Evolution of Viral Load and Genome Sequence in a Clinical Trial of Tenofovir/Emtricitabine Combination Versus Tenofovir Monotherapy for Patients with Previous Adefovir Dipivoxil Failure

44th Annual Meeting of the
European Association for the Study of the Liver
April 22 - 26, 2009
Copenhagen, Denmark

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Introduction

- Hepatitis B virus (HBV) is responsible for nearly 350 million chronic infections worldwide. More than 1 million people die each year following complication of the disease (cirrhosis or hepatocellular carcinoma)
- Two types of therapies are approved for treatment of chronic hepatitis B:
  - Immunomodulation using interferon alpha. Only one third of patients respond to this treatment and inconvenient side effects are observed
  - Inhibition of viral polymerase using nucleos(t)ide analogues. However, prolonged treatment with most nucelos(t)ide analogues results in emergence of resistant virus harboring mutations within the HBV polymerase gene associated with resistance to therapy

Background

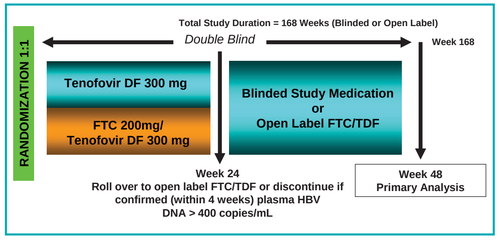
- Tenofovir disoproxyl fumarate (TDF) is a nucleotide analogue recently approved for HBV treatment. Currently, no resistance mutation has been described for TDF but adefovir dipivoxil (ADV) resistance mutations may confer some level of cross-resistance to TDF in vitro
- Combination therapy represents an emerging strategy for treating chronic HBV infection, although its added benefit is debated

Objective

- In patients with ADV failure, it is therefore important to determine the potential benefit of a combination therapy over a switch strategy in patients with HBV DNA greater than 1000 copies/mL on ADV
- The aim of the present study is to compare viral kinetics and polymerase gene resistance mutation evolution during antiviral therapy with TDF or TDF + emtricitabine (FTC) in patients with HBV DNA greater than 1000 copies/mL on ADV

Methods

- 105 patients with chronic hepatitis B and refractory to ADV therapy were randomized and treated in a controlled trial of TDF versus TDF + FTC
- 63 patients had also been exposed to lamivudine before the trial



Methods (cont'd)

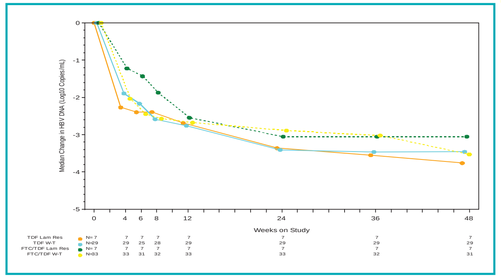
- Serum HBV DNA was quantified by real time PCR
- Resistance mutations (rtA181V/T, rtN236T, and rtL180M, rtM204V/I) were analyzed by direct sequencing of PCR products (population sequencing) and by specific hybridization assay (LiPA) at baseline and on all samples with viral load (VL) > 1000 copies/mL
- A simple logistic regression model was fit comparing baseline viral load between the slow and rapid responders

Results

Table 1. Patients mutations detected either by direct sequencing or by InnoLiPA assay

Patient Population	Direct Sequencing	Inno-LiPA assay
Randomized and treated	N=105	
Subjects with ADV-resistance mutations only	10 (9.5%)	18 (17.1%)
rtA181T	2	4
rtA181V	2	1
rtA181V/T	0	1
rtN236T	2	4
rtA181V/T + rtN236T	4	8
Subjects with LAM-resistance mutations only	13 (12.4%)	14 (13.3%)
rtM204V/I	1	1
rtL180M + M204V/I	12	13
Subjects with both LAM and ADV-resistance mutations	0	11 (10.5%)
All subjects with mutations	23 (22%)	43 (41%)

Figure 2. Median Change in HBV DNA by Baseline LAM-R (LiPA)



Results (cont'd)

Figure 3. Median Change in HBV DNA by Baseline ADV-R (LiPA)

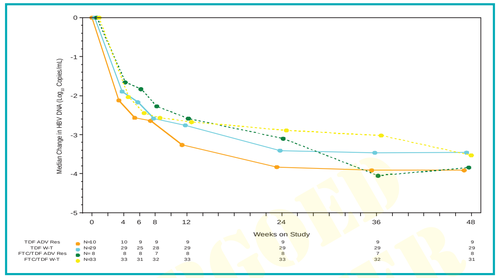


Figure 4. Median Change in HBV DNA by Baseline LAM-R + LAM-R/ADV-R (LiPA)

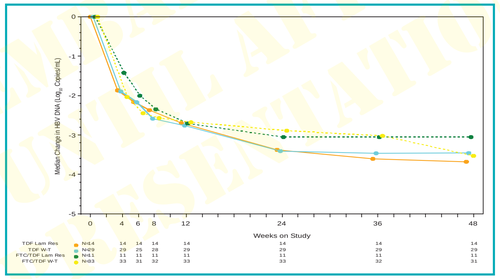


Figure 5. Median Change from Baseline in HBV DNA by ADV-R + ADV-R/LAM-R (LiPA)

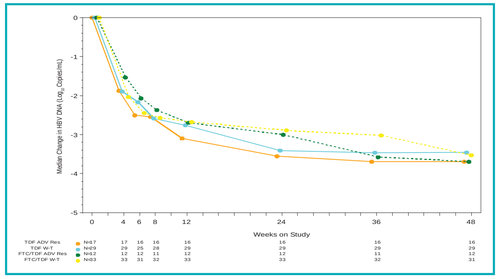


Figure 6. Median Change from Baseline in HBV DNA; All ADV-R and LAM-R by LiPA

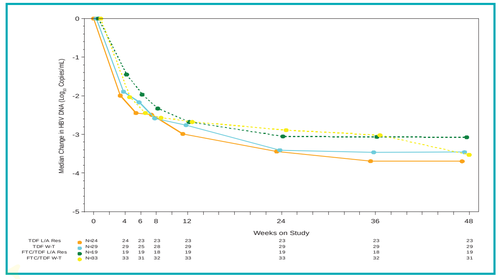


Table 2. Evolution of mutant populations

- After 24 and 48 weeks of treatment, 5 and 3 patients, respectively, maintained mutations whereas the majority of viremic patients became wild type again

Patient Population	Baseline	Week 24	Week 48
VL> 1000 copies/mL	105	23	16
Subjects with wild-type profile	62 (59%)	18 (78.3%)	13 (81.2%)
Subjects with ADV-resistance mutations only	18 (17.1%)	4 (17.4%)	2 (12.5%)
rtA181T	4	0	1
rtA181V	1	0	0
rtA181T/V	1	0	0
rtN236T	4	1	0
rtA181T/V + N236T	8	3	1
Subjects with LAM-resistance mutations only	14 (13.3%)	1 (4.3%)	1 (6.3%)
M204V/I	1	0	0
L180M + M204V/I	13	1	1
Subjects with both LAM and ADV-resistance mutations	11 (10.5%)	0	0
All subjects with mutations	43 (41%)	5 (21.7%)	3 (18.8%)

Table 3. Patients type of response according to their baseline mutations profile

- Three patterns of virologic response were found among patients who completed up to 24 weeks of blinded therapy :
  - A rapid response with a VL lower than 400 copies/mL before week 12 (W12)
  - An intermediate response with a VL lower than 400 copies/mL between W12 and W24
  - A slow response with a VL lower than 400 copies/mL after W24

	Response		
Baseline Mutations by LiPA	Rapid	Intermediate	Slow
ADV-R (N=18)	5	4	9
LAM-R (N=14)	5	4	5
ADV+LAM-R (N=11)	6	2	3
Wild Type (N=62)	24	15	23
Median Baseline Viral Load (log)	4.84	5.76	6.89
Median Decrease Viral Load BL vs W4 (log)	1.88	1.82	1.88
Median Decrease Viral Load BL vs W12 (log)	2.56	2.86	3.02

The median baseline VL was significantly lower for Slow vs. Rapid responders (P < 0.05)
There were no differences in viral load decreases at Week 4 or Week 12 between response patterns

Figure 7. Viral load evolution by type of response

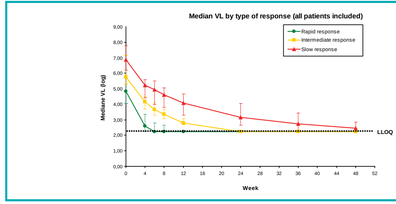
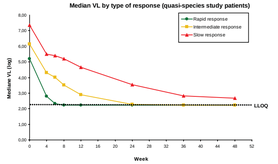


Figure 8. Characteristics of the patients selected for the quasi-species study



	Response		
	Rapid	Intermediate	Slow
Baseline Mutations by LiPA	3	3	6
ADV-R (N=12)	1	0	1
LAM-R (N=2)	2	0	1
Median Baseline Viral Load (log)	5.19	6.14	7.35
Median Decrease Viral Load BL vs W4 (log)	2.43	1.84	1.64
Median Decrease Viral Load BL vs W12 (log)	2.99	3.24	2.88

Conclusions

- **InnoLiPA is significantly more sensitive to detect resistance mutations than population sequencing**
- **Evolution of viral load was not different whether patients received combination or monotherapy**
- **Baseline resistance patterns were not associated with type of response. A rapid response to < 400 copies/mL was correlated with low baseline viral load (p<0.05)**
- **At Week 48, ADV-R and LAM-R mutations were found to persist by LiPA in two and one patient, respectively**
- **17 patients have been selected for clonal analysis. These patients have been chosen for their different type of response, treatment regimen and baseline mutations**
- **The viral quasi-species study and longer follow-up of these patients are ongoing to better understand viral kinetics and fitness during combination therapy and to evaluate the potential for cross resistance**