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Protein–Protein Interactions Occurring During HIV Capsid Assembly in a Cell-free Protein Synthesizing System

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Immature HIV capsid formation has previously been achieved upon cell-free protein synthesis (CFPS) programmed with HIV Gag p55 mRNA. Capsid formation by CFPS has been studied in considerable detail with a number of assembly intermediates identified, ordered into a pathway, and corroborated in HIV-infected mammalian cells. The protein–protein interactions occurring in this system are complex, with numerous host proteins implicated in capsid formation. We established an ELISA-based small molecule screen of the entire pathway, and have used it to evaluate over 20,000 drug-like small molecules conforming to Lipinski's rules. A number of hits from this screen have been validated against a variety of infectious cell culture systems, including infected T cell lines. Ongoing studies using CFPS and chronically infected H9 cells suggest that some of these compounds work through blockade of virion release, while others result in formation of aberrant capsids. Both phenotypes are consistent with disruption of steps in a host factor catalyzed capsid assembly pathway. Current efforts also involve use of a subset of the active drugs coupled to resins as affinity ligands to determine which drug targets are host proteins, through use of a variety of fractionation/reconstitution protocols. These studies have implications for novel anti-HIV drug discovery and for an understanding of the role of host proteins in the process of capsid formation.

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An Intrabody based on a Llama Single-domain Antibody Targeting the N-terminal α-Helical Multimerization Domain of HIV-1 Rev Inhibits Viral Replication

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Nuclear export of late viral mRNA from the nucleus to the cytoplasm of the cellular host is a crucial step in the cycle of HIV-1 that is mediated by the viral Rev protein. Rev forms large organized multimeric protein–protein complexes on the Rev response element (RRE) of these viral mRNA species and transports them from the nucleus to the cytoplasm exploiting the CRM1-mediated cellular machinery. Multimerization is required for efficient Rev function. Previously, we have employed the small size fully functional antibodies produced by *Camelidae* and composed of heavy-chains only to identify a multimerization inhibitor of Rev. Our strategy consisted of producing single-domain nanobodies against HIV-1 Rev by immunizing a llama with recombinant Rev protein. Using a FRET-based multimerization assay we have selected one nanobody that efficiently inhibits the assembly of Rev multimers. In the presence of this nanobody we observed an accumulation of dimeric Rev species, supporting a head-to-head/tail-to-tail molecular model for Rev assembly. Here, we demonstrate that the binding site of this nanobody is mapped to Rev residues Lys-20 and Tyr-23 located in the N-terminal α-helical multimerization domain, independently confirming its anti-multimerization effect. Using co-localisation experiments of Rev mutants we show that the nanobody also inhibits Rev multimerization in cells. This nanobody inhibits HIV-1 replication and specifically suppresses the Rev-dependent expression of partially spliced and unspliced HIV-1 RNA. It is the first known inhibitor directed against the Rev N-terminal α-helical multimerization domain. Our findings indicate that the oligomeric assembly of Rev is a potential target for anti-HIV therapy and identify specific amino acid residues in Rev that could guide strategies for the development of novel therapeutic anti-HIV-1 agents.

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Discovery of GS-9350: A Novel and Selective Pharmacoenhancer without Anti-HIV Activity

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Background: The HIV protease inhibitor (PI) ritonavir (RTV) is a potent, mechanism-based inhibitor of cytochrome P450 3A (CYP3A), an enzyme responsible for metabolizing most HIV PIs. Low, subtherapeutic doses of RTV improve the pharmacokinetic (PK) profiles of concomitant PIs, and RTV boosting has become the standard-of-care in PI-containing HAART regimens. Coadministration of RTV with the investigational integrase inhibitor elvitegravir (EVG) enhances the PK profile of EVG, and EVG boosted with RTV is currently in Phase 3 studies administered once-daily. However chronic use of RTV has been associated with gastrointestinal and metabolic side effects, and its use as a pharmacoenhancer at a subtherapeutic dose could potentially induce PI resistance mutations if administered in the absence of a fully active PI.

Methods: CYP3A inhibition data were generated using midazolam as the probe substrate. Inhibition of HIV protease was determined with a fluorescent substrate. Antiviral activity was assessed in a standard 5-day HIV-1 cytopathic assay. Induction studies were performed by receptor transactivation analysis.

Results: A series of novel 1,4-diamine carbamates with various P2 and P3 moieties was synthesized and the SARs with respect to human CYP3A inhibition, HIV protease inhibition, anti-HIV activity and pregnane X receptor (PXR) activation were established. Optimization of P2 moieties led to the discovery of GS-9350, which is a potent mechanism-based CYP3A inhibitor. Compared to RTV, GS-9350 has no antiviral activity at concentrations up to 90 μM. In addition, GS-9350 has less off-target effects including inhibition of CYP2D6 and activation of PXR, it has high aqueous solubility and can be co-formulated with other agents. It has recently been shown to enhance the PK of several CYP3A substrates (midazolam, EVG and atazanavir) in the clinic.

Conclusions: GS-9350 is a potent and selective inhibitor of human CYP3A without antiviral activity, and may exhibit reduced metabolic adverse events. It has been co-formulated with EVG and the NRTI backbone emtricitabine/tenofovir DF. GS-9350 is currently being evaluated as a pharmacoenhancer in Phase 2 efficacy studies in HIV patients.

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