post-infection, to ducks inoculated with 5×10^8 DHBV genomes. The antiviral effect of REP 9AC treatment was assessed by monitoring serum viremia by quantitative PCR and serum and liver DHB sAg by ELISA or immunohistochemistry. Short-term daily (14 days) REP 9AC treatment starting 1 day prior to infection resulted in undetectable DHBV infection in 80% of ducks 53 days after cessation of treatment. The same duration of daily REP 9AC treatment starting 4 days after DHBV infection, when 2–6% of hepatocytes are typically infected led to undetectable levels of DHBV DNA in 100% of ducks within 10 days after the initiation of REP 9AC treatment and remained undetectable in 75% of ducks for 49 days after treatment. REP 9AC treatment in fully infected ducks was started 14 days after DHBV infection. Ducks received 7 daily, followed by 7 weekly doses. This treatment resulted in rapid decreases (>3 log) in serum DHBV DNA at the end of the daily treatment in all ducks. However, only 20% of the ducks had a sustained virological response 49 days after cessation of treatment. These data show that REP 9AC treatment can control post-infection spread of DHBV in Pekin ducks and can result in sustained virologic responses after treatment is halted. Thus, REP 9AC might be a valuable monotherapy or an adjunct to nucleoside analogue therapy for HBV infection. An expanded therapeutic study with longer treatment duration and followup is in progress.

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Characterization of the Mechanism of Action of PG-301029: A Novel Late Stage Inhibitor of HCV Replicon Replication

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PG-301029 is a small molecule that was identified as an effective inhibitor (EC_{50} value of 2.8 μM) of bovine viral diarrhea virus (BVDV). Subsequent research suggested a novel mechanism of antiviral action that resulted in a highly significant reduction of viral RNA synthesis that was not related to inhibition of virus entry, IRES translation initiation, inhibition of the NS2/3 or NS3/4A viral proteinase activities, or the NS5B viral RNA-dependent RNA polymerase. PG-301029 was 100- to 200-fold less toxic than ribavirin to cells infected with BVDV, and facilitated a reduction in the toxicity of ribavirin in combination anti-BVDV assays. PG-301029 was also shown to be nontoxic to fresh human hepatocytes at the highest concentration tested (1.33 mM). These data suggest the possibility of using the compound as a monotherapy or in combination with ribavirin, or ribavirin plus interferon for treatment of HCV. In addition, serial passage of BVDV-infected cells in the presence of escalating doses of PG-301029 failed to select for resistant virus, suggesting a high genetic barrier for resistance and that the antiviral activity may be mediated through a cellular target. Subsequent research demonstrated that PG-301029 was efficacious in an HCV replicon system, yielding an EC_{50} value of 1.5 μM and a TC_{50} greater than 42 mM. Attempts to select PG-301029-resistant HCV replicons containing a neomycin selectable marker have demonstrated that culture of the replicon-producing cells in the presence of G418 and 7.5 μM PG-301029 resulted in replicons with three potential resistance engendering mutations—one in NS3 and two in NS5A. Based on these data, we hypothesize that PG-301029 inhibits HCV replication through a novel late stage mechanism of action. Hypothesis driven research is currently focused on the identification of the mechanism of action of PG-301029 using the HCV replicon system. Results will be presented detailing characterization of HCV replicons containing the putative resistance engendering mutations, the effect of PG-301029 on HCV RNA synthesis and stability, and gene expression examining the differential expression of host cell genes in PG-301029 treated Huh-7 cells and Huh-7 cells transfected with HCV replicons.

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Predictive Factors to Response to Interferon Therapies for Treatment-naive Patients Infected with Hepatitis C Virus in Islamabad and Vicinity Areas: A Study from Pakistan

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Viral load determinations can give an idea of disease severity. The initial viral load before any treatment is needed to check the response of any interferon therapy. Hepatitis C is a silent, systemic disease that has many extrahepatic manifestations in addition to hepatic inflammation and fibrosis, some of which may result in a poor health-related quality of life. Fatigue perhaps the most frequent and disabling extrahepatic symptom of hepatitis C virus (HCV) reported in almost one-half of all chronically infected individuals. This large study (4324 patients) was conducted at Nuclear Medicine Oncology and Radiotherapy Institute on the HCV patients referred from major hospitals of Islamabad and its vicinity during the year 2006–2007. Based on their personal history data, a questionnaire was filled by all the patients visiting the hospital stating their demographic data, treatment information, biochemical tests results (ALT, bilirubin and ALK), symptoms/complaints, any family history of hepatitis, any past history of drug abuse, dialysis, blood transfusion, surgery, multipricks, dental surgery, barber and stomach problem. Interestingly almost 82% (3546) patients reported as they have the fatigue, general body ache, and leg pains. Significant number of patients 75% (3244/4324) belong to families where any of their family member was suffering from HCV. 118 patients were found to have their counterpart HCV positive which may be the case of sexually transmitted disease but it was not clear who caught the first. Naïve viral load was quantified on Corbett Research Real Time PCR system using aj Roboscreen kit. It was found on average 8×10^6 IU/ml HCV incidence is increasing in Pakistan and it is very important to have baseline data for effective treatment. This will be the first study from Pakistan stating the prevalence of treatment-naïve patient viral load.

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Design, Molecular Modelling Studies of Specific Targeted Candidate Inhibitors of HCV NS5B RNA Polymerase

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Hepatitis C virus (HCV) a member of the *Flavivirus* genus, is a negative strand RNA virus that is estimated to have infected 170 million people globally. Ribavirin and interferon combined chemotherapy is now available to treat HCV viral infection. Due to high rate of viral drug resistance, we need new targetted therapy to combat HCV. Structure-based drug design methods utilize knowledge of three-dimensional structure of an enzyme to develop some novel inhibitors of HCV. NS5B protein encoded RNA depen-