

particularly when nevirapine (NVP) is combined with Truvada (emtricitabine/tenofovir). However, there is very limited data on the efficacy of NVP + Kivexa, which seems to be a promising HAART combination for several reasons: very low cost; limited metabolic and lipid disturbances,^{5,6} and low pill burden (3 pills daily now but will reduce to 2 when the new once-daily extended-release NVP formulation becomes available). Provided that a patient is HLA B57 negative and the plasma HIV-1 RNA viral load is undetectable, the safety of initiating this HAART combination is expected to be good.

To assess this, we retrospectively looked at the clinical records of 118 patients who initiated NVP + Kivexa between 2003 and 2010. Ninety-eight patients were simplifications: 76 from NVP plus 2 different nucleoside analogues; 20 from a protease inhibitor-based HAART plus Kivexa; 1 from a protease inhibitor plus 2 different nucleoside analogues; and 1 from enfuvirtide plus Kivexa. The main reasons for simplification were as follows: lipodystrophy and lipid abnormalities (36 patients); pill-burden reduction (15p); and other causes including D4T and didanosine substitution (47p). Twenty patients were treatment naive.

The mean age of patients was 44 years (range: 20–69 years) and 76.3% were males. Thirty-seven percent were coinfecting with hepatitis C virus and 2% were carriers of hepatitis B surface antigen. The median follow-up was 27 months (range: 5–84 months).

Overall, 20 patients (17%) discontinued treatment due to the following reasons: virologic failure (3p); toxicity (12p) including rash (5p, 1 was HLAB57 positive), and gastrointestinal disturbances as the most frequent events; changes in treatment strategy (3p); loss to follow-up (1p); and death from non-HIV-related causes (1p). Of the 3 patients who experienced virologic failure, 2 were naive with basal HIV-1 RNA viral load above 5 log copies per milliliter and the other was a simplification. The observed percentage of virologic failure of the NVP + Kivexa combination as a simplification strategy was 1% (1 of 98).

The mean total plasma cholesterol and triglyceride levels did not change during the follow-up period, and the mean CD4 increment was 44 cells per microliter.

In summary, we have observed that the combination of nevirapine plus Kivexa seems to be effective and safe, particularly as a simplification strategy for patients with undetectable viral load and negative HLA B57. Approximately 1 of 10 patients might discontinue the treatment due to side effects, but only 1% is expected to have virologic failure.

Patient adherence might improve with the new extended-release NVP formulation due to the low pill burden and the possibility of a once-a-day regimen. Countries with limited funding resources might also benefit from this combination, as it is one of the cheapest available on the market.

Alfonso Cabello Úbeda*

José Sanz Moreno†

Frances Williams*

Miguel Górgolas*

*Infectious Diseases Division, Fundación Jiménez Díaz, Madrid, Spain

†Internal Medicine Department, Hospital Universitario Príncipe de Asturias

REFERENCES

1. Gil P, Górgolas M, Estrada V, et al. Long-term efficacy and safety of protease inhibitor switching to nevirapine in HIV-infected patients with undetectable virus load. *Clin Infect Dis*. 2004;39:1024–1029.
2. Górgolas M, Hidalgo C, Williams F, et al. Discontinuation rate of nevirapine-based HAART. *AIDS Res Hum Retroviruses*. 2011; [e-pub ahead of print].
3. Giacometti A, Butini L, Cirioni O, et al. Durability and tolerability of long-term nevirapine-based HAART. *Infez Med*. 2010;18:20–26.
4. Calmy A, Vallier N, Nguyen A, et al. Swiss HIV Cohort study; HAART Observational Medical Evaluation and Research Cohort; Netherlands ATHENA Observational Cohort. Safety and efficacy of once-daily nevirapine dosing: a multicohort study. *Antivir Ther*. 2009;14:931–938.
5. Kesselring AM, Wit FW, Sabin CA, et al. Nevirapine Toxicity Multicohort Collaboration. Risk factors for treatment-limiting toxicities in patients starting nevirapine-containing antiretroviral therapy. *AIDS*. 2009;23:1689–1699.
6. Barragan P, Fisac C, Podzamczar D. Switching strategies to improve lipid profile and morphologic changes. *AIDS Rev*. 2006;8:191–203.

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

To the Editors:

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.

This work was supported by an investigator-initiated grant from Bristol-Myers Squibb [grant number AI463-162].

Presented in part at 17th Conference on Retroviruses and Opportunistic Infections. 2/2010, Abstract 636, San Francisco, CA.

A.L. has received research grant support from Bristol-Myers Squibb (manufacturer of entecavir). C.B.H. has served as a consultant and been a member of speakers' bureaus for Gilead and Bristol-Myers Squibb. M.G.P. has served on advisory boards for Roche, Vertex, and Merck.

D.H., D.B., and A.S. have no conflict of interest to disclose.

© 2011 Lippincott Williams & Wilkins

METHODS

Ten HIV-HBV-coinfected individuals with HIV RNA <75 copies per milliliter and detectable HBV DNA ($\geq 1.6 \log_{10}$ 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 non-intensified 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 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 $< 1.6 \log_{10}$ IU/mL, and 1 (#3) had attained HBV $< 1.6 \log_{10}$ 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 $< 1.6 \log_{10}$ 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 ($< 2.0 \log_{10}$ IU/mL) at screening/entry had HBV DNA $< 1.6 \log_{10}$ 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 $2.3 \log_{10}$ IU/mL in 1 patient (#2) and a slight increase $0.4 \log_{10}$ IU/mL in the other (#3). No participant experienced adverse events associated with ETV or ART administration, ALT flares (ALT

TABLE 1. Baseline Characteristics and Response to Randomized Treatment

	Previous HBV Response	HBV DNA Before TDF	Months TDF	Months 3tc/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 \times 24 weeks)							ETV Added \times 24 Weeks				
1	Never < 1.6	7.8	28	28	Not able to sequence	1.9	< 1.6	< 1.6	N/A	N/A	
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 \times 48 weeks							ETV \times 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 $< 1.6 \log_{10}$ IU/mL with subsequent detectable HBV DNA.

§L217R is a genotype A2 polymorphism.

>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 possibly 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 virologic 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-coinfecting 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.

Anne F. Luetkemeyer, MD*

Edwin D. Charlebois, PhD, MPH†

C. Bradley Hare, MD*

Douglas Black, MA*

Anna Smith, RN*

Diane V. Havlir, MD*

Marion G. Peters, MD‡

*Division of HIV/AIDS,
San Francisco General Hospital,
University of California,
San Francisco, CA

†Center for AIDS Prevention Studies,
HIV/AIDS Division,
San Francisco General Hospital,
University of California,
San Francisco, CA

‡Division of Gastroenterology,
University of California,
San Francisco, CA

ACKNOWLEDGMENTS

The authors would like to thank the study participants as well Deborah Zeitschel for her efforts in the initial study preparations.

REFERENCES

1. Department of Health and Human Services. January 10, 2011;1–166. Available at: <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>. Accessed September 23, 2011.

2. European Association for Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B. *J Hepatol*. 2009; 50:227–242.
3. de Vries-Sluijs TE, Reijnders JG, Hansen BE, et al. Long-term therapy with tenofovir is effective for patients co-infected with human immunodeficiency virus and hepatitis B virus. *Gastroenterology*. 2010; 139:1934–1941.
4. Martin-Carbonero L, Teixeira T, Poveda E, et al. Clinical and virological outcomes in HIV-infected patients with chronic hepatitis B on long-term nucleos(t)ide analogues. *AIDS*. 2011;25:73–79.
5. Benhamou Y, Bochet M, Thibault V, et al. Long-term incidence of hepatitis B virus resistance to lamivudine in human immunodeficiency virus-infected patients. *Hepatology*. 1999;30:1302–1306.
6. Ratcliffe L, Beadsworth MB, Pennell A, et al. Managing hepatitis B/HIV co-infected: adding entecavir to truvada (tenofovir disoproxil/ emtricitabine) experienced patients. *AIDS*. 2011;25:1051–1056.
7. Amini-Bavil-Olyaei S, Sheldon J, Lutz T, et al. Molecular analysis of an HBsAg-negative hepatitis B virus mutant selected in a tenofovir-treated HIV-hepatitis B virus co-infected patient. *AIDS*. 2009;23:268–272.
8. Bottecchia M, Madejon A, Sheldon J, et al. Hepatitis B virus genotype A2 harbours an L217R polymorphism which may account for a lower response to adefovir. *J Antimicrob Chemother*. 2008;62:626–627.
9. Snow-Lampart A, Chappell B, Curtis M, et al. No resistance to tenofovir disoproxil fumarate detected after up to 144 weeks of therapy in patients mono-infected with chronic hepatitis B virus. *Hepatology*. 2011; 53:763–773.
10. Colonna R. Assessment at three years shows high barrier to resistance is maintained in entecavir-treated nucleoside naive patients while resistance emergence increases over time in lamivudine refractory patients. *Hepatology*. 2006;44:229A. Abstract 110.
11. Lacombe K, Massari V, Girard PM, et al. Major role of hepatitis B genotypes in liver fibrosis during coinfection with HIV. *AIDS*. 2006;20:419–427.
12. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA*. 2006;295:65–73.
13. Iloeje UH, Yang HI, Jen CL, et al. Risk and predictors of mortality associated with chronic hepatitis B infection. *Clin Gastroenterol Hepatol*. 2007;5:921–931.
14. Iloeje UH, Yang HI, Su J, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology*. 2006; 130:678–686.
15. Di Marco V, Marzano A, Lampertico P, et al. Clinical outcome of HBeAg-negative chronic hepatitis B in relation to virological response to lamivudine. *Hepatology*. 2004; 40:883–891.
16. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med*. 2004;351:1521–1531.

Antibody Response to Inactivated Influenza A (H1N1) 2009 Monovalent Vaccine in Patients With and Without HIV

To the Editors:

In 2009, an outbreak of H1N1 influenza A virus infection led the World Health Organization to raise its pandemic alert to the highest level.^{1,2} Worldwide, laboratory confirmed cases of pandemic influenza H1N1 2009 led to more than 18,000 deaths,^{3,4} including 47 confirmed fatal cases in New York City.⁵

The Advisory Committee on Immunization Practices recommended that the 2009 H1N1 vaccination efforts focus initially on persons in 5 target groups: pregnant women; persons who live with or provide care to infants aged <6 months; health care and emergency medical services personnel; persons aged 6 months to 24 years; persons aged 25–64 years who have medical conditions that put them at higher risk for influenza-related complications, for example, chronic heart, lung, renal, liver disease, cancer or immunosuppression, including immunosuppression caused by medications or by human immunodeficiency virus.⁶

The influenza vaccine is the single best way to protect against influenza illness.⁷ Studies have shown that seasonal influenza vaccination is effective in HIV-1-infected individuals,^{8,9} including those with CD4 counts <200 cells per cubic millimeter, who are antibody positive for influenza who develop an antibody response.¹⁰ However, HIV-infected individuals may have an impaired antibody response to influenza vaccination compared with healthy controls, and lower CD4 cell counts were associated with greater impairment in antibody response.¹¹

The 2009 H1N1 vaccine was immunogenic in healthy adults.¹² However,

The authors have no funding or conflicts of interest to disclose.

the efficacy of this vaccine in patients infected with HIV is not well elucidated; studies have reported the protective effect of this vaccine in patients with malignancy,¹³ in HIV-infected youth and children,¹⁴ and HIV-infected adult pregnant women.¹⁵ One study has reported that the H1N1 2009 vaccine produced protective antibody titers by 3 weeks after vaccination, in only 60% of HIV adults whose median current CD4 cell count was 502 cells per cubic millimeter.¹⁶ To our knowledge, few, if any, studies on the 2009 H1N1 vaccine have followed antibody titers beyond 4 weeks postvaccination, particularly in HIV patients with very low CD4 cell counts. We studied the immunogenicity of this H1N1 influenza vaccine in patients with HIV, with CD4 counts >200 cells per cubic millimeter and those with CD4 counts less than 200 cells per cubic millimeter for up to 12 weeks after immunization.

We conducted a prospective observational study to determine the antibody response to the 2009 H1N1 vaccine (Sanofi Pasteur, Swiftwater, PA) at 4–6 weeks and at 8–12 weeks in patients with and without HIV. Written informed consent was obtained from all participants. We recruited study participants from our outpatient HIV clinic with documented HIV infection, and healthy non-HIV-infected controls were recruited from the hospital staff. Participants were then grouped as: healthy controls (group A); HIV patients with CD4 >200 cells per cubic millimeter (group B); and HIV patients with CD4 <200 cells per cubic millimeter (group C). All but 3 of HIV patients were on highly active antiretroviral therapy. Exclusion criteria were as follows: age younger than 18 years, inability to provide informed consent, pregnancy, acute febrile illness, anaphylactic egg allergy, previous allergic reaction to influenza vaccine, or previous Guillain Barre Syndrome. The study was approved by the Maimonides Medical Center Research Committee.

We enrolled 103 participants and collected peripheral blood samples between October 2009 and February 2010. Samples were obtained at baseline or prevaccination, then at 4–6