Poster Number

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Week 96 Resistance Surveillance for HBeAg Positive and Negative Subjects with Chronic HBV Infection Randomized to Receive Tenofovir DF 300 mg QD

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Introduction

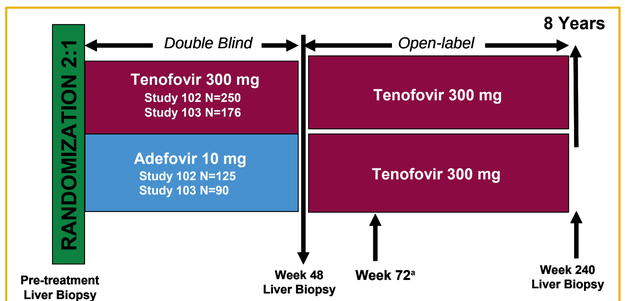
- Tenofovir DF (TDF) is a nucleotide analog with potent antiviral activity in patients mono-infected with HBV and co-infected with HIV/HBV
- HBV pol/RT resistance mutations have been identified following administration of oral anti-HBV agents (lamivudine, adefovir dipivoxil, entecavir, and telbivudine)
- The rtA194T substitution was observed in two HIV/HBV co-infected patients[1], however in a recent study the presence of this mutation did not result in reduced efficacy of TDF[2]
- No amino acid substitutions associated with resistance to tenofovir were detected during the first 48 weeks of Studies 102 and 103

Objectives

- To identify amino acid substitutions in the HBV pol/RT following 96 weeks of therapy with TDF 300 mg QD
- To evaluate the effects of these substitutions on the clinical response to TDF mono-therapy
- To determine whether these substitutions alter susceptibility to tenofovir using *in vitro* HBV replication assays and to evaluate the cross-resistance profile of these substitutions

Methods

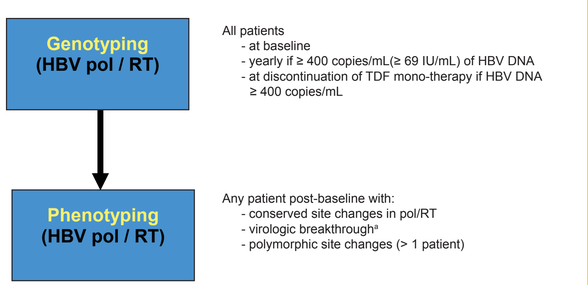
Figure 1. Design of HBeAg Negative Study 102 and HBeAg Positive Study 103 of TDF in Chronic Hepatitis B Patients



a. Patients could add emtricitabine 200 mg to tenofovir DF 300 mg if confirmed to be viremic at Week 72 or beyond

- Patients were enrolled in one of two double-blind, randomized studies of TDF [GS-US-174-0102 (HBeAg-) or GS-US-174-0103 (HBeAg+)]
- Population di-deoxy sequencing of serum HBV pol/RT
  - Covers AA 1-344 of pol/RT (AA 1-266 of HBsAg)
  - Able to detect AA substitutions present at ≥ 25% of viral quasi-species population
- Phenotypic analyses were conducted in HepG2 cells transiently transfected with a pool of recombinant HBV plasmid DNA derived from patient serum HBV
- Plasma HBV DNA levels were determined by Roche COBAS TaqMan assay (LLOQ = 169 copies/mL; 29 IU/mL)

Figure 2. Virology Analysis Plan for Studies 102 and 103



a. Defined as a confirmed 1log10 increase in HBV DNA and/or confirmed HBV DNA >400cp/ml after having <400 cp/mL

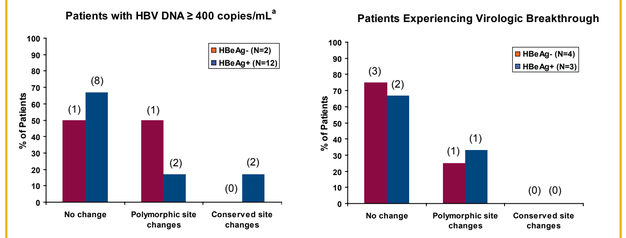
Results

Table 1. Summary of Resistance Surveillance Conducted at Week 96/last on TDF Among HBeAg- and HBeAg+ TDF Treated Patients

HBV DNA ≥ 400 copies/mL	HBeAg- (N=235)	HBeAg+ (N=154)	Total (N=389)
Without virologic breakthrough	2	15	17
With virologic breakthrough	4	3	7
Total number of patients included in week 96 resistance surveillance	6	18	24
Category			
After 96 weeks of TDF mono-therapy	2	3	5
Discontinued TDF mono-therapy between week 48 and week 96[a]	2	0	2
Added emtricitabine to open-label TDF between week 72 and week 96[a]	2	15	17

a. Median duration of TDF mono-therapy at time of discontinuation/addition of emtricitabine was 80 weeks

Figure 3. Genotypic Changes Observed at Week 96/last on TDF Among HBeAg- and HBeAg+ TDF Treated Patients



a. Three HBeAg+ patients with HBV DNA ≥ 400 copies/mL were unable to be genotyped (PCR negative)

Conserved site changes observed in one patient each at positions rtL101L/F and rtV173L + rtL180M + rtM204V. No two patients developed the same polymorphic site changes.

Table 2. Phenotypic Analysis of HBV DNA Obtained from HBeAg+ (Study 103) TDF Treated Patients Harboring Conserved Site Changes in HBV pol/RT (N=2)

Patient	pol/RT	Tenofovir EC_{50} (μM)	Fold Change[b]
8356 – Baseline	Wild-type	12.4 ± 3.6	
8356 – Week 72	rtL101L/F	13.8 ± 0.6	1.1
8356 – Week 72 (clone)	rtL101F	10.0 ± 6.2	0.7
7916 – Baseline[a]	Wild-type	9.9 ± 3.4	
7916 – Week 72	rtV173L, rtL180M, rtM204V	12.5 ± 6.3	1.3

a. Clonal analysis of the baseline sample demonstrated the presence of the LAM-R mutations at a frequency of 6.5%
b. Fold change = last on TDF EC_{50}/ Baseline EC_{50}. Fold changes < 2X are within the assay variability

Development of conserved site changes was not associated with phenotypic resistance to tenofovir.

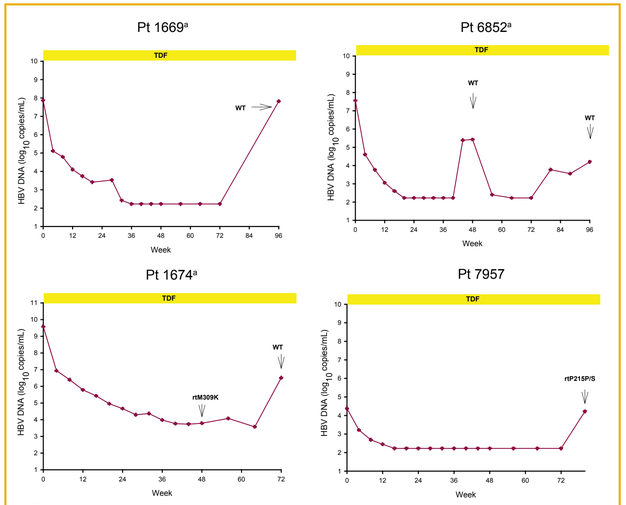
Table 3. Phenotypic Analysis of Clinical Isolates from HBeAg- and HBeAg+ TDF Treated Patients who Experienced Virologic Breakthrough on TDF (N=7)

Patient	Tenofovir EC_{50} (μM)	Fold Change[a]
1674 – Baseline (Study 102)	8.0 ± 1.0	
1674 – Week 72	7.7 ± 1.5	1.0
1669 – Baseline (Study 102)	9.7 ± 4.1	
1669 – Week 96	11.1 ± 7.7	1.1
6852 – Baseline (Study 102)	12.2 ± 4.7	
6852 – Week 96	10.5 ± 4.4	0.9
7957 – Baseline (Study 102)	10.3 ± 0.7	
7957 – Week 80	8.3 ± 1.5	0.8
1553 – Baseline (Study 103)	11.2 ± 5.3	
1553 – Week 96	11.3 ± 5.7	1.0
3958 – Baseline (Study 103)	11.3 ± 4.0	
3958 – Week 88	11.1 ± 2.5	1.0
4957 – Baseline (Study 103)	12.2 ± 0.8	
4957 – Week 88	11.6 ± 4.6	1.0

a. Fold change = last on TDF EC_{50}/ Baseline EC_{50}. Fold changes < 2X are within the assay variability

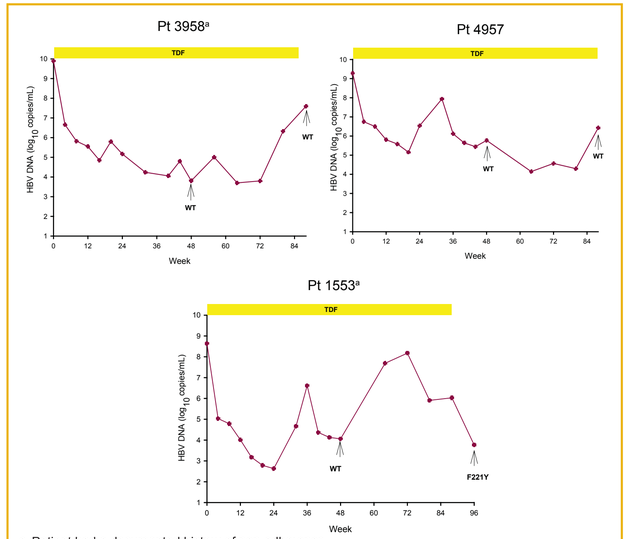
Virologic breakthrough was not associated with phenotypic resistance to tenofovir.

Figure 4. Patients in Study 102 Experiencing Virologic Breakthrough on TDF



a. Patient had a documented history of non-adherence
WT=wild type

Figure 5. Patients in Study 103 Experiencing Virologic Breakthrough on TDF



a. Patient had a documented history of non-adherence
WT=wild type

Conclusions

- **No HBV pol/RT amino acid substitutions associated with resistance to tenofovir were detected through 96 weeks of tenofovir DF mono-therapy**
  - **Annual resistance surveillance on-going through year 8 (week 384)**
- **Virologic breakthrough was infrequent and not associated with phenotypic resistance to tenofovir**
  - **The majority of patients experiencing virologic breakthrough had evidence of non-adherence**
- **Development of conserved site changes was rare and not associated with phenotypic resistance to tenofovir**

References

1. Sheldon et al. Antiviral Therapy, 10:727, 2005
2. Fung et al. AASLD 2008, Poster #880