Oral Session 2: Hepatitis Viruses

Chairs: Phillip Furman, Ph.D. and Klaus Klumpp, Ph.D., 2:00–4:00 pm, Grand A

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Diphenylcarboxamides as Inhibitors of HCV Non-Structural Protein NS5a

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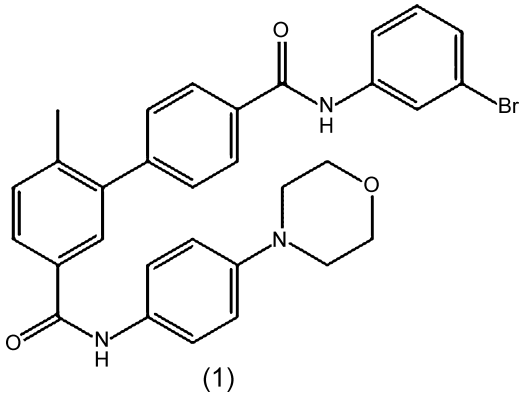
In order to identify novel agents for the treatment of hepatitis C (HCV) a series of analogues were screened in a genotype 1b replicon assay. Rather than directly targeting the protease or polymerase genes, we set about testing families of compounds known to bind to ATP recognition sites (i.e. biphenyl-dicarboxamide derivatives), as it is known that a number of processes vital to viral replication require ATP. The SAR of these molecules will be discussed in detail. Subsequent to the identification of these molecules, their anti-viral mechanism of action was determined by sequencing mutant replicons generated in the presence of compound. Using a simple 2D amide array the first micromolar inhibitor (1) was identified. Subsequent structural modification over a number of iterative cycles gave nanomolar inhibitors. When mutant replicon was generated and then sequenced, the major changes were found to occur in Domain 1 of the NS5a gene. The resistance phenotype was due to Y93C or H mutation, and this mechanism of action has been confirmed by reverse genetics. A compound from this series has been progressed into clinical trials, the results of which will be published in due course.



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Selection of Clinically-relevant Protease Inhibitor Resistant Viruses using the HCV Infection System

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Treatment of HCV patients with direct acting antivirals can lead to the emergence of drug-resistant variants which may pose a long term threat to viral eradication. HCV replicons have been used to select resistance mutations; however, genotype 2 JFH-based viruses provide the opportunity to perform resistance selection in a bona fide HCV infection system. In this study, we used J6/JFH-1 virus to select resistance to the NS3 protease inhibitors BILN-2061 and VX-950. Lunet-CD81 cells were infected with an adapted J6/JFH-1 virus and maintained in the presence of inhibitors until high-titer viral supernatant was produced. Viral supernatants were passaged over naïve cells at escalating drug concentrations and the resulting viruses were then characterized. Phenotypic analyses indicated the selected viruses had comparable infection kinetics to the parental virus but were significantly less susceptible to BILN-2061 (>33-fold) and VX-950 (>10-fold). Biochemical assays using NS3-containing lysates from infected cells confirmed resistance was due to phenotypic changes in the protease. Three NS3 mutations were identified in BILN-2061 resistant viruses: A156G, D168A and D168V. Interestingly, D168A and D168V, but not A156G, were selected in parallel using a genotype 2a replicon. More strikingly, from multiple selections with VX-950, three NS3 mutations were identified in the virus (T54A, A156S, and V102A) but only A156T/V emerged in genotype 2a replicon selections. Of note, resistance mutations selected from virus infection, including T54A, A156S, and A156G, have all been observed in the clinic. We hypothesized that resistant viruses may need to balance fitness and resistance to a greater extent than resistant replicons. To investigate this, each major viral resistance mutation was introduced into the parental J6/JFH-1 virus. Viral kinetic studies demonstrated that the mutant viruses expanded at similar or slightly delayed rates but reached similar peak titers to the parental virus. In conclusion, the HCV infection system is an efficient tool for drug-resistance selections and has advantages to rapidly identify and characterize clinically-relevant resistance mutations.

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In Vitro Selection and Characterization of Hepatitis C Virus Replicons Double or Triple Resistant to Various Non-nucleoside HCV Polymerase Inhibitors

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To prevent, delay or avoid the development of HCV resistance, combination therapies will be necessary. We determined the antiviral efficacy of various combinations of non-nucleoside polymerase inhibitors and the barrier towards resistance development. Short-term antiviral combination assays were performed in replicon containing cells. For resistance selection of mono- and double resistant replicons, a stepwise selection procedure was used. Triple resistant replicons were selected starting with a replicon already resistant to thiophene carboxylic acid (TCA) and the benzofuran HCV-796. All pair wise combinations of elicit an additive anti-HCV effect in short-term antiviral assays. Resistant replicons were selected for three non-nucleoside polymerase inhibitors (TCA, benzimidazole JT-16 and benzofuran HCV-796) and for each pair wise combination. Triple resistant replicons were selected for the following combinations: TCA + HCV-796 + VX-950 (protease inhibitor), TCA + HCV-796 + 2′-C-methylcytidine and TCA + HCV-796 + JT-16. The identified genotype of double and triple resistant replicons is the sum of the single resistance mutations. Cross-resistance selection experiments revealed that combinations of non-nucleoside inhibitors (HCV-796 + TCA and HCV-796 + JT-16)