Tenofvir Is Effective Alone or With Emtricitabine in Adefovir-Treated Patients With Chronic-Hepatitis B Virus Infection

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BACKGROUND & AIMS: We compared treatments for patients with chronic hepatitis B virus (HBV) infection who had an incomplete response to adefovir dipivoxil (ADV). We evaluated a combination of fixed-dose emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) from the start (early combination) versus TDF as monotherapy. METHODS: Patients (n = 105) were randomly assigned to groups given TDF (n = 53) or FTC/TDF (n = 52). End points included HBV DNA suppression, biochemical and serologic response, and response by baseline or developed resistance mutations through 48 weeks of treatment. Patients given TDF monotherapy had the option to receive FTC, as fixed-dose FTC/TDF, if viremia persisted after week 24. RESULTS: At baseline, patients’ mean HBV DNA level was 5.97 log10 copies/mL, and 58% had received lamivudine (LAM); LAM- and ADV-associated mutations were detected in 13 and 10 patients, respectively, by population sequencing and in 14 and 18 patients, respectively, by reverse hybridization line probe assay (INNO-LiPA HBV DR). Through week 24 (direct comparison of blinded therapy), viral decay curves were identical between groups. At week 48, 81% of patients initially given TDF or FTC/TDF had HBV DNA levels below 400 copies/mL. The presence of baseline LAM- or ADV-associated mutations did not affect response. Adherence to therapy appeared to be the primary factor associated with HBV DNA levels below 400 copies/mL at week 48. CONCLUSIONS: TDF monotherapy and the combination of FTC and TDF had similar efficacy in patients with incomplete viral suppression after therapy with ADV; response was not influenced by the presence of baseline LAM- or ADV-associated mutations. Initial monotherapy followed by combination therapy was as effective as early combination therapy.

Keywords: INNO-LiPA HBV DR; Emtricitabine/Tenofovir Disoproxil Fumarate; Combination Therapy; Second-Line Therapy.

Chronic hepatitis B virus (HBV) infection affects an estimated 400 million people worldwide and continues to be an important cause of morbidity and mortality, as well as a source of potential new infections.1 An estimated 1 million people die annually from the complications of hepatitis B.2 Although there is no cure for chronic hepatitis B, therapeutic agents that can effectively suppress HBV replication are available. Current guidelines3,4 advocate sustained HBV DNA suppression as the most effective way to improve quality of life and reduce the consequences of chronic infection, such as cirrhosis, hepatic failure, and death. Achieving these goals requires long-term antiviral therapy. Therefore, antiviral treatments should have the following properties: good safety and tolerability, convenience so as to increase adherence, potency, and durable efficacy. Because durable suppression of viral replication is affected by both the potency of the agent and the genetic barrier to resistance, optimal HBV therapy should also have a high genetic barrier to resistance.5

Adefovir dipivoxil (ADV) was approved in 2002 as an HBV therapy and is effective in the setting of lamivudine-resistance (ideally as add-on combination therapy6) and is indicated in patients with decompensated liver disease.7 Its key limitation is a relatively slow rate of viral decline. Patients on ADV who experience primary nonresponse or viral breakthrough/rebound in the setting of confirmed compliance are candidates for alternative therapy with or without confirmation of ADV-associated mutations. Such therapy may involve addition of a second drug, switching to a more potent drug, or combina-

Abbreviations used in this paper: ADV, adefovir dipivoxil; ADV-R, adefovir-resistant; AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FTC, emtricitabine; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; INNO-LiPA HBV DR, HBV resistance detection Line Probe Assay; LAM, lamivudine; LAM-R, lamivudine-resistant; NC = F, noncompleters = failure; SAE, serious adverse event; SD, standard deviation; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.

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tion therapy with 2 drugs with different resistance profiles.

Tenofovir disoproxil fumarate (TDF) was approved for treatment of chronic hepatitis B in adults in several countries in 2008 (European Union countries, Australia, United States, Canada, and Turkey). This approval was based on 2 prospective, ongoing randomized trials showing the superiority of TDF 300 mg/day compared with ADV 10 mg/day in both hepatitis B e antigen (HBeAg)-positive (GS-US-174-0103) and -negative patients (GS-US-174-0102); the majority of these patients were treatment naïve. However, a subset of patients from these studies were lamivudine (LAM) experienced, and TDF demonstrated potent antiviral efficacy (92% complete suppression through week 96).9 Fung et al have confirmed the high efficacy in LAM-resistant (LAM-R) patients treated with TDF.10

There are limited clinical data regarding the use of TDF in the setting of ADV resistance or primary nonresponse/breakthrough, and in vitro studies have shown that HBV strains expressing the ADV resistance-associated substitutions rtA181V and/or rtN236T showed reductions in susceptibility to tenofovir ranging from 2.9-fold to 10-fold that of wild-type virus,11–13 raising the question of a potential clinical cross resistance. In one retrospective analysis, among 20 patients who had virologic breakthrough on LAM and suboptimal response after 12 months of ADV (but no ADV-associated mutations) who were subsequently treated with TDF, HBV DNA suppression was achieved in 19 of 20 patients after a median treatment time of 3.5 months.14 In contrast, in a second retrospective study from the same group of researchers,15 only 6 of 20 patients with ADV resistance mutations (30%) achieved HBV DNA <400 copies/mL after 12 months of treatment with TDF. In the absence of phenotypic evidence of reduced HBV sensitivity to TDF in these patients, it is unclear whether the observed responses were due to ADV-associated mutations or other factors, such as treatment adherence or baseline HBV DNA. To address whether TDF monotherapy or combination therapy was the best option for ADV suboptimal responders, the present study compared 2 treatment strategies for patients with a suboptimal response to ADV: initial TDF monotherapy, with the option to intensify to emtricitabine (FTC)/TDF after 24 weeks for incomplete response (deferred combination), versus FTC/TDF from treatment start. This is a 168-week blinded study, and week 48 results are reported.

Patients and Methods

The study enrolled patients 18–69 years of age with HBeAg+ or HBeAg− chronic HBV currently treated with ADV and showing persistent viral replication defined as plasma HBV DNA levels >1000 copies/mL at screening after an ADV treatment duration of at least 24 and up to 96 weeks. Further eligibility criteria were serum alanine aminotransferase (ALT) levels <10 × the upper limit of normal (ULN) and no evidence of decompensated liver disease (ascites, jaundice, encephalopathy, or variceal hemorrhage) or hepatocellular carcinoma (eg, α-fetoprotein >50 ng/mL or by any other standard-of-care measure) or coinfection with human immunodeficiency virus, hepatitis C virus, or hepatitis D virus. Patients were required to be naïve to TDF and entecavir, to have not received interferon or pegylated interferon within 6 months of the screening visit, and to have reported being adherent to their current ADV therapy. Prior or current LAM use (ie, ADV and LAM combination therapy) was allowed. Baseline and diseases characteristics are shown in Table 1.

Patient adherence to study drug was measured by a count of pills returned at each visit. The number of pills taken (calculated based on number of pills returned) was divided by the number of pills prescribed and multiplied by 100 to obtain the adherence percentage at each visit. Treatment interruptions approved by the investigator were not considered to be nonadherence. If a study drug bottle was not returned, then the number of days covered by that bottle was subtracted from both the numerator and denominator for the calculation. This is equivalent to assuming that adherence is constant throughout the treatment period. No inferential statistics were computed.

Patients were enrolled from 28 enrolling study centers: 10 in the United States, 10 in Germany, 7 in France, and 1 in Spain. The first patient was screened on March 30, 2006, and the last week 48 observation occurred on January 31, 2008.

The protocol was approved by each investigator’s Independent Ethics Committee or Institutional Review Board before study initiation. Informed consent was obtained from all patients. The studies described in this manuscript were funded in full by Gilead Sciences (clinicaltrials.gov identifier NCT00307489). The writing/preparation of this paper was completed by the primary author with input from all other authors but without additional funding from any source. No additional personnel performed any data analyses.

Study Design

This was a randomized, double-blind, double-dummy, 168-week study. Patients were required to be on ADV at study enrollment and were randomized 1:1 to either TDF 300 mg once daily monotherapy or the fixed-dose combination of FTC 200 mg once daily/TDF 300 mg once daily; inactive placebo tablets resembling TDF or FTC/TDF were used to facilitate blinding. Randomization was stratified by history of LAM experience (<12 weeks vs ≥12 weeks of LAM therapy) and hepatitis B e antigen (HBeAg) status at screening. A centralized randomization procedure was used whereby numbered bot-
The primary efficacy end point for this analysis was HBV DNA <400 copies/mL at week 48, measured by the proportion of patients achieving this end point. Serum HBV DNA was measured using the Roche COBAS TaqMan 48 HBV assay (Roche Molecular Systems, Inc, Pleasanton, CA), which has a lower limit of quantitation of 169 copies/mL (equivalent to 29 IU/mL).

Secondary efficacy end points were HBV DNA and ALT change over time, HBV DNA <169 copies/mL, normalized ALT, HBeAg loss or seroconversion to antibody to hepatitis B e antigen, HBsAg loss or seroconversion to antibody to hepatitis B e antigen, as well as percentage of resistance mutations detected in the HBV polymerase (by population sequencing or by INNO-LiPA HBV DR v2/v3 [Innogenetics, NV, Gent, Belgium] assay, each with viral load requirement of at least 400 copies/mL). Efficacy end points were also evaluated within LAM- and ADV-resistant (ADV-R) subgroups.

The safety analyses included all patients who received at least 1 dose of a study drug and all events that occurred during double-blind treatment. Adverse events (AEs), serious adverse events (SAEs), laboratory abnormalities, discontinuation of the study drug because of
AEs, and deaths were evaluated. Hepatitis flares were defined as elevation of ALT >2 × baseline and 10 × ULN OR abnormal laboratory parameters suggestive of worsening hepatic function (abnormal bilirubin ≥2 mg/dL above baseline, abnormal prothrombin time >2 seconds above baseline, abnormal albumin ≥1 g/dL decrease from baseline or elevated serum lactate levels >2 × ULN along with any ALT elevation [ie, grade shift or 2 × previous value]). Specific markers of renal abnormalities along with any ALT elevation [ie, grade shift or 2] from baseline or elevated serum lactate levels >2 × ULN above baseline, abnormal albumin >2 seconds above baseline, abnormal prothrombin time >2 seconds above baseline, abnormal albumin ≥1 g/dL decrease from baseline or elevated serum lactate levels >2 × ULN along with any ALT elevation [ie, grade shift or 2 × previous value]). Specific markers of renal abnormalities included confirmed (defined as 2 consecutive visits, or last value on drug) increases in serum creatinine of at least 0.5 mg/dL above baseline, serum phosphorus values <2 mg/dL, and creatinine clearance <50 mL/min.

Statistical Analyses

The primary analysis set for safety and efficacy analyses was defined as all randomized patients who received at least 1 dose of study medication. Patients who withdrew after randomization but prior to receiving study medication were not included in the analysis. The percentages of patients in the efficacy analysis set with plasma HBV DNA <400 copies/mL or <169 copies/mL, normalized ALT (of patients with baseline ALT > ULN) HBeAg loss, HBsAg loss, or seroconversion to antibody to hepatitis B e antigen, or antibody to hepatitis B e antigen (respectively) were summarized by randomized treatment arm, and the differences in proportions between the 2 groups were evaluated at weeks 24 and 48 using a Cochran–Mantel–Haenszel test, controlling for randomization strata (baseline HBeAg status and prior LAM use). Patients who discontinued the study prior to week 48 were considered failures for all antiviral end points after the time of discontinuation (noncompleters = failure; NC = F). Using this analysis method, a patient with confirmed HBV DNA ≥400 copies/mL after week 24 who was switched (from blinded TDF or FTC/TDF) to open-label FTC/TDF was considered a treatment success if week 48 HBV DNA was <400 copies/mL (ie, the treatment strategy was successful for this patient). In a secondary analysis of the primary efficacy end point, patients who discontinued the study prior to week 48 or began open-label FTC/TDF (regardless of original treatment assignment) were considered failures (noncompleter/switch = failure; NC/S = F). The study was designed to enroll 45 patients per arm, with at least 76% power to detect a 30% difference in response rates between the 2 arms. With actual enrollment of 53 treated patients in the TDF group and 52 in the FTC/TDF group, there was at least 83% power to detect the same preplanned difference.

Plasma HBV DNA levels and serum ALT levels over time and their changes from baseline were summarized descriptively (number, mean, median, standard deviation [SD], interquartile range, minimum, and maximum), and treatment group differences were evaluated using the van Elteren test controlling for randomization strata (baseline HBeAg status and prior LAM use).

Baseline genotypic data were summarized for all patients. The proportions of patients with any genotypic changes from baseline at conserved sites within the HBV polymerase were summarized for all patients who were viremic at early discontinuation after week 24, who switched from blinded study medication to open-label FTC/TDF, who were viremic at week 48, or who experienced virologic breakthrough.

Efficacy end points were also evaluated within LAM-R and ADV-R subgroups as well as overall. Subgroups were determined based on the resistance profile at baseline, regardless of any changes that may have developed during treatment. LAM-R patients were defined as those with the rtM204V/I mutation in the HBV polymerase gene at baseline with or without additional mutations at positions rtL180M and rtV173L. ADV-R patients were defined as those harboring the rtN236T and/or the rtA181V/T mutation at baseline.

All safety data were collected on or after the date that study medication was first dispensed. Clinical and laboratory AEs were coded using the Medical Dictionary for Regulatory Activities.

Results

Patients

One hundred forty-seven patients were screened, 108 were randomized, and 105 were treated with at least 1 dose of blinded study medication (53 in the TDF group and 52 in the FTC/TDF group). Of the 105 patients randomized and treated, 48 were in Germany, 33 were in the United States, 22 were in France, and 2 were in Spain. A total of 44 patients had less than 12 weeks of LAM experience, including 29 HBeAg+ and 15 HBeAg− patients, and 61 patients had >12 weeks of LAM experience, including 47 HBeAg+ and 14 HBeAg− patients.

Of the 105 patients randomized and treated, 80 patients (37 in the TDF group and 43 in the FTC/TDF group) completed 48 weeks of double-blind treatment without meeting the protocol-defined criteria for switch to open-label FTC/TDF. Twenty-three patients discontinued double-blind study drug and received open-label FTC/TDF through week 48 (15 in the TDF group and 8 in the FTC/TDF group; 1 of the 8 patients in the open-label FTC/TDF group discontinued prior to week 48). Three patients (including 1 who began open-label FTC/TDF) discontinued the study prior to week 48 (1 in the TDF group and 2 in the FTC/TDF group). Reasons for discontinuation were investigator’s discretion, lost to follow-up, and withdrawal of consent in 1 patient each. The results of screening, enrollment, and cohort definition for analyses are displayed in Supplementary Figure 1.
Virologic Response

The first 24 weeks of therapy provided a head-to-head comparison of TDF versus FTC/TDF in this patient population. As can be seen in Figure 1, there was no difference in antiviral potency of these 2 regimens through week 24, with essentially superimposable viral decay curves. In addition, the proportion of patients at week 24 with HBV DNA <400 copies/mL was similar (66% TDF, 69% FTC/TDF; Figure 2). For those patients with HBV DNA >400 copies/mL at week 24, a repeat HBV DNA quantification was required, and, if a result ≥400 copies/mL was confirmed, therapy was changed to open-label FTC/TDF (by design, to address concerns at the time of protocol development that resistance emergence might be observed without therapy intensification). Among week 24 viremia patients undergoing an HBV retest, a larger number of patients initially randomized to FTC/TDF subsequently dropped below 400 copies/mL. Therefore, although viral kinetics between the groups were very similar, a greater proportion of TDF-randomized patients switched to open-label FTC/TDF (n = 15 vs 8, respectively).

At week 48, using the intent-to-treat analysis, 81% of patients in each treatment arm had HBV DNA <400 copies/mL (Figure 2). The mean reduction from baseline in plasma HBV DNA over 48 weeks of treatment was similar in the 2 treatment groups; at week 48, the mean (SD) change was −3.58 (1.29) log_{10} copies/mL in the TDF group and −3.34 (1.75) log_{10} copies/mL in the FTC/TDF group. Patients who switched to open-label FTC/TDF after week 24 were included in the analysis as part of their original randomized treatment group. When considering intensification to open-label FTC/TDF as a primary efficacy failure in the intent-to-treat analysis (NC/S = F), 66% and 77% of patients in the TDF and FTC/TDF groups, respectively, had HBV DNA <400 copies/mL at week 48 (P = .234). As can be seen in Supplementary Figure 2, which represents HBV DNA over time for the 15 patients who switched from blinded TDF to open-label FTC/TDF, the addition of FTC did not appear to alter the slope of the decline in the majority of patients. Eight percent of patients in either of the randomized arms achieved HBeAg loss at week 48. HBsAg seroconversion was observed in 1 TDF patient (male Asian patient with HBV genotype C infection).

Virologic Response According to Resistance Profile

Population sequencing. At baseline, 10% of the patients entered the study with ADV-associated mutations (specifically rtN236T and/or the rtA181V/T), including 2 patients who entered the study harboring the rtA181T mutation alone without the rtN236T. Twelve percent of the patients entered the study with LAM-associated mutations (specifically rtM204V/I with or without the rtL180M and rtV173L). Results are summarized by treatment group in Table 2.

At week 48, 92% of LAM-R patients and 79% of patients without LAM-resistance mutations had HBV DNA <400 copies/mL (NC = F analysis). In the ADV-resistant patients, 8 of the 10 patients were randomized to TDF monotherapy. By week 48, 80% of patients with ADV-associated mutations and 81% of patients without ADV mutations had HBV DNA <400 copies/mL. Figure 3A and B show median change in HBV DNA over time by baseline mutation type (by population sequencing) compared with patients without evidence of these mutations. The presence of ADV-R at baseline did not have an effect on the rate of initial HBV DNA decline on TDF or FTC/TDF. Subjects with ADV-R tended to have a higher viral load at baseline (6.75 log_{10}) compared with subjects without ADV-R (5.75 log_{10}), which resulted in a larger decrease in HBV DNA at week 24 and week 48 in this group.
HBV DR v2/v3 INNO-LiPA assay. Given the relatively low incidence of ADV-associated mutations observed at baseline (10%) by population sequencing (particularly because these were heavily pretreated patients), baseline isolates were also evaluated with the HBV DR v2/v3 assay. The HBV DR line probe assay can detect specific mutations in HBV polymerase/reverse transcriptase that are present at levels below the 20%-25% cutoff typical of most population-based sequencing assays. The current INNO-LiPA assay is designed to detect mutations at positions rtL80, rtV173, rtL180, rtA181, rtT184, rtA194, rtS202, rtM204, rtL250, rtI233, rtN236, and rtL250 when present at levels as low as 1%-5% of the virus population.16 Data were obtained for 101 of 105 patients at baseline, with all of the mutations that were detected by population-based sequencing, with the exception of 1 sample, were also detected by INNO-LiPA. As expected for this population of treatment experienced patients, the incidence of baseline mutations was higher using the INNO-LiPA assay as compared with standard population-based sequencing; overall 43 of 105 (41%) patients were shown to harbor mutations associated with resistance to lamivudine at baseline. Furthermore, in contrast to the results obtained by population sequencing, several patients were shown to harbor virus containing both ADV- and LAM-associated mutations. Results are summarized by treatment group in Table 2.

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Consistent with the results obtained by population sequencing, the majority of patients harboring ADV- or LAM-associated mutations achieved HBV DNA <400 copies/mL by week 48. At week 48, 93% of LAM-R patients and 79% of patients without LAM-R mutations had HBV DNA <400 copies/mL (NC = F analysis). In the ADV-R patients, 72% of patients with ADV-associated mutations and 83% of patients without ADV mutations had HBV DNA <400 copies/mL. Figure 4A and B show mean change in HBV DNA over time by baseline mutation type (as determined by INNO-LiPA) compared with patients with wild-type virus. As observed with the analyses based on baseline population-based sequencing, response to TDF or FTC/TDF therapy based on the presence at baseline of ADV-R or LAM-R mutations detected by INNO LiPA was comparable with patients without such mutations.

Resistance Surveillance
Genotypic testing was attempted for 18 viremic patients out of the 105 patients in the study through week 48. Among the 18 viremic patients, 1 discontinued the study after week 24 with HBV DNA ≥400 copies/mL, 12 (11%) were viremic at week 48 but did not experience viral breakthrough during the study, and 5 (5%) were viremic at week 48 after experiencing viral breakthrough. Six patients (all on open-label FTC/TDF) harbored conserved-site changes (by population sequencing) at week 48 as compared with baseline (4 originally randomized to TDF and 2 to FTC/TDF); no specific amino acid substitution occurred in more than 1 patient, and none of these amino acid substitutions was known to confer resistance to any approved anti-HBV drug. No additional mutations were observed by INNO-LiPA testing; there was a trend toward wild-type virus population during therapy. Furthermore, clinical isolates from these patients with conserved site changes remained phenotypically sensitive to tenofovir and FTC (Supplementary Tables 1 and 2).

Biochemical Response
Approximately half of the patients in either treatment group had normal baseline ALT values (Table 1). At week 48, the percentage of patients with normal ALT increased to 67% and 73%, in the TDF and FTC/TDF groups, respectively. There was no significant difference between groups (P = .423). The mean reduction (SD) from baseline in serum ALT at week 48 was greater in the FTC/TDF group (42.9 [147.03 SD] U/L) than in the TDF group (21.6 [54.53 SD] U/L), likely because of higher baseline ALT in the FTC/TDF group (Table 1), and this difference was not statistically significant (P = .694). The
median changes from baseline in ALT over time were similar between the 2 groups.

**Adherence Assessments**

Median adherence to the active component of the treatment regimen was similar in the 2 groups (84% in the TDF group and 81% in the FTC/TDF group). As shown in Supplementary Figure 3, the proportion of patients with HBV DNA <400 copies/mL was higher in patients with high (≥94%) adherence than among patients with low (≤68%) adherence.

**Safety**

Table 3 presents an overall summary of AEs. There were no statistically significant differences in any overall AE parameter (eg, total AEs, grades 2–4, grades 3 or 4, or SAEs), and the overall incidence of AEs was similar in the 2 treatment groups (77% in the TDF group and 71% in the FTC/TDF group). There were no deaths or discontinuations, dose interruptions, or dose modifications because of AEs. The most frequent AEs in both treatment groups (TDF and FTC/TDF) were nasopharyngitis (23% and 17%, respectively), headache (19% and 15%, respectively), and fatigue (11% and 13%, respectively). The only statistically significant difference in the incidence of AEs was for upper abdominal pain, which occurred in 2% of patients in the TDF group and 13% in the FTC/TDF group (P = .031). One fracture was reported (rib fracture in a patient in the TDF group), which resulted from a fall and was moderate in severity and considered not related to study drug. One patient (2%) in the TDF group and 4

![Figure 3](A) Median change in HBV DNA by baseline ADV-R (A) or LAM-R (B) detected by population sequencing.
patients (8%) in the FTC/TDF group had at least 1 SAE. No SAE was reported in more than 1 patient. Only 1 SAE (ALT increased; discussed below) was considered related to study drug.

There were 2 on-treatment flares that met the protocol criteria of an SAE. One patient had an increase in ALT from 80 U/L at baseline to a grade 4 value at week 8 (432 U/L), which was concomitant with a 3-log decrease in HBV DNA. The only concurrent laboratory abnormality was grade 3 AST (244 U/L peak value). The ALT and aspartate aminotransferase (AST) elevations resolved to grade 1 by week 24, and both analytes were in the normal range at week 48. HBV DNA continued to decrease during the flare and was undetectable at weeks 24, 36, and 48. This SAE was considered related to study drug. A second patient had an increase in ALT from 97 U/L at baseline to a grade 4 value of 470 U/L at week 48, along with grade 4 AST (328 U/L) and creatine kinase (14,013 U/L) and grade 1 serum glucose (128 mg/dL). The HBV DNA level decreased initially (nadir of 2.84-log_{10} copies/mL at week 12) but then increased and was similar to the baseline level at the time of the flare, possibly because of low adherence to the active component of the treatment regimen. This event was considered unrelated to study drug. Neither patient showed any signs of hepatic decompensation. No patient had a confirmed increase from the baseline serum creatinine concentration of at least 0.5 mg/dL, a confirmed creatinine clearance rate of <50 mL/min, or a confirmed serum phosphorus concentration <2 mg/dL.

Discussion

Tenofovir disoproxil fumarate has been shown to produce potent viral suppression in large phase 3 pivotal
The present study (GS-US-174-0106) compared TDF monotherapy (with option to add FTC [deferred combination]) versus combination FTC/TDF therapy from start in patients with chronic HBV infection who had incomplete virologic response after receiving ADV for at least 6 months. In both blinded treatment arms, patients were permitted to change to open-label FTC/TDF after 24 weeks to increase the barrier to resistance if persistent viremia was confirmed. Thus, this study compares 2 treatment strategies: TDF monotherapy with option to add FTC after 24 weeks for incomplete response versus FTC/TDF from treatment start.

In this heavily ADV pretreated population, in which the majority of enrolled patients also had prior or concomitant LAM exposure, both treatments were equally effective in suppressing HBV DNA in the majority of patients by week 24, and the majority of patients maintained HBV DNA <400 copies/mL at week 48. Among those patients who began open-label FTC/TDF, HBV levels continued to decline, and most achieved HBV DNA <400 copies/mL. Of those who did not have confirmed decline in HBV DNA, most were documented as nonadherent to therapy (adherence was high variable, ranging from 25% to 100% [interquartile range, 56%-97%]); clinicians should consider nonadherence in their patients who do not respond virologically to TDF. Thus, the strategy of initial monotherapy was clearly effective in the majority of patients. Moreover, such a strategy did not appear to impact subsequent response with combination therapy in instances in which inadequate virologic response was observed with monotherapy. However, evaluation beyond 1 year is needed to determine whether the similarity of the 2 strategies will be confirmed with durable viral suppression.

The present results in ADV-experienced patients with suboptimal response are also consistent with those seen through 96 weeks in the pivotal studies 102 (HBeAg−) and 103 (HBeAg+). In these studies, patients were initially randomized to receive either blinded TDF or ADV, and all patients who continued beyond 48 weeks received open-label TDF. Among those initially treated with ADV (n = 215), 4 harbored ADV-associated mutations at 48 weeks of ADV treatment; clonal analysis indicated that these 4 patients had subpopulations of LAM-R mutations (including rtA181T but not the rtN236T) at baseline. In study 102, 112 patients (35 who were viremic and 72 with HBV DNA <400 copies/mL) switched from blinded ADV to open-label TDF at study week 48. By week 96 (after 48 weeks of open-label TDF therapy), 100% of patients who were on study had HBV DNA <400 copies/mL. In study 103, 84 patients (72 with HBV DNA >400 copies/mL) switched from blinded ADV to open-label TDF at week 48. Of the patients who remained on study at week 96 (after 48 weeks of open-label TDF), 82% of patients who were viremic on ADV and 100% who were <400 copies/mL at week 48, respectively, were <400 copies/mL at week 96. In this population, it is clear that TDF monotherapy was highly efficacious in the majority of these ADV pretreated patients.

A limitation to the interpretation of data from the current study was the protocol-specified provision that patients should switch to open-label FTC/TDF if viremia was confirmed after week 24. This limits a direct, blinded comparison of the antiviral efficacy of TDF versus FTC/TDF beyond the week 24 time point. The study design decision to switch after the week 24 time point was made at a time when there were limited data regarding the effectiveness of TDF in patients infected with ADV-R virus, and results from the pivotal trials demonstrating the efficacy and high genetic barrier to resistance of TDF had not yet been obtained. In the interest of patient safety and long-term benefit, it was felt that mandating initiation of combination therapy after 24 weeks of monotherapy would minimize the risk of virologic failure for those patients without full responses. Although spec-
In summary, both TDF monotherapy and FTC/TDF combination therapy were well tolerated and resulted in complete viral suppression in the majority of patients by week 48 in this study population with incomplete viral suppression on ADV (most with prior and/or current LAM use). The treatment strategy of initial TDF monotherapy was effective in the majority of patients and did not impact the effectiveness of subsequent combination therapy when incomplete viral suppression was noted. Virologic response was independent of preexisting ADV- or LAM-associated mutations but may have been influenced by adherence to study medication(s). Evaluation beyond 1 year is necessary to confirm these initial results in this heavily pretreated population, with specific focus on development of resistance.

**Supplementary Material**

Note: To access the supplementary material accompanying this article, visit the online version of Gastroenterology at [www.gastrojournal.org](http://www.gastrojournal.org), and at doi: 10.1053/j.gastro.2010.06.053.

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The statistical analysis of the entire data sets pertaining to efficacy (specifically primary and major secondary efficacy end points) and safety (specifically, serious adverse events as defined in federal guidelines) have not been independently confirmed by a biostatistician who is not employed by the corporate entity. The corresponding author had full access to all of the data and takes full responsibility for the veracity of the data and analysis.

Conflicts of interest
A. Snow-Lampart, D. Frederick, J. Sorbel, K. Borroto-Esoda, D. Oldach, and F. Rousseau are/were employees of Gilead Sciences. All other authors received payment for their participation in the conduct of the trial.

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Supported by Gilead Sciences, and all non-Gilead employees received payment for their participation in the conduct of trial.
Supplementary Figure 1. Disposition of study patients.

*Two subjects were granted waivers to remain on blinded therapy (which was FTC/TDF) and one subject discontinued prior to Week 48 and is counted under Discontinued Study.
Supplementary Figure 2. HBV DNA $\log_{10}$ copies/mL by study visit for patients randomized to blinded tenofovir disoproxil fumarate treatment who switched to open-label emtricitabine/tenofovir disoproxil fumarate treatment after week 24.

Supplementary Table 1. Phenotypic Evaluations of Viruses With Conserved Site Changes

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Viral isolate</th>
<th>Change from BL in HBV pol/RT</th>
<th>Tenofovir: fold change from BL(^a)</th>
<th>FTC: fold change from BL(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF/TVD</td>
<td>Patient A week 48</td>
<td>M204L(^b)</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>TDF/TVD</td>
<td>Patient B week 48</td>
<td>H55Q, R153Q, V191I, L267Q, G295A(^b)</td>
<td>1.3</td>
<td>0.5</td>
</tr>
<tr>
<td>TDF/TVD</td>
<td>Patient C week 48</td>
<td>S116A, Q153R</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>TDF/TVD</td>
<td>Patient D week 48</td>
<td>K125Q, N236T, H238N</td>
<td>1.3</td>
<td>1.8</td>
</tr>
</tbody>
</table>

FTC, emtricitabine; TDF, tenofovir disoproxil fumarate; TVD, truvada (fixed dose combination of TDF + FTC). \(^a\)Values represent an average of 2 or 3 independent assays and values ≤2-fold are not statistically significant. \(^b\)Conserved site changes.

Supplementary Table 2. Phenotypic Evaluations for Viruses With Conserved Site Changes

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Laboratory isolate</th>
<th>Change from BL in HBV pol/RT</th>
<th>Tenofovir: fold change from control(^a)</th>
<th>FTC: fold change from control(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF/TVD</td>
<td>pCMVHBV-G304D (Patient E week 48)</td>
<td>G304D(^b)</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>TDF/TVD</td>
<td>pCMVHBV-G258E (Patient F week 48)</td>
<td>G258E(^b)</td>
<td>1.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

FTC, emtricitabine; TDF, tenofovir disoproxil fumarate; TVD, truvada (fixed dose combination of TDF + FTC). \(^a\)Values represent an average of 2 or 3 independent assays and values ≤2-fold are not statistically significant. \(^b\)Conserved site changes.
Supplementary Figure 3. Percentage of patients with HBV DNA <400 copies/mL by adherence. High adherence was defined as ≥94%, and low adherence was defined as ≤68%. Patient adherence to study drug was measured by a count of pills returned at each post-baseline visit.