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Vaccine Escape Mutants and Suboptimal Hepatitis B Virus Response in HIV HBV coinfected patients

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Introduction

Among the antivirals with anti-hepatitis B virus (HBV) activity, the two most often used treatments in first-line regimens for HIV-HBV co-infected patients are the nucleos(-t)ide analogues lamivudine (LAM) and tenofovir (TDF). One major hurdle in using these treatments is suboptimal response, where a patient continues to have replicating HBV-DNA despite long periods of time.[1]

With LAM-use, suboptimal response is mainly attributed to resistance.[2] Due to the overlapping genes on the HBV genome, these mutations are known to induce mutations on the surface (S) gene, which are associated with reduced binding to anti-HBs antibody and vaccine failure. Incidence data of these mutations specifically from Sub-Saharan Africa only extend to small groups of patients, reporting similar incidence as compared to industrialized countries[3,4] while much lower in others.[5]

TDF is ideal for treating individuals harboring these muations[6]; however, the reasons for suboptimal response during TDF-use are less known in both industrialized and resource-constrained settings.

Objectives

To determine the incidence of vaccine-escape mutations during suboptimal HBV response, specifically among HIV-HBV co-infected patients from Sub-Saharan Africa initiating anti-HBV containing antiretroviral therapy (ART).

Methods and Patients

Study Design

Prospective, nested cohort study including HIV-HBV infected patients from two randomized, open-label, multi-center trials in Abidjan, Côte d'Ivoire.

- Trivacan ANRS 1269 study (NCT00158405) evaluating the clinical impact of continuous combined ART, CD4-guided combined ART (with interruption and reintroduction thresholds at 350 and 250 cells per mm³, respectively), or 2-months-off/4-months on combined ART. ***(LAM-containing ART)***
- Temprano ANRS 12136 (NCT00495651) evaluating the clinical impact of early or regular ART-initiation in conjunction with isoniazid prophylaxis. ***(TDF-containing ART)***

Patients

Participants were enrolled in the present study according to the following inclusion criteria:

- HIV-1 or mixed HIV-1/2 infection
- HBsAg seropositivity
- ≥18 years old
- ART-naïve (with the exception of short course antiretroviral treatment for the prevention of mother-to-child transmission of HIV in the Trivacan study)
- CD4+ cell count between 150-350/mm³ (in the Trivacan study) or <800/mm³ and not indicated for initiating ART based on current recommendations (in the Temprano study)
- No ongoing active tuberculosis (in the Temprano study)

Quantification of HBV-parameters

- **HBV-DNA** was obtained at baseline and every 12 months during follow-up and quantified using an adapted PCR assay (Light Cycler 480; Roche Diagnostics; Meylan, France) with a detection limit of <12 copies/mL
- **preS/S regions** were sequenced on all samples with detectable HBV-DNA.

Statistical analysis

Determinants for time to undetectable HBV-DNA viral load were modeled using Cox proportional hazards regression.

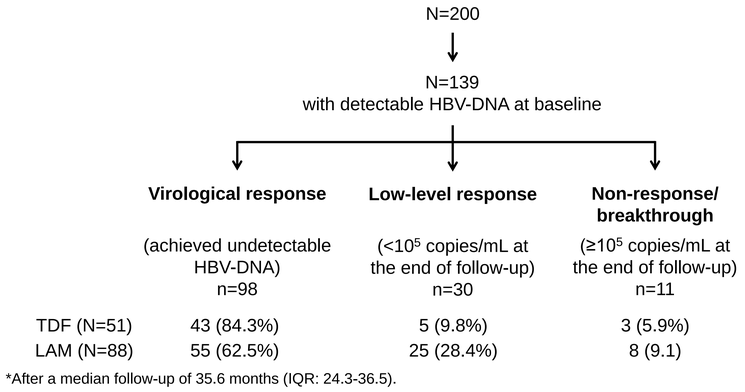
Results

Table 1. Description of the study population

	Total (N=200)	Trivican study (n=103)	Temprano study (n=97)	p^1
Demographic characteristics				
Gender, male/female (% males)	67/133 (33.5)	35/68 (34.0)	32/65 (33.0)	0.9
Age, years*	35 (30-41)	35 (31-37)	35 (30-41)	0.6
BMI, kg/m²*	21.5 (19.5-24.1)	21.4 (19.8-23.1)	21.7 (19.4-25.0)	0.20
HIV characteristics				
WHO clinical stage**				<0.001
Stage I/II	143 (71.5)	58 (56.3)	85 (87.6)	
Stage III/IV	57 (28.5)	45 (43.7)	12 (12.4)	
HIV-RNA >300 copies/mL**	192 (97.5)	97 (97.0)	95 (98.0)	0.9
HIV-RNA $\log_{10}$ copies/mL*	5.03 (4.52-5.50)	5.14 (4.64-5.53)	4.87 (4.37-5.45)	0.04
CD4+ cell count, /mm³*	293 (204-405)	222 (166-305)	369 (291-493)	0.0001
HBV characteristics				
HBeAg-positive**	39 (19.5)	24 (23.3)	15 (15.5)	0.16
Anti-HBe antibody positive**	143 (71.5)	70 (68.0)	73 (75.3)	0.3
HBV-DNA >12 copies/mL**	139 (69.5)	88 (85.4)	51 (51.6)	<0.001
HBV-DNA $\log_{10}$ copies/mL*	3.86 (3.11-7.43)	3.75 (3.02-7.28)	4.17 (3.29-7.79)	0.14
ALT, IU/mL*	25 (16-38)	25 (18-38)	24 (16-39)	0.4
ALT >35 IU/mL**	51 (29.0)	24 (30.4)	27 (27.8)	0.7

*Median (IQR), **Number (%) [1]Significance determined using Kruskal-Wallis test for continuous variables and Pearson χ^2 test or Fisher's exact test for categorical variables.

Figure 1. Flow chart of the study population



*After a median follow-up of 35.6 months (IQR: 24.3-36.5).

Figure 2. Cumulative proportion of patients with undetectable HBV-DNA per study

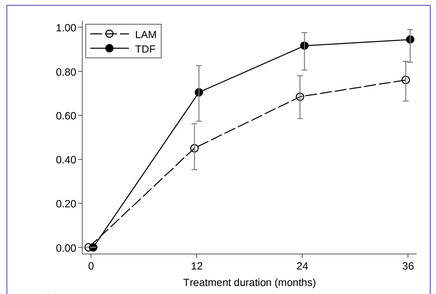


Table 2. Determinants of time-to-undetectable HBV-DNA (multivariable analysis)

	HR (95%CI)	p
CD4 cell count at baseline		
>500mm³	1.00	
350-500mm³	1.07 (0.67-1.71)	0.8
250-350mm³	0.62 (0.34-1.11)	0.11
<250mm³	0.41 (0.19-0.90)	0.03
Treatment arm		
LAM	1.00	
TDF	1.64 (1.01-2.66)	0.046
HBeAg-positive	0.68 (0.34-1.39)	0.3
Baseline HBV-DNA (per $\log_{10}$ copies/mL)	0.92 (0.81-1.05)	0.2
On-treatment duration (per 10%)*	0.93 (0.82-1.05)	0.3

*Time-varying covariate.

Table 3. Genotypic analysis of patients with low-level response (patients with available follow-up sequence)

Patient ID	Treatment	HBV-RNA ($\log_{10}$ cps/mL) BL	HBV-RNA ($\log_{10}$ cps/mL) Last	Genotypic findings (S gene) Baseline	Genotypic findings (S gene) Changes during follow-up
RB065	LAM-NRC	3.04	3.60	sT140L, sK160R, sS174N, sV224A	sI110L, sT126I, sI213L, sF219G, sF220C
PF003	LAM-Low	3.55	2.44	--	sM133L
PF004	LAM-Low	7.57	3.97	sK160N, sE164G, sI226S	None
PF069	LAM-Low	7.71	4.37	sS174N	None
PF073	LAM-Low	7.30	1.76	sQ129P, sG130R, sY206C	sA128D, sG130K
RB023	LAM-Low	8.53	2.81	sV224A	None
US022	LAM-High	3.14	2.59	sL216P, sP217I, sI218F, sF220C, sC221L, sL222W, sW223V, sV224Y, sY225I	None
NS086	TDF	9.24	3.12	wildtype (wt)	None

Among patients with low detectable HBV DNA levels at the end of follow-up, no incident amino acid changes were noted at positions s120, s145, s164, or s195. Low=low on-treatment duration, High=high on-treatment duration, NRC=not randomized and undergoing continuous treatment.

Table 4. Genotypic analysis of patients with viological breakthrough or non-response (patients with available follow-up sequence)

Patient ID	Treatment	HBV-DNA ($\log_{10}$ copies/mL) BL	Nadir	Last	Genotypic findings (S gene) Baseline	Changes during follow-up
A) Virological breakthrough						
PF162	LAM-Low	8.33	3.29	5.22	sI110L	None
US100	LAM-High	8.34	<0.3	7.99	sS154L, sI226S	None
US140	LAM-RC	8.47	2.53	7.76	wt	sE164D, sI195M
ST017	TDF	8.54	2.68	8.62	wt	wt
UC014	TDF	8.05	<0.3	8.35	wt	wt
B) Non-response						
US168	LAM-NRC	8.45	6.79	8.17	wt	None
PF016	LAM-Low	7.82	5.71	5.71	sP120A, sS143L, sS154P, sE164V, sL175S, sV177A	None
CN110	LAM-High	6.33	5.71	5.71	sP127S	None
PF105	LAM-RC	8.93	6.33	8.38	wt	sE164D, sI195M
HB090	TDF	8.79	8.18	8.18	wt	wt

Among patients with virological breakthrough/non-response, the only incident *S*-gene mutations observed during follow-up were at both positions sE164D+sI195M in two LAM-treated patients (while also harboring rtV173L+rtL180M+rtM204V/I mutations). Low=low on-treatment duration, High=high on-treatment duration, RC=continuous, NRC=not randomized and undergoing continuous treatment.

Conclusion

- **In HIV-HBV co-infected patients, undetectable HBV-DNA was achieved in 84.3% and 62.5% of patients treated with TDF and LAM, respectively, and was associated with higher levels of CD4+ cell count.**
- **Immune and antiviral-associated *S* gene mutations were not observed during low-level response and rare during virological breakthrough or non-response.**

References and Acknowledgements

[1]Matthews GV et al. *Clin Inf Dis*, 2013; [2]Lacombe K et al., *Hepatology*, 2013; [3]Hamers RL, et al. *J AIDS*, 2013; [4]Kouanfack C et al. *Antivir Ther*, 2012; [5]Kim HN et al. *J Viral Hepatol*, 2011. [6]Fung S, *Gastroenterology*, 2014 [in press]

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