

detecting viral expressed reporter genes to measure viral expression. In this case, the enhanced green fluorescent protein was inserted into the human parainfluenza virus type 3 genome. The rescued, recombinant virus (rHPIV3-EGFP) was plaque purified three times in MA104 cells and titered, by plaque assay, to $1.9 \times 10^7 \pm 4.9 \times 10^6$ PFU/mL, whereas the wild-type 14702 strain (HPIV-3 WT) was titered to $2.9 \times 10^7 \pm 4.1 \times 10^6$ PFU/mL. 50% efficacy values were calculated from neutral red-based antiviral assays with rHPIV3-EGFP and HPIV-3 WT viruses using ribavirin (16 ± 0.58 μ g/mL and 35 ± 2.5 μ g/mL, respectively) and 2-thio-6-azauridine (0.63 ± 0.075 μ g/mL and 1.5 ± 0.2 μ g/mL, respectively). Cytopathic effect (CPE) of each virus was measured by neutral red over a 7-day period; no differences were seen. Even though the plaque titers and antiviral data suggest a slightly attenuated EGFP virus, the CPE data suggests that *in vitro* differences are minimal. To determine when maximum GFP levels were obtained, rHPIV3-EGFP was infected at various MOIs and fluorescence was read each day for 8 days. GFP expression reached its maximum at day 3 in a dose responsive manner. Comparison of a 3-day GFP-based antiviral assay to a 7-day neutral-based antiviral assay yielded Z'-factor values of 0.83 and 0.70, respectively, signal-to-background ratios of 241 and 65, respectively, and signal-to-noise ratios of 4057 and 301, respectively. These data suggest the superiority of the GFP-based antiviral assay to the neutral red-based assay. A 3-day GFP-based assay and a 7-day neutral red-based assay were run side-by-side and significant indexes (SI) were calculated. Using a SI threshold of 10, the GFP-based antiviral assay had a sensitivity of 89% and a specificity of 47%. The use of a GFP-based antiviral assay for testing potential antiviral compounds against HPIV-3 in a high throughput format has been justified for initial screening purposes.

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NGO Analysis of Impact of HIV Infection and Antiretroviral Therapies in Resource Poor Nations Are We on Right Path to Control HIV

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Objectives: AIDS activists have argued against poor availability, high-cost of ARV drugs. Past studies proved that HAART non-cost-effective outside industrialized nations. Our Indian NGO explored/analyzed economic impact of HIV infection and antiretroviral therapies in rural/tribal communities. Building strategic alliances with NGOs and government agencies to provide discounted ARV's is propagated.

Methods: Analysis of available 16 studies on HIV-Rx cost, WHO publications/declarations by IAS/UNAIDS/global-AIDS fund. Time frame for statistical analysis taken from April 2004 till todate. Nine corporate, seven NGO and five Govt-sector programs undertaken for review.

Results: NGOs had limited opportunity to learn about HIV best practice and acquire technical skills and develop organizational capacity. Poor institutional infrastructure/systems to support program implementation raises questions about effective ways to develop community-base responses. Factors like Per capita income, social-standing change outcome of HIV drug therapy/compliance. Economic effects included low productivity, increased medical consultations/hospitalizations. Average cost of ARV therapy in subsidized centre US\$ 1200 Vs opportunistic infections US\$ 1385 per patient for 1 month. Additional costs for nutrition, supportive therapy/palliative care comes to US\$ 800. Considering family income at US\$ 400 in rural India, current HIV therapy is out of reach for >84% population.

Conclusion: HIV policy makers must form collaborative efforts to reduce cost, increase access to ARV drugs. Community NGO representatives must be made part of decision-making body of IAVI/IAS/UNAIDS. Realizing divergent versions of cost analysis a multicentre study on this burning issue be carried out in developing nations. ARV-drug development is a long-term commitment that must also consider financial constraints of population from south. At 21st ICAR-2008 conference, we shall form group of NGO activists and researchers from USA/Europe to substantially improve Anti-Retroviral-Drug-service provision policy.

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A Cell-based High-throughput Screening Approach for the Discovery of New Inhibitors of the Influenza H5N1 Virus

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Using a highly reproducible and robust cell-based HTS assay, we screened the NIH Molecular Libraries Screening Centers Network (MLSCN) 100,000 compound library at 50 μ M compound concentration against influenza strain AV/VN/1203/2004 (H5N1). The "hit" rate (>25% inhibition of the viral cytopathic effect) from the single dose screen was 0.32%. The hits were evaluated for their antiviral activity, cell toxicity and selectivity in dose response experiments. The screen yielded five active compounds (SI_{50} value > 3). One compound showed an SI_{50} value of greater than 3.45, three compounds had SI_{50} values ranging from greater than 13.84 to 34.29, while the most active compound displayed an SI_{50} value of 94.64. The active compounds represent two different classes of molecules, benzoquinazolinones and thiazoloimidazoles which have not been previously identified as having anti-viral/anti-influenza activity.

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