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Three-Year Efficacy and Safety of Tenofovir Disoproxil Fumarate Treatment for Chronic Hepatitis B

E. Jenny Heathcote, Patrick Marcellin, Maria Buti, Edward Gane, Robert A. de Man, Zahary Krastev, George Germanidis, Samuel S. Lee, Robert Flisiak, Kelly Kaita, Michael Manns, Iskren Kotzev, Konstantin Tchernev, Peter Buggisch, Frank Weilert, Oya Ovunc Kurdas, Mitchell L. Shiffman, Huy Trinh, Selim Gurel, Andrea Snow-Lampart, Katyna Borroto-Esoda, Elsa Mondou, Jane Anderson, Jeff Sorbel, Franck Rousseau

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Abbreviations used in this paper:

Tenofovir disoproxil fumarate (TDF); hepatitis B virus (HBV); adefovir dipivoxil (ADV); chronic hepatitis B (CHB); hepatitis B surface antigen (HBsAg); hepatitis B e antigen (HBeAg); emtricitabine (FTC); upper limit of normal (ULN); alanine aminotransferase (ALT); deoxyribonucleic acid (DNA); intent-to-treat (ITT); Long term evaluation analysis (LTE); Open label evaluation analysis (OLE); hepatocellular carcinoma (HCC); lamivudine (LAM); polymerase/reverse transcriptase (pol/RT).

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Independent Statistical Analysis: The statistical analysis for this manuscript was performed by the sponsor and then independently evaluated by Steven C. Grambow, PhD (Department of Biostatistics and Bioinformatics, Duke University Medical Center). Dr. Grambow was provided with the original study protocols, the long term statistical analysis plans, the manuscript, the CONSORT diagrams, the published article of 48 week study results, prior data presentations at professional meetings, the clinical virology report, and a data CD containing raw SAS data sets, analytic SAS data sets, SAS program code, SAS log files and SAS statistical analysis output reports. Dr. Grambow found the statistical methods used for the efficacy and safety analyses presented in the manuscript to be appropriate and confirmed the primary efficacy and select secondary efficacy and safety results presented in the results section, tables, and figures of the manuscript. Dr. Grambow received compensation from Gilead Sciences to perform this independent statistical evaluation

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Background & Aims. Tenofovir disoproxil fumarate (TDF), a nucleotide analogue and potent inhibitor of hepatitis B virus (HBV) polymerase, showed superior efficacy to adefovir dipivoxil (ADV) in the treatment of chronic hepatitis B (CHB) through 48 weeks. We evaluated the long-term efficacy and safety of TDF monotherapy in patients with CHB that were positive or negative for HB e antigen (HBeAg⁺ or HBeAg⁻).

Methods: After 48 weeks of double-blind comparison of TDF to ADV, patients who underwent liver biopsy were eligible to continue study on open-label TDF for 7 additional years; data presented were collected up to 3 years (Week 144), from 85% of participants. The primary efficacy endpoints at week 144 included levels of HBV DNA and alanine aminotransferase (ALT), development of resistance mutations, and presence of HBeAg or HBsAg in blood samples.

Results: At week 144, 87% of HBeAg⁻ and 72% of HBeAg⁺ patients treated with TDF had levels of HBV DNA <400 copies/mL. Among patients who had previously received ADV and then received TDF, 88% of the HBeAg⁻ and 71% of the HBeAg⁺ patients had levels of HBV DNA <400 copies/mL; overall, 81% and 74%, respectively, maintained normalized levels of ALT and 34% had lost HBeAg. Amino acid substitutions in HBV DNA polymerase that are associated with resistance to tenofovir were not detected in any patient. Cumulatively, 8% of HBeAg⁺ patients lost HBsAg. TDF maintained a favorable safety profile for up to 3 years.

Conclusion: TDF was safe and effective in the long-term management of HBeAg⁺ and HBeAg⁻ patients with CHB. TDF continuously suppressed the virus; there was an increasing percentage of patients with loss of HBsAg up to 3 years, without development of resistance mutations in HBV polymerase.

Key Words: liver disease, virology, drug resistance, viral replication, nucleotide

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INTRODUCTION

Chronic infection with hepatitis B virus results in substantial morbidity and mortality worldwide claiming up to a million deaths annually¹. Chronic hepatitis B (CHB) can be a silent disease for decades, but cirrhosis, liver failure and hepatocellular carcinoma may result from untreated infection^{2,3}. Persistent HBV replication with active hepatitis leads to disease progression and conversely treatments that suppress viral replication forestall disease progression^{4,5}. Loss of detectable hepatitis B surface antigen (HBsAg) in serum correlates with improved long-term clinical outcomes^{6,7,8}.

Oral antivirals effectively suppress viral replication; however the development of resistance limits efficacy. Therefore potent antivirals without the propensity to select for resistance are desirable⁹. Safe and well tolerated potent oral antivirals that are able to induce HBsAg loss are increasingly attractive as a therapeutic option since it allows patients to be treated for a finite period of time with safe and well tolerated drugs. Currently, treatment with oral antivirals is response-based whereas treatment with interferon is per a predefined period (per published guidelines for the treatment of CHB). To this point, up to 3 year post treatment efficacy data (including HBsAg loss) has been published in two observational follow-up protocols and observed sustained virological response, defined as either HBV DNA <400 copies/mL or HBV DNA \leq 10,000 copies/mL, in 19% and 28% and HBsAg loss in 11% and 8.7% of patients, respectively^{10,11}.

Tenofovir disoproxil fumarate (TDF), the oral prodrug of tenofovir, is a nucleotide analog with potent activity against HBV DNA polymerase and was recently shown to be superior to adefovir dipivoxil (ADV) in HBeAg⁻ and HBeAg⁺ CHB patients¹². After 48 weeks of double-blind treatment, HBV DNA was <400 copies/mL in 93% of HBeAg⁻ and 76% HBeAg⁺ patients on

TDF; no resistance mutations developed and notably 3% of HBeAg+ patients also lost HBsAg. By week 96, 6% of HBeAg+ patients lost HBsAg and no resistance mutations developed¹³.

Studies 102 (HBeAg- patients) and 103 (HBeAg+ patients) were designed to continue assessing the safety and efficacy of TDF treatment through 8 years and although not comparative post week 48, this longer term data may provide some data (albeit limited) into the effect of prolonged viral suppression on clinical outcomes. This manuscript presents results at 3 years of treatment and includes a description of patients initially randomized to ADV after they rolled over to TDF.

METHODS

Study conduct and methods have been previously described¹² and only methods unique to the open-label period are presented herein.

STUDY DESIGN

After the 48 weeks of double blind comparison, patients who underwent a week 48 liver biopsy, regardless of the therapeutic response at week 48, were eligible to receive open-label TDF for an additional 7 years. Optionally at the discretion of the investigator, emtricitabine (FTC) could be added to TDF as a fixed dose combination tablet (FTC/TDF) from week 72 onward for confirmed HBV DNA ≥ 400 copies/mL at two consecutive visits. After week 48, patients with confirmed HBsAg loss were eligible to discontinue treatment at their next scheduled visit and be followed off treatment for the remainder of the study. Monitoring for adverse events (AEs), serum chemistries, and HBV DNA occurred every 8 to 12 weeks, and hepatitis B serology (HBeAg and HBsAg) every 12 to 16 weeks. Laboratory tests were conducted by Covance Laboratories and Esoterix Laboratories.

Data after week 96 was captured using electronic case report forms. Gilead Sciences performed all statistical analyses (and independently evaluated by an external statistician) and the manuscript was written in collaboration with the lead authors (E. Jenny Heathcote, P Marcellin and F. Rousseau). The academic authors vouch for the veracity of the data and all authors approved the final draft of the manuscript.

EFFICACY

Efficacy endpoints were: HBV DNA <400 copies/mL, normalized ALT (upper limit of normal = 34U/L [females] and 43U/L [males]), HBeAg and HBsAg loss and seroconversion and resistance surveillance. HBV DNA was measured using the Roche COBAS TaqMan assay (lower limit of quantitation 169 copies/mL or 29 IU/mL) and HBsAg levels quantified using the Abbott Architect assay; samples exceeding the upper limit of 250 IU/mL were diluted according to the package insert (up to a dilution of 1:999).

SAFETY

Safety analyses included all patients who received at least one dose of open-label TDF and included all events during open-label treatment (including FTC/TDF treatment) up to week 144. AEs, serious AEs (SAEs), laboratory abnormalities, discontinuations due to AEs and deaths were evaluated. Serum phosphorus, serum creatinine and creatinine clearance were evaluated as categorical endpoints (confirmed phosphorus <2 mg/dL, serum creatinine increase from baseline ≥ 0.5 mg/dL and creatinine clearance <50 mL/min) and creatinine as a continuous variable over time.

RESISTANCE SURVEILLANCE

Changes from pre-TDF treatment in the HBV DNA pol/RT region were assessed annually (and at the time of TDF monotherapy discontinuation and early discontinuation of FTC/TDF) from all patients with persistent viremia and/or virologic breakthrough (confirmed HBV DNA ≥ 400 copies/mL after being < 400 copies/mL or confirmed $\geq 1 \log_{10}$ increase from nadir). Substitutions in the amino acid sequence of the HBV pol/RT domain were evaluated to determine if these substitutions occurred at polymorphic or conserved sites. The population-based di-deoxy sequencing assay required an HBV DNA concentration ≥ 400 copies/mL (69 IU/mL). All conserved site substitutions were phenotypically assessed in cell culture assays to measure *in vitro* susceptibility to tenofovir. Phenotyping was also done for polymorphic changes if they were observed in more than 1 patient and for all viruses isolated from patients with virologic breakthrough on study drug. Resistance surveillance testing was conducted by Gilead Sciences (Durham, NC).

STATISTICAL ANALYSES

Efficacy analyses were performed comparing the originally randomized treatment groups, ADV 10 mg and TDF 300 mg. Virologic or HBV DNA response was analyzed using three methods for handling missing data and modifications to the treatment regimen. In the primary analysis, i.e., long term evaluation (LTE) analysis, using a modified intent-to-treat (ITT) approach, patients discontinuing the study early due to any reason with HBV DNA > 400 copies/mL, or due to death, tolerability, loss to follow-up or had an ongoing AE at the time of discontinuation were all considered failures regardless of HBV DNA suppression. Data considered missing completely at random were excluded from statistical calculations. Patients with HBsAg loss were counted as a success at every visit after HBsAg loss even if their HBV DNA data were missing. The open-label evaluation analysis (OLE) is a modified ITT (missing=failure) analysis conducted in the population of patients who took at least one dose of open-label TDF. In both the

LTE and the OLE analyses, patients who added FTC were considered as treatment failures. The third analysis is an on-treatment analysis which includes all patients on study with non-missing data, regardless of the actual treatment received; all missing data were excluded. Subgroup analyses were performed on the lamivudine and adefovir dipivoxil-experienced patients. Normalized ALT and HBeAg loss were assessed using an on-treatment method. Analysis of the HBsAg loss was performed using the Kaplan-Meier method. Characteristics of the patients with HBsAg loss are described however due to insufficient numbers of TDF-TDF patients with HBsAg loss outcome (N=13) no multivariate modeling was conducted.

RESULTS

STUDY POPULATION

Study 102 (HBeAg⁻). Of the 375 patients enrolled (250 randomized to TDF and 125 to ADV), 365 completed week 48 and 347 (93%) entered the open-label period and 328 completed week 144. Year 3 retention was 87%.

Study 103 (HBeAg⁺). Of the 266 patients enrolled (176 randomized to TDF and 90 to ADV), 250 completed week 48 and 238 (89%) entered the open-label period and 214 completed week 144. Year 3 retention was 80%.

Patient disposition for both studies is shown in Figure 1.

VIROLOGIC RESPONSE

In the LTE analysis, 87% (313/359) of HBeAg⁻ and 71% (181/254) of HBeAg⁺ patients had HBV DNA <400 copies/mL at week 144 (Figure 2a and 2b). Similar results were obtained with the OLE analysis, 90% (313/347) of HBeAg⁻ (88%TDF-TDF; 96% ADV-TDF) and 74% (177/238) of HBeAg⁺ (75% TDF-TDF; 74% ADV-TDF) patients had HBV DNA <400

copies/mL (69 IU/mL) at week 144. In the on-treatment analysis, 99% (316/319) of HBeAg⁻ and 93% (194/208) HBeAg⁺ had HBV DNA <400 copies/mL. A similar proportion of patients also achieved HBV DNA <169 copies/mL (limit of quantification) by week 144: 99% (316/319) of HBeAg⁻ and 89% (186/208) HBeAg⁺ patients. Disposition data for the three analyses are included in Table 1. All patients with HBV DNA <400 copies/mL on ADV at week 48 remained suppressed through week 144 after switching to TDF. Additionally, 100% (33/33) of HBeAg⁻ and 89% (57/64) of HBeAg⁺ patients viremic on ADV at week 48 achieved viral suppression by week 144 on TDF. Among patients with prior lamivudine experience at study entry (N=75), 100% had HBV DNA <400 copies/mL on treatment at week 144.

Three of 11 (HBeAg⁻) and 31/40 (HBeAg⁺) viremic patients added FTC to TDF on or after week 72 (5% of the randomized and treated population). All 3 HBeAg⁻ patients and 17/31 HBeAg⁺ patients had HBV DNA <400 copies/mL at week 144 (in study 103, HBeAg⁺ patients, 9 were viremic at week 144 with all but one patient having HBV DNA levels <10,000 copies/mL, and 5 discontinued prior to week 144). Of the 17 patients with confirmed viremia beyond week 72 who chose to remain on TDF monotherapy, 12 had HBV DNA <400 copies/mL at week 144, 3 discontinued prior to week 144 and 2 were viremic at week 144 (HBV DNA 437 and 1356 copies/mL).

SEROLOGICAL RESPONSE and HBsAg QUANTIFICATION RESULTS

In HBeAg⁺ patients across both treatment groups, HBeAg loss occurred in 34% (34% TDF-TDF, 35% ADV-TDF) and HBeAg seroconversion in 26% of patients (on-treatment analysis). In HBeAg⁺ patients, the Kaplan Meier probability of losing HBsAg over 3 years was 8% in each treatment group (N=20 total) (Figure 3a); probability of seroconversion to anti-HBs was 6% and 7% in the TDF-TDF and ADV-TDF groups, respectively (N=15 total). Fourteen of twenty

patients discontinued treatment following HBsAg loss; follow up data off therapy is available for 12 patients and all but one remained HBsAg and HBV DNA negative (median follow-up 169 days; range 1-484). One patient regained HBsAg off-treatment, restarted TDF treatment and then experienced HBsAg loss again followed by seroconversion to anti-HBs 15 weeks later. No patient achieved HBsAg loss after switching to FTC/TDF and no HBeAg⁻ patient has experienced HBsAg loss as of week 144.

HBsAg over time for both HBeAg⁺ and HBeAg⁻ patients is presented in Figure 3b and Figure 3c. Overall, HBeAg⁺ patients who cleared HBsAg by week 144 had higher median baseline HBsAg levels than those who did not (5.11 IU/mL versus 4.50 IU/mL). Patients initially randomized to TDF who cleared HBsAg had a greater median change from baseline in HBsAg plasma levels at week 24 (-2.41 log₁₀ IU/mL versus -0.20 log₁₀ IU/mL) compared to patients who did not clear HBsAg; a similar profile was observed for ADV-treated patients after switching to TDF. Patients who did not clear HBsAg had a smaller rate of decline (approximately 0.5 log₁₀ IU/mL) by week 96 with an additional 0.1 log₁₀ IU/mL decline by week 144. In HBeAg⁻ patients the HBsAg level at baseline was about 5 fold lower than observed at baseline in HBeAg⁺ patients and declined less over 3 years (at week 144 mean reduction from baseline was -0.20 log₁₀ IU/mL). Characteristics of patients who achieved HBsAg loss by week 144 are shown in Table 2. Patients who achieved HBsAg loss had a high baseline Knodell necroinflammatory score and higher HBV DNA and HBsAg levels, were genotype A (60%), D (35%) or F (5%) and all were non-Asian, 65% had bridging fibrosis or cirrhosis and the majority were male. These data are descriptive only and would require a larger sample size to support a robust analysis for predictors of HBsAg loss.

BIOCHEMICAL RESPONSE

At week 144 in study 102, 81% of HBeAg⁻ patients (83% TDF-TDF, 77% ADV-TDF) had normalized ALT and mean ALT was 33 U/L while in study 103, 74% of HBeAg⁺ patients (73% TDF-TDF, 76% ADV-TDF) on treatment at week 144 had normalized ALT and mean ALT was 39 U/L. Ninety-three percent of the elevated ALT concentrations at week 144 were grade 1 or less ($\leq 2.5 \times \text{ULN}$).

RESISTANCE SURVEILLANCE

None of the amino acid substitutions in HBV DNA pol/RT that developed through 3 years of treatment were associated with decreased phenotypic sensitivity to tenofovir in either study 102 or 103. Table 3a summarizes the surveillance for resistance during the open label phase.

Phenotypic analysis of clinical isolates from patients with conserved site changes in HBV DNA pol/RT are presented in Table 3b. Data on conserved site changes observed during year one of the studies has previously been published¹³.

Three of the 29 patients on TDF who had persistent viremia ≥ 400 copies/mL harbored distinct conserved site changes (rtR51K; rtV173L_±L180M _±M204V and rtL101F). Clonal analysis of the HBV DNA pol/RT from the patient with lamivudine-associated mutations revealed the presence of these mutations at a frequency of 6.5% at baseline. Phenotypic analysis of these viruses showed full susceptibility to tenofovir *in vitro*. Eight patients had virologic breakthrough (7/8 due to non-adherence); in all 8 cases the virus isolated remained phenotypically sensitive to inhibition by tenofovir *in vitro* (data not shown).

Three of 19 patients initially randomized to ADV who were then treated for 2 additional years with TDF monotherapy had distinct conserved site changes (rtG152E; rtN236T + rtR274Q and rtA307T). The rtN236T mutation was observed as a subpopulation (9.3% of the virus population) prior to initiation of TDF therapy; treatment with TDF resulted in a decline of 3.8 \log_{10} copies/mL in the rtN236T population and the patient achieved HBV DNA < 400 copies/mL

on continued TDF monotherapy. The other two viruses with conserved site changes were fully sensitive to tenofovir *in vitro*.

Of the 34 patients who added FTC, 2 of the 12 viremic patients evaluated for resistance had virus with conserved site changes at week 144 (rtL180M± rtA181T±rtM204V and rtR192H) in the absence of virologic breakthrough and neither was associated with resistance to tenofovir. Clonal analysis of the baseline isolate demonstrated that the lamivudine-associated mutations (rtL180M± rtA181T±rtM204V) were present at levels ranging from 3.7 to 7.4% of the total viral population at baseline.

SAFETY

The safety profile observed for up to 3 years of TDF was consistent with the known safety profile of the drug¹⁴. Treatment emergent AEs occurring in ≥5% of the patients during open-label treatment were upper abdominal pain, nasopharyngitis, headache and influenza. The frequency of the gastrointestinal events, upper abdominal pain, nausea, and diarrhea were observed at a frequency of 5%, 2% and 3%, respectively. Fractures occurred infrequently and none were considered related to study drug (0.8% year 1, 1.7% year 2 and 1% year 3).

During open-label treatment 3 patients died of causes considered unrelated to TDF (carcinoma of the cervix, nasopharyngeal carcinoma and cholangiocarcinoma). Overall, 8.4% of the patients experienced an SAE and <1% were considered related to TDF (ALT flare, n=3; facial spasm, n=1; mild renal impairment, n=1). The renal impairment episode resolved following dose reduction to every other day and the patient (with arterial hypertension for the past 15 years) continued on study through week 144. Overall, ALT flares occurred infrequently during open-label treatment (n=7) with 4 attributed to a lack of study drug compliance and associated with rebounding HBV DNA and 3 cases associated with a change in therapy and a concurrent decline

in HBV DNA. None of the ALT flares resulted in hepatic decompensation. Hepatocellular carcinoma was diagnosed in <1% of patients (n=5; all with bridging fibrosis or cirrhosis at week 48). The following AEs led to discontinuation of TDF: hepatocellular carcinoma (n=1), serum creatinine increased (n=1), fatigue, dizziness and disturbance in attention (n=1), and septic shock (n=1). The patient with increased creatinine experienced an unconfirmed 0.5 mg/dL increase in creatinine.

Creatinine and creatinine clearance remained stable over 3 years with a change in creatinine of +0.02 mg/dL at week 144 (Figure 4a and 4b). Overall, two patients (originally randomized to ADV) experienced a ≥ 0.5 mg/dL increase in creatinine. Both remained on study through week 144, after a dose reduction in TDF. Four patients (<1%) experienced a reduction in serum phosphorus <2mg/dL which resolved on continued TDF therapy without intervention.

DISCUSSION

With an overall patient retention rate of 85% across the two studies, these 3 year data show 1) durable 3 year viral suppression with TDF; 2) effective viral suppression of TDF in both viremic and virologically-controlled patients on ADV treatment; 3) continued normalization of ALT and 4) increasing HBeAg and HBsAg loss in HBeAg+ patients. No resistance to tenofovir developed following up to 3 years of TDF therapy. Consistent with recent literature, TDF produced potent viral suppression in both ADV-viremic and lamivudine-experienced patients (including a small subset of patients with pre-existing ADV and LAM-associated mutations)^{15,16,17,18}. The previously described rtA194T amino acid substitution was not observed in these studies^{19,20}.

Continued viral suppression has been correlated to improved clinical response and outcomes^{21,22,23}. Although these data support a correlation between viral suppression and improved clinical outcomes it is virtually impossible to definitively demonstrate this within the

confines of a clinical study. More recently a 25-year longitudinal cohort of untreated patients showed that in addition to older age, male gender and cirrhosis, the risk of liver related mortality was strongly associated with sustained high viral replication independent of HBeAg status²⁴.

Although no HBsAg loss was observed among HBeAg⁻ patients the increasing probability of HBsAg loss among HBeAg⁺ patients over 3 years is encouraging especially given the significant positive impact HBsAg loss has on outcomes such as cirrhosis, liver failure, HCC and overall mortality^{6,7}. The lack of HBsAg loss in HBeAg⁻ patients may be explained by the characteristics of HBeAg⁻ CHB, which is for the most part characterized by milder, intermittent fluctuations in HBV DNA and ALT without evidence of sustained immune control making HBsAg clearance difficult to achieve with oral agents, though sometimes observed with long term follow-up pegylated interferon treatment¹¹.

Quantitative HBsAg levels have been used to understand the kinetics of this marker. In HBeAg⁻ patients treated with pegylated interferon alpha-2a for 48 weeks, early kinetics of HBsAg decline was correlated with subsequent loss of HBsAg off treatment^{25,26,27} and over 7 years the strongest prognostic variable of HBsAg seroconversion was low pretreatment HBsAg levels²⁵. In a prospective study, a 0.5 to 1.0 log decline in HBsAg levels at Weeks 12 and 24 were highly predictive of a sustained response following pegylated interferon alpha 2a treatment²⁶. HBsAg loss induced by treatment with pegylated interferon has been correlated with HBV genotype in HBeAg⁺ patients (genotypes A and B), but not HBeAg⁻ patients (all major genotypes)^{28,10,11}. HBeAg⁺ patients treated with TDF who lost HBsAg included genotypes A (60%), D (35%) and F (5%). Additionally, HBeAg⁺ patients who lost HBsAg had high Knodell necroinflammatory scores and high (rather than low) baseline HBsAg and HBV DNA levels. Although baseline HBsAg levels in HBeAg⁻ patients were lower than in HBeAg⁺ patients, the rate of decline of HBsAg levels from week 96 to 144 in HBeAg⁺ patients was 10 times greater suggesting a

considerably shorter period of time required to clear HBsAg in patients with active HBeAg+ hepatitis. While it is interesting that treatment with TDF induces loss of HBsAg in a growing percentage of patients which could be used as an endpoint for stopping drug therapy, the majority of patients are unlikely to lose HBsAg for a long time, even if given a year of pegylated interferon, calling for exploration of novel immune interventions in these patients.

TDF was well tolerated in both HBeAg- and HBeAg+ patients up to week 144 with few discontinuations due to AEs. No change was noted in the side-effect profile of TDF with long term treatment and in particular renal tolerability was good. Serum creatinine remained stable over the 3 year period and <1% of patients had a confirmed 0.5 mg/dL increase in creatinine. These data provide long term, prospective clinical renal safety for TDF monotherapy consistent with previously published data establishing renal safety in HIV-1 infected patients^{29,30}.

The open-label, 7 year extension design of these studies is clinically important given the necessity of prolonged treatment, potentially lifetime treatment in some, to maintain viral suppression. The rate of HBsAg clearance (generally recognized as a 'cure' for HBV infection) with this oral antiviral in HBeAg+ patients occurs at a rate similar to that published for the immunomodulator, pegylated interferon alpha-2b¹⁰ and greater than the 0.5-0.8% published reports of spontaneous HBsAg clearance³¹. The switch from ADV to TDF after 48 weeks is reassuring showing that both virologically controlled and uncontrolled patients on ADV maintained or achieved a complete viral suppression and started losing HBsAg after initiating TDF. Data from our studies demonstrate that TDF treatment has durable efficacy and good tolerability with no development of resistance over 3 years. TDF maintained ALT normalization in HBeAg- and HBeAg+ patients and increasing HBsAg loss in HBeAg+ patients.

Open-label data were initially presented in part at the 59th and 60th annual meeting of the American Association for the Study of Liver Diseases (AASLD), November 2008 and November 2009 and the 44th annual meeting of the European Association for the Study of the Liver (EASL), April 2009.

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Figure Legends

Figure 1 Study Flow Diagram

Figure 1a. HBeAg Negative Patients (Study 102)

^a 10 of the 14 patients who withdrew consent did not have a biopsy and were therefore not eligible to continue in the open-label extension

^b 3 patients were at sites that did not participate in the open-label extension

^c 4 patients discontinued due to the adverse events 1) liposarcoma, 2) hepatocellular carcinoma, 3) dizziness, fatigue and disturbance in attention and 4) septic shock. Three patients died during the study (2 patients had been lost to follow-up and the third had discontinued due to the adverse event, septic shock)

Figure 1b. HBeAg Positive Patients (Study 103)

^a 5 of the 7 patients who withdrew consent did not have a biopsy and were therefore not eligible to continue in the open-label extension

^b Patient was at a site that did not participate in the open-label extension

^c Patient discontinued due to the adverse event, creatinine increased

Figure 2 HBV DNA Suppression Over Time. The proportion of patients (95% CI) with HBV DNA suppression over time is plotted. HBV DNA suppression was defined as HBV DNA level <400 copies/mL (69 IU/mL). In this modified intent-to-treat analysis (LTE) patients who discontinued for safety and efficacy reasons (including patients who discontinued with HBV DNA >400 copies/mL or an ongoing AE at the time of discontinuation) and patients who added emtricitabine are considered failures after discontinuation or the time of emtricitabine addition.

Figure 2a. HBeAg Negative Patients (Study 102). Eighty seven percent (206/238) of patients originally randomized to tenofovir DF (TDF-TDF) and 88% (107/121) of patients originally randomized to adefovir dipivoxil (ADV-TDF) achieved HBV DNA <400 copies/mL at week 144.

Figure 2b. HBeAg Positive Patients (Study 103). Seventy-two percent (118/165) of TDF-TDF patients and 71% (63/89) of ADV-TDF patients achieved HBV DNA <400 copies/mL at week 144.

Figure 3. HBsAg Loss Over Time

Figure 3a. Kaplan Meier Probability of HBsAg Loss.

Figure 3b & c. HBsAg Quantification Over Time in HBeAg Negative

HBsAg was measured quantitatively for all patients using the Abbott Architect assay at 12 to 16 week intervals through Week 144. The dynamic range of the Architect assay was 0.05-250 IU/mL and HBsAg concentrations >250 IU/ml were diluted according to the package insert.

Figure 3b. HBeAg Negative Patients (Study 102)

Figure 3c. HBeAg Positive Patients (Study 103)

Figure 4. Serum Creatinine (95% CI) Over Time

Creatinine was measured at regular intervals throughout the 3 years. Creatinine clearance was calculated using the Cockcroft-Gault equation. For both studies combined, the overall mean change (min,max) from baseline at week 144 in serum creatinine was 0.02 mg/dL (-0.70, 0.60).

Figure 4a. Serum Creatinine (95% CI) Over Time

Figure 4b. Creatinine Clearance (95% CI) Over Time

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Table 1. Disposition of Patients for the HBV DNA Analyses at Week 144

	Study 102			Study 103		
	TDF-TDF	ADV-TDF	Overall	TDF-TDF	ADV-TDF	Overall
Randomized and Treated	250	125	375	176	90	266
Long Term Evaluation Analysis (LTE)						
Patients with missing HBV DNA at week 144 excluded from the Denominator	12	4	16	11	1	12
HBV DNA missing completely at random	7	2	9	2	1	3
Patients discontinued due to non-protocol endpoint with HBV DNA <400 copies/mL with no ongoing AE	5	2	7	9	0	9
Denominator at Week 144 for Long Term Evaluation Analysis	238	121	359	165	89	254
Open Label Evaluation Analysis (OLE)						
Patients entering the Open Label Phase	235	112	347	154	84	238
Denominator at Week 144 for Open Label Analysis	235	112	347	154	84	238
On-Treatment Analysis						
Patients with an HBV DNA value at Week 144 ^a	212	107	319	132	76	208
Patients who added FTC on or before Week 144	3	0	3	17	14	31
With HBV DNA <400 copies/mL at Week 144	3	0	3	10	7	17
Missing HBV DNA at Week 144	0	0	0	1	4	5
With HBV DNA ≥ 400 copies/mL at Week 144	0	0	0	6	3	9
Denominator at Week 144 for On-Treatment Analysis	212	107	319	132	76	208

In order to deal with the challenges inherent in appropriate management of missing data in long-term efficacy studies, a pre-specified intent-to-treat algorithm (LTE) was defined to allow for subjects withdrawing from the study for administrative reasons to be removed from the denominator for the calculation of virologic response by study visit. When the probability of an observation being missing does not depend on observed or unobserved measurements, then mathematically the observation is missing completely at random. If data are missing at random, then the analysis of only those subjects with complete data gives valid inferences without bias. As a sensitivity-type analysis the open label evaluation analysis was conducted whereby only patients who entered the open label phase are included in the denominator. Patients with missing HBV DNA data at each visit for any reason are considered failures. Lastly, an on-treatment analysis was conducted whereby only patients with data at that visit are included in the denominator. This latter analysis provides relevant virological response data to the clinician regarding patients who remain on treatment.

Table 2. Baseline Disease and Demographics Characteristics by Week 144 HBsAg Response in HBeAg-Positive Patients

	HBsAg Positive (N=243) ^a	HBsAg Negative (N=20)
Median Age (yrs) (min, max)	32 (18, 63)	37 (20, 64)
Race, N (%)		
Asian	94 (39)	0
White	118 (49)	19 (95)
Black	17 (7)	1 (5)
Gender		
Male	167 (69)	16 (80)
Baseline Genotype		
A	46 (19)	12 (60)
B	16 (19)	0
C	34 (14)	0
D	68 (29)	7 (35)
E	78 (33)	0
F	7 (3)	1 (5)
Missing/Unevaluable	5	0
Median Baseline HBV DNA (log ₁₀ copies/mL) (min, max)	8.81 (4.67, 10.92)	9.47 (8.47, 9.64)
Median Baseline ALT (U/L) (min, max)	109.0 (23, 964)	145.5 (75, 425)
Median Baseline HBsAg (log ₁₀ IU/mL) (min, max)	4.50 (1.01, 5.40)	5.11 (4.62, 5.40)
Median Baseline Knodell Necroinflammatory Score (min, max)	9.0 (1.0, 12.0)	9.0 (5.0, 12.0)
Baseline Knodell Fibrosis Score		
= 3 (bridging fibrosis)	89 (38)	8 (42)
= 4 (cirrhosis)	45 (19)	5 (26)

^aExcludes three patients without HBsAg quantitative data.

Key baseline demographic and disease characteristics are provided for HBeAg positive patients who achieved HBsAg loss by week 144 and are shown relative to HBeAg positive patients who remained HBsAg positive through week 144.

Table 3a. Summary of Resistance Surveillance during Open Label Treatment through Year 3 by Treatment

Category	TDF-TDF N=389	ADV-TDF N=196	TDF/FTC N=34
Patients included in Year 2 and Year 3 resistance surveillance	29	19	12
Genotypic Results:			
No change	16	11	5
Polymorphic change	6	4	4
Conserved Site Change	3	3	2
Could not be evaluated	4	1	1

Table 3b. Phenotypic analysis of clinical isolates from patients developing conserved site changes in HBV pol/RT

Treatment Group	Viral Isolate	Fold Change ^a	HBV DNA at Week 144 (log ₁₀ copies/mL)
TDF	Week 72 (rtL101F)	1.1	<2.23
TDF	Week 72 (rtV173L+rtL180M+rtM204V)	1.3	<2.23
TDF	Week 144 (rtR51K)	1.4	2.67
TDF-TVD	Week 144 (rtA181T) (rtL180M+rtM204V) ^c	0.7 ND ^d	2.91
ADV-TDF	Week 144 (rtN236T)	8.2	4.05
ADV-TDF	Week 144 (rtR274Q)	1.9	
TDF-TVD	Week 144 (rtR192H)	RD ^e	3.93
ADV-TDF	Week 72 (rtA307T) ^b	ND	2.68
ADV-TDF	Week 144 (rtG152E)	1.8	2.64

^a Defined as EC₅₀ of mutant virus/EC₅₀ of baseline/parent virus

^b Unable to clone

^c Viral clones containing the L180M+M204V were also present in the serum but could not be cloned for phenotypic analysis

^d ND = Not determined

^e RD = Replication Defective, virus containing the mutation did not grow in cell culture

Figure 1 Study Flow Diagram

Figure 1a. HBeAg Negative Patients (Study 102)

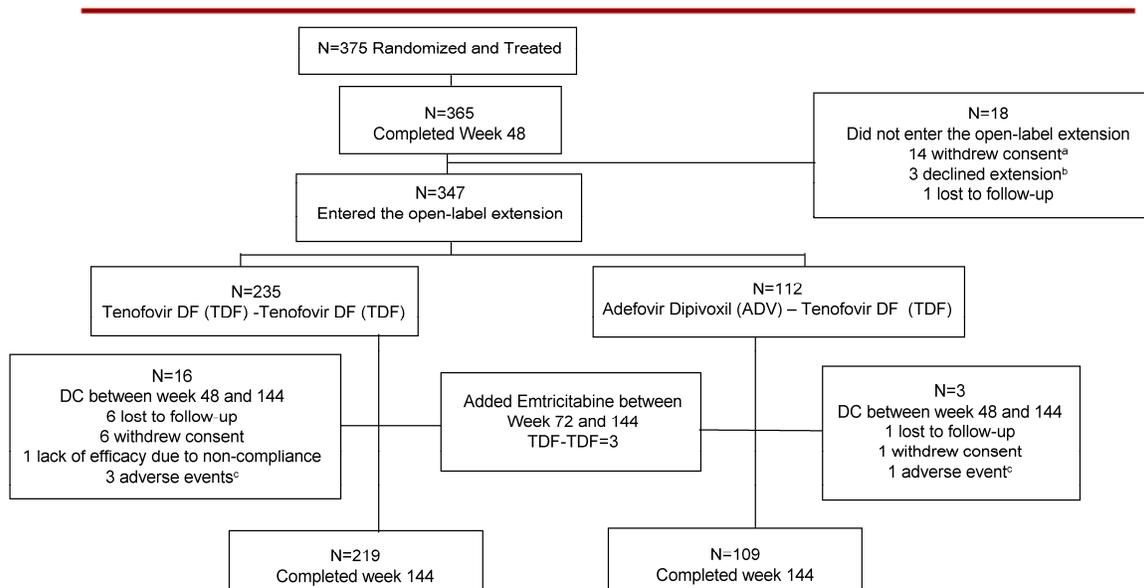


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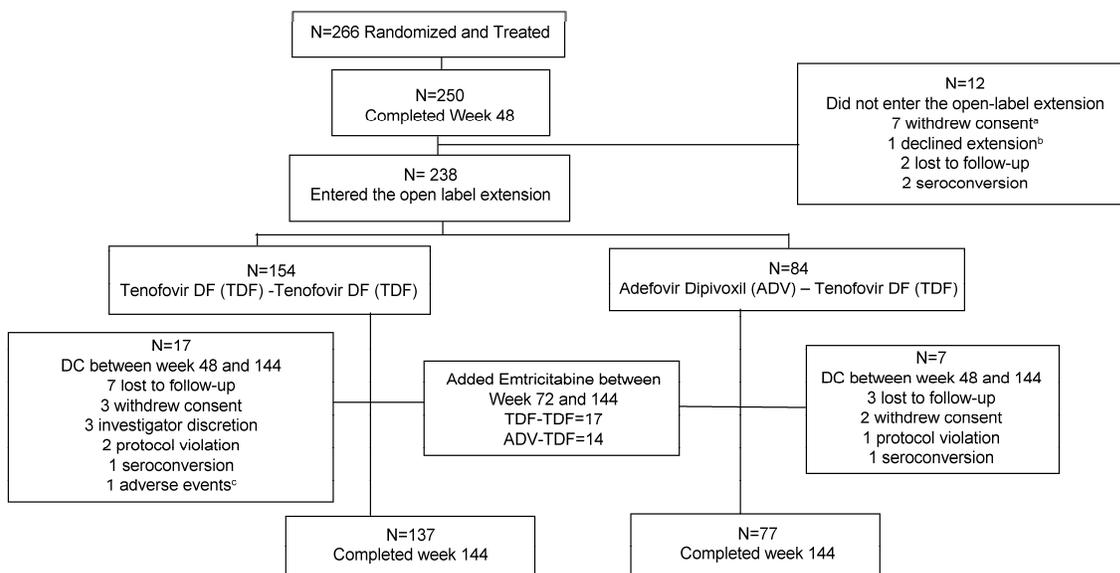


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Figure 2a. HBeAg Negative Patients (Study 102)

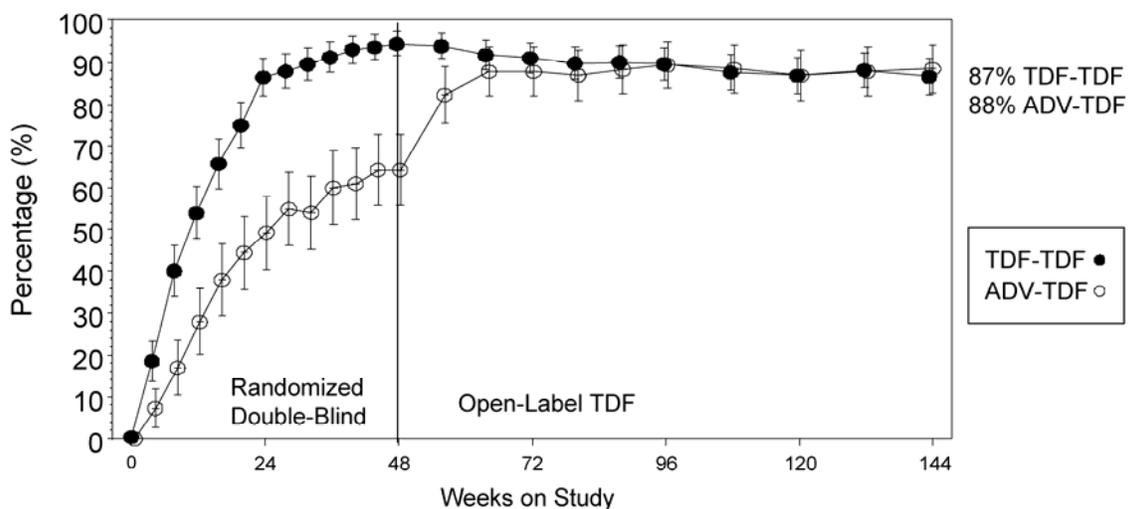


Figure 2b. HBeAg Positive Patients (Study 103)

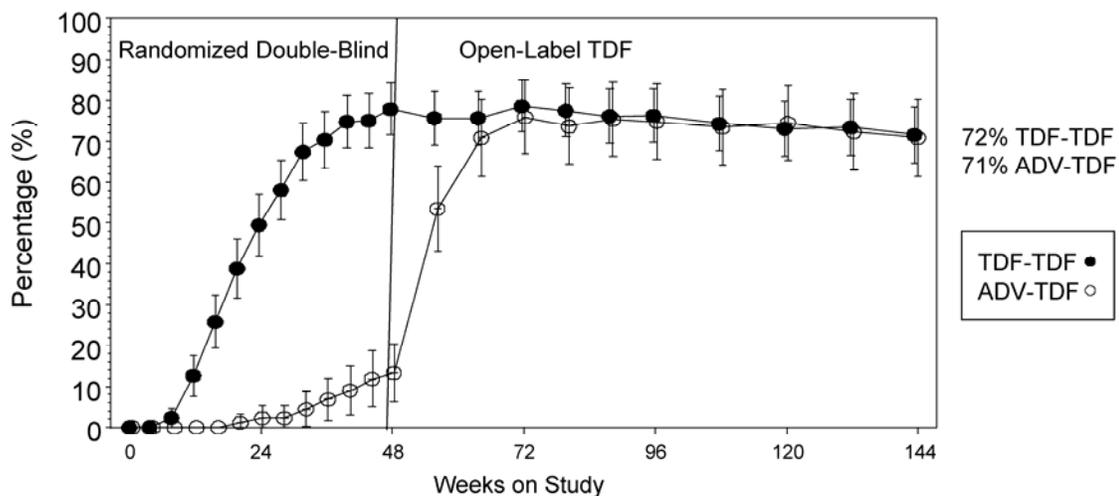
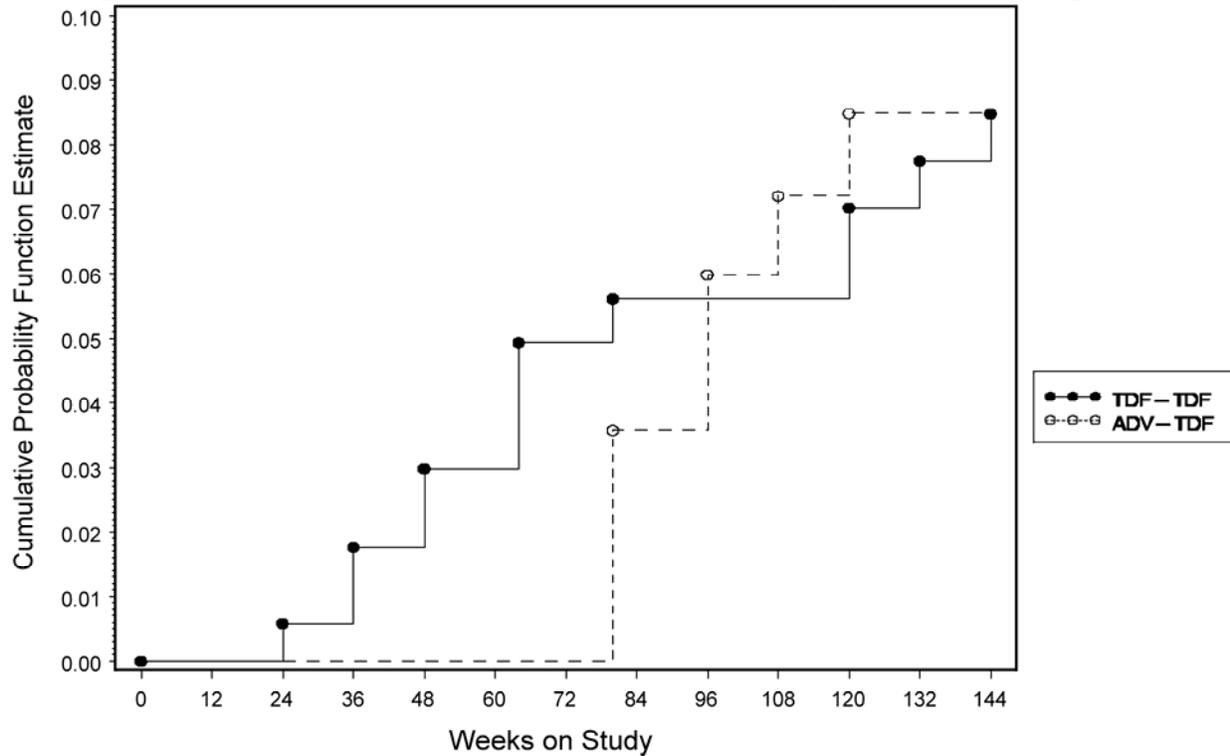


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Figure 3a. Kaplan Meier Probability of HBsAg Loss



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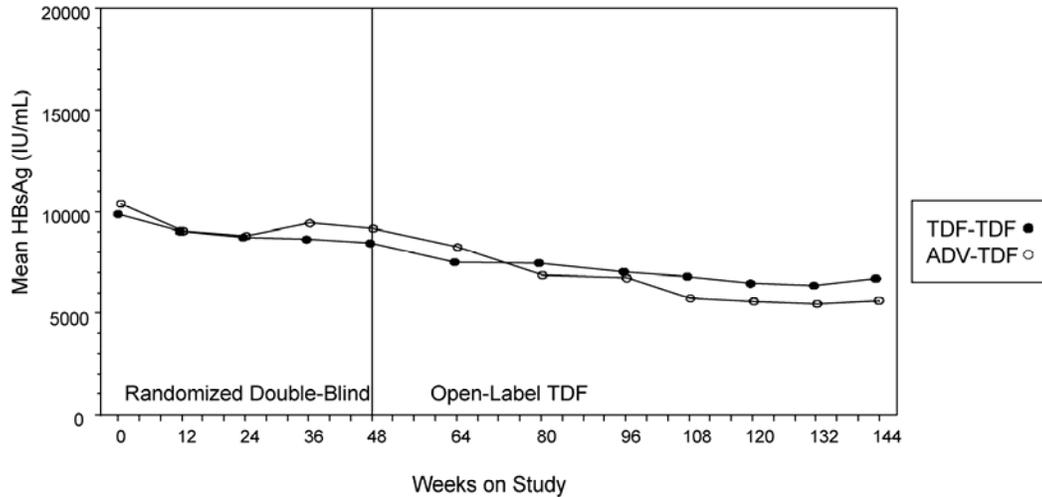


Figure 3c. HBeAg Positive Patients (Study 103)

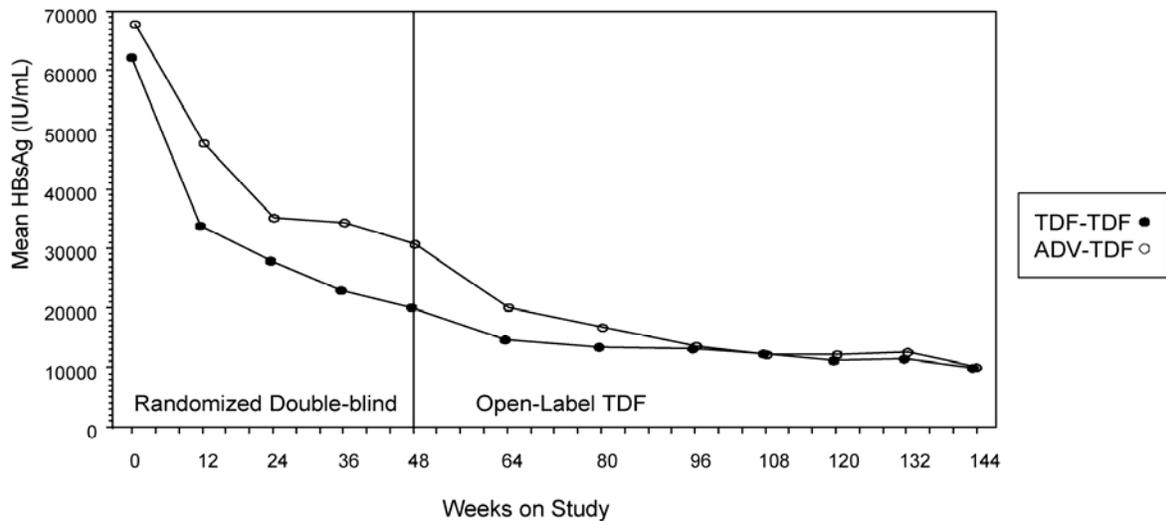


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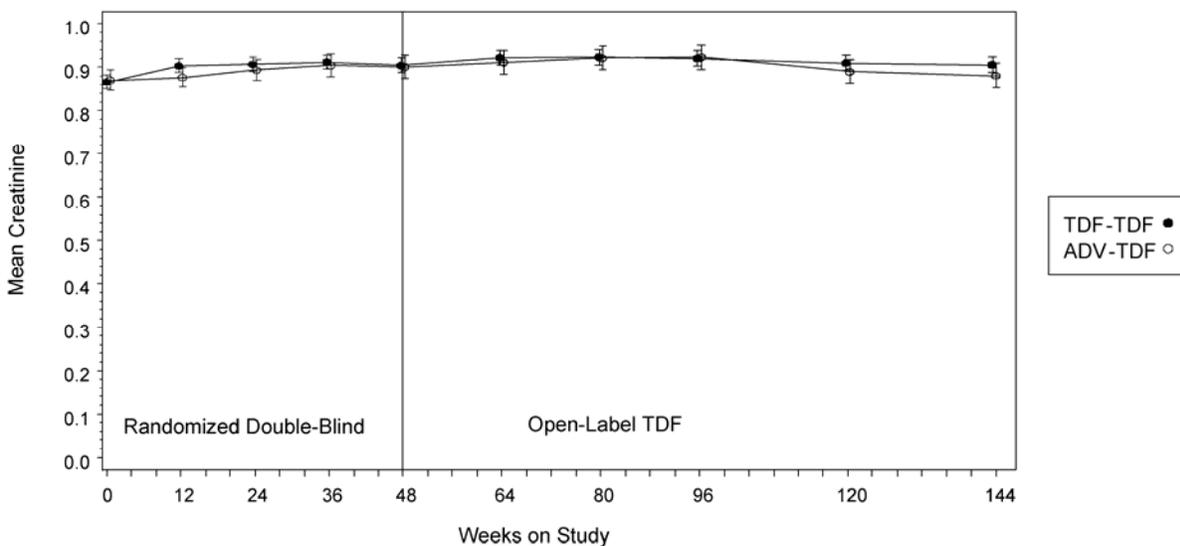


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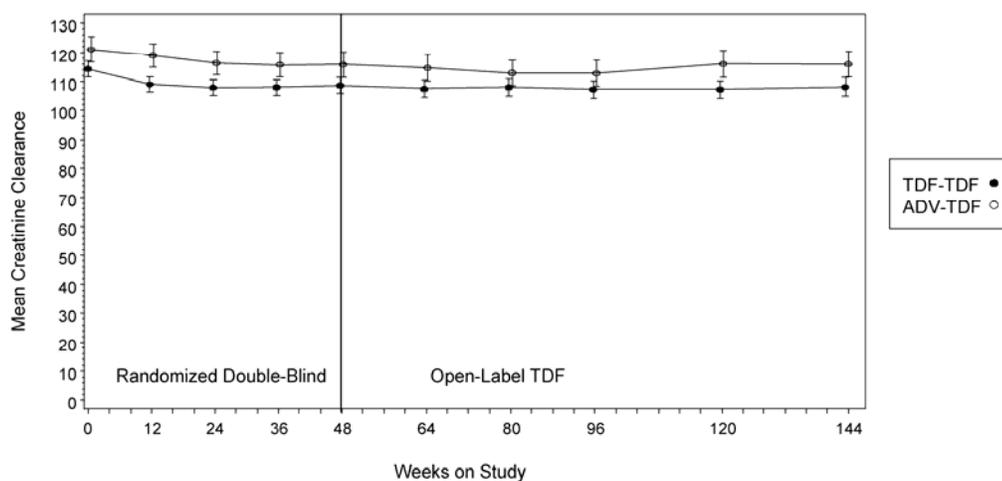


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