

144 but were not associated with HBV DNA rebound; in vitro phenotypic analysis of these mutations is ongoing.

Conclusion: ADV resistance mutations (rtN236T and rtA181V) emerged at a delayed rate and with a low frequency (cumulative probability of 3.9%) after 144 weeks of therapy in all patients in this analysis.

58 **INHIBITION OF HEPATITIS C VIRUS TRANSLATION AND SUBGENOMIC REPLICATION BY SMALL INTERFERING RNAS DIRECTED AGAINST CELLULAR RNA BINDING PROTEINS**

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Hepatitis C Virus (HCV) translation and replication is supported by several cellular RNA binding proteins. Polypyrimidine tract-binding protein (PTB) has previously been shown to stimulate HCV IRES-mediated translation by binding to the HCV 3' untranslated region (3'UTR). Hu antigen R (HuR) binds to A+U rich regions and protects mRNA against degradation. Modulation of the HCV IRES activity by proteasome α -subunit PSMA7 has been shown previously. RNA interference for destruction of these genes might substantially inhibit HCV replication and translation. The aim of our study was to examine the effects of siRNA-mediated inhibition of cellular RNA binding proteins on HCV IRES-mediated translation and HCV subgenomic replication. A panel of siRNA molecules directed against PTB, HuR and PSMA7 were expressed from a U6-promoter within a retroviral vector construct. SiRNA plasmids were cotransfected with a bicistronic reporter construct (Renilla-HCV IRES-Firefly-poly(U/C) tract-3'UTR) into Huh7 cells. Compared with a control siRNA, a reduction in luciferase activity of up to 40% was observed for selected siRNAs. SiRNA expression plasmids were transfected in Huh7 cells expressing monocistronic subgenomic HCV replicon. Northern blot analysis revealed reduction of up to 60% in HCV replicon RNA by siRNAs targeting HuR and PSMA7 RNA. In contrast, siRNAs targeting PTB mRNA revealed no significant effects. PSMA7- and HuR-directed siRNAs reduced HCV NS5B protein levels in Western blotting analyses up to 40%. These results demonstrate that HCV IRES-mediated translation and HCV subgenomic replication can be inhibited by depletion of HCV cofactors, especially PSMA7 and HuR.

59 **VX-950, A NOVEL HCV PROTEASE INHIBITOR, RETAINS POTENCY AGAINST BILN-2061 RESISTANT REPLICON CELLS: COMPUTATIONAL ANALYSIS INDICATES THAT RESISTANCE DEVELOPS VIA DIFFERENT MECHANISMS**

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We have used a structure-based approach to design small molecule inhibitors of the HCV NS3A protease as potential candidates for new, more effective anti-HCV therapies. VX-950 was recently selected as a clinical development candidate for treatment of Hepatitis C infection. In this report, we describe in vitro resistance studies on VX-950 and BILN-2061, another HCV protease inhibitor from Boehringer Ingelheim, using a sub-genomic replicon system. Distinct drug-resistant mutations were identified for both HCV serine protease inhibitors. The major BILN-2061-resistant mutations remain fully susceptible to VX-950, and the primary resistance mutation against VX-950 remains sensitive to BILN-2061. Our structural analysis suggests that resistance develops in response to VX-950 and BILN 2061 via different mechanisms. Cross-resistance mutations were identified under selection with both HCV protease inhibitors. Characterization of the enzymatic and anti-viral properties of the mutations that confer cross-resistance to both VX-950 and BILN-2061 will be presented.

60 **METABOLIC SIGNIFICANCE OF HEPATIC STEATOSIS IN NON ALCOHOLIC FATTY LIVER DISEASE AND HCV CHRONIC HEPATITIS**

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Background: Steatosis, the hallmark of Non Alcoholic Fatty Liver Disease (NAFLD), is a common histological finding also in patients with chronic HCV hepatitis, but the mechanism(s) of fat accumulation might be different.

Aims: To compare insulin sensitivity, glucose and lipid metabolism in HCV and NAFLD patients with similar degree of liver steatosis and fibrosis.

Methods: Insulin sensitivity, glucose and glycerol turnover were evaluated by euglycemic clamp technique (insulin infusion rate: 1 mU/kg.min) combined with tracers infusion (2H₂glucose and 2H₅glycerol) in 4 lean, non-diabetic HCV patients, 5 matched NAFLD patients and 4 healthy controls.

Results: Insulin stimulated glucose disposal was markedly reduced in NAFLD compared with HCV patients (M=2.96±1.62 mg/kg.min vs 6.10±1.80 mg/kg.min, P=0.017) and controls (M=7.91±0.34 mg/kg.min, P=0.00001). Hepatic glucose production (HGP) was similar in the three groups in the basal state, but less efficiently inhibited by insulin both in HCV and NAFLD (HGP during clamp=0.31±0.36 and 0.29±0.15 mg/kg.min vs 0.09±0.03 in controls; P=0.06). In the basal state, glycerol Ra was increased in NAFLD compared with both HCV and controls (2.58±0.48 vs 1.77±0.76 and 1.50±0.23 respectively, P=0.05) and less suppressed by insulin. The amount of hepatic steatosis was directly related to basal glycerol turnover in NAFLD (r=0.63) and inversely in HCV (r=-0.60).

Conclusions: Liver steatosis a) in NAFLD is associated with peripheral insulin resistance and increased systemic lipolysis, b) in HCV with normal peripheral insulin sensitivity and reduced lipid turnover. These findings suggest that fat accumulation is secondary to increased lipid delivery to the liver in NAFLD, to reduced lipid dismission from the liver in HCV.