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Resistance Surveillance for up to 144 Weeks in HBeAg+ and HBeAg- Hepatitis B Patients Treated with Tenofovir DF Showed No Relationship Between Virologic Breakthrough and Emergence of Genotypic Changes in HBV Polymerase

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Introduction

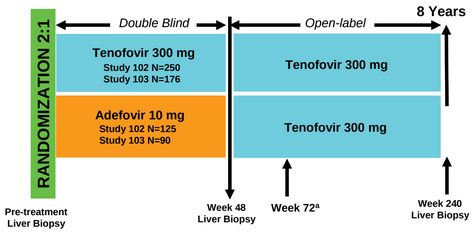
- Tenofovir disoproxil fumarate (tenofovir DF, TDF) is a nucleotide analog with potent antiviral activity in patients mono-infected with HBV and co-infected with HIV-1 and HBV
- HBV pol/RT resistance mutations have been identified following administration of other oral anti-HBV agents (lamivudine, adefovir dipivoxil, entecavir, and telbivudine)
- No amino acid substitutions associated with resistance to tenofovir DF were detected in the HBV pol/RT during the first 96 weeks of TDF treatment of HBeAg- and HBeAg+ patients in Studies 102 and 103[1]

Objectives

- To identify amino acid substitutions in the HBV pol/RT following up to 144 weeks of therapy with TDF 300 mg once daily
- To evaluate the effects of these substitutions on the clinical response to TDF monotherapy in chronic hepatitis B
- 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

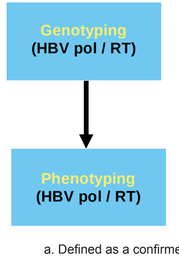
Figure 1. Design of HBeAg- Study 102 and HBeAg+ Study 103 of TDF in Chronic Hepatitis B Patients



a. Patients had the option at the discretion of the investigator to 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 [Study 102 (HBeAg-) or Study 103 (HBeAg+)]
- Genotypic analysis by 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 pol/RT or
  - Mutant virus created by site-directed mutagenesis in the pCMVHBV (genotype D) or pHY92 (genotype A) backbone
- 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



- All patients:
  - At baseline
  - Yearly if HBV DNA ≥ 400 copies/mL (≥ 69 IU/mL)
  - At discontinuation of TDF mono-therapy if HBV DNA ≥ 400 copies/mL
- Any patient post-baseline with:
  - Conserved site changes in pol/RT
  - Virologic breakthrough (VB)[a]
  - Polymorphic site changes (> 1 patient)

a. Defined as a confirmed 1 $\log_{10}$ increase in HBV DNA from nadir and/or confirmed HBV DNA >400 copies/mL after having <400 copies/mL

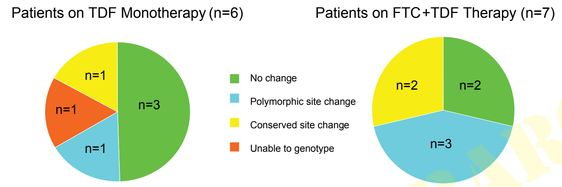
Results

Table 1. HBeAg- and HBeAg+ Patients Evaluated During Year 3

	TDF-TDF Group	ADV-TDF Group
Patients entering Year 3	364/426 (85%)	192/215 (89%)
Patients with HBV DNA >400 copies/mL	13/364 (4%)	9/192 (5%)
Patients on TDF monotherapy	6 (4 HBeAg-, 2 HBeAg+)	5 (all HBeAg+)
Patients with VB	1	2
Patients on FTC/TDF combination therapy	7 (all HBeAg+)	4 (all HBeAg+)
Patients with VB	3	1

VB = Virologic Breakthrough defined as a confirmed 1 $\log_{10}$ increase in HBV DNA from nadir or confirmed HBV DNA >400 copies/mL after having been <400 copies/mL

Figure 3. Genotypic Changes in HBeAg- and HBeAg+ TDF-TDF Treated Patients During Year 3



Conserved site changes observed in 3/13 subjects
Patient A (TDF): rtR51K
Patient B (FTC+TDF): rtL180M±rtM204V±rtA181T
Patient C (FTC+TDF): rtR192H
None of the changes were associated with Virologic Breakthrough

Figure 4. HBV DNA Profile for the TDF-TDF Treated Patients with Conserved Site Changes

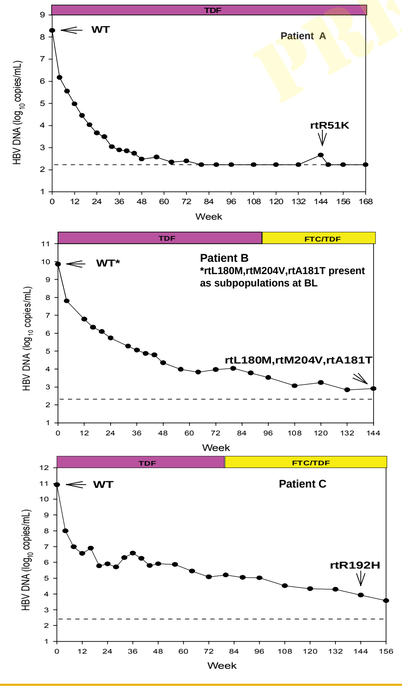
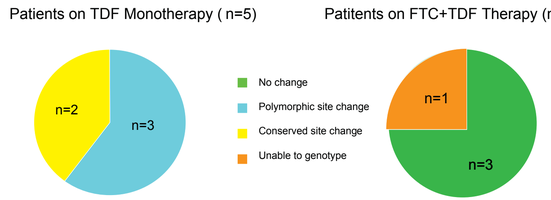
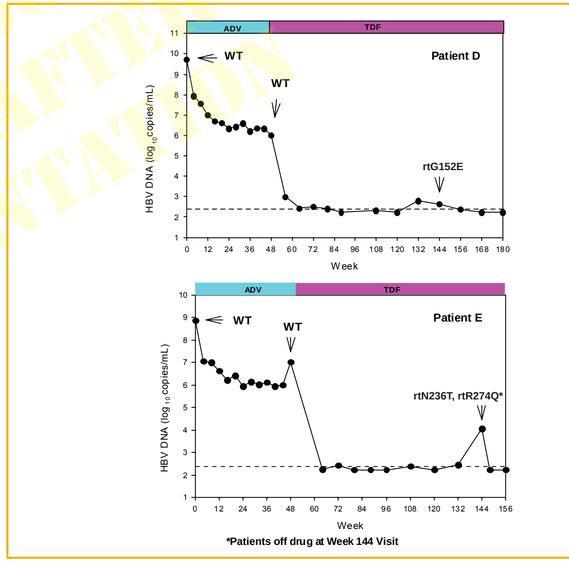


Figure 5. Genotypic Changes in HBeAg- and HBeAg+ ADV-TDF Treated Patients During Year 3



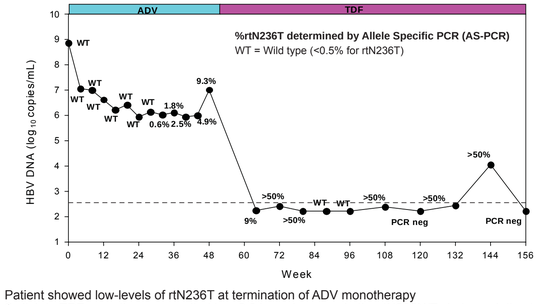
Conserved site changes observed in 2/9 subjects
Patient D (ADV-TDF): rtG152E
Patient E (ADV-TDF): rtN236T±rtR274Q
The rtG152E change was observed during a transient Virologic Breakthrough

Figure 6. HBV DNA Profile for the ADV-TDF Treated Patients with Conserved Site Changes



*Patients off drug at Week 144 Visit

Figure 7. Evolution of Low Levels of rtN236T on ADV-TDF Therapy: Case Study of Patient E by Allele-Specific PCR



- Patient showed low-levels of rtN236T at termination of ADV monotherapy
- Switch to TDF monotherapy resulted in a 3.8 $\log_{10}$ decrease in the rtN236N/T viral population to below detection limits of HBV DNA PCR assay at most visits

Table 2. Development of Conserved Site Changes in HBV pol/RT did not Impact Phenotypic Sensitivity to Tenofovir *in vitro*

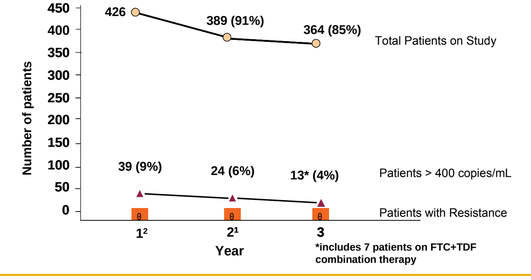
Treatment Group	Viral Isolate	Change from BL in HBV pol/RT	Fold Change from BL[a]
TDF	Patient A Week 144_pool	rtR51K	1.4
TDF-FTC/TDF	Patient B Week144_pool[b]	rtA181T[b]	0.7
TDF-FTC/TDF	Patient C Week 144_pool/clones[c]	rtR192H	Replication Defective
ADV-TDF	Patient E Week 144_pool Week 144_clone 1 Week 144_clone 2	rtN236N/T±rtR274R/Q rtN236T rtR274Q	1.9 8.2 1.9
Treatment Group	**Laboratory Isolate**	**Change from control in HBV pol/RT**	**Fold Change from control**
TDF-FTC/TDF	pCMVHBV rtR192H	rtR192H	Replication Defective
ADV-TDF	pHY92 rtG152E	rtG152E[d]	1.8

a. Values ≤ 2-fold are not statistically significant
b. Constructs containing the rtL180M+rtM204V were not obtained in clonal analysis for phenotypic evaluation
c. Seven clones containing the rtR192H were also tested, all were replication defective (i.e. did not grow in cell culture)
d. Site-directed recombinant expressing rtG152E created for patient D as patient serum failed to generate a phenotyping vector

Results Summary

- Conserved site changes in HBV pol/RT observed in 5 of 556 patients across both arms of Studies 102 and 103 during Year 3
  - Not associated with persistent virologic breakthrough
  - Not associated with altered susceptibility to tenofovir *in vitro*
- Polymorphic site changes observed in 7 patients
  - Represent natural polymorphic changes as observed historically among placebo-treated patients
  - The presence of these substitutions at baseline did not impact clinical response to TDF
- Virologic breakthrough observed in 7 patients
  - Associated with non-adherence in the majority of cases
  - Not associated with *in vitro* resistance to tenofovir (data not shown)

Figure 8. Summary of Resistance Analyses of TDF-Treated Patients Through Year 3



Conclusions

- **No resistance to TDF developed following up to 3 years of TDF monotherapy in 364 patients**
  - **Similar data observed among the 20 patients who added FTC**
- **No resistance to TDF developed among 192 ADV treated patients following up to 2 years of TDF monotherapy**
  - **Similar data observed among the 14 patients who added FTC**
- **Patient retention remained high, 86.7% (556/641) across both arms of Studies 102 and 103**

References & Acknowledgements

1. Snow-Lampart et. al. AASLD 2008, Poster #977
2. Snow-Lampart et. al. EASL Monothematic conference on Hepatitis B and C Virus Resistance to Antiviral Therapy 2008, Poster #4

Jeff Sorbel – Biostatistics department GSI; Members of the Bioanalytical department GSI

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