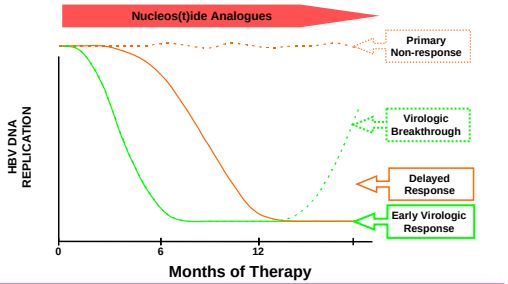
IN VITRO SUSCEPTIBILITY OF HBV STRAINS ISOLATED FROM HIV-HBV COINFECTED PATIENTS WITH DELAYED RESPONSE TO TENOFOVIR DF

[1]Olivier Lada, [2]Anne Gervais, [3]Michel Branger, [4]Gilles Peytavin, [3]Gilles Colin, [2]Gil Fraqueiro, [1]Michèle Martinot-Peignoux, [2]Sophie Matheron, [1]Patrick Marcellin.
[1]Service d'Hépatologie and INSERM CRB3, AP-HP Hopital Beaujon, Clichy, France.[2]Service des Maladies Infectieuses , [3]Service de Virologie, [4]Service de Pharmacie Clinique, and Bichat Claude Bernard, Paris, France.

Introduction

- More than 400 million persons are chronic carriers of hepatitis B virus (HBV) and as many as 10% are coinfected with HIV.
- Individuals coinfected with HIV and HBV tend to have higher incidence of liver-related cirrhosis and cirrhosis-related death than HIV-negative patients.
- HBV antiviral resistance is a major concern in the treatment of chronic hepatitis B carriers.
- Tenofovir disoproxil fumarate (TDF) is a nucleotide analogue approved for treatment of HIV and chronic hepatitis B.

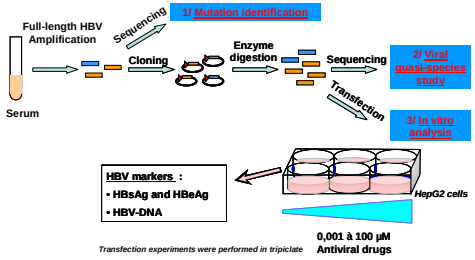
Response to Treatment



- Early virologic response (EVR): HBV DNA level below 2.3 log IU/mL at 6 months of therapy
- Primary non-response: HBV DNA reduction < 2 log IU/mL after 6 months of therapy*
- Delayed response (DR): HBV DNA reduction < 2 log IU/mL after 6 months of therapy and became undetectable after several months of therapy
- Virologic breakthrough: 1 log HBV DNA IU/mL increase in serum HBV DNA level from nadir in two consecutive samples 1 month apart in patients who have responded

** Lok et al. Hepatology 2007*

Methods



Transfection experiments were performed in tripicate

Methods

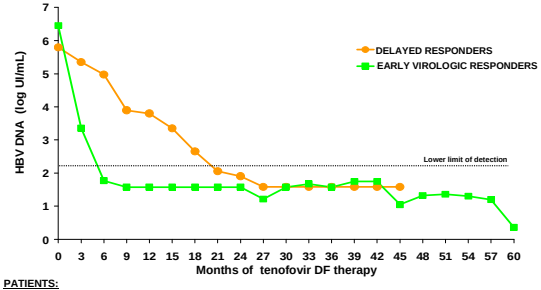
➢ ***WT**: 1 non-treated patient was infected with wild-type (WT) HBV .*

➢ ***EVR**: 2 patients infected with lamivudine resistant HBV with early virologic response to TDF.*

➢ ***DR**: 4 patients infected with lamivudine resistant HBV with delayed response to TDF.*

Aim of the Study

➢ ***Among the 141 HIV/HBV co-infected patients treated with TDF and followed from 2003-2007 at Bichat Claude Bernard Hospital, we observed 7 adherent patients with delayed response (DR) to TDF.***



PATIENTS:

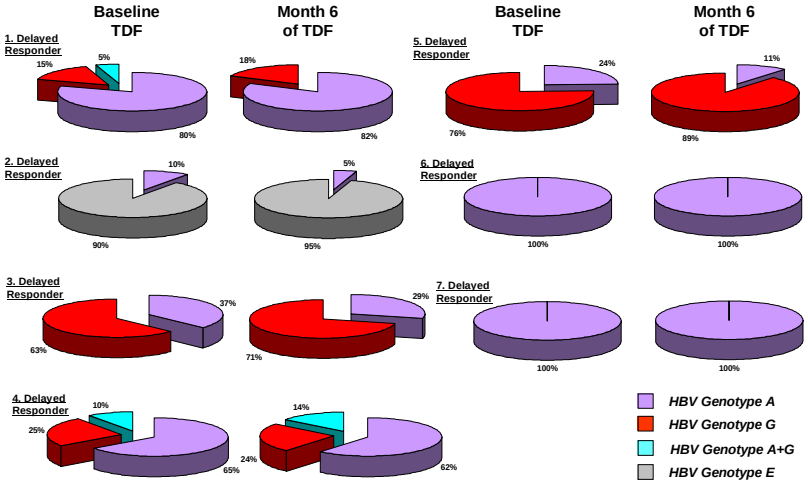
Early Virologic Responders	47	27	47	22	46	21	42	12	37	5	31	4	15	2	6	1	3	1	1	1	1
Delayed Responders	7	5	7	4	7	3	5	3	6	3	3	3	3	2	1	1					

The aim of this study was to evaluate the in vitro sensitivity to HBV approved antiviral drugs of baseline HBV strains isolated from patients with Delayed Response (DR) and Patients with Early Virologic Response (EVR) to TDF therapy

Results/Discussion

- All 7 Delayed Responders (DR) developed a subsequent response to TDF after a median of 20 (17-24) months of therapy.
- Adherence to therapy of these 7 DR were assessed by pharmacological analysis.
- Quasi-species analysis showed that DR infected with HBV genotype G were always coinfected with HBV genotype A and/or genotype A+G recombinant.
- In vitro testing of the main HBV strains isolated from DR 3, 4, 5 and 6 at TDF initiation were performed.
- In absence of antiviral drugs, we observed a lower level of replication and protein production on LAM-R HBV strain from patient DR and EVR 3 vs WT.
- As expected, LAM-R HBV strains isolated from DR and EVR were resistant in our in vitro system to LAM and FTC, compared to wt.
- For ADV and TDF, EVR and DR HBV IC50 was increased compared to wt.
- In vitro ADV activity was similar on EVR compared to DR HBV whereas in vitro TDF activity seems to be lower on DR than EVR HBV.
- HBV genotype G from DR 3 and 5 had lower in vitro sensibility to TDF than HBV genotype A from DR 4 and 6

Genotyping Analysing of Delayed Responders Quasi-Species



In vitro Analysis

➢**Full length HBV DNA genome were not amplified from patient 7 because of a low baseline HBV DNA level and several clones from patient 2 were tested but no replication was observed.**

					3TC	FTC	ADV	TDF
Patient	**Geno**	**Known mutations**	**Polymorphism mutations**	**Others**	**FR***	**FR***	**FR***	**FR***
Control	A	WT	WT		1	1	1	1
1.EVR	A	V173L/ L180M/ M204V			>10000	>16000	2.3	2.9
2.EVR	A	L180M/ M204V			>10000	>16000	2.2	2.3
3.DR	G	L180M/ M204V	R153W/I163V		>10000	>16000	2.7	5.4
4.DR	A	V173L/ L180M/ M204V	I103V/ N122H/ W153R/A223S	L229V	>10000	>16000	3.1	5.0
5.DR	G	L180M/ M204I	V103I/I163V/ R153W/L217R	V190E/ S219F/ L229W	>10000	>16000	3.0	5.8
6.DR	A	L180M/ M204V		V191L/ S202I	>10000	>16000	2.9	4.9

**FR : fold reduction, mutant IC50/ wt IC50; DR: Delayed Responders; EVR: Early Virologic Responders.*

Conclusions/Perspectives

Presence of mutations and unknown reverse transcriptase mutations in delayed responders could have a possible impact on delayed response to TDF. The role of these mutations needs to be further investigated.

Acknowledgments: this work was supported by a research grant from Gilead Sciences, Inc. And Sidaction