver Fibrosis and Hepatitis C Virus-related Parameters in Patients Coinfected with Human Immunodeficiency Virus. Collazos J, Cartón JA, Asensi V. *Curr HIV Res.* 2011 Aug 9. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21827383>

OBJECTIVES: To evaluate gender differences in liver fibrosis and hepatitis C virus-related parameters in patients coinfecting with human immunodeficiency virus. **METHODS:** Transversal study of 782 patients who underwent a complete clinical and laboratory evaluation. Fibrosis was measured by transient elastometry (TE) and by commonly used laboratory-derived fibrosis indexes. **RESULTS:** Men were older, had higher rates of alcohol abuse, HCV viral load and liver tests, lower platelet values, poorer CDC clinical stages, longer duration of HCV infection, shorter time on successful antiretroviral therapy (ART) and had appreciably more advanced fibrosis than women. Multivariate analysis revealed that male gender ($P<0.0001$), longer time since HCV acquisition ($P<0.0001$), alcohol abuse ($P<0.0001$), HCV genotype 3 ($P=0.01$), shorter time on successful ART ($P=0.005$) and worse CDC clinical stages ($P=0.03$) were independently associated with significant or higher stages of fibrosis. Male gender was also independently predictive of advanced or higher stages of fibrosis ($P=0.06$) or cirrhosis ($P=0.02$). In patients with no alcohol abuse, men had worse fibrosis parameters than women ($P<0.01$ for each), but these differences decreased in patients with alcohol abuse and became non-significant. **CONCLUSIONS:** HIV-HCV-coinfecting women have more favorable HCV virological and clinical profile than men and, particularly, lower degrees of fibrosis. Alcohol abuse seemed to result more deleterious in women than in men. The reportedly poorer outcomes of liver disease in HIV-HCV-coinfecting patients, as compared with their HCV-monoinfecting counterparts, could be ameliorated by addressing these cofactors, some of them preventable or treatable.

Randomized Trial Comparing Dose Reduction and Growth Factor Supplementation for Management of Hematological Side Effects in HIV/HCV Patients Receiving Pegylated-Interferon and Ribavirin. Talal AH, Liu RC, Zeremski M, et al. *J Acquir Immune Defic Syndr.* 2011 Aug 26. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21876446>

INTRODUCTION: Pegylated-interferon (PEG-IFN) and ribavirin (RBV), current standard treatment for hepatitis C virus (HCV) infection, is frequently associated with neutropenia and anemia, leading to high treatment discontinuation rates in HIV/HCV coinfecting patients. Our objective was to compare the effectiveness of intervening with hematologic growth factors versus dose reductions of standard HCV therapy for the management of treatment-induced hematologic disorders. **METHODS:** Ninety-two HIV/HCV coinfecting, therapy-naive subjects received PEG-IFN alfa-2b 1.5 $\mu\text{g}/\text{kg}/\text{wk}$ and RBV 13 + 2 $\text{mg}/\text{kg}/\text{day}$ for up to 48 weeks. Before treatment initiation, subjects were randomized to subsequently receive growth factors, recombinant human erythropoietin (rHuEPO) and/or granulocyte-colony stimulating factor (G-CSF), or dose reduction (RBV and/or PEG-IFN) for anemia and neutropenia management, respectively. We analyzed the ability of each management strategy to control anemia and

neutropenia and the percentage of subjects who achieved a successful treatment outcome among subjects according to the different management strategies. **RESULTS:** During treatment, 43 subjects developed anemia (HuEPO, n=24; dose reduction, n=19) while 25 subjects developed neutropenia (G-CSF, n=10; dose reduction, n=15). Following the intervention, the increase in both hemoglobin and absolute neutrophil counts also did not differ between the two side effect management strategies. Sustained response percentages were similar comparing anemic and neutropenic subjects regardless of management strategy (anemia: rHuEPO, 29% versus dose reduction, 21%, p=0.92; neutropenia: G-CSF, 40% versus dose reduction, 20%, p=0.46). **CONCLUSIONS:** Growth factor supplementation and dose reduction do not appear to differ as management strategies for anemia and neutropenia in HIV/HCV co-infected individuals treated with PEG-IFN/RBV.

COMPLEMENTARY AND ALTERNATIVE MEDICINE

The green tea polyphenol epigallocatechin-3-gallate (EGCG) inhibits hepatitis C virus (HCV) entry. Ciesek S, von Hahn T, Colpitts CC, et al; Hepatology. 2011 Aug 11. doi: 10.1002/hep.24610. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21837753>

Hepatitis C virus (HCV) is a major cause of liver cirrhosis and hepatocellular carcinoma. Current antiviral therapy fails to clear infection in a substantial proportion of cases. Drug development is focused on non-structural proteins required for RNA replication. Individuals undergoing orthotopic liver transplantation face rapid and universal reinfection of the graft. Therefore, antiviral strategies targeting the early stages of infection are urgently needed for the prevention of HCV infection. **In this study**, we identified the polyphenol epigallocatechin-3-gallate (EGCG) as an inhibitor of HCV entry. Green tea catechins, such as EGCG and its derivatives epigallocatechin (EGC), epicatechingallate (ECG) and epicatechin (EC), have been previously found to exert antiviral and anti-oncogenic properties. EGCG had no effect on HCV RNA replication, assembly or release of progeny virions. However, it potently inhibited HCVcc entry into hepatoma cell lines as well as primary human hepatocytes. The effect was independent of the HCV genotype and both infection of cells by extra-cellular virions and cell-to-cell spread were blocked. Pretreatment of cells with EGCG before HCV inoculation did not reduce HCV infection while application of EGCG during inoculation strongly inhibited HCV infectivity. Moreover, treatment with EGCG directly during inoculation strongly inhibited HCV infectivity. Expression levels of all known HCV (co-)receptors were unaltered by EGCG. Finally, we showed that EGCG inhibits viral attachment to the cell, thus disrupting the initial step of HCV cell entry. **CONCLUSION:** the green tea molecule EGCG potently inhibits HCV entry and could be part of an antiviral strategy aimed at the prevention of HCV reinfection after liver transplantation.

EPIDEMIOLOGY, DIAGNOSTICS, AND MISCELLANEOUS WORKS

Risk Factors for Liver Disease and Associated Knowledge and Practices Among Mexican Adults in the US and Mexico. Flores YN, Lang CM, Salmerón J, Bastani R. J Community Health. 2011 Aug 30. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21877109>

Latinos in the US are disproportionately affected by chronic liver disease, which is the sixth most common cause of death among this group. In Mexico, cirrhosis and other liver diseases are the fourth leading cause of general mortality. **The objective** of this study was to contrast the liver

disease risk factors, knowledge, and prevention practices reported among separate samples of Mexicans living in Los Angeles, CA and in Cuernavaca, Mexico. **We assessed** the prevalence of specific risk factors (body mass index, waist circumference, and alcohol consumption), the level of knowledge about liver disease in general, hepatitis B (HBV), and hepatitis C (HCV), as well as prevention activities such as screening and vaccination. Data were collected from in-person interviews and anthropometric measures obtained from Mexican adults aged 18-70 years. Chi-square and t tests were used to compare the results between groups. Numerous similarities were observed in the bi-national samples, including high prevalence of obesity, abdominal obesity, and high levels of alcohol consumption. Most participants in both countries recognized that excessive alcohol consumption is a risk factor for liver disease, but only 60% correctly identified hepatitis C, being overweight or obese, or having diabetes as risk factors. Few participants reported having been screened for HBV or HCV, vaccinated for HBV, or having the intention of getting screened for HBV or HCV. US participants reported significantly higher levels of prevention activities and screening intentions than those in Mexico. Identifying the specific risk factors, levels of knowledge and prevention activities that affect specific racial/ethnic populations is important in order to effectively target efforts to prevent liver disease.

Temporal changes in HCV genotype distribution in three different high risk populations in San Francisco, California. Telles Dias P, Hahn JA, Delwart E, et al. BMC Infect Dis. 2011 Aug 2;11(1):208. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21810243>

BACKGROUND: Hepatitis C virus (HCV) genotype (GT) has become an important measure in the diagnosis and monitoring of HCV infection treatment. In the United States (U.S.) HCV GT 1 is reported as the most common infecting GT among chronically infected patients. In Europe, however, recent studies have suggested that the epidemiology of HCV GTs is changing.

METHODS: We assessed HCV GT distribution in 460 patients from three HCV-infected high risk populations in San Francisco, and examined patterns by birth cohort to assess temporal trends. Multiple logistic regression was used to assess factors independently associated with GT 1 infection compared to other GTs (2, 3, and 4). **RESULTS:** Overall, GT 1 was predominant (72.4%), however younger injection drug users (IDU) had a lower proportion of GT 1 infections (54.7%) compared to older IDU and HIV-infected patients (80.5% and 76.6%, respectively). Analysis by birth cohort showed increasing proportions of non-GT 1 infections associated with year of birth: birth before 1970 was independently associated with higher adjusted odds of GT 1: AOR 2.03 (95% CI: 1.23, 3.34). African-Americans as compared to whites also had higher adjusted odds of GT 1 infection (AOR: 3.37; 95% CI: 1.89, 5.99). **CONCLUSIONS:** Although, HCV GT 1 remains the most prevalent GT, especially among older groups, changes in GT distribution could have significant implications for how HCV might be controlled on a population level and treated on an individual level.

Usefulness of a novel serum proteome-derived index FI-PRO (fibrosis-protein) in the prediction of fibrosis in chronic hepatitis C. Cheung KJ, Tilleman K, Deforce D, et al. Eur J Gastroenterol Hepatol. 2011 Aug;23(8):701-10.

<http://www.ncbi.nlm.nih.gov/pubmed/21623191>

BACKGROUND: Liver biopsy is an imperfect standard for the assessment of chronic hepatitis C liver fibrosis. In this study, the diagnostic role of proteome-derived protein markers and the usefulness of a protein-based index were assessed. **METHODS:** Characteristics, clinical biochemistry, and protein markers of patients with chronic hepatitis C from a study (n=62) and validation group (n=73) were statistically assessed according to fibrosis severity. Multivariate models were built using linear discriminant analysis for the prediction of minor fibrosis (F0-F1),

moderate fibrosis (F2-F3), and cirrhosis (F4). The best model was validated and diagnostic performance was compared with the aspartate aminotransferase-to-platelet ratio index based on their receiver operator characteristic curves. **RESULTS:** Statistical analysis resulted in significant outcomes for both clinical and protein markers. The best multivariate model was based on four protein markers: α -2-macroglobulin (A2M), haptoglobin, hemopexin, and galectin-3-binding protein. A2M and hemopexin were the primary predictors according to this model. A novel index A2M/hemopexin [fibrosis-protein (FI-PRO) index] showed a diagnostic performance rate of 0.80-0.92 for the detection of significant fibrosis (F2-F4) and advanced fibrosis (F3-F4) in the validation group, which was better compared with aspartate aminotransferase-to-platelet ratio index. FI-PRO had an overall positive predictive value of 86% for significant fibrosis and a negative predictive value of at least 90% for advanced fibrosis. **CONCLUSION:** Proteome-derived protein markers were successfully implemented in clinical diagnosis of hepatitis C fibrosis, which resulted in the FI-PRO index. The efficiency and usability of FI-PRO should be validated in large-scale, prospective studies.

IL28B polymorphism and genetic biomarkers of viral clearance in hepatitis C virus

infection. Holmes JA, Sievert W, Thompson AJ. *Biomark Med.* 2011 Aug;5(4):461-78.

<http://www.ncbi.nlm.nih.gov/pubmed/21861668>

Infection with hepatitis C virus (HCV) is a major global health issue. Only a small proportion of patients clear the virus spontaneously and the majority develop chronic hepatitis C infection. Chronic hepatitis C is one of the most common causes of advanced liver disease in the western world and is now the leading indication for liver transplantation. Unfortunately, the standard treatment, consisting of pegylated-interferon and ribavirin, is suboptimal. Less than 50% of patients infected with HCV genotype 1 are cured, treatment is costly and is associated with significant toxicity. Therefore, there has been a need to identify accurate predictors of treatment outcome to facilitate treatment decision-making. Four independent genome-wide association studies have recently confirmed an association between genetic variation in the region of the IL28B gene and treatment outcome in HCV-1 patients. Patients who carry the good response variant are two- to three-fold more likely to be cured. The difference in the frequency of the good response variant between patients of different ethnic background explains much of the recognized ethnic disparity in treatment response rates. The IL28B variants are also associated with likelihood of spontaneous clearance of HCV infection. This discovery represents a significant advance in our ability to personalize HCV therapy, as well as suggesting novel avenues for research into viral pathogenesis and therapeutic development.

Peripheral blood monocyte subsets predict antiviral response in chronic hepatitis C.

Rodríguez-Muñoz Y, Martín-Vílchez S, López-Rodríguez R, et al. *Aliment Pharmacol Ther.*

2011 Aug 17. doi: 10.1111/j.1365-2036.2011.04807.x. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21848603>

BACKGROUND: Hepatitis C virus infection evolves into chronic progressive liver disease in a significant percentage of patients. Monocytes constitute a diverse group of myeloid cells that mediate innate and adaptive immune response. In addition to proinflammatory CD16+ monocytes, a Tie-2+ subgroup - Tie-2 expressing monocytes (TEMs) - that has robust proangiogenic potential has been recently defined. **AIM:** To study the heterogeneity of peripheral blood monocytes in chronic hepatitis C (CHC) patients and to examine their proposed pathophysiological roles on disease progression and response to antiviral therapy. **Methods** We studied CD16+ and Tie-2+ peripheral monocyte subpopulations in 21 healthy subjects and 39 CHC patients in various stages of disease and responses to antiviral treatment using flow cytometry. Expression profiles of proangiogenic and tissue remodelling factors in monocyte

supernatants were measured using ELISA and protein arrays. Intrahepatic expression of CD14, CD31 and Tie-2 was analysed using immunofluorescence. **RESULTS:** Increases of certain peripheral monocyte subsets were observed in the blood of CHC patients, wherein those cells with proinflammatory (CD16+) or proangiogenic (TEMs) potential expanded ($P < 0.005$, both). Notably, TEMs were significantly increased in nonresponders, particularly those with lower CD16 expression. In addition, many angiogenic factors were differentially expressed by peripheral monocytes from control or CHC patients, such as angiopoietin-1 and angiogenin ($P < 0.05$). Interestingly, intrahepatic TEMs were distinguished within portal infiltrates of CHC patients. **CONCLUSIONS:** These findings suggest for the first time the relevance of peripheral monocytes phenotypes for the achievement of response to treatment. Hence, the study of monocyte subset regulation might effect improved CHC prognoses and adjuvant therapies.

Identification of improved IL28B SNPs and haplotypes for prediction of drug response in treatment of hepatitis C using massively parallel sequencing in a cross-sectional European cohort. Smith KR, Suppiah V, O'Connor K, et al. *Genome Med.* 2011 Aug 31;3(8):57. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21884576>

BACKGROUND: The hepatitis C virus (HCV) infects nearly 3% of the World's population, causing severe liver disease in many. Standard of care therapy is currently pegylated interferon alpha and ribavirin (PegIFN/R), which is effective in less than half of those infected with the most common viral genotype. Two IL28B single nucleotide polymorphisms (SNPs), rs8099917 and rs12979860, predict response to (PegIFN/R) therapy in treatment of HCV infection. These SNPs were identified in genome wide analyses using Illumina genotyping chips. In people of European ancestry, there are 6 common (more than 1%) haplotypes for IL28B, one tagged by rs8099917 minor allele, four tagged by rs12979860. **METHODS:** We used massively parallel sequencing of the IL28B and IL28A gene regions generated by polymerase chain reaction (PCR) from pooled DNA samples from 100 responders and 99 non-responders to therapy, to identify common variants. Variants that had high odds ratios and were validated were then genotyped in a cohort of 905 responders and non-responders. Their predictive power was assessed, alone and in combination with HLA-C. **RESULTS:** Only SNPs in the IL28B linkage disequilibrium block predicted drug response. Eighteen SNPs were identified with evidence for association with drug response, and with a high degree of confidence in the sequence call. We found that two SNPs, rs4803221 (homozygote minor allele positive predictive value (PPV) of 77%) and rs7248668 (PPV 78%), predicted failure to respond better than the current best, rs8099917 (PPV 73%) and rs12979860 (PPV 68%) in this cross-sectional cohort. The best SNPs tagged a single common haplotype, haplotype 2. Genotypes predicted lack of response better than alleles. However, combination of IL28B haplotype 2 carrier status with the HLA-C C2C2 genotype, which has previously been reported to improve prediction in combination with IL28B, provides the highest PPV (80%). The haplotypes present alternative putative transcription factor binding and methylation sites. **CONCLUSIONS:** Massively parallel sequencing allowed identification and comparison of the best common SNPs for identifying treatment failure in therapy for HCV. SNPs tagging a single haplotype have the highest PPV, especially in combination with HLA-C. The functional basis for the association may be due to altered regulation of the gene. These approaches have utility in improving diagnostic testing and identifying causal haplotypes or SNPs.

FibroTest(®) and Fibroscan(®) performances revisited in patients with chronic hepatitis C. Impact of the spectrum effect and the applicability rate. Poynard T, de Ledinghen V, Zarski JP, et al. *Clin Res Hepatol Gastroenterol.* 2011 Aug 16. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21852224>

BACKGROUND: Two widely used biomarkers of fibrosis, FibroTest(®) and liver stiffness measurement (LSM), have been mostly validated in patients with chronic hepatitis C (CHC) using the standard area under the ROC curve (sAUROC) which is not the most appropriate method due to the risk of fibrosis spectrum effect. Furthermore the performance of these biomarkers have not been assessed in "intention to diagnose" which takes into account the failures and non-reliable results. **AIM:** The aim was to compare the accuracy of FibroTest(®) and LSM for the diagnosis of fibrosis using sAUROC, the pairwise comparison of fibrosis stages by Obuchowski measure (wAUROC), and these AUROCs reassessed after taking into account the applicability rates. **METHODS:** One thousand two hundred and eighty-nine patients with CHC and 604 healthy volunteers were analyzed. The performances of biomarkers assessed were compared in a patients-only group (P1: n=1289), in a population combining both patients and healthy volunteers (P2: n=1893) and in a simulated population (P3: n=1893) with the prevalence of stages observed in a reference population, to demonstrate the impact of spectrum effect. Applicability rates were estimated prospectively in 24,872 consecutive FibroTest(®) and in 13,669 consecutive LSM examinations. **RESULTS:** Using wAUROC, the conclusions of studies with reliable results in P1 were different than in those of P2 and in P3. There was a lower performance of FibroTest(®) versus LSM in P1 (0.864 [0.855-0.873] vs. 0.883 [0.874-0.892]; P=0.002) which was not found in P2 (0.893 [0.887-0.900] vs. 0.894 [0.887-0.901]; P=0.86) and in P3 (0.899 [0.893-0.905] vs 0.902 [0.895-0.909]; P=0.60). Using the sAUROC, in P1, P2 and P3, there was no significant difference between FibroTest(®) and LSM performance for advanced fibrosis and a lower performance of FibroTest(®) versus LSM for cirrhosis. In intention to diagnose, using wAUROCs performances were higher for FibroTest(®) vs. LSM in P1 (0.857 [0.848-0.866] vs. 0.814 [0.807-0.821]; P<0.0001) and P2 (0.885 [0.879-0.892] vs. 0.743 [0.737-0.749]; P<0.0001), without difference in P3 (0.891 [0.885-0.897] vs. 0.894 [0.887-0.901]; P=0.90). Using sAUROC, the significant differences in favor of FibroTest(®) vs LSM persisted also for the diagnosis of advanced fibrosis, both in P1 and P2 (P<0.0001) and for the diagnosis of cirrhosis in P1 (P<0.001). **CONCLUSION:** When the spectrum effects and applicability rates were taken into account, LSM had lower performance results than FibroTest(®) for the diagnosis of fibrosis stages.

Relative performances of FibroTest, Fibroscan and biopsy for assessing the stage of liver fibrosis in patients with chronic hepatitis C: a step toward the truth in the absence of a gold standard. Poynard T, de Ledinghen V, Zarski JP, et al. J Hepatol. 2011 Aug 31. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21889468>

BACKGROUND: Liver fibrosis stage is traditionally assessed with biopsy, an imperfect gold standard. Two widely used techniques, FibroTest®, and liver stiffness measurement (LSM) using Fibroscan® have been validated using biopsy, and therefore the true performances of these estimates are still unknown in the absence of a perfect reference. The aim was to assess the relative accuracy of FibroTest, LSM, and biopsy using methods without gold standard in patients with chronic hepatitis C (CHC) and controls. **METHODS:** A total of 1289 patients with CHC and 604 healthy volunteers, with assessment of fibrosis stage by the 3 techniques, and alanine aminotransferase (ALT) taken as a control test, were analyzed by latent class method with random effects. In the volunteers, the false positive risk of biopsy was obtained from a large surgical sample of four normal livers. **RESULTS:** The latent class model with random effects permitted to conciliate the observed data and estimates of tests' performances. For advanced fibrosis the specificity/sensitivity was for FibroTest 0.93/0.70, LSM 0.96/0.45, ALT 0.79/0.78 and biopsy 0.67/0.63, and for cirrhosis FibroTest 0.87/0.41, LSM 0.93/0.39, ALT 0.78/0.08 and biopsy

0.95/0.51. The analysis of the discordances between pairs suggested that the variability of the model was mainly related to the discordances between biopsy and LSM (residuals>10; P<0.0001). **CONCLUSION:** A method without the use of a gold standard confirmed the accuracy of FibroTest and Fibroscan for the diagnosis of advanced fibrosis and cirrhosis in patients with chronic hepatitis C. The variability of the model was mostly due to the discordances between Fibroscan and biopsy.

Hepatorenal Syndrome: Are We Missing Some Prognostic Factors? Martinez MO, Sayles H, Vivekanandan R, D' Souza S, Florescu MC. Dig Dis Sci. 2011 Aug 18. [Epub ahead of print] <http://www.ncbi.nlm.nih.gov/pubmed/21850494>

BACKGROUND: Hepatorenal syndrome (HRS) is the functional renal failure associated with advanced cirrhosis and has also been described in fulminant hepatic failure. Without liver transplantation its prognosis is dismal. Our study included patients with type 1 HRS associated with cirrhosis, who were not liver transplant candidates. **AIM:** To identify variables associated with improved survival. **METHODS:** Sixty-eight patients fulfilled the revised Ascites Club Criteria for type 1 HRS. None of them was suitable for liver transplantation. All the patients were treated with combinations of: albumin, midodrine and octreotide, pressors, and hemodialysis. **RESULTS:** Median survival was 13 days for the whole group. Survival varied with the end-stage liver disease (ESLD) etiology: autoimmune, 49 days, cardiac cirrhosis, 22 days, idiopathic, 15.5 days, viral, 15 days, hepatitis C and alcohol, 14.5 days, alcohol 8 days, and neoplasia 4 days (p = 0.048). Survival of HRS associated with alcoholic liver disease versus other etiologies was not statistically significant (p = 0.1). Increased serum creatinine (p = 0.02) and urinary sodium 6-10 mEq/l (p = 0.027) at the initiation of therapy were prognostic factors for mortality. HRS treatment modalities (p = 0.73), use of dialysis (p = 0.56), dialysis modality (p = 0.35), use of vasopressors (p = 0.26), pre-existing renal disease (p = 0.49), gender (p = 0.90), and age (p = 0.57) were not associated with survival. **CONCLUSIONS:** We report for the first time ESLD etiology as a prognostic factor for survival. The renal function (expressed as serum creatinine) and urinary Na (<5 mEq/l) at the time of diagnosis were found to be associated with survival, suggesting that early treatment might increase survival.

Dermatological side effects of hepatitis c and its treatment: Patient management in the era of direct-acting antivirals. Cacoub P, Bourlière M, Lübke J, et al. J Hepatol. 2011 Aug 29. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21884670>

Dermatological adverse events (AEs) are an existing concern during hepatitis C virus (HCV) infection and peginterferon/ribavirin treatment. HCV infection leads to dermatological and muco-cutaneous manifestations including small-vessel vasculitis as part of the mixed cryoglobulinemic syndrome. Peginterferon/ribavirin treatment is associated with well-characterized dermatological AEs tending towards a uniform entity of dermatitis. New direct-acting antivirals have led to significant improvements in sustained virologic response rates, but several have led to an increase in dermatological AEs versus peginterferon/ribavirin alone. In telaprevir trials, approximately half of treated patients had rash. More than 90% of these events were Grade 1 or 2 (mild/moderate) and in the majority (92%) of cases, progression to a more severe grade did not occur. In a small number of cases (6%), rash led to telaprevir discontinuation, whereupon symptoms commonly resolved. Dermatological AEs with telaprevir-based triple therapy were generally similar to those observed with peginterferon/ribavirin (xerosis, pruritus and eczema). A few cases were classified as severe cutaneous adverse reaction (SCAR), also referred to as serious skin reactions, a group of rare conditions that are potentially life-threatening. It is therefore important to distinguish between telaprevir-related dermatitis and

SCAR. The telaprevir prescribing information does not require telaprevir discontinuation for Grade 1 or 2 (mild/moderate) rash, which can be treated using emollients/moisturizers and topical corticosteroids. For Grade 3 rash, the prescribing information mandates immediate telaprevir discontinuation, with ribavirin interruption (with or without peginterferon) within 7 days of stopping telaprevir if there is no improvement, or sooner if it worsens. In case of suspicion or confirmed diagnosis of SCAR, all study medication must be discontinued.

Economic Burden Associated With Patients Diagnosed With Hepatitis C. McCombs JS, Yuan Y, Shin J, Saab S. Clin Ther. 2011 Aug 11. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21840056>

BACKGROUND: New therapies for Hepatitis C virus (HCV) are under development that will augment pegylated interferon-alpha plus ribavirin to improve patient outcomes. Data documenting the incremental economic and health burden of patients with HCV relative to those who are not infected with HCV will be required to evaluate the comparative effectiveness of these new therapies. **OBJECTIVE:** The objective of this study was to estimate the incremental impact of HCV infection on health care costs and risk of adverse health events. **METHODS:** Paid claims data for commercially insured patients in the United States were used to identify 2 matched cohorts of 8861 patients with and without HCV infection. Propensity score matching was used to adjust for patient demographics, diagnostic mix, prior use, and drug profile. Patients with prior cirrhosis, liver cancer, or liver transplantation were excluded. Differences in the first postindex year associated with the diagnosis of an HCV infection were estimated for adverse event risk (logistic regression), costs (ordinary least square regression), and utilization counts (generalized linear models), controlling for patient demographics, prior use, comorbidity profile, and prescription drug profile. **RESULTS:** The costs of treating patients infected with HCV and a matched sample not infected with HCV were \$37,390 and \$13,575, respectively. The incremental cost of HCV infection was estimated at +\$23,406, primarily because of higher costs for ambulatory care (+\$6531), hospital services (+\$1827), and prescription drugs (+\$6935). The presence of HCV was associated with a significantly higher risk of hospitalization (odds ratio [OR] = 2.5) and number of hospital admissions (+186%); depression (OR = 2.2); cirrhosis (OR = 65.8); hepatic cancer (OR = 28.1), and liver transplantation (OR = 46.1; $P < 0.0001$ for all estimates). **CONCLUSIONS:** A diagnosis of HCV infection was correlated significantly with increased adverse event risk and increased health care costs. New alternative treatments are needed that are more efficacious and less burdensome for the patient. Limitations of this study are that only 1 year was used to screen for preexisting conditions and events and that paid claims data do not capture indirect HCV infection costs such as time lost from work.

LIVER CANCER

Integrative network analysis identifies key genes and pathways in the progression of hepatitis C virus induced hepatocellular carcinoma.

Zheng S, Tansey WP, Hiebert SW, Zhao Z. BMC Med Genomics. 2011 Aug 8;4(1):62. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21824427>

BACKGROUND: Incidence of hepatitis C virus (HCV) induced hepatocellular carcinoma (HCC) has been increasing in the United States and Europe during recent years. Although HCV-associated HCC shares many pathological characteristics with other types of HCC, its molecular mechanisms of progression remain elusive. **METHODS:** To investigate the underlying pathology, we developed a systematic approach to identify deregulated biological networks in HCC by integrating gene expression profiles with high-throughput protein-protein interaction

data. We examined five stages including normal (control) liver, cirrhotic liver, dysplasia, early HCC and advanced HCC. **RESULTS:** Among the five consecutive pathological stages, we identified four networks including precancerous networks (Normal-Cirrhosis and Cirrhosis-Dysplasia) and cancerous networks (Dysplasia-Early HCC, Early-Advanced HCC). We found little overlap between precancerous and cancerous networks, opposite to a substantial overlap within precancerous or cancerous networks. We further found that the hub proteins interacted with HCV proteins, suggesting direct interventions of these networks by the virus. The functional annotation of each network demonstrates a high degree of consistency with current knowledge in HCC. By assembling these functions into a module map, we could depict the stepwise biological functions that are deregulated in HCV-induced hepatocarcinogenesis. Additionally, these networks enable us to identify important genes and pathways by developmental stage, such as LCK signalling pathways in cirrhosis, MMP genes and TIMP genes in dysplastic liver, and CDC2-mediated cell cycle signalling in early and advanced HCC. CDC2 (alternative symbol CDK1), a cell cycle regulatory gene, is particularly interesting due to its topological position in temporally deregulated networks. **CONCLUSIONS:** Our study uncovers a temporal spectrum of functional deregulation and prioritizes key genes and pathways in the progression of HCV induced HCC. These findings present a wealth of information for further investigation.

Level of Alpha-Fetoprotein Predicts Mortality among Patients with Hepatitis C-Related Hepatocellular Carcinoma. Tyson GL, Duan Z, Kramer JR, Davila JA, Richardson PA, El-Serag HB. Clin Gastroenterol Hepatol. 2011 Aug 3. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21820396>

BACKGROUND & AIMS: Hepatocellular carcinoma (HCC) can result from hepatitis C (HCV)-related liver disease and is the fastest-growing cause of cancer-related death in the United States. Alpha-fetoprotein (AFP) has been used as a prognostic factor for HCC, but the value of AFP as a prognostic factor for HCV-related HCC in the United States is unknown. We investigated whether higher levels of AFP at the time of diagnosis are associated with increased mortality of patients with HCV-related HCC. **METHODS:** In a retrospective study, we collected data from a cohort of HCV-infected veterans, identifying incident HCC cases from October 1, 1998 to January 1, 2007 (n=1480 patients). Mean serum levels of AFP, obtained within 60 days before to 30 days after HCC diagnosis, were determined for 1064 patients and categorized as <10 ng/ml (18%), 10-<100 ng/ml (30%), 100-<1000 ng/ml (22%), or ≥1000 ng/ml (29%). Cox proportional hazard models were used to associate serum levels of AFP with mortality, adjusting for demographic features, clinical factors, and treatment. **RESULTS:** The median survival times were significantly lower among patients with higher levels of AFP: 709 d for patients with <10 ng/ml, 422 d for 10-<100 ng/ml, 208 d for 100-<1000 ng/ml, and 68 d for ≥1000 ng/ml. In the multivariate analysis, increased levels of AFP (10-<100, 100-<1000, ≥1000) were significantly associated with increased mortality, compared to a serum level of AFP <10; hazard ratios were 1.50, 2.23, and 4.35, respectively. **CONCLUSIONS:** Serum level of AFP at the time of diagnosis with HCV-related HCC is an independent predictor of mortality.

Occurrence of Hepatocellular Carcinoma Was Not a Rare Event during and Immediately after Antiviral Treatment in Japanese HCV-Positive Patients. Kanda T, Imazeki F, Mikami S, et al. Oncology. 2011 Aug 3;80(5-6):366-372. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21822027>

Advanced chronic hepatitis C patients with sustained virological response by antivirals remain at risk for hepatocellular carcinoma (HCC). We investigated the incidence of HCC during and immediately after peginterferon-alfa-2a and ribavirin (RBV) treatment in patients with chronic

hepatitis C in Japan. HCC was detected in 8 of 238 patients during and after these treatments (mean follow-up period: 572 ± 252 days). In conclusion, occurrence of HCC is not a rare event during and immediately after peginterferon-alfa-2a plus RBV treatment. In cases with cirrhosis, higher α -fetoprotein levels, old age, or a previous history of HCC treatment, clinicians should be especially alert for the possible development of HCC during and immediately after peginterferon-alfa-2a and RBV treatment. Clinicians should regularly check for the possible development of HCC even in chronic hepatitis C patients under treatment.

Amino Acid substitutions in the hepatitis C virus core region are associated with postoperative recurrence and survival of patients with HCV genotype 1b-associated hepatocellular carcinoma. Toyoda H, Kumada T, Kaneoka Y, Maeda A. *Ann Surg.* 2011 Aug;254(2):326-32.

<http://www.ncbi.nlm.nih.gov/pubmed/21709542>

OBJECTIVE: We researched the molecular marker for prognosis of postoperative patients with hepatocellular carcinoma (HCC). **BACKGROUND:** The association of amino acid substitutions in the hepatitis C virus (HCV) core region and hepatocarcinogenesis has recently been explored. We investigated if these amino acid substitutions are associated with recurrence or survival in patients with HCC after attempted curative treatment by hepatectomy.

METHODS: A total of 163 patients infected with HCV genotype 1b who previously underwent hepatectomy for primary, not recurrent HCC were analyzed. Amino acid substitutions in the HCV core region were measured by direct sequencing. Postoperative recurrence or survival rates were compared according to tumor characteristics, tumor markers, and amino acid substitutions in the core region. **RESULTS:** Recurrence rates after hepatectomy were higher in patients bearing a methionine at residue 91 of the HCV core region than in patients with leucine ($P = 0.0002$). Survival was also decreased in patients with methionine at this residue from that seen in patients with leucine at this position ($P = 0.0061$). The associations between amino acid substitutions at residue 91 of the HCV core region and either recurrence or survival rates were independent of liver function, progression of HCC, or tumor marker levels. **CONCLUSIONS:** Amino acid substitutions at residue 91 of the HCV core region are associated with postoperative recurrence or survival in patients infected with HCV genotype 1b who developed HCC and treated by hepatectomy. This factor should be taken into consideration for the postoperative management of patients with HCC.

Occult and previous hepatitis B virus infection are not associated with hepatocellular carcinoma in United States patients with chronic hepatitis C. Lok AS, Everhart JE, Di Bisceglie AM, et al. *Hepatology.* 2011 Aug;54(2):434-42. doi: 10.1002/hep.24257.

<http://www.ncbi.nlm.nih.gov/pubmed/21374690>

Previous studies have suggested that prior exposure to hepatitis B virus (HBV) infection may increase the risk of development of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C. **The aim of this study** was to compare the prevalence of previous or occult HBV infection in a cohort of hepatitis B surface antigen-negative patients with histologically advanced chronic hepatitis C in the United States who did or did not develop HCC. Stored sera from 91 patients with HCC and 182 matched controls who participated in the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial were tested for hepatitis B core antibody (anti-HBc), hepatitis B surface antibody, and HBV DNA. Frozen liver samples from 28 HCC cases and 55 controls were tested for HBV DNA by way of real-time polymerase chain reaction. Anti-HBc (as a marker of previous HBV infection) was present in the serum of 41.8% HCC cases and 45.6% controls ($P = 0.54$); anti-HBc alone was present in 16.5% of HCC cases and 24.7% of controls. HBV DNA was detected in the serum of only one control subject and no patients with

HCC. HBV DNA (as a marker of occult HBV infection) was detected in the livers of 10.7% of HCC cases and 23.6% of controls (P = 0.18). **CONCLUSION:** Although almost half the patients in the HALT-C Trial had serological evidence of previous HBV infection, there was no difference in prevalence of anti-HBc in serum or HBV DNA in liver between patients who did or did not develop HCC. In the United States, neither previous nor occult HBV infection is an important factor in HCC development among patients with advanced chronic hepatitis C.

Building a comprehensive genomic program for hepatocellular carcinoma. Haring TR, Guiteau JJ, Nguyen NT, et al. *World J Surg.* 2011 Aug;35(8):1746-50.

<http://www.ncbi.nlm.nih.gov/pubmed/21225260>

BACKGROUND: Hepatocellular carcinoma (HCC) is the most common primary liver cancer, causing approximately 660,000 deaths worldwide annually. The preferred treatment of HCC is surgical resection or orthotopic liver transplantation (OLT) for patients meeting specific criteria. For patients outside these criteria, options are limited and include medical therapy, radio-frequency ablation, chemoembolization, or palliative measures, and these result in poor outcomes. Various centers at Baylor are elucidating the genomics of HCC to improve treatment options, with a focus on three etiologies: hepatitis C virus, hepatitis B virus, and non-viral.

METHODS: Through collaborative efforts, we have established an effective specimen biobanking protocol, and we are using several techniques to analyze HCC, including whole genome sequencing, whole exome sequencing, gene-specific analysis, gene expression, and epigenetic analysis. **RESULTS:** We have completed whole genome sequencing on two patient samples, whole exome sequencing on 47 patient samples, gene-specific analysis on 94 patient samples, gene expression on 4 patient samples, and epigenetic analysis on 1 patient sample.

CONCLUSIONS: We hope to use these results to define novel genetic therapeutic strategies that may work in conjunction with surgical approaches to improve long-term patient and graft survival rates in patients with HCC. We also aim to provide a functional framework of a comprehensive program for genomic analysis that may be imitated by other institutions and for other tumors in the global quest toward personalized genomic medicine.