out using either this fused NS4A-NS3 protease or the NS3 protease-NS4A peptide complex. BI 201335 was successfully soaked into unliganded NS3 protease-NS4A peptide crystals, and the resulting 1.9 Å crystal structure of the ternary complex clearly shows all interactions of the bound inhibitor with the target. Of particular interest are the C-terminal acid, which interacts with residues of both the catalytic triad and the oxyanion hole, and the quinoline-substituted proline, which induces the formation of a large flat surface partially comprised of catalytic triad residues. This arrangement affects the protonation state of the active site histidine, as demonstrated by NMR. These findings provide detailed insight into the mechanism of action for this promising new candidate for treatment of hepatitis C infection.

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MK-5172, a Novel Macrocyclic Inhibitor of NS3/4a Protease Demonstrates Efficacy Against Viral Resistance in the Chimpanzee Model of Chronic Hepatitis C Virus Infection

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MK-5172 is a potent macrocyclic inhibitor of NS3/4a protease enzyme (IC_{50} < 0.016 nM, replicon IC_{50} = 2 nM) optimized for an improved pharmacokinetic profile and increased potency against resistant virus. As part of its pre-clinical characterization, three chimpanzees chronically infected with HCV were dosed with MK-5172; plasma samples were periodically collected and evaluated for efficacy and viral resistance. Two chimpanzees (gt1a/gt1b) were burdened with high viral load infections ($\sim 10^6$ IU/ml) while a third sustained a modest viral load of 10^4 IU/ml that was homogenous for R155K virus. All three chimpanzees had previously undergone experimental treatment with the first generation protease inhibitor MK-7009. Both high viral load chimps experienced rapid, robust reductions in viral load of 4 and 5 logs, respectively. The R155K infected chimpanzee experienced a two log viral load reduction within 2 days and remained similarly suppressed through the duration of the dosing period. All three demonstrated viral load reductions one log or greater compared to their responses to MK-7009 at equal or higher doses. Sequence analysis of NS3/4a demonstrated no emergence of additional homogenous resistance or genetic polymorphisms. MK-5172 possesses an attractive pharmacokinetic and potency profile, and demonstrates suppression of resistant virus in vivo. Clinical studies with MK-5172 are currently in progress.

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Oral Session 3: Retroviruses and Herpesviruses

Chairs: Rhonda Cardin, Ph.D. and Masanori Baba, Ph.D., 9:00–11:30 am, Grand A

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Identification of a N-linked Glycan on HIV-1 gp120 that is Indispensable for Viral Entry

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Carbohydrate-binding agents (CBAs) inhibit HIV-1 entry into susceptible cells by binding to the N-linked glycans that make up the protective glycan shield on the viral envelope. Under increasing pressure of the CBAs, HIV-1 deletes a variety of N-linked envelope glycans to escape drug pressure. More than 50 virus strains were already selected under CBA pressure, but none of them showed glycan deletions at the N239, N260, N354 and N461 amino acid positions of gp120. To reveal why certain N-glycosylation sites were so far not deleted under CBA pressure in HIV-1 infected cell cultures, the plasmid pBlue-Env which encodes the HIV-1 env gene was used to generate mutant virus envelope gp120 with disrupted glycosylation sites at positions N239, N260, N354 and N461, where Asn was replaced by Gln. A variety of mutant viruses were constructed by env chimeric virus technology with the pNL4.3-Denv-EGFP plasmid and subsequent transfection of 293T cells. Neither the mutant N260Q/A nor the S262C/A gp120 HIV-1 strains in which the 260NGS262 glycosylation motif was destroyed were able to infect the U87.CD4.CCR5.CXCR4 cells. The replication capacity of the 3 other mutant monodeglycosylated gp120 virus strains (N239Q, N354Q and N461Q) was reduced but not annihilated. Whereas 293T cells expressing the HIV-1 wild-type gp120 envelope were able to induce syncytia when cocultured with U87.CD4.CCR5.CXCR4 cells, giant cell formation was not observed when 293T cells expressed the mutant N260Q HIV-1 gp120 envelope. This loss of infection potential and capacity to form giant cells in co-cultures was not due to a lethal defect in Env processing. Instead, the mutant N260Q gp120 virus displayed a significantly decreased CD4 binding ability. In conclusion, deletion of the N-linked glycan at amino acid position N260 of HIV-1 gp120 results in the complete loss of infectivity and capacity to induce giant cell formation. This is probably caused by a significant lower CD4-binding compared to wild-type virus. Since N260 is a highly conserved glycosylation site among the different clades of HIV-1, the crucial role of the N-linked glycan at amino acid position 260 of HIV-1 gp120 for viral infectivity makes it an interesting target for antiviral therapy or vaccine development against HIV-1.

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