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Preclinical Development of the Amphipathic DNA Polymer REP 9AC for the Treatment of Hepatitis B Virus Infection

Faseeha Noordeen[1,*], Andrew Vaillant[2], Jean-Marc Juteau[2], Allison Jilbert[1,3]

[1] School of Molecular and Biomedical Science, The University of Adelaide, Adelaide, Australia; [2] REPLICor Inc., Laval, Canada; [3] Infectious Diseases Laboratories, Institute of Medical and Veterinary Science, Adelaide, Australia

Amphipathic DNA polymers (APs) are a novel class of nucleic acid-based medicines that have a broad spectrum activity against enveloped viruses and are effective *in vivo* against hepatitis C virus, HSV, CMV, influenza, RSV and Ebola infections. The assessment of the AP REP 9AC in the treatment of duck hepatitis B virus (DHBV) is presented as a model for human hepatitis B virus (HBV) infection. The antiviral assessment of APs was carried out in vitro using the infection of primary duck hepatocytes (PDH) with DHBV and *in vivo* using the Pekin duck DHBV model. The activity of APs and their analogs suggested a large amphipathic domain related to those found in type 1 fusion glycoproteins is involved in the entry or release of DHBV. For *in vivo* evaluation, 14-day-old ducks were infected with a defined dose of DHBV and treated with the REP 9AC (10 mg/(kg day) by IP injection) for 14 days. REP 9AC was well tolerated by the ducks with no detectable side effects. Liver biopsies collected on day 4 post-inoculation (p.i.) showed a small percentage of $DHBV^+$ hepatocytes in all REP 9AC treated and control ducks. However, by day 14 p.i. 100% of ducks treated with REP 9AC showed no evidence of DHBV infection in the liver. In contrast, in all control ducks treated with normal saline DHBV infection had spread to >95% of hepatocytes. REP 9AC was similarly effective at doses as low as 1 mg/(kg day). REP 9AC could be an effective treatment for HBV infection either as mono or combination therapy, which can likely be given once a week. More importantly, the novel mode of action of APs strongly suggest that treatment with REP 9AC will not result in the development of antiviral drug resistance. The compounds may also address the still uncertain role of virus spread in maintenance of chronic HBV infections as well as preventing the spread of antiviral drug-resistant HBV mutants in treated patients.

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Oral Session 4: Retroviruses

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Characterization of a Novel Series of gp120 Inhibitors

Tanya Parkinson*, Peter Stephenson, Chris Pickford, Thien Duc Tran, David Williams, David Fenwick, Juin Fok-Seang, Iain Gardner, Mike Westby, Don Middleton, Manos Perros

Pfizer Global R&D, Sandwich, United Kingdom

Despite recent advances in treatment of HIV infection, there remains a high need for new classes of antiretrovirals. Reports of virological and clinical efficacy of viral entry inhibitors suggest that this process may be an attractive point of intervention. A cell-based viral protein-mediated fusion assay was used to identify novel inhibitors of HIV entry. Subsequent assays were performed to identify the molecular target of the compounds, including gp120/CCR5 binding, gp120/CD4 binding and Biacore molecular interaction studies. Antiviral activity of compounds was assessed in a Phenosense™ assay (Monogram Biosciences) and in PBMC assays with primary isolates. The compounds were tested against a range of gp120 proteins containing mutations previously shown to confer resistance to small molecule gp120 inhibitors. Pharmacokinetic properties were assessed in rat and dog. A novel series of small molecule inhibitors of HIV entry was identified. The compounds inhibited the interaction of purified gp120 with soluble CD4 and were shown to bind to the gp120 protein. Antiviral activity was demonstrated against a range of clade B viruses, although activity against other clades was limited. The compounds were unable to inhibit fusion mediated by gp120 proteins containing mutations previously shown to confer resistance to gp120 inhibitors. Pre-clinical pharmacokinetic studies demonstrated favourable properties, including low protein binding, high oral bioavailability and low clearance. Our data indicate that the novel series of gp120 inhibitors we have identified is likely interacting with the viral protein in a similar way to previously described compounds. While our series may offer an attractive starting point for development of novel antiretrovirals, improvements in spectrum and resistance profile may be required prior to further progression.

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Novel Mechanism of Action of Pyrimidinediones Yields Enhanced Sensitivity to Multi-Drug Resistant Virus Strains

Robert W. Buckheit Jr.*, Lu Yang, Tracy L. Hartman, Karen M. Watson

ImQuest BioSciences, Inc., Frederick, USA

A diverse series of 74 HEPT-like compounds with homocyclic substitutions at the N-1 of the pyrimidinedione have been evaluated in structure–activity relationship assays. Twelve congeners with subnanomolar efficacy and therapeutic indices greater than 1 million were evaluated to define their mechanism of anti-HIV action. The pyrimidinediones inhibit HIV-1 RT through direct interaction at the hydrophobic NNRTI-binding site. The compounds have no activity against HIV-2 RT and the selection of resistant HIV-1 results in typical NNRTI mutations with an accumulation of amino acid changes required to achieve high-level resistance. Unlike the NNRTIs, the pyrimidinediones possess a second mechanism of action which extends their range of action to include HIV-2 and results in inhibition of virus entry and/or maturation; this entry inhibition is more potent against clinical virus strains and prevents resistance mutations in the RT from accumulating to engender greater than 100-fold levels of resistance. Mechanistic assays with HIV-1 and HIV-2 suggest that the compounds act early to inhibit virus entry prior to fusion through interaction with a complex conformational