interfere with the formation of the HCV replication complex. This approach represents a valuable tool for screening of HCV inhibitors with a novel mode of action as well as for the characterization of replicon hits.

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Novel HCV Replication Mouse Model Using Human Hepatocellular Carcinoma Xenografts

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Background: Hepatitis C virus (HCV) infection causes chronic liver diseases and is a global public health problem. The only established animal model of HCV infection is the chimpanzee. In view of the limited availability of these animals, expense and ethical aspects, the establishment of a small-animal model for the study of HCV infection is a high priority. In absence of an immunocompetent mouse model for HCV replication, we developed a convenient xenograft mouse model that produces infectious viral particles.

Methods: To produce xenograft human hepatocellular carcinoma (HCC), we developed a highly tumorigenic Huh7 cells population able to promote the formation of HCC subcutaneously in SCID/beige mice. Huh7-7 cells are permissive cell line for cell culture of HCV particles (HCVcc) and were obtained through successive *in vivo* passages of tumor cells performed by subcutaneous transplantation of tumor fragments from the previous *in vivo* passage.

Results: Following injection with HCV-infected Huh7-7 cells, HCV RNA rose in the mouse sera and plateau at 10^3–10^5 GE/ml. Quantitative RT-PCR showed that up to 10^7 GE/μg of total RNA are present within tumors. Furthermore, a direct correlation between the size of tumors and the level of HCV RNA in the tumor was observed. Immunohistochemistry analysis of infected tumor tissue showed that the virus is widely spread within the tumor. Moreover, virus recovered from infected mice is infectious in cell culture. Finally, we showed that interferon-α and the protease inhibitors BILN-2061 both inhibited HCVcc strain JFH1 replication *in vivo*.

Conclusions: Human trials are realized solely based on efficacy data collected *in vitro* and on safety and pharmacokinetic profiles. The simplicity and the convenience of the model present here, should allow its utilization at an early stage in the compound profiling and give a more accurate indication of the compounds ability to block viral replication and infection in an *in vivo* setting.

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In Vitro Activity and In Vivo Pharmacokinetics of Highly Potent Phosphoramidate Nucleoside Analogue Inhibitors of Hepatitis C NS5B

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Phosphoramidate nucleoside analogues, or pronucleotides (ProTides), possess a number of pharmacological advantages over their parent nucleoside including a significant increase in antiviral activity, higher concentrations of triphosphate in liver, and potentially less toxicity due to reduced systemic nucleoside exposure. The in vitro and in vivo properties of a series of aminoacyl ProTides of 2′-C-methyl guanosine (2′-C-MeG) have been characterized. ProTides exhibited anti-HCV replicon activity as much as 20-fold greater than the parent nucleoside, with EC90's ranging from 200 to 800 nM. The compounds were synergistic when combined with ribavirin or interferon-2α. Cytotoxicity was not observed in Huh-7 cells (CC50 > 100 μM). In the more sensitive MT-4 cell line, CC50 values ranged from 20 to >100 μM. Culturing CEM cells with ProTides for 3 days at 100 μm or 13 days at 5 μM had no significant effect on mitochondria copy number. In primary human hepatocytes, conversion of ProTides to the triphosphate was measured, with C_{max} = 78 pmol/10E6 cells which is approximately 20-fold greater than the IC90. Therefore, ProTides of 2′-C-MeG exhibited excellent therapeutic indices and conversion to 2′-C-MeGTP in primary human hepatocytes exceeding the IC90. In PK experiments designed to measure plasma concentrations of the ProTides and parent nucleoside in the peripheral circulation and portal vein of cannulated cynomologus monkeys, efficient extraction by the liver was observed as indicated by low systemic levels of the ProTides. Triphosphate levels exceeding the IC90 were measured in primate liver biopsies following oral dosing. The primate PK study data indicate delivery of the ProTides to the liver and subsequent conversion to the triphosphate after oral administration. The findings of these studies support the continued development of 2′-C-MeG ProTides for the treatment of HCV infections.

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Hepatitis C Virus NS5A Protein In Vitro Modulates Template Selection by the RNA-dependent RNA Polymerase

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Hepatitis C virus (HCV) infection is one of the most dangerous human diseases. The HCV replication complex is composed of viral nonstructural proteins including NS5B (RNA-dependent RNA polymerase, RdRp) and NS5A and of several cellular proteins. Since the recombinant NS5A protein can directly interact with NS5B and with viral RNA, it was proposed that NS5A plays an important role in virus replication.

NS5A is presented in infected cells in an unphosphorylated and two phosphorylated forms (basal and hyperphosphorylated). Basal NS5A phosphorylation occurs in the C-terminus and is catalyzed by casein kinase (CK) II, whereas hyperphosphorylation is accounted for by CKI. Although basal NS5A phosphorylation has no effect on HCV replication, its effect on the protein interaction with HCV RdRp and with RNA is unknown.

Here we demonstrate that unphosphorylated NS5A protein inhibits HCV RdRp activity in vitro in an artificial polyA-oligoU system but has only minor inhibitory activity on synthesis of viral RNA. In contrast, the phosphorylated CKII NS5A protein does not block polyA-dependent polyU synthesis but completely abolishes viral (−)-3′UTR replication and significantly inhibits (+)-3′UTR synthesis. The NS5A phosphorylation with CKI does not change the RdRp activity in any system. Phosphorylation of NS5A with CKII has no effect on the protein affinity to RdRp or RNA. By UV-crosslinking and RNA filter-binding experiments we revealed that NS5A prevented binding of the template to the polymerase. The presented mecha-