prophylaxis and therapy of respiratory virus infections cause by IAV or HRV.

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Development of Resistance to the Natural HIV-1 Entry Virus Inhibitory Peptide (VIRIP)

Emmanuel Gonzalez*, Maria Pau Mena, Mercedes Armand-Ugon, Ester Ballana, Bonaventura Clotet, Jose Esté *IrsiCaixa, Badalona, Spain*

The virus inhibitory peptide (virip) was identified as a component of human hemofiltrate and shown to have anti-HIV activity through the inhibition of HIV-1 gp41-dependent fusion. We confirmed the anti-HIV activity of virip and the optimized virip-derived peptide vir353 in lymphoid cells. Virip and vir-353 showed a dose-dependent activity with 50% effective concentrations of 16 and 0.7 mM respectively and a time of addition experiment showed that virip and vir353 target a time/site of action that corresponds to gp41-dependent fusion. Sequential passage of HIV-1 NL4-3 in lymphoid MT-4 cells in the presence of increasing concentration of different anti-HIV drugs led to the generations of virus resistant to nevirapine (10 days), the entry inhibitor BMS-155 (30 days), the fusion inhibitors, enfuvirtide (90 days), sifuvirtide (180 days) and vir-353 (260 days) suggesting a high genetic resistance for the virip-related compound. The resulting vir-353 resistant virus was completely cross-resistant (>200-fold) to virip but remained sensitive to the fusion inhibitors enfuvirtide and C34 as well as other HIV inhibitors targeting virus entry (AMD3100) or reverse transcriptase (AZT, nevirapine). Recombination of gp41 of the virip resistant virus into a wild-type HxB2 backbone partially recovered the resistant phenotype but both resistant gp120 and gp41 were necessary to recover full resistance to virip. Mutations were found in both gp120 and gp41 of the virip-resistant virus. However, no mutations were found in the fusion peptide of gp41 the alleged target of virip. The time needed to generate a virip resistant virus and the position of mutations found suggest that virip may target an essential part of gp41 and highlight possible interactions between gp41 and gp120 required during the fusion process.

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Single-dose Intranasal Delivery with DEF201 (Adenovirus Vectored Mouse Interferon-α) Protects Against Phlebovirus and Sars Coronavirus Challenge

Brian Gowen[1,*], Dale Barnard[1], Min-Hui Wong[1], Deanna Larson[1], Josh Wu[2], Jane Ennis[3], John Morrey[1], Jeffery Turner[3] [1] *Institute for Antiviral Research and Department of Animal, Dairy, and Veterinary Sciences, Logan, USA* [2] *Defence R&D Canada – Suffield, Medicine Hat, Canada* [3] *Defyrus Inc., Toronto, Canada*

Interferon (IFN)-α is an effective and safe recombinant human protein with broad clinical appeal. While recombinant IFN-α has great therapeutic value, its utility for biodefense is hindered by its short *in vivo* half-life and costly production. Here we describe the use of Ad5-mIFN-α (DEF201) to address these limitations as a prophylactic countermeasure in two murine viral challenge models, Punta Toro virus (PTV; *Bunyaviridae*, *Phlebovirus*) and SARS coronavirus (CoV). Significant protection ($p < 0.001$) against PTV and SARS-CoV infections was observed in mice from a single dose of DEF201 administered 1 day to 3 weeks prior to challenge. DEF201 was delivered intranasally to stimulate mucosal immunity at the probable site of infection and bypass any preexisting immunity. Intramuscular inoculation with DEF201 rapidly increased (~3 h) IFN-α concentrations in unchallenged mice and persisted for extended periods of time. In contrast, a control Ad5 construct elicited only small amounts of IFN-α that were short-lived. Studies investigating the kinetics of mucosal and systemic IFN-α levels following intranasal administration of DEF201 are underway. Effective medical countermeasures that are highly stable, easily administered, and elicit long lasting protective immunity are much needed. The DEF201 technology has the potential to address all of these issues and serves as a broad-spectrum approach to enhance host defense against a number of viral pathogens.

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Evaluation of the Contribution of Amantadine, Ribavirin, and Oseltamivir in a Triple Combination Antiviral Drug (TCAD) Regimen to Suppressing the Emergence of Resistance using a Novel Quantitative Approach

Justin Hoopes[1,*], Minh Le[3], Elizabeth Driebe[2], Erin Kelley[2], David Engelthaler[2], Amy Patick[3], Jack Nguyen[3] [1] *Utah State University, Logan, USA* [2] *TGen North, Flagstaff, USA* [3] *Adamas Pharmaceuticals, Emeryville, USA*

Background: We have previously demonstrated that a triple combination antiviral drug (TCAD) regimen comprised of amantadine, ribavirin, and oseltamivir carboxylate was highly active and synergistic against susceptible and resistant influenza A viruses *in vitro*. To determine the contribution of each drug in TCAD to preventing the emergence of resistance, we have developed a novel assay to quantify the development of resistance following serial passage under drug pressure.

Methods: MDCK cells in 96-well plates were infected with influenza A/Hawaii/31/2007 (H1N1) in the presence of a clinically achievable, fixed concentration of two drugs alone, or in triple combination with varying concentrations of the third drug, using 12 replicates for each condition. Following 5 serial passages, the percentage of wells for each condition having virus breakthrough (>50% cytopathic effect) and the presence of resistance-associated mutations (>1% total population of variants bearing the V27A, A30T, and S31N substitution in M2, and the H274Y substitution in neuraminidase) was determined by neutral red staining and mismatch amplification mutational analysis, respectively.

Results: Treatment of infected cells with any double combination resulted in virus breakthrough in up to 12 of 12 wells (100%) and virus resistance in up to 10 of 11 wells (91%). Addition of each third drug (TCAD) resulted in concentration dependent reductions in the percentage of wells with virus breakthrough and virus resistance. Importantly, the contribution of each drug in preventing the emergence of resistance was shown by a statistically greater ($P < 0.05$) reduction in virus breakthrough and/or emergence of influenza resistant variants compared to all double combinations at clinically achievable concentrations.