Table 1
Effect of peramivir treatment (IM) on weight loss and survival in mice infected with H1N1 virus (prophylaxis model).

Treatment	Dose level (mg/kg)	Percent survival	Mean weight change (±SEM) day 4
Vehicle, uninfected	0	100	0.32 ± 0.22
Vehicle, infected saline treated	0	20	−3.41 ± 0.17
Peramivir	1.0	60*	−2.11 ± 0.22**
Peramivir	3.0	60*	−1.51 ± 0.33**
Peramivir	10.0	90**	−1.84 ± 0.19**
Peramivir	30.0	100**	−1.83 ± 0.17**

* $p < 0.03$ vs. vehicle, infected control group.
** $p < 0.001$ vs. vehicle, infected control group.

Conclusion: These data demonstrate efficacy of parenterally administered peramivir against the recently isolated pandemic flu virus.

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In Vitro Dose Ranging Studies for Serine Protease Inhibitor, MK-4519, Against a Hepatitis C Virus (HCV) Replicon using the Bellocell System

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Background: Development of new anti-HCV agents has been limited due to the inability to effectively grow HCV in cell culture. This problem is obviated by use of a HCV replicon system. We have developed an in vitro system to examine the pharmacodynamically-linked parameters and monitor the exposure response of MK-4519 against HCV using a HCV replicon-bearing cell line.

Methods: The HCV replicon cell line, 2209–23, was obtained from Roche (Palo Alto, CA). The replicon contains a Renilla luciferase reporter gene that was used to monitor HCV replication kinetics. We inoculated 2209–23 cells into four BelloCell bottles at a concentration of 6×10^7 cells per bottle. One bottle served as a control and three bottles were treated with various concentrations of MK-4519, which was obtained from Merck. Medium, with or without drug, was infused into the system for 14 days. Replicon kinetics and cell growth were monitored daily by harvesting 6 carrier flakes in quadruplicate. Three sets of 6 flakes were trypsinized to remove 2209–23 cells from the flakes and viable cells were counted. RNA was later extracted from these trypsinized cells and used for sequencing of the NS3 gene. The remaining flakes were immersed in Renilla luciferase lysis buffer and frozen until the end of the study. Luciferase activity was quantified from all samples simultaneously to determine the effect of each drug on the HCV replicon.

Results: In the BelloCell system, MK-4519 significantly reduced luciferase activity in the 2209–23 cells in a dose dependent manner. Cell viability assays confirmed that the suppression of luciferase activity was not due to cytotoxicity. Sequencing analysis detected genotypic changes in the replicon as a result of drug exposure. Mutation A156T, which is associated with phenotypic drug resistance, was present only in replicons exposed to drug.

Conclusion: These studies indicate that the BelloCell system is an effective and relevant in vitro method to model in vivo pharmacodynamics for antiviral agents active against HCV. This system can be used to optimize dosing for anti-HCV compounds for inhibition of viral replication and suppression of resistance.

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Maporal Hantavirus ββ-Integrin Utilization and Sensitivity to Favipiravir

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Hantaviruses are members of the Bunyaviridae family of viruses. Pathogenic hantaviruses are the etiologic agents of hemorrhagic fever with renal syndrome (HFRS), a disease principally endemic in the Old World, and hantavirus pulmonary syndrome (HPS), a disease primarily restricted to the Americas. Maporal virus (MPRLV), a recently isolated hantavirus, has been found to cause disease in hamsters that resembles HPS in humans. However, the virus has not been linked to human cases of HPS. Considerable evidence suggests that ββ-integrin usage mediating infection may serve to distinguish pathogenic from non-pathogenic hantaviruses, but this receptor usage pattern information is not yet available for MPRLV. Although ribavirin has been shown to be effective in treating HFRS, it lacks specificity and has toxicity. Moreover, there are no effective antivirals for the treatment of HPS. Considering the above, we have investigated MPRLV (1) susceptibility to favipiriavir (T-705), (2) ββ-integrin-mediated mechanism of entry, and (3) genetic determinants of pathogenicity. Favipiravir, a pyrazine derivative reported to be active against related bunyaviruses, was found to be active against MPRLV, Dobrava virus (DOBV), and Prospect Hill virus (PHV) (EC_{50} = 65–93 μM) with therapeutic indexes of 77, 65, and 82, respectively. Using antibodies targeting specific integrin chains, we found infection of Vero E6 cells with MPRLV to be dependant on ββ3 integrins, similar to that reported for other pathogenic hantaviruses such as DOBV included in our studies. ββ1-Integrin chain-specific antibodies and fibronectin did not block MPRLV or DOBV infectivity as observed with the non-pathogenic PHV. Phylogenic analysis of characteristic degron sequences and ITAM motifs in the G1 cytoplasmic tails of MPRLV and other hantaviruses emphasizes the close genetic proximity of MPRLV to other HPS-causing hantaviruses. The data presented suggests that MPRLV may be pathogenic to humans and that it and other hantaviruses tested are sensitive to favipiravir in cell culture.

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Small Molecule Inhibitors of Dengue Virus Replication are Active *In Vivo*

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Flaviviruses, including dengue virus, West Nile virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, are a group of viruses transmitted by mosquitoes or ticks in the Flaviviridae family, that are significant pathogens responsible for emerging infectious disease in both humans and animals. Dengue virus is considered to be one of the most important viruses