Sensitive Detection of Y448H NS5B Mutant Viruses in Patients Infected with Genotype 1a and 1b HCV

A Bae1, J Jackson1, K Ku1, J Harris1, M Miller2, K Borroto-Esoda1, H Mo2, E Svarovskaia1
Gilead Sciences, Inc., 1Durham, NC, and 2Foster City, CA, USA

Poster 1897
161st Annual Meeting of the American Association for the Study of Liver Diseases
October 29 – November 2, 2010
Boston, Massachusetts, USA

Introduction

- Genetic diversity of HCV results from rapid virus replication and nucleotide misincorporation by the error-prone NS5B polymerase
- HCV antiviral drug resistance mutations may pre-exist in a viral population in antiviral treatment-naive patients
- The NS5B Y448H mutant confers resistance to various non-nucleoside NS5B inhibitors in vitro and has been observed in patients who received GS-9190
- Standard population sequencing is limited in detecting minor variants (<25%) within a viral population

Methods

- Standard curves were generated for both GT1a and GT1b, using fixed combinations of mutant and wild-type clones
- AS-PCR was performed using standard TopoTA cloning
- Population sequencing of 65/65 subjects were Y448Y wild-type. Subjects >0.5% Y448H mutant by AS-PCR ranged from 0.5%-3.0%

Results

- An AS-PCR assay has been developed capable of detecting the NS5B Y448H mutant in genotype 1a or 1b HCV replicons or infected patients when present at levels as low as 0.5%
- HCV replicons treated with GS-9190 showed by AS-PCR 1.6% to 19.2% Y448H by passage 3
- AS-PCR showed a good correlation with single-genome sequencing and clonal analysis

Conclusions

- An AS-PCR assay has been developed capable of detecting low levels of the NS5B Y448H mutant down to 0.5% in genotype 1a and 1b HCV
- This assay can be used to monitor the selection and decay of the Y448H mutant in HCV infected patients during and off treatment with GS-9190 and other “Site 3” NS5B inhibitors
- Naturally occurring low levels of Y448H variants were detected in 5/6 (83%) treatment-naive patients infected with HCV GT1 by AS-PCR

References & Acknowledgements

1. Reduction and Resurgence of HCV Infected Patients Receiving Single and Multiple Doses of GS-9190. Poster B10 3612 E02, October 30 – November 1, 2010, Boston, MA

© 2010 Gilead Sciences, Inc. All rights reserved