anti-HCV agent 2′-β-C-methylguanosine. A phosphoramidate (ProTide) motif and a C^6-methoxy base pro-drug moiety are combined to generate lipophilic prodrugs of the monophosphate of the guanine nucleoside. Extensive DMPK studies in multiple species which supported the selection of the lead compound will be discussed. Details of the pre-clinical development of INX-08189 including radiolabeled metabolism studies will be described. INX-08189 has completed investigational new drug enabling studies and has been progressed into human clinical trials for the treatment of chronic HCV infection.

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Oral Session 3: Retroviruses and Herpesviruses *Chairs: Rhonda Cardin, Ph.D. and Masanori Baba, Ph.D.* 8:45–11:45 am Sofia 1 and 2

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Mechanism of HIV-1 Neutralization by an Antibody: Reversible Binding Stalls Entry

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Despite structural knowledge of broadly neutralizing monoclonal antibodies (NMAbs) complexed to the HIV-1 envelope glycoproteins gp120 or gp41 1 and tools to analyze the HIV-1 cell entry pathway, a mechanism for neutralization has never been proven. In part, this deficiency derives from ambiguities in neutralization assays, with different assays giving widely discrepant results using identical antibodies and viruses. We inferred from these discrepancies that NMAbs might impair virions rather than inactivate them, in which case their residual infectivities would depend on factors limiting specific assays. Using the most common neutralization system, which employs a HeLa-CD4/CCR5 cell clone made in our laboratory, we recently found that HIV-1 titers are determined by a race between entry of cell-attached virions and competing kinetic processes leading to inactivation. Here we show that the widely studied model NMAb, which efficiently inhibits infection after passive transfer into patients, neutralizes by slowing entry of adsorbed virions. Specifically, it slows the assembly and lowers the steady-state concentration of virus complexes with CD4 and coreceptors that control entry rates. Further analysis revealed the stoichiometry of the NMAb required for neutralization and the specific entry step that is slowed. Surprisingly, removing the NMAb from culture media caused its dissociation from virions coupled to accelerated entry and restored infectivity. We believe this is the first evidence that neutralization of viruses can be reversible. These results reveal a kinetic mechanism for HIV-1 neutralization and demonstrate that antibodies able to control infection in patients can function by reversibly slowing entry, a mechanism that has not been previously proposed for any virus.

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Beta5 integrin is the major contributor to the alphaV integrin-mediated blockade of HIV-1 replication

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Monocytes and macrophages are targets of HIV-1 infection and play critical roles in multiple aspects of viral pathogenesis. During the differentiation of monocytes to macrophages, adhesion molecules such as integrins are upregulated, therefore providing signals that control the process and subsequently may render macrophages more susceptible to HIV-1 infection. Integrins are a family of transmembrane cell adhesion receptors that recognize cell-surface and extracellular matrix ligands. Previous work demonstrated that blocking alphaV containing integrins triggered a signal transduction pathway leading to the inhibition of NF-kappaB dependent HIV-1 transcription. However, very little is known regarding the contribution of the different beta integrins to HIV-1 infection in macrophages. Here, we show the influence of the different alphaV-coupled beta integrins in HIV-1 replication in macrophages, by evaluating the antiviral effect of anti-beta integrin antibodies, small RGD mimetic compounds and RNA interference. Expression of beta integrins was evaluated in monocyte derived macrophages (MDM) by flow cytometry. siRNAs specifically targeting beta integrins that dimerize with alphaV were used to transiently downregulate the corresponding integrin expression in MDMs. MDMs treated or not with siRNA were infected using R5-tropic virus. Inhibition of beta integrins either by specific monoclonal antibodies, small RGD mimetic compounds or RNA interference, showed that integrin beta5 was the major contributor to the integrin-mediated blockade of HIV-1 replication. Importantly, such inhibition did not induce changes in cell adhesion to the substrate. In conclusion, we demonstrate that HIV-1 infection in MDMs is influenced by integrin function, especially by the integrin dimer alphaVbeta5. In addition, these results highlight the use of RNA interference as a powerful tool to study and identify cellular factors associated to HIV replication and disease, the first step towards the identification and characterization of potential novel antiviral targets.

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Amido tyrosine esters: a promising new approach to antiviral nucleoside phosphonate prodrugs

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Acyclic nucleoside phosphonates (ANPs) are effective antivirals, but their low permeability limits their therapeutic applications. In a continuing project to increase the oral bioavailability of ANPs, we have synthesized a series of water soluble amido tyrosine ester prodrugs of cyclic (*S*)-HPMPC, (*S*)-HPMPA and also of PMEA