

and NIM811 to affect HBV replication and viral particle secretion from the cells.

Method HepG2215 cells, stably transfected with the full HBV genome and supporting the production of both infectious virions and HBsAg particles, and the parent cells (HepG2) were cultured for 7 days (baseline), prior to treatment with DEB025 or NIM811 at 0.25; 1.0 and 5.0 mg/ml. The cells and supernatants were harvested separately at baseline; 6, 24, 48 and 72 h after addition of cyclophilin inhibitors. HBV DNA levels - both intracellular and in culture supernatants—were quantitated by Taqman qPCR (ABI7500). Western blot and ELISA were used to assess intracellular and secreted HBsAg, respectively. PLC/PRF/5 cells, expressing only HBsAg, were also tested. Cyclophilin expression in the cells was silenced by transfection separately with siRNA for cyclophilins A, B, C or D to determine the role of individual cyclophilins in HBV replication.

Results Cyclophilin inhibition with either DEB025 or NIM811 significantly reduced cytoplasmic core-particle associated HBV DNA levels in the cells, between 2 and 10-fold as compared with the control cells. The most pronounced reduction of intracellular HBV DNA (by 10-fold at 72 h) was observed with DEB025 5 mg/ml, which was greater than the reduction observed with NIM811. Similarly, DEB025 (at 1 and 5 mg/ml) showed a greater impact in reducing HBV virion secretion in the supernatants, compared with NIM811. HBsAg secretion from the cells was also reduced by up to 50% when compared to controls. Cyclophilin-A expression was markedly reduced after transfection with corresponding siRNA, which led to a rapid decrease of intracellular HBV DNA by 2 logs. HBV-DNA was reduced further when the cyclophilin-A silenced cultures were treated with NIM811 or DEB025.

Conclusion These results demonstrate that cyclophilin A is directly involved in HBV replication. Cyclophilin inhibition by DEB025 or NIM811 interferes with HBV replication within liver cells and reduces the secretion of infectious virions and HBsAg particles from the cells, with DEB025 having a greater antiviral activity than NIM811.

P44 USE OF INTRALIPID INFUSION TO ANALYSE APOLIPOPROTEIN B (APOB) AND HCV RNA KINETICS IN CHRONIC INFECTION

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Introduction Production of infectious HCV is dependent on hepatocytes VLDL assembly, maturation, and secretory machinery. ApoB-100 is the structural apolipoprotein of large VLDL (VLDL1), small VLDL2, and LDL. Although HCV production is dependent on VLDL, chronic HCV infection clinically manifests in lower VLDL and LDL levels, particularly in genotype 3.

Aim To determine the production and clearance rates of apoB and triglyceride (TG) in VLDL1 in chronic HCV infected patients compared to uninfected volunteers. In addition, to observe if altering the VLDL1 kinetics would affect low-density HCV RNA quantities.

Method VLDL1 kinetics were analysed using a protocol involving an IV infusion of a chylomicron-like lipid emulsion (Intralipid) for 120 min to prevent the clearance of VLDL1 by lipoprotein lipase.¹ Multiple blood samples were taken during and for 4 h after the infusion. Lipoprotein kinetics were examined by cumulative flotation ultracentrifugation and the clearance of HCV RNA

from different density fractions was studied by iodixanol gradient ultracentrifugation.²

Results VLDL1 TG production rate was lower for HCV patients [n=6] compared to healthy subjects [n=10], but the production rate of VLDL1 apoB was similar. Chronic HCV patients cleared Intralipid at a slower rate than uninfected controls.

Plasma HCV RNA accumulated linearly in the serum during the 6 h experiment [14% increase per hour], indicating that Intralipid infusion either stimulated virion production, diminished virion clearance or both. Immediately after the Intralipid infusion ceased, triglyceride cleared exponentially, but at a slower rate than in uninfected individuals. HCV RNA in a very-low density fraction (VLDL, d<1.025 g/ml) also immediately cleared, but linearly, paralleling VLDL1 clearance (t_{1/2}=77 min). However, HCV RNA in the high density fraction continued to accumulate [19.8% increase per hr] during the post-infusion period.

Conclusion VLDL1 TG production and clearance rates are impaired in patients with chronic HCV infection. HCV associates with large TG-rich lipoproteins in vivo, and clears from the plasma via this route. However, competitive inhibition of lipoprotein clearance results in accumulation of HCV particles in the vascular compartment, particularly those that lack association with TG-rich lipoproteins. Intralipid effectively uncouples the interaction of higher density de novo produced particles, with VLDL. We hypothesise that HCV particles need to transfer onto very low density 'acceptor' particles to facilitate clearance via the remnant pathway.

REFERENCES

1. Al-Shayji IAR, et al. *J Lipid Res* 2007;**48**:2086.
2. Felmllee DJ, et al. *Gastro* 2010;**139**:1774.

P45 NATURAL KILLER CELL CYTOTOXICITY IS ENHANCED IN INJECTION DRUG USERS WITH APPARENT RESISTANCE TO HEPATITIS C VIRUS INFECTION

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Introduction We have identified a cohort of injection drug users (IDU) who, despite long-term high risk sharing of drug injection equipment, remain seronegative and aviraemic for HCV. These exposed uninfected (EU) IDU appear resistant to HCV infection. This resistance is associated with the same Natural Killer (NK) immunoglobulin-like receptor (KIR)/HLA genotype known to favour spontaneous HCV clearance. Whether there is a functional alteration in NK cell cytotoxicity in these individuals is unknown.

Aim To investigate cytotoxic function of NK cells in EU.

Method Peripheral blood mononuclear cells (PBMC) were isolated from 16 EU (HCV-Ab positive and HCV-RNA negative), 10 IDU with untreated chronic HCV (cHCV) and five IDU with spontaneous resolution (SR) of HCV (HCV-Ab positive but HCV RNA negative). NK cytotoxicity was assessed against the NK-sensitive K562 cell line. PBMC incubated for 48 h with or without interleukin-2 (IL-2) were co-cultured with K562 for 4 h at an E:T ratio of 10:1. Flow cytometry was used to assess the frequency of NK cells [CD56(+) CD3(-)] in PBMC and cytotoxicity quantified by CFSE/7-AAD co-staining and expressed as the percentage of cells lysed.

Results Natural killer cell cytotoxicity, in the absence of IL-2 stimulation, was no different between the groups. With IL-2 stimulation, EU demonstrated significantly higher cytotoxicity compared to cHCV ($32.8 \pm 4.4\%$ vs $17.6 \pm 3.2\%$, $p=0.023$), with similar levels to SR ($27.7 \pm 9.9\%$, $p=0.50$). The proportion of NK cells in PBMC was not significantly different between the groups.

Conclusion The current findings point to enhanced NK cytotoxicity in EU cases compared to those with chronic infection and suggests a role for NK cells in early viral clearance and resistance to HCV infection.

12.48 and 12.69%) compared to median CAF scores in patients with no outcomes (8.59, 8.32 and 8.10%) at years 3, 5 and 7 respectively.

Conclusion Clinical outcomes represent realistic and meaningful end-points for future trials evaluating anti-fibrotic agents once advanced fibrosis has developed. Further development and validation of morphometry within advanced fibrosis could enable better identification of patients at risk of more rapid progression of liver disease than Ishak stage alone.

P46 THE PREDICTION OF LIVER RELATED OUTCOMES USING HISTOLOGICAL TOOLS AS AN ENDPOINT FOR STUDIES EVALUATING ANTI-FIBROTIC THERAPIES

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Introduction Liver related outcomes (LRO) represent a meaningful end-point for future anti-fibrotic therapies. Staging of liver fibrosis on histology is a surrogate for these outcomes but may not be an ideal tool.

Aim Our initial aim was to determine liver related outcomes and survival from the individual stages of significant fibrosis. Our second aim was to assess the value of morphometric collagen quantification within cirrhosis to predict clinical outcomes.

Method The study cohort was selected from a single centre within the Trent HCV study, a prospective cohort which began in 1991 to address the natural history of chronic hepatitis C. Inclusion criteria for this study were the presence of significant fibrosis (at least Ishak Stage (IS) three on biopsy) and at least 3-year follow-up post biopsy. LRO was defined as decompensation (variceal bleeding, ascites, encephalopathy), HCC, liver transplant and liver-related death. Automated morphometry was performed to measure the Collagen Area Fraction (CAF). Survival at 3, 5 and 7 years respectively was evaluated.

Results The study cohort comprised 155 patients (70% male; mean age 49 years). The median follow-up time was 78 months. A LRO occurred in 48 patients (31.0%, estimated annual incidence 5.2%). HCC developed in 16 patients (10.6%, estimated annual incidence 1.6%) liver-related death occurred in 34 patients (21.9%, estimated annual incidence 3.3%); clinical decompensation developed in 20 patients (13.3%, estimated annual incidence 2.1%). See Abstract P46 table 1. CAF was measured in a subgroup of 89 patients. The median CAF was calculated for each Ishak stage and increased progressively towards the more advanced stages. (IS 3: median CAF 3.7%, IQR 1.5–5.1; IS 4: median CAF 5.2%, IQR 2.8–7.4; IS 5: median CAF 6.8%, IQR 3.4–9.5; IS 6: median CAF 9.9%, IQR 6.2–15.7). Within Ishak stage 6 the median CAF scores predicting LRO were higher (13.89,

P47 DE-NOVO ANTIVIRAL THERAPY WITH NUCLEOS(T)IDE ANALOGUES IN 'REAL-LIFE' PATIENTS WITH CHRONIC HEPATITIS B INFECTION: COMPARISON OF VIROLOGICAL RESPONSES BETWEEN LAMIVUDINE + ADEFOVIR VS ENTECAVIR VS TENOFOVIR THERAPY

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Introduction Several nucleos(t)ide analogues (NA) are approved for the treatment of chronic hepatitis B (CH-B); all aim to control HBV replication with minimal risk of drug-resistance and toxicity. Limited comparative data exist assessing differences between viral responses to different de-novo therapeutic regimens in real-life cohorts.

Aim To assess and compare virological and serological responses in 3 real-life CH-B de-novo therapeutic cohorts—lamivudine 100 mg/d + adefovir 10 mg/d (LAM+ADV) combination therapy vs entecavir 0.5 mg/d (ETV) vs tenofovir 245 mg/d (TDF) monotherapies.

Method Patients: NA therapy naive 406 CH-B patients treated at a single-centre practice [median 30 months (m), range 3–72] were split into three groups according therapy regimen: LAM+ADV (n=192, 78% males, median age 40 y, 35% HBeAg+, 34% cirrhosis, median duration 36 months), ETV (n=154, 79% males, median age 42 y, 31% HBeAg+, 34% cirrhosis, median duration 28 months) and TDF (n=60, 50% males, median age 40 y, 23% HBeAg+, 25% cirrhosis, median duration 9 months). HBV DNA viral load tested by real-time PCR [\log_{10} IU/ml], serology for HBeAg/HBsAg were compared between baseline, months 3, 6, 9 and 12. Five responses, evaluated by change in serum HBV DNA, were recorded: (1) complete (CR) <12 IU/ml; (2) partial (PR), fall $>3\log_{10}$ but >12 IU/ml; (3) slow (SR), fall 2–3 \log_{10} ; (4) non-response (NR), fall $<1 \log_{10}$; (5) viral breakthrough (VB), rise $>1 \log_{10}$ from nadir. HBV genotypic resistance was tested pre-treatment and at the time of SR, NR or VB by direct sequencing.

Results Baseline HBV DNA was similar in all cohorts (median \log_{10} 4.6 vs 4.4 vs 4.2 IU/ml), higher proportions achieved CR in TDF cohort than LAM+ADV and ETV (m3: 78% vs 48% and 53%, $p<0.01$; m6: 82% vs 60% and 65%, $p=0.02$; m9: 86% vs 62% and 55%, $p<0.01$), but were similar at m12: 80% vs 73% and 76% and there were no differences in PR, SR and NR in all groups. HBV DNA

Abstract P46 Table 1 LRO and survival by Ishak Stage

Ishak Stage	Annual Incidence of LRO (%)	HR of LRO (95% CI, p value)	3 year survival (%)	5 years survival (%)	7 years survival (%)
3	0.7%	Ref.	97.7 (84.6, 99.7)	97.7 (84.6, 99.7)	94.1 (77.6, 98.5)
4	3.2%	4.76 (0.87 to 26.016, 0.0715)	93.8 (63.2, 99.1)	87.1 (57.3, 96.6)	78.4 (46.0, 92.6)
5	5.1%	6.996 (1.578 to 31.019, 0.0105)	94.4 (79.6, 98.6)	82.8 (65.6, 91.9)	68.2 (48.6, 81.7)
6	11.0%	15.986 (3.816 to 66.961, 0.0001)	68.8 (54.6, 79.3)	53.3 (38.6, 66.0)	41.8 (26.9, 56.0)