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Development of Anti-Infective Topical Microbicides 3. Combination Microbicidal Approaches Targeting Multiple Virus Targets or Utilizing Multi-Functional Anti-Infective Agents

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

ImQuest BioSciences, Inc., Frederick, MD, USA

Topical microbicides represent an important potential strategy for preventing the transmission of HIV through sexual intercourse, the predominant mode of HIV transmission worldwide. The number of women with HIV infection and AIDS has been increasing steadily worldwide, accounting for 46% of all adults living with HIV worldwide, and for 57% in sub-Saharan Africa. Thus the dynamics of the epidemic demand the development of safe, effective and acceptable female-controlled chemical and physical barrier methods, including topical microbicides, to reduce HIV transmission. We have been actively developing several novel classes of microbicides which could be used alone or in combination, targeting early steps of HIV replication such as entry, fusion and reverse transcription, as well as agents which result in the direct inactivation of HIV. We have also identified agents which target multiple sexually transmitted organisms. Our strategy requires the development of microbicides which attack HIV at multiple targets through the development of a formulated product which will place the right drug(s) at the right concentration at the right place at the right time to prevent the infection of target cells in the vaginal vault. Agents currently under development include the pyrimidinediones (inhibitors of virus entry and reverse transcription), the phosphorothioate oligonucleotide ISIS 5320 (inhibits CD4-gp120 interactions), the NCp7 zinc finger inhibitors (directl viral inactivation) and a novel natural product derived from a plant (inhibits both HIV and herpes virus). We have evaluated the in vitro microbicidal activity of each of these agents and have shown them to be both safe and effective at preventing virus transmission. We have evaluated potential combination microbicide development approaches with this group of agents to define and prioritize combination therapy strategies. Our formulation strategy exploits the mechanism of action of each compound to deliver each agent to the appropriate site of action upon introduction of the viral inoculum to the vaginal vault. The results of our developmental studies will be presented.

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Peptidomimetic Dimerization Inhibitors of HIV-1 Protease: Further Insights into Structural Variations and Mechanism of Action

Ludovic Bannwarth[1,*], Thierry Rose[2], Silvia Frutos[3], Ernest Giralt[3], Regis Vanderesse[4], Brigitte Jamart-Grégoire[4], Anamaria Vidu[5], Sandrine Ongeri[5], Sames Sicsic[5], Christophe Pannecouque[6], Erik De Clercq[6], Michèle Reboud-Ravaux[1]

[1] Laboratory of Molecular and Functional Enzymology, FRE2852, CNRS-University Paris 6, Paris, France; [2] Macromolecular Biophysics Facility, Institut Pasteur, Paris, France; [3] Institut di Recerca Biochèmica, Parc Scientific de Barcelona, Barcelona, Spain; [4] Laboratory of Macromolecular Chemistry and Physics, UMR7568, ENSIC, Nancy, France; [5] Laboratory of Molecular Recognition and Synthesis, Biocis-CNRS, UMR C8076, Faculty of Pharmacy, Châtenay-Malabry, France; [6] Rega Institute, Leuven, Belgium

Mutations that occur in response to the HIV-1 protease inhibitors (PIs) are responsible for the development of multi-drug cross-resistance to PIs in AIDS treatment. Virtually all PIs act through the same mechanism: they are transition-state analogs that target the active site of the homodimeric enzyme located at the junction of the two monomers. The emergence of resistance to one PI usually results in cross-resistance to other PIs. One alternative to inhibiting the active site of HIV-1 protease is to target the dimer interface of the enzyme at the antiparallel beta-sheet formed by the interdigitation of the C- and N-ends of each monomer. This region is highly conserved and is responsible for about 75% of the dimer stabilization energy. Here we describe new dimerization inhibitors in which new structural molecular variations have been introduced and the peptidic characteristics have been decreased by introducing peptidomimetic groups that have peptide-like hydrogen bonding properties. This led to an increase of the in vitro efficiency (subnanomolar level) against HIV-1 protease activity. Our dimerization inhibitors proved equally active in vitro against both wild-type and mutated proteases. The mechanism of inhibition was established using a combination of kinetic and biophysical methods. Using analytical ultracentrifugation and NMR, we obtained direct experimental evidence of non-covalent dissociative mode of interaction of the HIV-1 protease dimerization inhibitors.

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A High Throughput HIV-1 Full Replication Assay that Includes HIV-1 Vif as an Antiviral Target

Joan Cao*, Jason Isaacson, Amy Patick, Wade Blair

Pfizer Global Research and Development, La Jolla, CA, USA

Background: Although clinically effective when used in combination, current HIV-1 therapies are less than ideal due to drug-related side effects, inconvenient dosing requirements,

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

Imma Clotet-Codina*, Berta Bosch, Ruth Peña, Bonaventura Clotet, Margarita Bofill, José A. Esté

Retrovirology Laboratory irsiCaixa, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, 08916 Badalona, Spain

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

Howard Elford[1,*], Ron Lee[2], Jadwiga Turchan[3], Vincent Gallicchio[4], Michael Ussery[5], John Hiscott[2], Avindra Nath[3]

[1] Molecules for Health, Inc., USA; [2] McGill University, Canada; [3] Johns Hopkins University, USA; [4] Clemson University, USA; [5] NIAID, National Institutes of Health, USA

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