and/or the emergence of drug resistant virus. The identification of HIV-1 inhibitors directed against new targets in the HIV-1 replication cycle represents one approach to address the issue of drug resistance. As part of an effort to search for inhibitors targeting new mechanisms, a high throughput antiviral screen was developed and reduced to practice on an industrial scale.

Methods: A high throughput antiviral screen was developed using the HIV-1 NL4-3 strain, MT-2 T-cells, and HeLa CD4 LTR/beta-Gal indicator cells.

Results: In this study, we describe an HIV-1 full replication assay (HIV-1 Rep) that incorporates all of the targets required for replication in T-cell lines. In the HIV-1 Rep assay, virus replication in infected T-cell lines is monitored using HeLa indictor cells that are co-cultured with the infected T-cell lines. We demonstrate the HIV-1 Rep assay is sensitive to known HIV-1 inhibitors of different classes targeting both early and late steps in the viral replication cycle. In addition, we show that HIV-1 virions containing a non-functional Vif gene exhibit a 79–93% reduction in replication signal in the HIV-1 Rep assay. These data strongly suggest that the HIV-1 Rep assay may be used to screen for novel HIV-1 inhibitors, including inhibitors targeting Vif. To demonstrate the utility of the HIV-1 Rep assay, we show that assay exhibits characteristics (e.g., a favorable Z' value) compatible with high throughput screening in a 384-well format and employ the assay in a high throughput screen of >2 million compounds.

Conclusions: The HIV Rep assay represents a simple antiviral screening method with the potential to identify novel target inhibitors that was executed on an industrial scale (>2 million compounds).

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DC-SIGN is Not Required for HIV-1 Transmission to CD4+ T Lymphocytes

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Dendritic cells (DCs) capture HIV-1 particles and promote efficient viral transfer and replication in CD4+ T lymphocytes. Cell to cell HIV-1 transfer from HIV-1 chronically infected T cells to primary CD4+ T lymphocytes occurs through a mechanism that depends on SUgp120-CD4 interaction. However, the mechanism of virus transfer by DCs and the exact role of the dendritic cell-specific ICAM-grabbing non-integrin (DC-SIGN) remains unclear.

In our study immature monocyte derived dendritic cells were used to assess the role of DC-SIGN in the capture of HIV-1 and its transfer to CD4+ T lymphocytes.

Two-hour cultures of DCs with HIV-1_{BaL} induced the capture of virus as measured by ELISA. This process was inhibited by an anti-DCSIGN mAb or mannan confirming the apparent role of DC-SIGN in HIV-1 capture. Coculture of these HIV-1_{BaL} loaded DCs with primary CD4+ T lymphocytes induced virus transfer and replication in the target cells, that could be prevented by the anti-gp120 mAb IgGb12, the fusion inhibitor C34 or the RT inhibitor AZT. Virus transfer was only prevented by anti-DCSIGN mAb or mannan if they were present during virus capture by DC. Virus replication in DC cultures was apparent after 9 days post-infection and could be blocked by C34 and AZT (>90% inhibition) and partially blocked by the anti-DCSIGN mAb ($68 \pm 20\%$) or mannan ($72 \pm 10\%$) suggesting a role of DC-SIGN in HIV entry to dendritic cells. However, cocultures of HIV-1_{BaL} productively infected DCs with CD4+ T lymphocytes showed the ability of DCs to transfer p24 antigen to the target cells, process that only could be blocked by IgGb12 mAb, but not by the anti-DCSIGN mAb or mannan.

In conclusion, our results suggest that DC-SIGN is not an absolute requirement for viral capture by DCs or their productive infection. Once infected, DC-SIGN appears to be irrelevant to the process of HIV-1 transfer to uninfected CD4+ T lymphocytes.

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The Virtues of Unique Ribonucleotide Reductase Inhibitors Didox and Trimidox for Retrovirus Therapy

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Ribonucleotide reductase inhibition (RRI) as a strategy to impair HIV replication functions by depleting the dNTP pools required for proviral DNA synthesis and potentiates NRTIs by lowering the competing natural dNTP pools. This strategy gained credibility by the success of hydroxyurea (HU) to enhance the NRTI ddI in clinical trials. HU in HIV therapy has not shown single agent activity. The RRIs Didox and Trimidox have shown more anti-retroviral activity than HU when used alone or with ddI or the NRTIs abacavir and tenofovir in murine models. We describe here two additional therapeutic attributes of the unique RRIs, Didox and Trimidox, that can contribute to HIV treatment.

Firstly, Didox and Trimidox have the capacity to downregulate NF-kB activation. Since the NF-kB transcription complex plays a crucial role in the intracellular efficiency of gene expression and replication of HIV, it is thought that impairing NF-kB activation should impede HIV infection. When Jurkat, Jurkat-Tat or Jurkat T cells transfected with HIV LTR were exposed to TNFα, NF-kB regulation was significantly downregulated. The data also indicate that IkBα phosphorylation was impaired.

Secondly, oxidative stress has been implicated in HIV dementia. Since Didox and Trimidox are potent free radical scavengers, they were examined for their efficacy in protecting cultured fetal

neurons against toxic substances in CSF from human infected patients with moderate to severe dementia. Mitochondrial potential was used to assess neuronal functionality. Didox nearly prevented the toxic CSF effect from moderate-severe dementia HIV patients on the mitochondrial potential of cultured fetal neurons.

These results demonstrate that this series of RRIs, particularly Didox, have multi-faceted actions that can be beneficial to HIV patients. They can impair HIV replication through inhibiting proviral DNA synthesis and potentiate NRTIs. Additionally, these RRIs can inhibit NF-kB activation. Lastly, they have the potential to impede the development of HIV dementia.

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Human Immunodeficiency Virus Type 1 Does Not Escape from Novel Single-Stranded Dnazyme Expression-Mediated Inhibition

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Recently, several groups reported that the antiviral activity of shRNA targeting the HIV-1 gene is abolished due to the emergence of viral quasispecies harboring a point mutation in the shRNA target region. This finding is particularly relevant for viruses that exhibit significant genetic variation due to error-prone replication machinery, and the risk might be more severe for RNA viruses and retroviruses than for DNA viruses. On the other hand, ribozyme technologies are also major tools for inactivating genes in gene therapy. One model, termed deoxyribozyme (Dz), is especially useful because it can bind and cleave any single-stranded RNA at purine/pyrimidine junctions. The DNAzyme is similar to hammerhead ribozymes, at least in terms of its secondary structure, with two binding arms and a catalytic loop that captures the indispensable catalytic metal ions. We designed a vector to produce single-stranded DNA. Human inmmunodeficiency virus type 1 (HIV-1) reverse transcription was used to construct a DNAzyme expression vector against the HIV-1 env V3 loop (Kusunoki et al., 2003). Initiation of HIV-1 reverse transcription requires the formation of a complex containing the viral RNA, tRNALys-3, and reverse transcriptase. The expression vector contains the HIV-1 primer binding site and tRNALys-3 at the 3′ end of its RNA transcript, thus enabling the synthesis of a single-stranded DNA by HIV-1 reverse transcriptase. We demonstrated that the lentiviral vector-mediated DNAzyme expression suppressed HIV-1 replication in SupT1 cells. Furthermore, HIV-1 did not escape from novel single-stranded DNAzyme expression-mediated inhibition. This lentiviral vector-mediated DNAzyme anti-genes are promising tools for HIV-1 gene therapy for the treatment of HIV/AIDS.

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Characterization of a New Class of Polycyclic RSV Inhibitors

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Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis and pneumonia in children under one year of age and is a leading cause of severe lower respiratory infections in infants and young children. Prophylactic antibodies such as Synagis® (palivizumab) effectively reduce the incidence and severity of RSV disease in high-risk pediatric populations but the only antiviral treatment available for patients with RSV disease is ribavirin, a nucleoside analog with suboptimal clinical efficacy and safety profile.

RSV enters cells in the lung using a fusion glycoprotein (RSV-F), found on the virus's outer envelope. Biota has developed small-molecule, orally available, synthetic drugs that specifically target RSV-F, preventing it from functioning and therefore stopping RSV infection from spreading.

We will present in vitro cellular data evaluating the antiviral activity and cytotoxicity of this potent class of RSV inhibitors. Mechanism of action will be reported including functional assays and genotypic and phenotypic analysis of resistant mutants. Cross-resistance data with known fusion inhibitors and modelling studies to establish the proposed binding site will be presented. The compounds display promising oral bioavailability and efficacy in rodent models of RSV infection.

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HIV Coreceptor Switch Induced by Antagonism to CCR5

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HIV resistance to CCR5 antagonists in cell culture has been observed in the absence of coreceptor switch, but it is unclear whether inhibition of HIV-1 replication with a CCR5 antagonist will lead to an increased rate of emergence of CXCR4 variants.