

# Caring Ambassadors Program

## Hepatitis C Newsletter

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### CLINICAL TRIALS, COHORT STUDIES, PILOT STUDIES

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**Controlled release recombinant human interferon- $\alpha$ 2b for treating patients with chronic hepatitis C genotype 1: a phase 2a clinical trial.** Dzyublyk I, Yegorova T, Moroz L, et al. J Viral Hepat. 2011 Apr;18(4):271-9. doi: 10.1111/j.1365-2893.2010.01298.x.

<http://www.ncbi.nlm.nih.gov/pubmed/20367790>

Better convenience and tolerability and sustained therapeutic concentrations might improve interferon (IFN) treatment for chronic hepatitis C virus (HCV) infection. In an open-label, randomized study, controlled release free (chemically unmodified) recombinant human IFN- $\alpha$ (2b) in poly(ether-ester) microspheres (CR-rhIFN- $\alpha$ (2b)), was injected at doses of 160, 320, 480 or 640  $\mu$ g every 2 weeks for 12 weeks with concomitant weight-based oral ribavirin in 32 treatment-naïve patients with chronic HCV genotype 1. Treatment was well tolerated, with 31 patients (97%) successfully completing the study. Full doses of CR-rhIFN- $\alpha$ (2b) were administered on 96% of scheduled occasions. Flu-like symptoms were generally mild and brief. Injection site reactions developed in 13 patients (41%), and neutropenia occurred in six of eight patients receiving 640  $\mu$ g. In the 320, 480 and 640  $\mu$ g groups, 62-75% of patients achieved a  $\geq 2$  log(10) HCV RNA reduction by 4 weeks and 88-100% by 12 weeks. For those groups, the pooled median time to  $\geq 2$  log(10) reduction was 11 days (95% confidence interval, 7-35 days). In those groups, viral reduction below the limit of detection was accomplished in 25% of patients by 4 weeks and in 62% by 12 weeks. The 160- $\mu$ g dose was less potent. After CR-rhIFN- $\alpha$ (2b) injection, stable plateau levels of serum IFN- $\alpha$ (2b) were generally reached within 72 h. Treatment-emergent neutralizing antibodies to IFN- $\alpha$ (2b) were observed in one patient. No antibodies to host plant proteins were detected. CR-rhIFN- $\alpha$ (2b) with ribavirin cotherapy was well tolerated and displayed potent early antiviral activity in patients with chronic HCV genotype 1.

**Virological response is associated with decline in hemoglobin concentration during pegylated interferon and ribavirin therapy in hepatitis C virus genotype 1.** Sievert W, Dore GJ, McCaughan GW, et al. Hepatology. 2011 Apr;53(4):1109-1117. doi: 10.1002/hep.24180.

<http://www.ncbi.nlm.nih.gov/pubmed/21480317>

Anemia may increase the likelihood of achieving a sustained virological response (SVR) during pegylated interferon and ribavirin treatment of hepatitis C virus (HCV) infection. To determine whether hemoglobin decline is associated with SVR, we retrospectively evaluated the CHARIOT study of 871 treatment-naïve HCV genotype 1 patients. Anemia (serum hemoglobin <100 g/L) occurred in 137 (16%) patients, of whom only 14 (10%) received erythropoietin. Hemoglobin

decline >30g/L from baseline occurred in 76% of patients overall, including 526 patients who did not become anemic. Virological responses were higher in anemic patients compared with those who did not develop anemia (end of treatment, 80% versus 65%,  $P = 0.003$ ; SVR, 61% versus 50%,  $P = 0.02$ ); these differences remained significant when patients receiving erythropoietin were excluded from analysis. SVR was also higher in patients with hemoglobin decline >30 g/L compared with patients without a similar decline. In multiple logistic regression analyses with treatment group and baseline characteristics, the odds ratio for SVR was 1.97 (95% confidence interval, 1.08-3.62) for anemia and 2.17 (95% confidence interval, 1.31-3.62) for hemoglobin decline >30 g/L. Patients who first developed a hemoglobin decline >30 g/L during weeks 5-12 and 13-48 were more likely to achieve SVR than those who first developed such changes in weeks 0-4 or who never experienced them. **CONCLUSION:** Patients with HCV genotype 1 infection who develop anemia or experience a hemoglobin decline >30 g/L during weeks 5-48 of therapy achieve higher virological responses to pegylated interferon and ribavirin therapy that are unrelated to erythropoietin use.

**Excess mortality in patients with advanced chronic hepatitis C treated with long-term peginterferon.** Di Bisceglie AM, Stoddard AM, Dienstag JL, et al. *Hepatology*. 2011 Apr;53(4):1100-1108. doi: 10.1002/hep.24169.

<http://www.ncbi.nlm.nih.gov/pubmed/21480316>

Chronic hepatitis C virus infection can cause chronic liver disease, cirrhosis and liver cancer. The Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial was a prospective, randomized controlled study of long-term, low-dose peginterferon therapy in patients with advanced chronic hepatitis C who failed to respond to a previous course of optimal antiviral therapy. The aim of this follow-up analysis is to describe the frequency and causes of death among this cohort of patients. Deaths occurring during and after the HALT-C Trial were reviewed by a committee of investigators to determine the cause of death and to categorize each death as liver- or nonliver-related and as related or not to complications of peginterferon. Rates of liver transplantation were also assessed. Over a median of 5.7 years, 122 deaths occurred among 1,050 randomized patients (12%), of which 76 were considered liver-related (62%) and 46 nonliver-related (38%); 74 patients (7%) underwent liver transplantation. At 7 years the cumulative mortality rate was higher in the treatment compared to the control group (20% versus 15%,  $P = 0.049$ ); the primary difference in mortality was in patients in the fibrosis compared to the cirrhosis stratum (14% versus 7%,  $P = 0.01$ ); comparable differences were observed when liver transplantation was included. Excess mortality, emerging after 3 years of treatment, was related largely to nonliver-related death; liver-related mortality was similar in the treatment and control groups. No specific cause of death accounted for the excess mortality and only one death was suspected to be a direct complication of peginterferon. **CONCLUSION:** Long-term maintenance peginterferon in patients with advanced chronic hepatitis C is associated with an excess overall mortality, which was primarily due to nonliver-related causes among patients with bridging fibrosis.

**Hepatitis C virus infection causes hypolipidemia regardless of hepatic damage or nutritional state: An epidemiological survey of a large Japanese cohort.** Miyazaki T, Honda A, Ikegami T, et al. *Hepatol Res*. 2011 Apr 19. doi: 10.1111/j.1872-034X.2011.00803.x. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21501354>

**AIM:** Infection with hepatitis C virus (HCV) is the leading cause of liver cirrhosis that develops into hepatocellular carcinoma. Previous studies have shown in vitro that lipids within hepatocytes are crucially important for a series of HCV infection-proliferation-release processes.

On the other hand, in the patients with HCV, the serum total cholesterol (Total-C) and low-density lipoprotein cholesterol (LDL-C) levels have been reported to be lower. We conducted an epidemiological survey of a large cohort and investigated whether the lower serum lipid levels were caused by a direct or the secondary effects of HCV infection (i.e. hepatic damage or nutritional disorder). **METHODS:** Among 146 857 participants (male, 34%; female, 66%) undergoing public health examinations between 2002 and 2007 in Ibaraki Prefecture, Japan, the HCV positive rates determined by HCV antibody/antigen and/or RNA tests were 1.37% and 0.67% in males and females, respectively. **RESULTS:** In addition to Total-C and LDL-C, serum high-density lipoprotein cholesterol and triglyceride concentrations were also significantly lower in the HCV positive subjects compared with the negative subjects, regardless of sex, age or nutritional state evaluated by body mass index. Multivariate analysis showed that HCV infection was the strongest among the factors to be significantly associated with the lower level of these lipids. Particularly, the hypolipidemia was also confirmed in the HCV positive subjects with normal aminotransferase levels (alanine aminotransferase  $\leq 30$  and aspartate aminotransferase  $\leq 30$ ). **CONCLUSION:** This epidemiological survey in a large Japanese cohort suggests that the HCV infection itself might directly cause hypolipidemia, irrespective of host factors including age, hepatic damage and nutritional state.

**A prospective study of the rate of progression in compensated, histologically advanced chronic hepatitis C (HEP-10-2210).** Dienstag JL, Ghany MG, Morgan TR, et al. Hepatology. 2011 Apr 21. doi: 10.1002/hep.24370. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21520194>

**BACKGROUND & AIMS:** The incidence of liver disease progression among subjects with histologically advanced but compensated, chronic hepatitis C is incomplete.

**METHODS:** The Hepatitis C Antiviral Long-term Treatment against Cirrhosis Trial was a randomized study of 3.5 years of maintenance peginterferon treatment on liver disease progression among patients who had not cleared virus on peginterferon and ribavirin therapy. Patients were followed subsequently off therapy. Because maintenance peginterferon treatment did not alter liver disease progression, we analyzed treated and control patients together. Among 1,050 subjects (60% advanced fibrosis, 40% cirrhosis), we determined the rate of progression to cirrhosis over 4 years and of clinical outcomes over 8 years. **RESULTS:** Among patients with fibrosis, the incidence of cirrhosis was 9.9% per year. 679 clinical outcomes occurred among 329 subjects. Initial clinical outcomes occurred more frequently among subjects with cirrhosis (7.5%/year) than with fibrosis (3.3%/year) ( $P < 0.0001$ ). Child-Turcotte-Pugh (CTP)  $\geq 7$  was the most common first outcome, followed by hepatocellular carcinoma. Following occurrence of a score CTP  $\geq 7$ , the rate of subsequent events increased to 12.9%/year, including a death rate of 10%/year. Age and sex did not influence outcome rates. Baseline platelet count was a strong predictor of all clinical outcomes. During the 8 years of follow-up, death or liver transplantation occurred among 12.2% of patients with advanced fibrosis and 31.5% of those with cirrhosis.

**CONCLUSIONS:** Among patients with advanced hepatitis C who failed peginterferon and ribavirin, the rate of liver-related outcomes, including death and liver transplantation, is high, especially once CTP reaches at least 7.

**Association of a single nucleotide polymorphism near the interleukin-28B gene with response to hepatitis C therapy in Asian Patients.** Sinn DH, Kim YJ, Lee ST, et al. J Gastroenterol Hepatol. 2011 Apr 18. doi: 10.1111/j.1440-1746.2011.06744.x. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21501223>

**BACKGROUND AND AIMS:** A single nucleotide polymorphism near the interleukin-28B (IL28B) gene has been shown to predict hepatitis C virus (HCV) treatment response. We aim to determine the role of the IL28B genotype in Asian patients. **METHODS:** A total of 118 patients (all Korean, 55 patients with genotype 1 infection and 63 patients with genotype 2 infection) were consecutively enrolled and analyzed. **RESULTS:** The sustained virological response (SVR) rate was 74% (87/118), while 26 patients (22%) relapsed and five patients were non-responders (4%). For rs8099917, the frequencies of major homozygotes (TT), heterozygotes (GT), and minor homozygotes (GG) were 0.85, 0.14 and 0.01, respectively. Of the 55 patients with HCV genotype 1 infection, the SVR rate was 67% and 44% ( $p = 0.19$ ) and the non-response rate was 2% and 22% ( $p = 0.015$ ) for the major allele and minor or hetero allele, respectively. Of the 63 patients with HCV genotype 2 infection, the SVR rate was 80% and 100% ( $p = 0.13$ ) and the non-response rate was 4% and 0% ( $p = 0.55$ ) for major allele and hetero allele, respectively. **CONCLUSIONS:** The IL28B genotype may help identify non-responding patients in HCV genotype 1, but not in HCV genotype 2. Because of the high frequency of favorable alleles and the low frequency of non-response, the IL28B polymorphism may play a smaller role in Asian patients.

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## BASIC AND APPLIED SCIENCE, PRE-CLINICAL STUDIES

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**A common polymorphism in the ABCB11 gene is associated with advanced fibrosis in hepatitis C but not in non-alcoholic fatty liver disease.** Iwata R, Baur K, Stieger B, et al. Clin Sci (Lond). 2011 Apr;120(7):285-6.

<http://www.ncbi.nlm.nih.gov/pubmed/20883210>

Chronic HCV (hepatitis C virus)-associated cirrhosis represents a major indication for liver transplantation. Bile acids contribute to hepatic stellate cell activation as a key event in fibrogenesis. The aim of the present study was to investigate the role of bile acids and polymorphisms in bile acid level-regulating genes on fibrosis progression. A total of 206 subjects with chronic HCV infection were included for ABCB11 (ATP-binding cassette, subfamily B, member II) 1331T>C and NR1H4 (nuclear receptor) -1G>T genotyping, 178 of which were analysed for fibrosis stage. Exclusion criteria were HBV (hepatitis B virus) or HIV coinfection, alcohol >40 g/day and morbid obesity. A total of 358 patients with NAFLD (non-alcoholic fatty liver disease) were genotyped for comparison with a non-viral liver disease. Caucasian individuals ( $n = 110$ ), undergoing liver resection for focal hepatic metastasis, served as controls. The ABCB11 1331C allele was significantly overrepresented in HCV patients compared with controls {allelic frequency 62.9%; OR (odds ratio), 1.41 [95% CI (confidence interval), 1.012-1.965]}. Median plasma bile acid levels were not significantly increased in the CC compared with TT genotype [7.2 (1-110)  $\mu\text{mol/l}$  compared with 3.5 (1-61)  $\mu\text{mol/l}$ ; values are medians (range)]. A significant association between the presence of cirrhosis and ABCB11 genotype (CC compared with CT or TT,  $P=0.047$ ) was observed in the  $\chi^2$  test and independent of other risk factors of age, gender, body mass index and disease duration in multivariate analysis ( $P = 0.010$ ). No such association could be observed in fatty liver patients with regard to advanced fibrosis ( $F \geq 2$ ). The common ABCB11 1331CC genotype, which is present in 40% of HCV patients and renders the carrier susceptible to increased bile acid levels, is associated with cirrhosis.

**Regulation of Infectious Genotype 1a Hepatitis C Virus Production by Domain III of NS5A.** Kim S, Welsch C, Yi M, Lemon SM. J Virol. 2011 Apr 27. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21525356>

Although hepatitis C virus (HCV) assembly remains incompletely understood, recent studies with the genotype 2a JFH-1 strain suggest that it is dependent upon phosphorylation of Ser

residues near the C-terminus of NS5A, a multifunctional nonstructural protein. Since genotype 1 viruses account for most HCV disease yet differ substantially in sequence from JFH-1, we studied the role of NS5A in production of H77S virus. While less efficient than JFH-1, genotype 1a H77S RNA produces infectious virus when transfected into permissive Huh-7 cells. Exchange of complete NS5A sequences between these viruses was highly detrimental to replication, while exchanges of the C-terminal domain III sequence (46% amino acid sequence identity) were well tolerated with little effect on RNA synthesis. Surprisingly, placing the H77S domain III sequence into JFH-1 resulted in increased virus yields; conversely, H77S yields were reduced by the introduction of domain III from JFH-1. These changes in infectious virus yield correlated well with changes in NS5A abundance in RNA-transfected cells, but not RNA replication or core protein expression levels. Alanine-replacement mutagenesis of selected Ser and Thr residues in the C-terminal domain III sequence revealed no single residue to be essential for infectious H77S virus production. However, virus production was eliminated by Ala substitutions at multiple residues, and could be restored by phosphomimetic Asp substitutions at these sites. **Thus**, despite low overall sequence homology, production of infectious virus is regulated similarly in JFH-1 and H77S viruses by a conserved function associated with a C-terminal Ser/Thr cluster in domain III of NS5A.

**Temporal Variations in the Hepatitis C Virus Intra-Host Population during Chronic Infection.** Ramachandran S, Campo DS, Dimitrova ZE, Xia GL, Purdy MA, Khudyakov YE. *J Virol.* 2011 Apr 27. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21525348>

The hepatitis C virus (HCV) intra-host evolution holds keys to understanding mechanisms responsible for establishment of chronic infections and development of a vaccine and therapeutics. In this study, intra-host variants of two variable HCV genomic regions, HVR1 and NS5A, were sequenced from four treatment-naïve chronically infected patients who were followed up from the acute stage for 9-18 years. Median joining network analysis indicated that majority of the HCV intra-host variants were observed only at certain time-points, but some variants were detectable at more than one time-point. In all patients, these variants were found organized into communities or subpopulations. We hypothesize that HCV intra-host evolution is defined by 2 processes: incremental changes within communities through random mutation, and alternations between coexisting communities. The HCV population was observed to incrementally evolve within a single community during the first ~3 years of infection followed by dispersion into several subpopulations. Two patients demonstrated this pattern of dispersion for the rest of the observation period, while HCV variants in the other two patients converged into another single subpopulation after ~9-12 years of dispersion. The final subpopulation in these two patients was under purifying selection. Intra-host HCV evolution in all four patients was characterized by a consistent increase in negative selection over time, suggesting the increasing HCV adaptation to the host late in infection. The data suggest specific staging of the intra-host HCV evolution.

**Resistance mutations define specific antiviral effects for inhibitors of the hepatitis C virus (HCV) p7 ion channel.** Foster TL, Verow M, Wozniak AL, et al. *Hepatology.* 2011 Apr 21. doi: 10.1002/hep.24371. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21520195>

The hepatitis C virus (HCV) p7 ion channel plays a critical role during infectious virus production and represents an important new therapeutic target. Its activity is blocked by structurally distinct classes of small molecules, with sensitivity varying between isolate p7 sequences. Though this is indicative of specific protein-drug interactions, a lack of high

resolution structural information has precluded the identification of inhibitor binding sites and their modes of action remain undefined. Furthermore, a lack of clinical efficacy for existing p7 inhibitors has cast doubt over their specific antiviral effects. We have identified specific resistance mutations that define the mode of action for two classes of p7 inhibitor, adamantanes and alkylated imino-sugars (IS). Adamantane resistance was mediated by an L20F mutation, documented in clinical trials. Molecular modelling revealed that L20 resided within a membrane-exposed binding pocket, where drug binding prevented low pH-mediated channel opening. The peripheral binding pocket was further validated by a panel of adamantane derivatives as well as a bespoke molecule designed to bind the region with high affinity. By contrast, an F25A polymorphism found in GT3a HCV conferred IS resistance and confirmed that these compounds intercalate between p7 protomers, preventing channel oligomerisation. Neither resistance mutation significantly reduced viral fitness in culture, consistent with a low genetic barrier to resistance occurring in vivo. Furthermore, no cross-resistance was observed for the mutant phenotypes and the two inhibitor classes showed additive effects against wild type HCV. These observations support the notion that p7 inhibitor combinations could be a useful addition to future HCV-specific therapies.

**Novel Evolved Immunoglobulin (Ig)-Binding Molecules Enhance the Detection of IgM against Hepatitis C Virus.** Cao J, Chen Q, Zhang H, et al. PLoS One. 2011 Apr 14;6(4):e18477. <http://www.ncbi.nlm.nih.gov/pubmed/21533225>

Detection of specific antibodies against hepatitis C virus (HCV) is the most widely available test for viral diagnosis and monitoring of HCV infections. However, narrowing the serologic window of anti-HCV detection by enhancing anti-HCV IgM detection has remained to be a problem. Herein, we used LD5, a novel evolved immunoglobulin-binding molecule (NEIBM) with a high affinity for IgM, to develop a new anti-HCV enzyme-linked immunosorbent assay (ELISA) using horseradish peroxidase-labeled LD5 (HRP-LD5) as the conjugated enzyme complex. The HRP-LD5 assay showed detection efficacy that is comparable with two kinds of domestic diagnostic kits and the Abbott 3.0 kit when tested against the national reference panel. Moreover, the HRP-LD5 assay showed a higher detection rate (55.9%, 95% confidence intervals (95% CI) 0.489, 0.629) than that of a domestic diagnostic ELISA kit (Chang Zheng) (53.3%, 95% CI 0.463, 0.603) in 195 hemodialysis patient serum samples. Five serum samples that were positive using the HRP-LD5 assay and negative with the conventional anti-HCV diagnostic ELISA kits were all positive for HCV RNA, and 4 of them had detectable antibodies when tested with the established anti-HCV IgM assay. An IgM confirmation study revealed the IgM reaction nature of these five serum samples. These results demonstrate that HRP-LD5 improved anti-HCV detection by enhancing the detection of anti-HCV IgM, which may have potential value for the early diagnosis and screening of hepatitis C and other infectious diseases.

**Hepatitis C Virus Protects Human B Lymphocytes from Fas-Mediated Apoptosis via E2-CD81 Engagement.** Chen Z, Zhu Y, Ren Y, et al. PLoS One. 2011 Apr 19;6(4):e18933. <http://www.ncbi.nlm.nih.gov/pubmed/21526201>

HCV infection is often associated with B-cell regulatory control disturbance and delayed appearance of neutralizing antibodies. CD81 is a cellular receptor for HCV and can bind to HCV envelope protein 2 (E2). CD81 also participates to form a B cell costimulatory complex. To investigate whether HCV influences B cell activation and immune function through E2-CD81 engagement, here, human Burkitt's lymphoma cell line Raji cells and primary human B lymphocytes (PHB) were treated with HCV E2 protein and cell culture produced HCV particles (HCVcc), and then the related cell phenotypes were assayed. The results showed that both E2 and HCVcc triggered phosphorylation of I $\kappa$ B $\alpha$ , enhanced the expression of anti-apoptosis Bcl-2

family proteins, and protected Raji cells and PHB cells from Fas-mediated death. In addition, both E2 protein and HCVcc increased the expression of costimulatory molecules CD80, CD86 and CD81 itself, and decreased the expression of complement receptor CD21. The effects were dependent on E2-CD81 interaction on the cell surface, since CD81-silenced Raji cells did not respond to both treatments; and an E2 mutant that lose the CD81 binding activity, could not trigger the responses of both Raji cells and PHB cells. The effects were not associated with HCV replication in cells, for HCV pseudoparticle (HCVpp) and HCVcc failed to infect Raji cells. Hence, E2-CD81 engagement may contribute to HCV-associated B cell lymphoproliferative disorders and insufficient neutralizing antibody production.

**Structural in silico analysis of cross-genotype-reactivity among naturally occurring HCV NS3-1073-variants in the context of HLA-A\*02:01 allele.** Antunes DA, Rigo MM, Silva JP, Cibulski SP, Sinigaglia M, Chies JA, Vieira GF. Mol Immunol. 2011 Apr 20. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21513985>

Cellular immune response plays a central role in outcome of Hepatitis C Virus (HCV) infection. While specific T-cell responses are related to viral clearance, impaired responses can lead to chronic infection, turning HCV variability into a major obstacle for vaccine development. In a recent work, Fytily et al. (2008) studied the cross reactive potential of HCV specific CD8+ T-cells and observed a large variation in immunogenicity among 28 naturally occurring NS3(1073) variants. In this work, we intend to evaluate this immunogenic variation at molecular level, through bioinformatics approaches. The D1-EM-D2 strategy was used to build in silico MHC:peptide complexes (pMHC) of these HCV-derived peptides in the context of HLA-A\*02:01 allele. The TCR-interacting surface of these complexes were evaluated using the GRASP2 program. Structural analysis indicated a sharing of topological and electrostatic features among complexes that induced strong response in vitro. Besides, complexes that induced low response presented an important positively charged spot in the center of TCR-interacting area. This spot was seen even in complexes with conservative amino acid changes and is consistent with the impairment of recognition by wild-type-specific T-cells, observed in vitro. Furthermore, the most remarkable difference in electrostatic potential was seen precisely in the only complex unable to induce in vitro stimulation. All these observations were confirmed by Principal Component Analysis (PCA) and this approach was also applied to a set of 45 non-related immunogenic viral epitopes, indicating possible new targets for cross-reactivity studies. **Our results suggest** structural in silico analysis of pMHC complexes as a reliable tool for vaccine development, affording to predict the impact of viral escape mutations and selection of epitopes with potential to induce cross-reactive immune responses.

**The hepatitis C virus NS5A inhibitor (BMS-790052) alters the subcellular localization of the NS5A non-structural viral protein.** Lee C, Ma H, Hang JQ, Leveque V, Sklan EH, Elazar <http://www.ncbi.nlm.nih.gov/pubmed/21513964>

The hepatitis C virus (HCV) non-structural (NS) 5A protein plays an essential role in the replication of the viral RNA by the membrane-associated replication complex (RC). Recently, a putative NS5A inhibitor, BMS-790052, exhibited the highest potency of any known anti-HCV compound in inhibiting HCV replication in vitro and showed a promising clinical effect in HCV-infected patients. The precise mechanism of action for this new class of potential anti-HCV therapeutics, however, is still unclear. In order to gain further insight into its mode of action, we sought to test the hypothesis that the antiviral effect of BMS-790052 might be mediated by interfering with the functional assembly of the HCV RC. We observed that BMS-790052 indeed altered the subcellular localization and biochemical fractionation of NS5A. Taken together, our

data suggest that NS5A inhibitors such as BMS-790052 can suppress viral genome replication by altering the proper localization of NS5A into functional RCs.

**Serum chemokine levels are associated with the outcome of pegylated interferon and ribavirin therapy in patients with chronic hepatitis C.** Yoneda S, Umemura T, Joshita S, et al. *Hepatol Res.* 2011 Apr 19. doi: 10.1111/j.1872-034X.2011.00802.x. [Epub ahead of print] <http://www.ncbi.nlm.nih.gov/pubmed/21504519>

**AIM:** Serum chemokine levels and amino acid substitutions in the interferon-sensitivity determining region (ISDR) and core region have been associated with treatment outcome of pegylated interferon and ribavirin therapy in genotype 1 hepatitis C virus (HCV)-infected patients. The present study was conducted to clarify the association between serum chemokines and treatment outcome in patients with chronic HCV-1 infection in a Japanese cohort.

**METHODS:** A total of six serum chemokines were quantified before, during and after pegylated interferon and ribavirin treatment in 79 genotype 1 chronic HCV patients using a multiple bead array system. Viral ISDR and core region variants were determined by direct sequencing. **RESULTS:** The baseline serum levels of eotaxin, IP-10 and RANTES were significantly higher in chronic HCV patients than in controls. High levels of eotaxin and macrophage inflammatory protein (MIP)-1 $\beta$  before therapy and more than two mutations in the ISDR were associated with a sustained virological response, and patients with more than two mutations in the ISDR also had significantly higher MIP-1 $\beta$  levels. Receiver-operator curve analysis showed a 77% sensitivity and 73% specificity for predicting an SVR using MIP-1 $\beta$  values. **CONCLUSION:** Serum MIP-1 $\beta$  levels may predict the response to HCV treatment with pegylated interferon and ribavirin and are associated with amino acid substitutions in the ISDR.

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## HIV/HCV COINFECTION

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### **The natural history of liver cirrhosis in HIV-hepatitis C virus-coinfected patients.**

López-Diéguez M, Montes ML, Pascual-Pareja JF, et al. *AIDS.* 2011 Apr 24;25(7):899-904. <http://www.ncbi.nlm.nih.gov/pubmed/21330908>

**OBJECTIVE:** To provide detailed information about the natural history of HIV-hepatitis C virus (HCV)-coinfected patients with cirrhosis. **METHODS:** Prospective cohort including 340 HIV-HCV-coinfected patients with compensated (n = 248) or decompensated (n = 92) cirrhosis. We evaluated predictors of survival and of first hepatic decompensation. **RESULTS:** The mortality rate for patients with decompensated and compensated cirrhosis was 27.14 deaths per 100 person-years [95% confidence interval (CI) 18.93-35.35] and 3.98 deaths per 100 person-years (95% CI 2.42-5.54), respectively. Rate of first hepatic decompensation in patients with compensated cirrhosis was 4.62 per 100 persons-years (95% CI 2.91-6.33). In the complete cohort, permanent HAART interruption during follow-up, CD4 cell count nadir and baseline Child-Pugh score (CPS) B or C were significantly associated with shorter survival. In patients with compensated cirrhosis factors significantly associated with decreased survival were having the first hepatic decompensation during follow-up, permanent HAART discontinuation, and CPS B and C at baseline. For patients with compensated cirrhosis, time since diagnosis of HCV infection, CPS B and C and permanent HAART discontinuation were significantly associated with the risk of first hepatic decompensation. Sustained viral response to anti-HCV therapy was not independently associated with better survival in patients with compensated cirrhosis. **CONCLUSION:** : HIV-HCV-coinfected patients with cirrhosis have a relatively good 3-year survival (87%). In contrast, 2-year survival of patients with decompensated liver cirrhosis is only 50%. Three-year survival was mostly impacted by liver-related factors and HAART maintenance.

**Peginterferon alfa and ribavirin for chronic hepatitis C in patients eligible for shortened treatment, re-treatment or in HCV/HIV co-infection: a systematic review and economic evaluation.** Hartwell D, Jones J, Baxter L, Shepherd J. *Health Technol Assess.* 2011 Apr;15(17):1-210.

<http://www.ncbi.nlm.nih.gov/pubmed/21473834>

**OBJECTIVE:** to assess the clinical effectiveness and cost-effectiveness of peginterferon alfa and ribavirin for the treatment of chronic hepatitis c virus (HCV) in three specific patient subgroups affected by recent licence changes: those eligible for shortened treatment courses [i.e. those with low viral load (LVL) and who attained a rapid virological response (RVR) at 4 weeks of treatment], those eligible for re-treatment following previous non-response or relapse, and those co-infected with human immunodeficiency virus (HIV). **DATA SOURCES:** Fourteen electronic bibliographic databases, including the Cochrane Library, MEDLINE and EMBASE, were searched up to October 2009. Key hepatitis C resources and symposia, bibliographies of related papers and manufacturer submissions to the National Institute for Health and Clinical Excellence were also searched and clinical experts were contacted. **REVIEW METHODS:** A systematic review and economic evaluation were carried out. Titles and abstracts were screened for eligibility by one reviewer. Inclusion criteria were defined a priori and applied independently by two reviewers to the full text of retrieved references. For the clinical effectiveness review, studies were included if they were randomised controlled trials (RCTs) of adults with chronic HCV, restricted to the patient groups described above. The intervention was standard peginterferon and ribavirin combination therapy compared with shortened duration courses (24 weeks for genotype 1, 16 weeks for genotype 2/3) or best supportive care (BSC). Outcomes included sustained virological response (SVR), relapse rate and adverse events. In addition, full economic evaluations and studies of health-related quality of life were sought for this subgroup of patients. Data extraction and quality assessment were undertaken by two reviewers independently. Studies were synthesised through a narrative review with tabulation of results. Our previously published Markov state-transition model was adapted to estimate the cost-effectiveness of treatment strategies in subgroups of adults with chronic HCV who were eligible for shortened treatment and re-treatment and those with HCV/HIV co-infection. The model extrapolated the impact of SVR on life expectancy, quality-adjusted life expectancy and lifetime costs for each subgroup of patients with HCV. Categories of costs included in the model were drug acquisition, patient management, on-treatment monitoring, management of adverse events, and health-state costs for disease progression. **RESULTS:** In total, 2400 references were identified. Six RCTs were included in the review of clinical effectiveness, all reporting peginterferon alfa and ribavirin therapy in patients eligible for shortened treatment. In general, these RCTs were of good quality. No RCTs comparing peginterferon and ribavirin with BSC were identified for the re-treatment or co-infection populations. The results suggest that chronic HCV patients who have LVL at baseline and who achieve an RVR can be treated with shortened courses of therapy (24 weeks for genotype 1, 16 weeks for genotype 2/3) and achieve SVR rates that are comparable to those who receive the standard duration of treatment (ranges 84%-96% vs 83%-100%, respectively). However, patient numbers in the LVL/RVR subgroups were small and none of the trials was powered for this subgroup analysis, so results should be interpreted with caution. In the one trial reporting virological relapse rates in the subgroup of patients with LVL/RVR, rates were low and not statistically significantly different between those treated for 24 versus 48 weeks [3.6% vs 0%, respectively, difference 3.6%, 95% confidence interval (CI) - 7.2% to 6.6%,  $p = 1.000$ ]. In the cost-effectiveness analysis of shortened treatment with peginterferon alfa-2a, incremental cost-effectiveness ratios (ICERs) ranged from £35,000 to £65,000 for patients with genotype 1, whereas in patients with genotypes 2 and 3 shortened

treatment dominated standard treatment. For patients with genotype 1 with LVL/RVR, shortened treatment with peginterferon alfa-2b dominated standard treatment. In patients with genotype 1 and those with genotype non-1 who were re-treated with peginterferon alfa-2a, the ICERs were £9169 and £2294, respectively. In patients with genotypes 1 and 4, who were re-treated with peginterferon alfa-2b, the ICER was £7681, whereas re-treatment dominated BSC for patients with genotypes 2 and 3. In patients co-infected with HCV/HIV, who were receiving peginterferon alfa-2a, the ICER was £7941 per quality-adjusted life-year (QALY) gained in patients with genotypes 1 and 4, whereas in patients with genotypes 2 and 3 peginterferon alfa-2a dominated BSC. In co-infected patients receiving peginterferon alfa-2b the ICER was £11,806 in genotypes 1 and 4, and £2161 in genotypes 2 and 3. **CONCLUSIONS:** The clinical trial evidence indicates that patients may be successfully treated with a shorter course of peginterferon combination therapy without compromising the likelihood of achieving an SVR. The economic evaluation shows that treatment with peginterferon alfa in the specified subgroups of patients with LVL/RVR will yield QALY gains, without excessive increases in costs, and may be cost saving in some situations. However, a judgement is required on the value of the QALY loss that may result from adopting a shorter treatment regimen, if shorter treatment is associated with a lower SVR than standard treatment duration. There is a need for further RCT evidence, particularly in people who have not responded to, or relapsed following, treatment. Phase II and Phase III trials are currently in progress, evaluating the safety and efficacy of protease inhibitors and nucleoside analogues for treatment-naïve and treatment-experienced people with chronic HCV.

**Influence of antiretroviral therapy on liver disease.** Kovari H, Weber R. *Curr Opin HIV AIDS*. 2011 Apr 20. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21508839>

**PURPOSE OF REVIEW:** Liver disease is a major cause of morbidity and mortality in HIV-infected persons. The long-term beneficial versus potentially harmful influence of antiretroviral therapy (ART) on the liver is debated. We review current data on factors contributing to liver disease in HIV-monoinfected as well as in HIV/viral hepatitis-coinfected patients, highlighting the role of ART, HIV itself, immunodeficiency, patient characteristics, and lifestyle risk factors.

**RECENT FINDINGS:** New ART-related clinical syndromes, including noncirrhotic portal hypertension and nonalcoholic fatty liver disease, have emerged, and observational data suggest long-term ART-associated liver injury. Recently, there is increasing evidence that HIV itself and immunosuppression are contributing to liver injury in both HIV-coinfected and HIV-monoinfected patients. In HIV-positive persons, ART attenuates progression of chronic viral hepatitis. **SUMMARY:** Current expert guidelines recommend earlier treatment of HIV infection in persons coinfecting with hepatitis B virus and possibly hepatitis C virus. It is unknown whether an earlier start of ART is beneficial for the liver in HIV-monoinfected patients. Future research should focus on long-term ART-related hepatotoxicity.

**Treatment of acute hepatitis C infection in HIV-infected patients.** Boesecke C, Rockstroh JK. *Curr Opin HIV AIDS*. 2011 Apr 23. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21519248>

**PURPOSE OF REVIEW:** Clinicians started to notice the cases of an outbreak of acute hepatitis C (AHC) infections among HIV-positive MSM in Europe almost 10 years ago. Similar reports from the USA and Australia soon followed. In the absence of randomized controlled treatment trials clinicians have to rely on published data from uncontrolled clinical and cohort studies to develop treatment algorithms in these patients and give recommendations on best clinical management. **RECENT FINDINGS:** Data from recent cohort studies indicate that the early

course of hepatitis C virus (HCV) RNA in the first 4 weeks after diagnosis may be a helpful predictor of spontaneous clearance of AHC in HIV-infected individuals. Additionally, single-nucleotide polymorphisms near the IL28B gene have been demonstrated to impact chances of spontaneous clearance. Pegylated interferon in combination with weight-adapted ribavirin is recommended as treatment of choice for all HCV genotypes. For patients developing a rapid virological response, defined as a negative HCV-RNA in an ultrasensitive assay, treatment duration of 24 weeks is recommended. Overall, high-sustained virological response rates of 60-80% have been observed if antiviral therapy was initiated within 24 weeks after diagnosis.

**SUMMARY:** The current epidemic of AHC particularly among MSM is still ongoing, and prevention and screening efforts have to be intensified to allow for control of viral dissemination. Concise recommendations for best clinical management of AHC in HIV infection have been developed on the basis of published observational data.

**Very early prediction of response to HCV treatment with PEG-IFN-alfa-2a and ribavirin in HIV/HCV-coinfected patients.** Araújo ES, Dahari H, Neumann AU, et al. *J Viral Hepat.* 2011 Apr;18(4):e52-60. doi: 10.1111/j.1365-2893.2010.01358.x.

<http://www.ncbi.nlm.nih.gov/pubmed/20738775>

The objective of this study was to find very early viral kinetic markers to predict nonresponse to hepatitis C virus (HCV) therapy in a group of human immunodeficiency virus (HIV)/HCV-coinfected patients. Twenty-six patients (15 HCV genotype-1 and 11 genotype-3) were treated with a 48-week regimen of peginterferon-alfa-2a (PEG-IFN) (180 µg/week) and weight-based ribavirin (11 mg/kg/day). Samples were collected at baseline; 4, 8, 12, 18, 24, 30, 36 and 42 h; days 2, 3, 4, 7, 8, 15, 22, 29, 43 and 57 then weekly and monthly. Five patients discontinued treatment. Seven patients (27%) achieved a sustained virological response (SVR). Nadir HCV RNA levels were observed 1.6 ± 0.3 days after initiation of therapy, followed by a 0.3- to 12.9-fold viral rebound until the administration of the second dose of PEG-IFN, which were not associated with SVR or HCV genotype. A viral decline <1.19 log for genotype-1 and <0.97 log for genotype-3, 2 days after starting therapy, had a negative predictive value (NPV) of 100% for SVR. The day 2 virological response had a similar positive predictive value for SVR as a rapid virological response at week 4. In addition, a second-phase viral decline slope (i.e., measured from day 2 to 29) <0.3 log/week had a NPV = 100% for SVR. **We conclude that** first-phase viral decline at day 2 and second-phase viral decline slope (<0.3 log/week) are excellent predictors of nonresponse. Further studies are needed to validate these viral kinetic parameters as early on-treatment prognosticators of nonresponse in patients with HCV and HIV.

**Antiretroviral treatment interruption leads to progression of liver fibrosis in HIV-hepatitis C virus co-infection.** Thorpe J, Saeed S, Moodie EE, Klein MB; for the Canadian Co-infection Cohort Study (CTN222). *AIDS.* 2011 Apr 24;25(7):967-975.

<http://www.ncbi.nlm.nih.gov/pubmed/21330904>

**OBJECTIVE:** Despite potential negative consequences, HIV/hepatitis C virus (HCV) co-infected patients may discontinue antiretroviral treatment (ART) for several reasons. We examined the impact of ART interruption on liver fibrosis progression in co-infected adults, using the aspartate aminotransferase-to-platelet ratio index (APRI) as a surrogate marker of liver fibrosis. **METHOD:** Data were analyzed from a multisite prospective cohort of 541 HIV-HCV co-infected adults. ART interruption was included as a time-updated variable and defined as the cessation of all antiretrovirals for at least 14 days. The primary endpoint was the development of an APRI score at least 1.5. Time-dependent Cox proportional hazards regression and inverse probability-of-treatment weighting (IPTW) in a marginal structural model were used to evaluate the association of baseline and time-varying covariates with developing significant fibrosis.

**RESULTS:** Patients were followed for a median of 1.02 years; 10% (n = 53) interrupted ART and 10% (n = 53) developed significant fibrosis. After accounting for potential confounders, including CD4 T-cell count, HIV viral load, baseline APRI score, age and gender, the hazard ratio for ART interruption was 2.52 (95% confidence interval 1.20-5.28). Use of IPTW resulted in a similar effect estimate, suggesting that mediation by time-varying confounders was negligible. **CONCLUSION:** ART interruption was associated with an increased risk of fibrosis progression in HIV-HCV co-infection that was only partially accounted for by HIV viral load and CD4 T-cell counts. Our findings suggest that liver disease progression observed in ART-treated co-infected patients is partly due to the consequences of treatment interruptions.

**IL28B gene polymorphisms and viral kinetics in HIV/hepatitis C virus-coinfected patients treated with pegylated interferon and ribavirin.** Rallón NI, Soriano V, Naggie S, et al. AIDS. 2011 Apr 18. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21505315>

**BACKGROUND:** A single nucleotide polymorphism (SNP) upstream of the IL28B gene (rs12979860) predicts sustained virological response (SVR) to peginterferon-ribavirin therapy in chronic hepatitis C patients. There is scarce information regarding the influence of this IL28B SNP on early viral kinetics during therapy, particularly in patients coinfecting with HIV, in whom treatment response is lower than in hepatitis C virus (HCV)-monoinfected patients. **METHODS:** We selected 196 HIV/HCV-coinfected individuals who had completed a course of peginterferon-ribavirin therapy, and a validated outcome for SVR. Association of IL28B SNPs with rapid, early and end-of-treatment virological responses [rapid virological response (RVR), early virological response (EVR) and end of treatment virological response, respectively] was assessed in univariate and multivariate analyses. **RESULTS:** Rate of SVR in the study population was 54%. Frequency of the IL28B CC genotype was 44%. The distribution of HCV genotypes was as follows: HCV-1 57%, HCV-2 1%, HCV-3 30% and HCV-4 12%. Compared to CT/TT, the CC genotype was associated with significantly higher rates of all on-treatment viral outcomes, after adjusting for other predictors of viral response as serum HCV-RNA, HCV genotype and liver fibrosis staging. IL28B CC genotype kept its predictive power of SVR in patients who did not achieve RVR or cEVR. The association between IL28B SNP and viral kinetics and treatment outcomes was significant only for HCV genotypes 1 and 4. **CONCLUSION:** IL28B CC genotype is a strong predictor of virological response to therapy in HIV/HCV-coinfected patients. This effect is mediated by an increase in viral clearance during the first 12 weeks of treatment and is mainly seen in patients infected with HCV genotypes 1 and 4.

**Felt and Enacted Stigma Among HIV/HCV-Coinfected Adults: The Impact of Stigma Layering.** Lekas HM, Siegel K, Leider J. Qual Health Res. 2011 Apr 15. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21498828>

The realization that many persons with HIV/AIDS are subjected to multiple layers of stigmatization because they belong to socially deviant and disenfranchised groups (e.g., injection drug users, racial/ethnic and sexual minorities) accounts for an increasing interest in the phenomenon of stigma layering. The stigma associated with hepatitis C virus (HCV) has also been conceptualized as layered. However, researchers have overlooked the fact that HCV adds a layer to the HIV stigma and vice versa. Qualitative interviews with 132 HIV/HCV-coinfected patients were analyzed to explore how they experience the two layers of stigma. Most participants hierarchically ordered the stigmas associated with each disease and regarded HIV as the more stigmatizing of the two. A small number perceived HIV and HCV as equally stigmatizing. The impact of the hierarchical and nonhierarchical ordering of the two stigmas on

coinfecting patients' felt and enacted stigmatization is explored and implications for interventions are discussed.

**Hepatitis B and C infection and liver disease trends among human immunodeficiency virus-infected individuals.** Buskin SE, Barash EA, Scott JD, Aboulaflia DM, Wood RW. *World J Gastroenterol.* 2011 Apr 14;17(14):1807-16.

<http://www.ncbi.nlm.nih.gov/pubmed/21528052>

**AIM:** To examine trends in and correlates of liver disease and viral hepatitis in an human immunodeficiency virus (HIV)-infected cohort. **METHODS:** The multi-site adult/adolescent spectrum of HIV-related diseases (ASD) followed 29 490 HIV-infected individuals receiving medical care in 11 U.S. metropolitan areas for an average of 2.4 years, and a total of 69 487 person-years, between 1998 and 2004. ASD collected data on the presentation, treatment, and outcomes of HIV, including liver disease, hepatitis screening, and hepatitis diagnoses.

**RESULTS:** Incident liver disease, chronic hepatitis B virus (HBV), and hepatitis C virus (HCV) were diagnosed in 0.9, 1.8, and 4.7 per 100 person-years. HBV and HCV screening increased from fewer than 20% to over 60% during this period of observation ( $P < 0.001$ ). Deaths occurred in 57% of those diagnosed with liver disease relative to 15% overall ( $P < 0.001$ ). Overall 10% of deaths occurred among individuals with a diagnosis of liver disease. Despite care guidelines promoting screening and vaccination for HBV and screening for HCV, screening and vaccination were not universally conducted or, if conducted, not documented. **CONCLUSION:** Due to high rates of incident liver disease, viral hepatitis screening, vaccination, and treatment among HIV-infected individuals should be a priority.

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#### COMPLEMENTARY AND ALTERNATIVE MEDICINE

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**A single arm phase II study of a Far-Eastern traditional herbal formulation (sho-sai-ko-to or xiao-chai-hu-tang) in chronic hepatitis C patients.** Deng G, Kurtz RC, Vickers A, Lau N, Yeung KS, Shia J, Cassileth B. *J Ethnopharmacol.* 2011 Apr 17. [Epub ahead of print]

Source

<http://www.ncbi.nlm.nih.gov/pubmed/21527335>

**OBJECTIVES:** Hepatitis C is a major public health problem internationally. Many patients cannot benefit from the current treatment regimen (interferon/ribavirin combinations) due to its side effects or ineffectiveness. Xiao-Chai-Hu-Tang or Sho-sai-ko-to (SST), a compound of seven botanical extracts used for liver diseases traditionally in East Asia, was shown to reduce transaminases and the incidence of hepatocellular carcinoma in hepatitis B patients. We conducted a phase II trial of SST in hepatitis C patients who were not candidates for interferon-based therapy to determine whether this agent is worthy of further study. **METHODS:** Twenty four chronic hepatitis C patients received SST at 2.5g per os (p.o.) three times daily (t.i.d.) for 12 months. Liver function, hepatitis C virus (HCV) viral load and liver biopsy histology were assessed before and after the intervention. **RESULTS:** Improvement of aspartate aminotransferase (AST) was observed in 16 (67%) of study participants. Improvement of alanine aminotransferase (ALT) was seen in 18 (75%) patients. Viral load response was mixed, with 7 patients showing reductions, 10 increases and 7 indeterminate due to assay limitations. Among the 9 (38%) subjects who showed improvement per Knodell's histology activity index (HAI) scores in paired comparison of pre- and post-treatment liver biopsy (the primary endpoints of the study), 5 (21%) showed an improvement of 2 points or greater, meeting the pre-defined criteria for "response. **CONCLUSIONS:** Sho-sai-ko-to (SST or Xiao Chai Hu Tang) may improve liver pathology in selected hepatitis C patients who are not candidates for interferon based treatment. Larger, controlled studies of this botanical formulation may be warranted.

[**Syndrome distribution among patients with chronic hepatitis C and interventions of integrated traditional Chinese and Western medicine: study protocol.**] [Article in Chinese] Nie HM, Gao YQ, Chen JJ. Zhong Xi Yi Jie He Xue Bao. 2011 Apr 15;9(4):365-373. <http://www.ncbi.nlm.nih.gov/pubmed/21486548>

**BACKGROUND:** Chronic hepatitis C is one of the major causes of end-stage liver disease with a high incidence rate, amounting to a grave and serious problem of public health. Currently, interferon-based (with or without ribavirin) antiviral therapy has limited use due to its stringent indications, possible contraindications and side effects. Traditional Chinese medicine (TCM) may have advantages in the prevention and treatment of chronic hepatitis C and it is of significant value to discover the advantages. Through this research, a safe and effective treatment protocol of TCM or integrated TCM and Western medicine for chronic hepatitis C can be formed. To this end, during China's Eleventh Five-Year Plan, special research projects on acquired immune deficiency syndrome (AIDS), viral hepatitis and the other major infectious diseases were established. Our studies on chronic hepatitis C constitute one of the major special research topics. **METHODS AND DESIGN:** Clinical information of patients with chronic hepatitis C will be first collected in a large, multicenter epidemiological survey. Positive symptoms will be analyzed by rapid cluster analysis, principal constituent analysis and factor analysis, and syndrome types will be diagnosed based on expert advice. Concurrently, a large, multicenter, randomized, parallel-group prospective study will be launched based on evidence-based medical principles to evaluate the effects and safety of the treatment protocol for chronic hepatitis C. The evaluated indexes will include the normalization rate of liver function, virological improvement and quality of life improvement for the short-term efficacy and the incidence of liver cirrhosis and (or) primary liver cancer and mortality for the long-term efficacy. **DISCUSSION:** This study will investigate the TCM syndrome differentiation norms and the syndrome distribution rules of chronic hepatitis C and evaluate the efficacy and safety of a treatment protocol for chronic hepatitis C based on TCM theory or combined treatment of TCM and Western medicine. The study results will be helpful to developing a TCM treatment program for chronic hepatitis C. Trial registration: *The research program was registered in the Chinese Clinical Trial Registry in English and Chinese in January 2010.*

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## EPIDEMIOLOGY, DIAGNOSTICS, AND MISCELLANEOUS WORKS

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**Racial differences in hepatitis C treatment eligibility.** Melia MT, Muir AJ, McCone J, et al. Hepatology. 2011 Apr 12. doi: 10.1002/hep.24358. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21488082>

**BACKGROUND AND RATIONALE:** Black Americans are disproportionately infected with hepatitis C virus (HCV) and are less likely than whites to respond to treatment with peginterferon (PEG- IFN) plus ribavirin (RBV). The impact of race on HCV treatment eligibility is unknown. We therefore performed a retrospective analysis of a phase 3B multicenter clinical trial conducted at 118 United States community and academic medical centers to evaluate the rates of and reasons for HCV treatment ineligibility according to self-reported race. **MAIN RESULTS:** 4469 patients were screened, of whom 1038 (23.2%) were treatment ineligible. While blacks represented 19% of treated patients, they were more likely not to be treated due to ineligibility and/or failure to complete required evaluations (40.2%) than were nonblack patients (28.5%;  $P < .001$ ). After the exclusion of persons not treated due to undetectable HCV RNA or non-genotype 1 infection, blacks were 65% less likely than nonblacks to be eligible for treatment (28.1% > 17.0%; relative risk, 1.65; 95% confidence interval, 1.46- 1.87;  $P < .001$ ). Blacks were

more likely to be ineligible due to neutropenia (14% vs 3%,  $P<.001$ ), anemia (7% vs 4%,  $P=.02$ ), elevated glucose (8% vs 3%,  $P<.001$ ), and elevated creatinine (5% vs 1%,  $P<.001$ ).

**CONCLUSIONS:** Largely due to a higher prevalence of neutropenia and uncontrolled medical conditions, blacks were significantly less likely to be eligible for HCV treatment. Increased access to treatment may be facilitated by less conservative neutrophil requirements and more effective care for chronic diseases, namely diabetes and renal insufficiency.

### **A New Combination of Blood Test and Fibroscan for Accurate Non-Invasive Diagnosis of Liver Fibrosis Stages in Chronic Hepatitis C.** Boursier J, de Ledinghen V, Zarski JP, et al.

<http://www.ncbi.nlm.nih.gov/pubmed/21468012>

**OBJECTIVES:** Precise evaluation of the level of liver fibrosis is recommended in patients with chronic hepatitis C (CHC). Blood fibrosis tests and Fibroscan are now widely used for the non-invasive diagnosis of liver fibrosis. Detailed fibrosis stage classifications have been developed to provide an estimation of the liver fibrosis stage from the results of these non-invasive tests. Our aim was to develop a new and more accurate fibrosis stage classification by using new scores combining non-invasive fibrosis tests. **METHODS:** In all, 729 patients with CHC (exploratory set: 349; validation set: 380) had liver biopsy for Metavir fibrosis (F) staging, and 6 fibrosis tests: Fibroscan, Fibrotest, FibroMeter, Hepascore, FIB-4, APRI. **RESULTS:** Exploratory set: Fibroscan and FibroMeter were the independent predictors of different diagnostic targets of liver fibrosis. New fibrosis indexes combining FibroMeter and Fibroscan were thus developed for the diagnosis of clinically significant fibrosis (CSF-index) or severe fibrosis (SF-index). The association of CSF- and SF-indexes provided a new fibrosis stage classification (CSF/SF classification): F0/1, F1/2, F2±1, F2/3, F3±1, F4. Validation set: CSF/SF classification had a high diagnostic accuracy (85.8% well-classified patients), significantly higher than the diagnostic accuracies of FibroMeter (69.7%,  $P<0.001$ ), Fibroscan (63.3%,  $P<0.001$ ), or Fibrotest (43.9%,  $P<0.001$ ) classifications. **CONCLUSIONS:** The association of new fibrosis indexes combining FibroMeter and Fibroscan provides a new fibrosis stage classification. This classification is significantly more accurate than Fibrotest, FibroMeter, or Fibroscan classifications, and improves the accuracy of the non-invasive diagnosis of liver fibrosis stages to 86% without any liver biopsy.

**Quality of life in patients with various liver diseases: patients with HCV show greater mental impairment, while patients with PBC have greater physical impairment.** Tillmann HL, Wiese M, Braun Y, et al. *J Viral Hepat.* 2011 Apr;18(4):252-61. doi: 10.1111/j.1365-2893.2010.01292.x.

<http://www.ncbi.nlm.nih.gov/pubmed/20337922>

Little is known comparing and contrasting quality of life (QoL) in patients with hepatitis C, compared to patients with other liver diseases. We performed two independent prospective cross-sectional studies including 511 and 284 patients with different forms of liver diseases. SF-36 was used in both studies. Fatigue Impact Score, WHO-BREF and Hospital Anxiety and Depression Scale (HADS) were used in either study only. In both studies, HCV-positive patients scored worse in the mental aspects of health-related QoL compared to other liver diseases, except for HBV in one study. Surprisingly, in both studies, quality of life was also significantly impaired in patients with viral clearance after interferon therapy but not after spontaneous clearance. Furthermore, patients with primary biliary cirrhosis showed significantly better mental health but significantly worse physical well-being. Liver diseases differ in their form of impaired QoL. In HCV, this impairment might not always return to normal after treatment-induced viral clearance. This may suggest that HCV either may not be involved in QoL impairment or may induce a process which persists after viral clearance in some patients.

**Barriers to Providing Health Services for HIV/AIDS, Hepatitis C Virus Infection and Sexually Transmitted Infections in Substance Abuse Treatment Programs in the United States.** Bini Deceased EJ, Kritz S, Brown LS Jr, Robinson J, Alderson D, Rotrosen J. J Addict Dis. 2011 Apr;30(2):98-109.

<http://www.ncbi.nlm.nih.gov/pubmed/21491291>

We sought to identify barriers to offering services for HIV/AIDS, hepatitis C virus, and sexually transmitted infections in substance abuse treatment programs. We surveyed treatment program administrators and clinicians within the National Drug Abuse Treatment Clinical Trials Network to evaluate the availability of medical and non-medical services for patients with or at risk for acquiring these infections. A substantial proportion of programs do not offer services (particularly medical services) for these infections. The most commonly cited barriers were funding, health insurance benefits, patient acceptance, and staff training. The findings highlight a missed opportunity to positively impact these infectious disease epidemics.

**Risk factors associated with Hepatitis C among female substance users enrolled in community-based HIV prevention studies.** Nurutdinova D, Abdallah AB, Bradford S, O'Leary CC, Cottler LB. BMC Res Notes. 2011 Apr 14;4(1):126. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21492467>

**BACKGROUND:** Hepatitis C virus (HCV) infection is one of the most frequent chronic blood-borne infections in the United States. The epidemiology of HCV transmission is not completely understood, particularly in women and minorities. **FINDINGS:** We examined the HCV associated risk factors in substance abusing females involved in National Institute on Alcohol Abuse and Alcoholism (NIAAA) and National Institute on Drug Abuse (NIDA) funded HIV prevention studies of street recruited women. As a part of the 12 month follow-up, participants were interviewed about substance use and sexual risk behaviors, including drug implement sharing practices, tattoos, body piercing and blood transfusions and the sharing of personal hygiene equipment including tweezers, toothbrushes and razors. Urine and blood testing for HCV antibody (Ab), HIV and Sexually transmitted diseases (STDs) was conducted at the time of assessment. Among 782 predominantly African American women, 162 (21%) tested positive for HCV Ab. Older age ( $p < 0.001$ ), history of injection drug use ( $p < 0.001$ ), lifetime crack cocaine use ( $p = 0.004$ ) and having a tattoo ( $p = 0.01$ ) were significantly associated with HCV Ab positivity. Other risk factors previously reported in association with HCV Ab positivity such as sexual risk behaviors were not significantly associated with the presence of a positive HCV Ab. **CONCLUSIONS:** This large community based sample of predominantly African American substance abusing women showed high prevalence of HCV Ab positivity and low awareness of their HCV serostatus. Our study demonstrated that in addition to intravenous drug use (IDU), other factors were significantly associated with HCV Ab positivity such as having a tattoo and a lifetime history of crack use. Other potential routes of HCV transmission should be further studied among high risk female populations.

**A comparison of four fibrosis indexes in chronic HCV: Development of new fibrosis-cirrhosis index (FCI).** Ahmad W, Ijaz B, Javed FT, et al. BMC Gastroenterol. 2011 Apr 21;11(1):44. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21507271>

**BACKGROUND:** Hepatitis C can lead to liver fibrosis and cirrhosis. We compared readily available non-invasive fibrosis indexes for the fibrosis progression discrimination to find a better combination of existing non-invasive markers. **METHODS:** We studied 157 HCV infected patients who underwent liver biopsy. In order to differentiate HCV fibrosis progression, readily

available AAR, APRI, FI and FIB-4 serum indexes were tested in the patients. We derived a new fibrosis-cirrhosis index (FCI) comprised of ALP, bilirubin, serum albumin and platelet count.  $FCI = [(ALP \times Bilirubin) / (Albumin \times Platelet \text{ count})]$ . **RESULTS:** Already established serum indexes AAR, APRI, FI and FIB-4 were able to stage liver fibrosis with correlation coefficient indexes 0.130, 0.444, 0.578 and 0.494, respectively. Our new fibrosis cirrhosis index FCI significantly correlated with the histological fibrosis stages F0-F1, F2-F3 and F4 ( $r = 0.818$ ,  $p < 0.05$ ) with AUROCs 0.932 and 0.996, respectively. The sensitivity and PPV of FCI at a cutoff value  $< 0.130$  for predicting fibrosis stage F0-F1 was 81% and 82%, respectively with AUROC 0.932. Corresponding value of FCI at a cutoff value [greater than or equal to]1.25 for the prediction of cirrhosis was 86% and 100%. **CONCLUSIONS:** The fibrosis-cirrhosis index (FCI) accurately predicted fibrosis stages in HCV infected patients and seems more efficient than frequently used serum indexes.

**Potential role of safer injection facilities in reducing HIV and Hepatitis C infections and overdose mortality in the United States.** Semaan S, Fleming P, Worrell C, Stolp H, Baack B, Miller M. *Drug Alcohol Depend.* 2011 Apr 22. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21515001>

**BACKGROUND:** Safer injection facilities (SIFs) reduce risks associated with injecting drugs, particularly public injection and overdose mortality. They exist in many countries, but do not exist in the United States. We assessed several ethical, operational, and public health considerations for establishing SIFs in the United States. **METHOD:** We used the six-factor Kass framework (goals, effectiveness, concerns, minimization of concerns, fair implementation, and balancing of benefits and concerns), summarized needs of persons who inject drugs in the United States, and reviewed global evidence for SIFs.

**RESULTS:** SIFs offer a hygienic environment to inject drugs, provide sterile injection equipment at time of injection, and allow for safe disposal of used equipment. Injection of pre-obtained drugs, purchased by persons who inject drugs, happens in a facility where trained personnel provide on-site counseling and referral to addiction treatment and health care and intervene in overdose emergency situations. SIFs provide positive health benefits (reducing transmission of HIV and viral hepatitis, bacterial infections, and overdose mortality) without evidence for negative health or social consequences. SIFs serve most-at-risk persons, including those who inject in public or inject frequently, and those who do not use other public health programs. It is critical to address legal, ethical, and local concerns, develop and implement relevant policies and procedures, and assess individual- and community-level needs and benefits of SIFs given local epidemiologic data. **CONCLUSIONS:** SIFs have the potential to reduce viral and bacterial infections and overdose mortality among those who engage in high-risk injection behaviors by offering unique public health services that are complementary to other interventions.

**Abbott RealTime PCR assay is useful for evaluating virological response to antiviral treatment for chronic hepatitis C.** Ikezaki H, Furusyo N, Ihara T, et al. *J Infect Chemother.* 2011 Apr 29. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21528383>

This study was done to evaluate the utility of the Abbott RealTime PCR assay (ART) for the monitoring of chronic hepatitis C patients. The serum samples of 183 patients infected with hepatitis C virus (HCV) genotype 1b who had completed a 48-week period of pegylated interferon (PEG-IFN) alpha-2b plus ribavirin treatment were prospectively analyzed. Serum HCV RNA levels were measured both by ART and by the Roche COBAS Amplicor Monitor test, version 2.0 (CAM) at baseline and at weeks 4, 12, 24, 36, and 48 of treatment, and at 24

weeks after the end of treatment (EOT). A significant positive correlation of pretreatment HCV RNA levels was found between ART and CAM ( $r = 0.595$ ,  $P < 0.0001$ ). Of the 183 patients, 66 (36.0%) achieved a sustained virological response (SVR). The logarithmic decline of the HCV RNA level from the pretreatment level determined by ART in SVR patients was significantly higher than that in non-SVR patients at all time points tested. The logarithmic decline determined by CAM in SVR patients was significantly higher than that in non-SVR patients only at week 4, but there was no significant difference at other weeks. Of 124 patients who were HCV RNA-negative at EOT by ART, 58 (46.8%) had a relapse of viremia at 24 weeks after EOT, whereas 77 of 143 patients (53.8%) who were HCV RNA-negative at EOT by CAM had a relapse. The relapse rate was lower when determined by ART than by CAM, but not significantly so. ART is more useful than CAM for evaluating the virological response to antiviral treatment for chronic hepatitis C.

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## LIVER CANCER

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**A comparison of prognosis between patients with hepatitis B and C virus-related hepatocellular carcinoma undergoing resection surgery.** Kao WY, Su CW, Chau GY, Lui WY, Wu CW, Wu JC. *World J Surg.* 2011 Apr;35(4):858-67.

<http://www.ncbi.nlm.nih.gov/pubmed/21207029>

**BACKGROUND:** The impact of viral factors on the prognosis of hepatocellular carcinoma (HCC) remains controversial because of heterogeneous populations included in previous reports. This study aims to compare clinicopathologic features and prognoses between patients with hepatitis B- and hepatitis C-related HCC who underwent resection surgery.

**METHODS:** We enrolled 609 patients with positive serum hepatitis B virus (HBV) surface antigen (HBsAg) and negative serum antibody against hepatitis C virus (anti-HCV) as the B-HCC group and 206 patients with negative serum HBsAg and positive anti-HCV as the C-HCC group. The overall survival rates and cumulative recurrence rates were compared between these two groups. **RESULTS:** B-HCC patients were significantly younger, predominantly male, had better liver functional reserve, but more advanced tumor stage than C-HCC patients. After a median follow-up period of 40.6 months, 427 patients had died. Furthermore, 501 patients had tumor recurrence after surgery. The postoperative overall survival rates ( $p = 0.640$ ) and recurrence rates ( $p = 0.387$ ) of the two groups were comparable. However, the overall survival rate was higher in the B-HCC group than in the C-HCC group in the cases of transplantable HCC ( $p = 0.021$ ) and Barcelona-Clinic Liver Cancer stage A HCC ( $p = 0.040$ ). **CONCLUSIONS:** Viral etiologies were not apparent in determining outcomes of HCC patients who underwent resection due to heterogeneous studied populations. In early-stage HCC, B-HCC patients had better outcomes than C-HCC patients did because of better liver reserve and less hepatic inflammation.

**Percutaneous Radiofrequency Ablation for Pulmonary Metastases from Hepatocellular Carcinoma: Results of a Multicenter Study in Japan.** Hiraki T, Yamakado K, Ikeda O, et al. *J Vasc Interv Radiol.* 2011 Apr 29. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21531575>

**PURPOSE:** To retrospectively evaluate technical success, effectiveness, complications, patient survival, and prognostic factors with percutaneous radiofrequency (RF) ablation for pulmonary metastases resulting from hepatocellular carcinoma (HCC).

**MATERIALS AND METHODS:** Thirty-two patients from six institutions were included, with a total of 83 pulmonary metastases treated in 65 sessions. RF ablation was always performed percutaneously with computed tomography (CT) guidance. Primary endpoints were technical

success and technique effectiveness. Technique effectiveness was evaluated based on sequential follow-up CT images. Secondary study endpoints were complications, patient survival, and determination of prognostic factors. Complications were classified as major or minor. Prognostic factors were determined by analyzing multiple variables with the log-rank test.

**RESULTS:** Technical success rate was 100%. Primary technique effectiveness rates were 92% each at 1, 2, and 3 years. Major and minor complications occurred after 16 (25%) and 23 (35%) of the 65 sessions, respectively. The median follow-up period was 20.5 months. Overall survival rates were 87% at 1 year and 57% each at 2 and 3 years (median and mean survival times, 37.7 mo and 43.2 mo, respectively). Significantly better survival rates were obtained in cases of (i) no viable intrahepatic recurrence ( $P < .001$ ), (ii) Child-Pugh class A disease ( $P < .001$ ), (iii) absence of liver cirrhosis ( $P < .001$ ), (iv) absence of hepatitis C virus infection ( $P = .006$ ), and (v)  $\alpha$ -fetoprotein level of 10 ng/mL or lower ( $P = .007$ ) at the time of RF ablation.

**CONCLUSIONS:** RF ablation appears effective, with an acceptable safety profile, in selected patients with pulmonary metastases resulting from HCC.

**A comprehensive analysis of the dynamic biological networks in HCV induced hepatocarcinogenesis.** He B, Zhang H, Shi T. PLoS One. 2011 Apr 19;6(4):e18516.

<http://www.ncbi.nlm.nih.gov/pubmed/21526182>

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver, which is closely related to hepatitis C and cirrhosis. The molecular mechanisms underlying the hepatocarcinogenesis induced by HCV infection remain clarified from a standpoint of systems biology. By integrating data from protein-protein interactions, transcriptional regulation, and disease related microarray analysis, we carried out a dynamic biological network analysis on the progression of HCV induced hepatocarcinogenesis, and systematically explored the potentially disease-related mechanisms through a network view. The dysfunctional interactions among proteins and deregulatory relationships between transcription factors and their target genes could be causes for the occurrence and progression of this disease. The six pathologically defined disease stages in the development and progression of HCC after HCV infection were included in this study. We constructed disease-related biological networks for each disease stage, and identified progression-related sub-networks that potentially play roles in the developmental stage of the corresponding disease and participate in the later stage of cancer progression. In addition, we identified novel risk factors related to HCC based on the analysis of the progression-related sub-networks. The dynamic characteristics of the network reflect important features of the disease development and progression, which provide important information for us to further explore underlying mechanisms of the disease.

**The toll-like receptor 2 (TLR2) -196 to -174 del/ins polymorphism affects viral loads and susceptibility to hepatocellular carcinoma in chronic hepatitis C.** Nischalke HD, Coenen M, Berger C, et al. Int J Cancer. 2011 Apr 15. doi: 10.1002/ijc.26143. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21500195>

Chronic hepatitis C virus (HCV) infection is a major risk factor for hepatocellular carcinoma (HCC). HCV proteins core and NS3 can bind to TLR2 and trigger inflammatory responses. Polymorphisms in the TLR2 gene predispose to various forms of malignancy but have not been studied in HCV-associated HCC. Here, we investigated whether single nucleotide polymorphisms (SNPs), rs4696480, rs5743708, rs5743704 and the -196 to -174del/ins polymorphism of the TLR2 gene affect the risk for HCC in chronic hepatitis C. The study involved 189 and 192 HCV genotype 1 infected patients with and without HCC, respectively, as well as 347 healthy controls. TLR2 alleles were determined by hybridisation probe assays and allele-specific short fragment polymerase chain reaction on a LightCycler system. All TLR2

polymorphisms matched the Hardy-Weinberg equilibrium in each study group. While TLR2 SNPs showed no effect, the frequency of the TLR2 -196 to -174del allele was significantly higher in patients with HCV-associated HCC (22.5%) than in HCV-infected patients without HCC (15.6%,  $p=0.016$ ) and healthy controls (15.3%,  $p=0.003$ ). HCV-infected carriers of a TLR2 -196 to -174del allele had significantly higher HCV viral loads than TLR2 -196 to -174ins/ins homozygous patients ( $p=0.031$ ). Finally, in carriers of the TLR2 -196 to -174del allele stimulation of monocytes resulted in significantly lower TLR2 expression levels and IL-8 induction than in individuals with the TLR2 -196 to -174ins/ins genotype ( $p<0.05$ ). Our data suggest the TLR2 -196 to -174del allele to affect HCV viral loads and to increase the risk for HCC in HCV genotype 1-infected patients.

**Altered cytokine levels and increased CD4+CD57+ T cells in the peripheral blood of hepatitis C virus-related hepatocellular carcinoma patients.** Shiraki T, Takayama E, Magari H, et al. *Oncol Rep.* 2011 Apr 12. doi: 10.3892/or.2011.1258. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/21491089>

Although CD57+ lymphocytes are closely correlated with prognosis in various cancers, the role of subsets of CD57+ cells in hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) is unclear. In the present study, peripheral blood (PB) from HCV-related HCC patients was analyzed. Plasma cytokine levels and in vitro cytokine-producing capabilities were analyzed with enzyme-linked immunosorbent assays, and CD57+ cell subsets were studied using a multi-color FACS system. Interferon (IFN)- $\gamma$  was undetectable in the plasma of patients with tumors at any stage, whereas the plasma levels of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-10 and IL-18, but not that of IL-12, were significantly higher in stage IV patients compared to patients with earlier-stage tumors. In contrast, the IFN- $\gamma$ -producing capability of PB was highest in stage I patients and gradually decreased with tumor progression. The IL-10-, IL-18- and IL-12-producing capabilities of PB increased from stage I to III. However, PB-TNF- $\alpha$ , IL-10- and IL-18-producing capabilities were reduced in stage IV patients, probably due to repeated anti-cancer treatments. The percentage of CD4+CD57+ $\alpha\beta$ TCR+ cells (CD4+CD57+ T cells) in peripheral blood lymphocytes (PBLs) increased with tumor progression. Moreover, the percentage of CD4+CD57+ T cells in PBLs and the ratio of CD4+CD57+ T cells to CD4+ $\alpha\beta$ TCR+ cells (CD4+ T cells), but not that of CD4+CD57+ T cells to CD57+ $\alpha\beta$ TCR+ cells (CD57+ T cells), showed a significant inverse correlation with PB-IFN- $\gamma$ -producing capability. **The present results suggest** that an increase in CD4+CD57+ T cells controls the capability of PB to produce the anti-tumor cytokine IFN- $\gamma$  and that PB-IFN- $\gamma$  production is impaired with HCC tumor progression.

**Impact of radiation and hepatitis virus infection on risk of hepatocellular carcinoma.**

Ohishi W, Fujiwara S, Cologne JB, et al. *Hepatology.* 2011 Apr;53(4):1237-45. doi: 10.1002/hep.24207.

<http://www.ncbi.nlm.nih.gov/pubmed/21480328>

In cohort studies of atomic bomb survivors and Mayak nuclear facility workers, radiation-associated increases in liver cancer risk were observed, but hepatitis B virus (HBV) and hepatitis C virus (HCV) infections were not taken strictly into account. We identified 359 hepatocellular carcinoma (HCC) cases between 1970 and 2002 in the cohort of atomic bomb survivors and estimated cumulative incidence of HCC by radiation dose. To investigate contributions of radiation exposure and hepatitis virus infection to HCC risk, we conducted a nested case-control study using sera stored before HCC diagnosis in the longitudinal cohort of atomic bomb survivors. The study included 224 HCC cases and 644 controls that were matched to the cases on gender, age, city, and time and method of serum storage, and counter-matched on radiation dose.

The cumulative incidence of HCC by follow-up time and age increased significantly with radiation dose. The relative risk (RR) of HCC for radiation at 1 Gy was 1.67 (95% confidence interval: 1.22-2.35) with adjustment for alcohol consumption, body mass index (BMI), and smoking habit, whereas the RRs for HBV or HCV infection alone were 63 (20-241) and 83 (36-231) with such adjustment, respectively. Those estimates changed little when radiation and hepatitis virus infection were fit simultaneously. The RR of non-B, non-C HCC at 1 Gy was 1.90 (1.02-3.92) without adjustment for alcohol consumption, BMI, or smoking habit and 2.74 (1.26-7.04) with such adjustment. **CONCLUSION:** These results indicate that radiation exposure and HBV and HCV infection are associated independently with increased HCC risk. In particular, radiation exposure was a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption, BMI, or smoking habit.