

POSTER 21**HBV DNA REPLICATION MEDIATED BY CLONED PATIENT RT GENES FROM HBV GENOTYPES A-H AND ITS USE IN ANTIVIRAL PHENOTYPIC ASSAYS**

Y. Zhu, M. Curtis, and K. Borroto-Esoda

Gilead Sciences, Durham, NC

Introduction: HBV RT is the target of all the nucleos(t)ide analogs developed for HBV treatment. As such, all known drug resistance mutations reside within the RT domain. The aim of this study is to establish a convenient assay to phenotypically analyze patient RT sequences for potential drug resistance.

Methods: The HBV RT/pol region (pol aa 304 to 715, including the entire RT) from HBV clinical isolates was amplified with primers containing the EcoRI and SphI sites, and ligated into a plasmid vector (pRTAN) that expresses a lab strain of HBV (genotype A) genome lacking the RT region. The RT region of woodchuck hepatitis virus (WHV, Kodama strain) was obtained by digesting pCMVWHV with AvrII and RsrII and cloned into the corresponding sites of pRTAN. HBV DNA replication of the recombinants was assessed by transient transfection into HepG2 cells and intracellular core DNA was analyzed by Southern blot. *In vitro* drug susceptibility was tested by transient transfection of the recombinant pools into HepG2 cells using a 96-well format for high-throughput testing and intracellular HBV DNA was quantified by real-time PCR.

Results: Cloning of the HBV RT gene from clinical isolates representing genotypes A-H into the lab strain, RT deleted HBV was successful and led to virus DNA replication. However, a recombinant of RT from WHV imbedded in HBV genome did not replicate. Recombinants containing patient derived RT genes containing L180M/M204V lamivudine resistance (LAM-R) mutations demonstrated a lamivudine-resistant phenotype ($EC_{50} > 100 \mu\text{M}$). Similarly, patient derived RT genes containing the adefovir resistance (ADV-R) A181V or N236T mutations demonstrated an adefovir-resistant phenotype. Recombinants containing HBV RT from paired patient samples (baseline and later time point) without drug resistance genotypic changes had similar EC_{50} values (≤ 2 -fold change in EC_{50}).

Conclusions: A high-throughput testing format was successfully developed for evaluating drug resistance of antiviral agents targeting HBV RT. Recombinants representing HBV genotypes A-H (but not WHV RT) led to successful virus DNA replication, allowing phenotypic evaluation of patient derived HBV RT region of all HBV genotypes and confirmed the drug resistance phenotype in samples containing LAM-R or ADV-R drug resistance mutations.