Poster Number

955

59th Annual Meeting of the American Association for the Study of Liver Diseases
October 31 - November 4, 2008
San Francisco, California, USA

Tenofovir is Equally Active *In Vitro* Against Wild-type HBV Clinical Isolates of Genotypes A-H

M Curtis[1], J Hinkle[1], J Harris[1], K Borroto-Esoda[1] and Y Zhu[1]

[1]Gilead Sciences, Inc., Durham, NC, USA

GILEAD
Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
Tel: (650)574-3000
Fax: (650)578-9264

Introduction

- Tenofovir is an acyclic nucleotide analog whose oral prodrug tenofovir disoproxil fumarate (TDF) was recently approved for the treatment of chronic hepatitis B (CHB)
- HBV is classified into eight different viral genotypes (A-H)
- Differences among the viral genotypes could affect susceptibility to antiviral agents

Objective

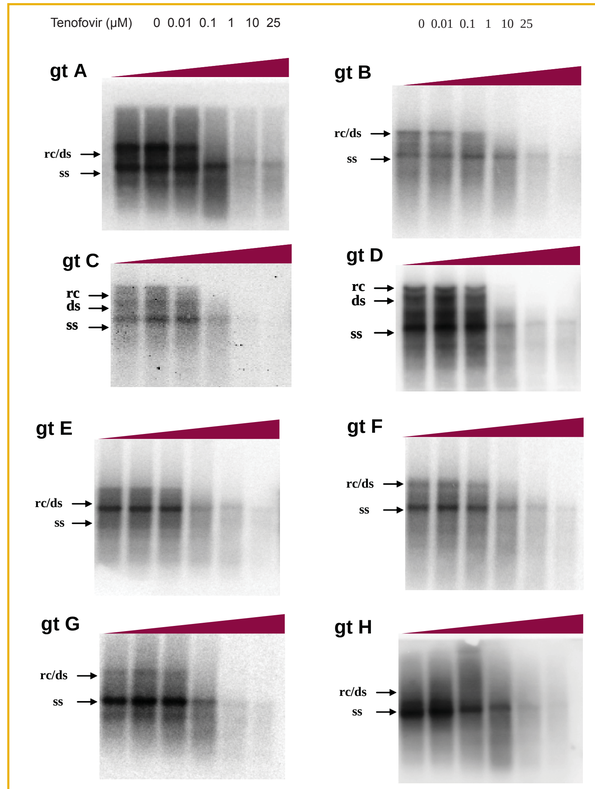
- To evaluate the *in vitro* tenofovir susceptibility of patient derived wild-type HBV representing viral genotypes A-H

Methods

- **Subjects and sera:** Baseline samples from treatment naïve, CHB patients enrolled in various Gilead sponsored clinical trials
- **Extraction and amplification:** HBV DNA was extracted and the full-length HBV genome was amplified as described by Gunther, et al[1]
- **Cloning of full-length quasi-species pools:** The full-length HBV genomes were cloned into the pHY106 plasmid vector[2]
- **Drug susceptibility testing:** HBV quasi-species pools were transfected into HepG2 cells in 6-well plates. A day later drug treatment started and lasted for 7 days, with replacement of fresh drug-containing media every 2-3 days. Intra-cellular core-associated HBV DNA was extracted and analyzed by Southern hybridization using ^{32}P-labelled HBV DNA as a probe. Tenofovir EC_{50} values were calculated based on the signal intensities of ds-HBV DNA using TableCurve 2D software (SYSTAT)
- **Statistical analysis:** Mixed effect ANOVA models were fit to log-transformed EC_{50} and HBV DNA data across patients and lab strain samples with a fixed effect for genotype and a random effect for sample-within-genotype

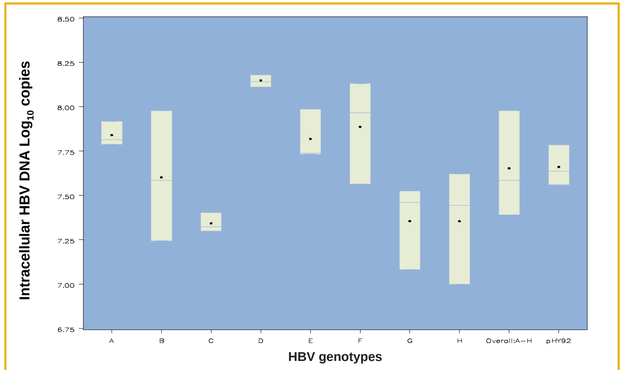
Results

Figure 1. HBV DNA replication of the full length wild-type HBV genomes representing viral genotypes A-H decreased in the presence of tenofovir



Shown are representative Southern blots of intracellular HBV DNA.
Relaxed circular (rc), double-stranded linear (ds) and single stranded (ss) forms are labeled

Figure 2. Levels of Intracellular Replicating HBV DNA Were Similar Across Genotypes A-H, Except for Genotype D*



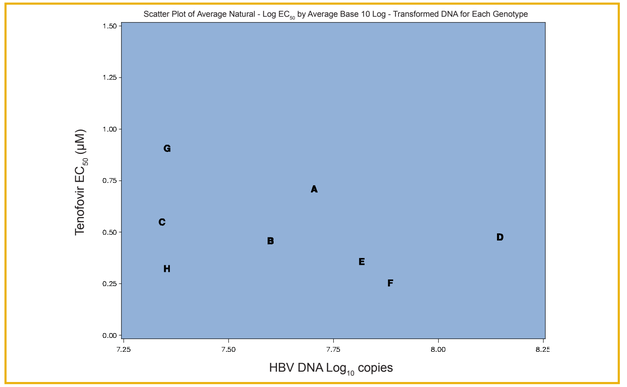
*Genotype D isolates had a significantly higher level of replication (p=0.006) compared to combined data

Table 1. HBV Viral Genotypes A-H Were Equally Susceptible to Inhibition by Tenofovir

Sample ID	Viral Genotype	Replicates (n=)	Tenofovir EC_{50} (uM) +/- STDEV
pHY92	A	11	0.7± 0.3
1	A	3	0.8 ± 0.2
2	B	3	0.4 ± 0.1
3	C	3	0.4 ± 0.3
4	C	3	0.7 ± 0.1
5	C	3	0.5 ± 0.1
6	D	3	0.3 ± 0.1
7	D	3	0.6 ± 0.1
8	E	3	0.3 ± 0.1
9	F	3	0.2 ± 0.1
10	G	3	0.9 ± 0.2
11	H	3	0.3 ± 0.2

Statistical analysis (mixed ANOVA) comparing tenofovir EC_{50} values across genotypes A-H, p=0.30

Figure 3. No Correlation Between Intracellular HBV DNA and Tenofovir EC_{50} Values by Genotype



Conclusions

- **Tenofovir was equally active in vitro against wild-type HBV clinical isolates representing viral genotypes A-H**
- **These results are consistent with observations from clinical studies which demonstrated similar virologic response across viral genotypes in patients treated with TDF[3]**

References

1. Gunther,S et al, A Novel Method for Efficient Amplification of Whole Hepatitis B Virus Genomes Permits Rapid Functional Analysis and Reveals Deletion Mutants in Immunosuppressed Patients, Journal of Virology, Sept. 1995, p. 5437–5444, Vol. 69, No. 9
2. Zhu,Y et al, In Vitro Drug Susceptibility Analysis of Hepatitis B Virus Clinical Quasispecies Populations. Journal of Clinical Microbiology, Oct. 2007, p. 3335-3341, vol. 45, No. 10
3. Gane, E. et al, Lack Of Influence Of Baseline Genotype On Antiviral Response In Subjects With Chronic Hepatitis B Infection Receiving Tenofovir DF 300 MG QD For 1 Year. Poster No. 2780, 43rd Annual EASL, April 23-27, 2008 Milan, Italy