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Resistance Patterns and Response to Entecavir Intensification Among HIV-HBV–Coinfected Adults With Persistent HBV Viremia

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Tenofovir disoproxil fumarate (TDF) is a potent anti-hepatitis B virus (HBV) agent and is recommended as the first-line therapy of HBV infection in HIV-coinfected patients.^{1,2} However, 8%–11% of patients will experience persistent HBV viremia despite up to 5 years of TDF treatment.^{3,4} The impact of persistent HBV viremia on the generation of HBV drug resistance during TDF treatment is not known. As lamivudine (3TC) monotherapy in HIV-HBV infection is associated with 3TC resistance in up to 90% of patients after 4 years,⁵ ongoing viremia despite HBV therapy raises concern for driving development of TDF and/or 3TC resistance. Therefore, intensification with entecavir (ETV) has been used by some clinicians with the rationale of reducing HBV drug resistance and preventing progression of liver disease associated with ongoing HBV viremia.⁶ In a pilot study, we characterized HBV resistance mutations among adults with persistent HBV viremia after 48 weeks of TDF and 3TC/emtricitabine (FTC) treatment and evaluated virologic response among patients with and without subsequent ETV intensification.

METHODS

Ten HIV-HBV–coinfected individuals with HIV RNA <75 copies per milliliter and detectable HBV DNA ($> 1.6 \log_{10}$ IU/mL HBV DNA, equivalent to 40 copies per milliliter HBV DNA) at the time of screening after at least 48 weeks of 3TC/FTC, and TDF-containing antiretroviral therapy (ART) were enrolled in the randomized controlled pilot study. Population-based Sanger sequencing, sensitive to 20%, of precore, basal core promotor, and reverse transcriptase regions of HBV (codons 96–250) was performed (Quest Diagnostics, San Juan Capistrano) upon entry. Subjects were randomized in an open-label fashion to receive 24 weeks of continued TDF-containing ART with or without intensification with 1 mg ETV daily. Serum was tested for HBV DNA using Cobas TAQman (lower limit of quantitation $1.6 \log_{10}$ IU/mL) every 12 weeks. At 24 weeks, those

in nonintensified arm with ongoing detectable HBV DNA started 1 mg ETV daily for an additional 24 weeks; those in the ETV arm continued ongoing ETV intensification for a total of 48 weeks. The protocol was approved by the University of California, San Francisco IRB and was registered with [clinical-trials.gov](https://clinicaltrials.gov) (NCT00662545).

RESULTS

All 10 enrollees were male, median age 43 (range 30–64), with median CD4⁺ 354 cells per cubic millimeter (range 189–499). All were HBeAg positive, and none had hepatitis C or hepatitis delta viremia. Median baseline ALT was 40.5 U/mL (21–146). Tenofovir had been administered for a median of 36.5 months (15–77) and 3TC/FTC for a median of 72.5 months (32–150) (Table 1). Before entry, 6 subjects had no recorded HBV DNA value <1.6 log₁₀ IU/mL, and 1 (#3) had attained HBV <1.6 log₁₀ followed by subsequent HBV rebound without interruption of HIV/ HBV treatment or HIV viremia.

HBV Baseline

Resistance Mutations—Seven participants had interpretable HBV sequencing at time of study entry. Of these, 5 lacked any *pol* mutations, despite a median of 52 months on 3TC/FTC with persistent HBV viremia. Two participants (#7, 10) had YMDD mutations (M204V). No mutations that have been putatively associated with TDF treatment were detected in any participant, including A181 T/V, A194T, or N236T. One patient (#7) had rtV191I, a mutation reported to be associated with HBsAg loss despite ongoing HBV viremia⁷; however, this subject remained persistently HBsAg positive. Three subjects had genotype A2 associated polymorphism L217R.⁸

Outcome of HBV Viremia—The 5 participants randomized to ETV intensification all had HBV <1.6 log₁₀ IU/mL by 48 weeks of treatment. In the 5 patients randomized to no ETV intensification, 3 participants (#1, 4, 5) with low-level HBV viremia (<2.0 log₁₀ IU/mL) at screening/entry had HBV DNA <1.6 log₁₀ IU/mL by week 12. These 3 subjects had received TDF for a range of 28–75 months before study entry. In the remaining 2 subjects (#2, 3), HBV DNA was detectable at 24 weeks, at which time ETV was started. Twenty four weeks of ETV treatment in these patients produced an HBV DNA decline of 2.3 log₁₀ IU/mL in 1 patient (#2) and a slight increase 0.4 log₁₀ IU/mL in the other (#3). No participant experienced adverse events associated with ETV or ART administration, ALT flares (ALT >3 times upper limit of baseline), or hepatic decompensation. No subjects discontinued ART or ETV. Median ALT in the ETV intensified arm was 45 U/mL (21–145) at baseline and 50 U/mL (18–107) at week 24; median ALT in the nonintensified arm was 50 U/mL (10–68) and 32 U/mL (10–61), respectively. HIV RNA remained <75 copies per milliliter in all participants.

DISCUSSION

In this small pilot study, we observed that that HBV resistance during persistent HBV replication in presence of TDF and 3TC/FTC was infrequently associated with 3TC resistance and was not associated with TDF resistance. Five patients had no HBV drug resistance at study entry despite a preceding median of 32 months of TDF and 70 months of

3TC/FTC treatment. Because 3TC resistance is nearly universal in the setting of detectable HBV DNA during prolonged administration of 3TC monotherapy, our observation suggests TDF may protect against development of 3TC resistance. Of note, the 2 subjects with M204V 3TC mutations had undergone lengthy 3TC/FTC monotherapy (55 and 37 months) before TDF treatment, allowing for the possibility that these mutations developed before TDF treatment. Further, sequencing of HBV *pol* did not demonstrate any novel mutations that could be attributed to TDF resistance or mutations previously associated with TDF treatment. To date, HBV mutations conferring TDF resistance have not been convincingly described,⁹ and these data further support that despite ongoing HBV viremia in the presence of TDF, emergence of tenofovir-related HBV drug resistance mutations does not explain persistent HBV replication. All participants were HIV infected with continued suppression of HIV RNA, suggesting either that poor adherence with TDF alone is an unlikely explanation for HBV viremia or that if suboptimal ART adherence contributed to HBV viremia, HBV may require more rigorous adherence to oral therapy for full viro-logic suppression than HIV in some individuals.

All patients suppressed after 48 weeks of ETV intensification; 3 patients without additional treatment also suppressed. Two patients with M204V suppressed HBV replication on ETV, despite the association of this mutation with a lower barrier to development of ETV resistance.¹⁰ ETV intensification was well tolerated and may require up to 48 weeks to suppress HBV DNA.

This study is limited by the small size and the limited follow-up, which do not permit evaluation of clinical impact of the intensification strategy on hepatic disease progression. Despite randomization, of the genotypable patients, all 3 in the ETV arm patients were HBV genotype A, and all 3 patients in TDF continuation arm were genotype G. Although these genotypes are not known to impact response to ETV or tenofovir, genotype G is associated with more advanced fibrosis than genotype A.¹¹

It remains unclear if intensification is indicated to treat persistent HBV viremia despite TDF therapy. The rationale for intensification is twofold; to prevent drug resistance and to reduce the adverse outcomes associated with HBV viremia. The pattern of 3TC-associated *pol* mutations encountered in this series suggests that development of 3TC mutations is not common in the presence of TDF and is contrary to what is seen with 3TC monotherapy, where the majority of patients develop 3TC resistance over time.⁵ There seems to be no emergence of TDF resistance, despite persistent viremia in the presence of drug pressure.

The second rationale for intensification is the putative clinical benefit of suppression of HBV viremia below the limit of detection. High levels of HBV viremia are associated with hepatocellular carcinoma and hepatic fibrosis,¹² and even lower levels of detectable HBV DNA confer an elevated risk of hepatocellular carcinoma, liver damage, and death.^{13,14} HIV-uninfected patients treated with 3TC for HBV had worse outcomes if HBV remained detectable compared with those with suppressed HBV.^{15,16} However, cohort data suggest that the majority of coinfecting patients treated with TDF will eventually suppress HBV replication, 92% after 5 years of TDF in one study.³ The question remains—is ongoing HBV viremia detrimental during the years required for suppression and if so, when should

intensification with a third agent be considered, if ever? And what should be done for the small percentage of patients who do not suppress with years of tenofovir-based therapy, despite good adherence? Although it is unknown if there is a clinical benefit to suppression of HBV replication in TDF-treated HIV-coin-fected patients with low-level HBV viremia, intensification to fully suppress HBV may be a consideration for patients at higher risk of adverse outcomes from ongoing viremia, such as those with persistent high levels of HBV DNA or advanced liver disease. Larger randomized controlled trials are warranted to address the optimal timing of intensification with a third agent and to further evaluate the impact of prolonged HBV viremia despite tenofovir treatment.

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TABLE 1

Baseline Characteristics and Response to Randomized Treatment

	Previous HBV Response	HBV DNA Before TDF	Months TDF	Months 3c/FTC	HBV Genotype	HBV rt mutations	HBV DNA (log ₁₀ IU/mL)				
							Week 0	Week 12	Week 24	Week 36	Week 48
Arm A: Continued TDF + 3TC/FTC (no ETV × 24 weeks)											
1	Never < 1.6	7.8	28	28	Not able to sequence		1.9	<1.6	<1.6	N/A	ETV Added × 24 Weeks
2	Never < 1.6	Not available	32	32	G	None	7.7	7.2	5.8	4.3	3.3
3	Never < 1.6	6.3	81	102	G	None	3.4	2.7	3.2	2.1	3.6
4	Unknown [*]	>8.3	12	12	G	None	1.9 [†]	<1.6	<1.6	N/A	N/A
5	Rebound [‡]	4.6	31	146	N/A	None	1.9 [‡]	<1.6	<1.6	N/A	N/A
Arm B: ETV in addition to TDF + 3TC/FTC × 48 weeks											
6	Never < 1.6	7.7	41	41	Not able to sequence		3.2	<1.6	<1.6	<1.6	<1.6
7	Never < 1.6	Not available	15	70	A	V191I, S106C L180M, M204V L217R [§]	3.9	2.2	2.5	<1.6	<1.6
8	Unknown	Not available	24	150	Not able to sequence		2.7	1.8	2.41	Not available	<1.6
9	Never < 1.6	Not available	52	52	A	L217R [§]	6.4	2.5	1.6	<1.6	<1.6
10	Unknown	Not available	77	114	A	V173V/L, L180M M204V, L217R [§]	2.4	2.6	2.4	1.8	<1.6

* HBV response to previous TDF-containing regimen unknown.

[†] Screening DNA presented, as entry DNA was <1.6

[‡] Rebound defined as at least one prior HBV DNA <1.6 log₁₀ IU/mL with subsequent detectable HBV DNA.

[§] L217R is a genotype A2 polymorphism.