SI of 100. Time of addition studies demonstrated activity of this compound when added as late as 16 h after virus challenge of Vero cells with an EC_{90} of 8.9 μg/ml. Significant improvement in survival, serum levels of ALT, and virus titer in the liver was observed after bid treatment with 120 mg/kg/d of 2′-C-MeC for 7 days beginning just prior to virus challenge. A 4-day bid treatment regimen with this dose beginning 4 h prior to virus challenge was also effective in significantly improving survival. A lower dose of 80 mg/kg/d was also effective in significantly improving survival and serum ALT. Treatment with the 120 mg/kg/d dose initiated beginning 2 dpi was effective in significantly improving survival and serum ALT. Due to the severe gastrointestinal effects associated with long-term treatment, this compound and its derivatives may not be clinically viable for the treatment of chronic HCV. Alternatively, short-term treatment of an acute flaviviral disease like YFV would likely minimize or eliminate deleterious side effects associated with long-term treatment, potentially making the use of 2′-C-MeC and active derivatives a viable option for therapeutic intervention.

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Immunosafety Assessment of CD4 MAB-based Bifunctional HIV Entry Inhibitor (CD4-BFFI) using *In Vitro* Immunoassays

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We have previously described a CD4 monoclonal antibody (mAb)-based bifunctional HIV entry inhibitor (CD4-BFFI). CD4-BFFI demonstrated highly potent anti-HIV activities and excellent *in vivo* stability. Since CD4-BFFI binds to CD4 and CD4 is involved in $CD4^+$ T cell activation and other immunological functions, it is important to assess the potential immunological liabilities of CD4-BFFI before it enters human studies. We evaluated the direct effects of CD4-BFFI on $CD4^+$ T cells to see if it activates T cells via cross-linking CD4 molecules on cell surface. Our results showed that CD4-BFFI did not activate Jurkat cells or peripheral blood mononuclear cells (PBMC). There have been reports that some antibodies, especially those targeting blood cells, induced quick and marked cytokine release (cytokine storm) when dosed in humans, which may result in severe complications and even deaths. To assess the risk, an *in vitro* assay was performed using human whole blood from multiple donors. CD4-BFFI was incubated with human blood for 6 h, no cytokine secretion was observed, while the control anti-CD52 antibody (alemtuzumab) caused significant release of cytokine TNF-α and neutrophil activation (elevated CD11 expression) in 11 of the 12 donor blood samples. To investigate whether CD4-BFFI interferes with the co-receptor function of CD4 on T cells, an in vitro T cell activation assay was performed using Jurkat T cells and MACSiBeads that mimic antigen-presenting cells (APC). Significant activation of Jurkat cells was observed after stimulation with MACSiBeads. Co-incubation with CD4-BFFI showed no effects on Jurkat cell activation. Similar results were obtained using primary human PBMC cultures. An antigen-specific T cell activation assay was then developed for further evaluation. Human PBMC from cytomegalovirus (CMV)-infected donors was stimulated with CMV pp65 protein and significant activation of $CD4^+$ T cells (elevated CD69 and CD25 expression) was observed. Co-treatment with CD4-BFFI showed no effect on T cell activation. In summary, by using several in vitro immunoassays, we have demonstrated that CD4-BFFI did not activate human whole blood or T cells, and it did not interfere with the co-receptor function of CD4 in T lymphocytes in APC-mediated T cell activations.

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Inhibition of Severe Acute Respiratory Syndrome Coronavirus Replication in a Lethal SARS-Cov Balb/C Mouse Model by Stinging Nettle Lectin, Urtica Dioica Agglutinin (UDA)

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Urtica dioica agglutinin (UDA) was tested for efficacy in a lethal SARS-CoV-infected BALB/c mouse model. UDA is a small plant monomeric lectin, 8.7 kDa in size, with an N-acetylglucosamine specificity and inhibits viruses from Nidovirales in vitro. In the current study, groups of BALB/c mice were infected with 2 LD50 of virus and treated intranasally with UDA at the doses of 20, 10, 5 and 0 mg/kg/day for 4 days beginning at 4 h post virus exposure. Treatment with UDA at 5 mg/kg significantly protected mice against a lethal infection with mouse-adapted SARS-CoV ($p < 0.001$), but did not significantly reduce virus lung titers. All mice receiving UDA treatments were also significantly protected against weight loss due to the infection ($p < 0.001$). UDA also effectively reduced lung pathology scores. All mice receiving poly IC:LC, the positive control drug, survived the infection ($p < 0.001$). At day 6 after virus exposure, all groups of mice receiving UDA or poly IC:LC had much lower lung weights than did the placebo-treated mice. Our data suggest that UDA treatment of SARS infection in mice leads to a substantial therapeutic effect that protects mice against death and weight loss.

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Viprolaxikine, a Novel Cytokine-like Protein from Insect Cell Cultures can Reduce Dengue-2 Virus Titres in Mammalian Cells

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Dengue virus (DEN) is an arthropod-born virus that causes dengue fever and dengue hemorrhagic fever in human hosts, but no disease in mosquito vectors. Viruses often persist in insects and other arthropods such as shrimp in either single, dual or multiple infections without gross signs of disease. From the supernatant solution of grossly normal C6/36 mosquito cell cultures persistently infected with DEN-2 virus, a novel antiviral agent was separated by ultrafiltration (5 kDa). Pre-incubation of mammalian (Vero) cell cultures with the ultrafiltrate reduced DEN-2 titres by up to 4 logs upon subsequent challenge. There was no reduction in titre for Vero cells pre-incubated with ultrafiltrate from uninfected C6/36 cells. Protease treatment of the protective ultrafiltrate removed its anti-DEN-2 activity while heating did not. Since 8-hr pre-incubation with the unltrafiltrate was required to obtain maximum protection against DEN-2, the active substance was called viprolaxikine,