

15th International HIV Drug Resistance Workshop



13-17 June 2006, Sitges, Spain

SELECTION AND CHARACTERIZATION OF HEPATITIS C VIRUS REPLICON VARIANTS DUALY RESISTANT TO THUMB AND PALM BINDING NON-NUCLEOSIDE POLYMERASE INHIBITORS

Antivir Ther. 2006, 11:S5 (abstract no. 3)

S Le Pogam, H Kang, SF Harris, V Leveque, AM Giannetti, S Ali, WR Jiang, S Rajyaguru, G Tavares, C Oshiro, T Hendricks, K Klumpp, J Symons, MF Browner, N Cammack and INájera

Roche Palo Alto LLC, Palo Alto, CA, USA

BACKGROUND: BACKGROUND: Multiple non-nucleoside inhibitor binding sites have been identified within the HCV polymerase, including in the palm and thumb domains. The high genetic heterogeneity of HCV, due to the error-prone nature of its RNA-dependent RNA polymerase, represents an opportunity for the virus to evade antiviral treatment. Therefore, the development of successful therapies based on inhibitors targeted against viral enzymes requires an understanding of the nature of resistant HCV variants likely to emerge upon treatment and their fitness.

METHODS: Using the HCV subgenomic replicon system, we selected and characterized HCV variants resistant to a thiophene-2-carboxylic acid (NNI-1, which binds to the thumb I site). Through combination studies of NNI-1 with a potent polymerase inhibitor which binds to the palm domain (NNI-3), we have studied the effect of targeting simultaneously different sites of the NS5B polymerase.

RESULTS: After single treatment with a thumb site inhibitor (thiophene-2-carboxylic acid, NNI-1), resistant HCV replicon variants emerged that contained mutations at residues Leu419, Met423 and Ile482 in the polymerase thumb domain. Binding studies using WT and mutant enzymes and structure-based modelling showed that the mechanism of resistance is through the reduced binding of the inhibitor to the mutant enzymes. Combined treatment with a thumb and a palm binding polymerase inhibitor had a dramatic impact on the number of replicon colonies able to replicate in the presence of both inhibitors. A closer characterization through molecular cloning showed that 97.7% of replicons contained amino acid substitutions that conferred resistance to either of the

inhibitors. Of those, 65% contained simultaneously multiple amino acid substitutions that conferred resistance to both inhibitors. Double mutant replicons Met414Leu/Met423Thr were predominantly selected, which showed reduced replication capacity compared to WT replicon.

CONCLUSIONS: These findings demonstrate the selection of replicon variants dually resistant to two NS5B polymerase inhibitors binding to different sites of the enzyme. Additionally, these findings provide initial insights into the *in vitro* mutational threshold of the HCV NS5B polymerase and the potential impact of viral fitness on the selection of multiple resistant mutants.

2006-06-13

3

Copyright © 2006 - [International Medical Press Ltd.](#) Reproduction of this abstract (other than one copy for personal reference) must be cleared through the International Medical Press Ltd. 2-4 Idol Lane, London EC3R 5DD UK.