Switching from entecavir to tenofovir disoproxil fumarate for HBeAg-positive chronic hepatitis B patients: a phase 4, prospective study

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Abstract

Background: Tenofovir disoproxil fumarate (TDF) is widely used and recommended as first-line treatment for patients infected with the hepatitis B virus (HBV). However, current data are limited regarding the efficacy and safety of switching to TDF for the treatment of chronic hepatitis B in hepatitis B e-antigen (HBeAg)-positive patients who are virologically suppressed with another nucleos(t)ide analogue. The primary objective of this study was to evaluate the hepatitis B surface antigen (HBSAg) reduction potential of switching from entecavir (ETV) to TDF at week 48 in HBeAg-positive chronic hepatitis B patients with undetectable serum HBV-DNA.

Methods: In this multicenter, single-arm, open-label, phase 4 clinical study, 75 participants currently treated with ETV 0.5 mg once daily were switched to TDF 300 mg once daily for 96 weeks.

Results: At week 48, 3/74 participants (4%) achieved 0.25 log10 reduction of HBsAg levels from baseline (the primary endpoint). Mean HBsAg reduction was −0.14 log10 IU/mL and 12% (9/74) achieved 0.25 log10 reduction by 96 weeks. No participants achieved HBsAg seroclearance. HBsAg reduction at weeks 48 and 96 was numerically greater in participants with higher alanine aminotransferase levels (≥ 60 U/L). Seventeen participants (25%) achieved HBeAg seroclearance up to week 96. No participants experienced viral breakthrough. All drug-related adverse events (18 participants [24%]) were mild in intensity, including an increase in urine beta-2-microglobulin (15 participants [20%]).

Conclusions: In conclusion, HBsAg reduction was limited after switching from ETV to TDF in this study population. Further investigation is warranted to better understand the clinical impact of switching from ETV to TDF.

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Background
Hepatitis B virus (HBV) infection is characterized by the serologic presence of hepatitis B e-antigen (HBeAg) and HBV-DNA, as well as hepatitis B surface antigen (HBsAg) [1]. Oral nucleos(t)ide analogues (NAs) such as lamivudine, entecavir (ETV), tenofovir alafenamide (TAF), and tenofovir disoproxil fumarate (TDF) effectively inhibit reverse transcription of pregenomic HBV-RNA, thereby lowering serum HBV-DNA levels [2, 3]. Achieving HBsAg seroclearance is associated with sustained immunologic and virologic control by appropriate treatments and a reduced risk of hepatocellular carcinoma (HCC) [4–6]. While the long-term treatment goal of HBsAg seroclearance is rarely achievable with current treatment options, including NAs [7, 8], several clinical trials have been conducted to seek better treatment options to approach HBsAg seroclearance [9–11].

Regarding HCC risk during treatment with NAs, a study in Korea indicated that treatment with TDF has been associated with a significantly lower risk of HCC compared with ETV in chronic hepatitis B (CHB) patients [12]. Moreover, previous studies in CHB patients conducted by our group demonstrated superior HBsAg reduction with TDF compared with other NAs in NA-naïve patients. In addition, reduction in HBsAg was previously achieved predominantly in HBeAg-positive patients with relatively high viral activity who were receiving TDF [4, 13].

Previous studies, mainly in HBeAg negative patients, have reported short-term efficacy after switching from ETV to TDF [11, 14]. However, current data are limited, focusing on HBeAg-positive patients with CHB who are virologically suppressed with another NA. Furthermore, the impact of patient factors on the reduction of HBsAg remains unclear [13, 15]. Based on previous studies showing a decline of HBsAg in patients with HBeAg-positive CHB and undetectable serum HBV-DNA who were treated with ETV therapy, we predicted that HBsAg reduction after switching from ETV to TDF could potentially be of clinical benefit.

Methods
Study design
This was a multicenter, single-arm, open-label clinical study conducted in HBeAg-positive CHB patients with undetectable serum HBV-DNA (initiated October 2, 2017 and completed November 25, 2019). Participants (N = 75) currently treated with ETV 0.5 mg once daily were switched to TDF 300 mg on day 1, following a 6-week screening period. TDF was administered once daily for 96 weeks.

The study was approved by the ethics committee at every participating institution (19 centers in Japan) and was conducted according to the recommendations of Good Clinical Practice and the Declaration of Helsinki (2013). All participants provided written informed consent to participate in the study.

Study participants
Male and female patients with CHB between 20 and 69 years of age at the time of informed consent who had been treated with ETV for at least two years were eligible for study inclusion. At screening, participants were required to test positive for serum HBeAg (local hospital or central laboratory), have an HBV-DNA level below the limit of quantitation (< 1.3 log10 IU/mL [<20 IU/mL]), and have a serum HBsAg level ≥ 800 IU/mL (or 80 to 800 IU/mL and fluctuation decrease within 0.1 log10 IU/mL per year to exclude participants with continuous HBsAg decrease). Additional inclusion criteria included creatinine clearance ≥ 70 mL/min, hemoglobin ≥ 8 g/dL, and white blood cell count ≥ 1000/mm3.

Patients who received any interferon (IFN) or HBV vaccine therapy within 24 weeks prior to initiation of the study treatment; or TDF, adefovir dipivoxil (ADV), or TAF within two years prior were excluded. Patients coinfected with HIV or hepatitis C virus (HCV) were excluded. Patients with a history of (or suspected of having) HCC were also ineligible. Other exclusion criteria included drugs causing renal impairment, competitors of Keywords: Tenofovir disoproxil fumarate, Chronic hepatitis B, HBsAg, HBeAg-positive, Entecavir
renal excretion, immunosuppressants, or glucocorticoids within eight weeks prior to study initiation; the presence of proximal tubulopathy; decompensated CHB (direct bilirubin > 1.5 × the upper limit of normal; prothrombin time < 60%; platelets < 75,000/mm3; albumin < 3.0 g/dL); or a history of alcohol or drug abuse.

Clinical evaluations
Virologic testing at the time of screening included HIV, HCV, HBV genotype, HBV-DNA levels, HBeAg/anti-HBe, and HBsAg/anti-HBs quantitation. HBV-DNA, HBeAg/anti-HBe, HBsAg/anti-HBs, and hepatitis B core-related antigen (HBcrAg) testing was performed at week 4, week 12, and every 12 weeks thereafter, by SRL Medisearch Inc. (Tokyo, Japan). Dual energy X-ray absorptiometry (DXA) testing was performed at screening, last visit, and at intervals as needed based on laboratory results, but not more frequently than every 4 months.

Study endpoints
The primary endpoint of this study was the proportion of participants achieving 0.25 log₁₀ HBsAg reduction from baseline at week 48 (defined as the HBsAg responder rate). The secondary endpoint was the proportion of participants achieving 0.25 log₁₀ HBsAg reduction from baseline at weeks 24 and 96. Additional secondary efficacy analyses at weeks 24, 48, and 96 included the proportion of participants achieving HBsAg seroclearance and HBsAg/anti-HBs seroconversion, reduction of HBsAg from baseline, the proportion of participants achieving HBeAg seroclearance and HBeAg/anti-HBe seroconversion, and reduction of HBcrAg from baseline.

Safety assessments included the monitoring of adverse events (AEs), as well as urinalysis, hematology, and clinical chemistry testing, which was performed at baseline and again at weeks 4 and 12, and every 12 weeks thereafter. Adverse events were summarized as study drug-related AEs, AEs by intensity, and those resulting in discontinuation of the study. Adverse events of special interest (AESIs) included those with renal, liver, or bone involvement.

Statistical analyses
Descriptive statistics were used to assess the efficacy and safety objectives. An estimation approach was used to address the efficacy objectives, where point estimates and corresponding 95% confidence intervals (CIs) were constructed.

Although no confirmatory hypotheses were tested in this study, the sample size was based on the expected responder rate and threshold rate. Results from the previous Japanese phase 3 study [13] showed that the proportion of participants expected to achieve 0.25 log₁₀ HBsAg reduction from the baseline at week 48 was 20%. Additionally, a 0.25 log₁₀ HBsAg reduction after 48 weeks of TDF therapy was considered to be clinically meaningful and defined as a primary endpoint in this study. The proportion of HBsAg responders in subjects not switched to TDF-based regimens on the basis of ETV treatment was assumed to be 6% (threshold rate), and the sample size with at least 90% power to detect a 14% difference against the threshold was calculated to be 57. Allowing for a participant dropout rate of 10%, the target sample size was set at approximately 65.

| Table 1 | Participant demographics and baseline characteristics (SP) |
|---|---|
| **Baseline characteristic** | **TDF (N = 75)** |
| Age (years), mean (± SD) | 48.4 (± 9.35) |
| Sex, n (%) |  |
| Male | 55 (73) |
| Female | 20 (27) |
| Race, n (%) |  |
| Japanese | 71 (95) |
| East Asian | 4 (5) |
| HBV genotype, n (%) |  |
| A | 1 (1) |
| B | 2 (3) |
| C | 72 (96) |
| HBV-DNA (log₁₀ IU/mL), mean (± SD) | 0.92 (± 0.12) |
| ALT (IU/L), mean (± SD) | 23.5 (± 18.13) |
| HBeAg, n (%) |  |
| Positive | 70 (93) |
| Negativea | 5 (7) |
| HBsAg (IU/mL), mean (± SD) | 5311.3 (± 5619.84) |
| HBcrAg (log₁₀ U/mL), mean (± SD) | 5.5 (± 0.55) |
| eGFR by JSN-CKDI (mL/min/1.73m²), n (%) |  |
| < 60 | 0 (0) |
| 60 to < 90 | 42 (56) |
| ≥ 90 | 33 (44) |
| Urine beta-2-microglobulin (µg/g Cr), mean (± SD) | 241.9 (305.58) |
| Protocol defined liver cirrhosis, n (%) |  |
| Yes | 0 (0) |
| No | 75 (100) |

ALT alanine aminotransferase, Cr creatinine, eGFR by JSN-CKDI estimated glomerular filtration rate calculated by the Japanese Society of Nephrology-Chronic Kidney Disease Initiatives equation, HBV hepatitis B virus, HBeAg hepatitis B e-antigen, HBsAg hepatitis B surface antigen, HBcrAg hepatitis B core-related antigen, SD standard deviation, SP safety population, TDF tenofovir disoproxil fumarate

a HBeAg-positive according to local in-hospital laboratory results, but HBeAg-negative according to central laboratory results at screening
Analyses of safety data were performed at screening, baseline, weeks 4, 12, 24, 36, 48 and week 96 using the safety population, defined as all participants who received study treatment. Analyses of efficacy data were performed at screening, baseline, weeks 4, 12, 24, 36, 48, and week 96 using the full analysis set (FAS), defined as all participants enrolled who received study treatment. A subset of participants, which excluded three participants in the FAS who met the protocol deviation criteria of receiving medication, was defined as the efficacy evaluable set (EES). Analysis of the EES was performed to evaluate the robustness of the efficacy results.

Results
A total of 92 patients were screened and 75 patients enrolled in the study (safety population) (Fig. 1).

The FAS included 74 participants, 72 of whom completed week 96. One participant withdrew from the study due to a study drug-related adverse event, one participant reached protocol-defined liver stopping criteria (bilirubin ≥ 2 × the upper limit of normal and direct bilirubin > 35%) on day 1 before receiving TDF, and one participant was withdrawn at the discretion of the investigator.

Participant characteristics
Of the 75 participants (SP), 70 were HBeAg-positive at baseline. Another five participants who were HBeAg-positive at screening according to local in-hospital laboratory results were HBeAg-negative at screening and baseline according to central laboratory results. For all participants, serum HBV-DNA level was undetectable (< 1.3 log10 IU/mL) at baseline except for one participant with 1.9 log10 IU/mL. No patients met protocol-defined criteria for liver cirrhosis at baseline. Baseline characteristics for the safety population are shown in Table 1.

Antiviral responses
The proportion of participants achieving 0.25 log10 HBsAg reduction from baseline at week 48 was 3/74 (4%) participants (Table 2). All three participants were
male, infected with HBV genotype C, and were similar in age (45, 44, and 42 years). One of these participants was HBeAg-negative at baseline but was judged HBeAg-positive according to a local laboratory at screening. At 96 weeks, the proportion of participants achieving > 0.25 log_{10} reduction from baseline was 12% (9/74, including the three participants who had responded by week 48).

The overall mean (± standard deviation [SD]) change from baseline in HBsAg level decreased, with a mean change of −0.11 (± 0.08) log_{10} IU/mL at week 48 and −0.14 (± 0.12) log_{10} IU/mL at week 96 (Table 2 and Fig. 2).

At weeks 48 and 96, HBsAg reduction from baseline was numerically greater in the higher alanine aminotransferase (ALT) group (defined as ≥60 U/L at least once from baseline) compared with the lower ALT group (defined as < 60 U/L from baseline). Mean (± SD; [95% CI]) change in HBsAg from baseline at week 48 was −0.25 (± 0.09; [−0.35, −0.15]) log_{10} IU/mL in the higher ALT group (N = 6), versus −0.10 (± 0.07; [−0.12, −0.08]) log_{10} IU/mL in the lower ALT group (N = 67). At week 96, mean change from baseline in HBsAg was −0.37 (± 0.14; [−0.52, −0.22]) in the higher ALT group (N = 6) and −0.12 (± 0.09; [−0.14, −0.09]) in the lower ALT group (N = 66). All three participants who achieved 0.25 log_{10} HBsAg reduction from baseline at week 48 demonstrated high serum ALT levels throughout the study period as shown in Fig. 3.

Of the additional six participants who achieved 0.25 log_{10} HBsAg reduction from baseline by week 96, two were in the higher ALT group. No patients achieved HBsAg seroclearance up to week 96 (Table 2).

HBeAg seroclearance was analyzed in the 69 participants who were HBeAg-positive at baseline. Eight participants (12%) achieved HBeAg seroclearance up to week 48 and 17 participants (25%) achieved HBeAg seroclearance by week 96. HBeAg/anti-HBe seroconversion was analyzed in the 53 participants who were HBeAg-positive and anti-HBe-negative at baseline, and was achieved by seven participants (13%) up to week 48 and 13 participants (25%) by week 96.

The mean (± SD) HBcrAg change from baseline was −0.17 (± 0.29) log_{10} U/mL at week 48 and −0.30 (± 0.36) at week 96. No virologic breakthrough was observed, and serum HBV-DNA levels remained undetectable (< 1.3 log_{10} IU/mL [< 20 IU/mL]) through week 96 in all participants.

### Safety

Adverse events and other key safety analyses are summarized in Table 3.

Overall, through week 96, most AEs were mild in severity. Two serious AEs, osteoarthritis (one participant) and appendicitis (one participant), were reported but neither was considered to be study drug-related. Drug-related AEs included renal tubular dysfunction and prothrombin time prolonged (one participant each), which were mild in intensity. One participant was withdrawn at the judgment of the investigator due to renal tubular disorder of mild intensity, which resolved. An increase in urine beta-2-microglobulin, considered a sensitive and specific indicator of renal tubular dysfunction associated with TDF
### Table 3  Key safety endpoints (SP)

| Adverse events (week 96) | TDF (N = 75) |
|--------------------------|-------------|
|                           | n (%)       |
| On-therapy AE            | 58 (77)     |
| On-therapy serious AE    | 2 (3)       |
| On-therapy AE leading to study withdrawal | 1 (1) |
| Drug-related AE          | 18 (24)     |
| Drug-related urine beta-2-microglobulin increase | 15 (20) |
| Renal tubular disorder   | 1 (1)       |
| Renal tubular dysfunction | 1 (1)     |
| Prothrombin time prolonged | 1 (1) |

| Other key safety endpoints | n | Median (min, max) | Median change from baseline (min, max) |
|----------------------------|---|-------------------|----------------------------------------|
| **Serum creatinine (mg/dL)** |   |                   |                                        |
| Baseline                   | 75 | 0.70 (0.38, 1.01)  | 0.01 (−0.14, 0.20)                      |
| Week 4                     | 75 | 0.71 (0.44, 1.02)  |                                        |
| Week 24                    | 74 | 0.74 (0.41, 1.05)  | 0.02 (−0.08, 0.15)                      |
| Week 48                    | 73 | 0.72 (0.42, 1.09)  | 0.04 (−0.11, 0.12)                      |
| Week 96                    | 72 | 0.76 (0.45, 1.02)  | 0.06 (−0.10, 0.17)                      |
| **eGFR by JSN-CKD1 (mL/min/1.73m²)** |   |                   |                                        |
| Baseline                   | 75 | 86 (60, 135)       |                                        |
| Week 4                     | 75 | 84 (60, 138)       | −2.0 (−21, 20)                         |
| Week 24                    | 74 | 81 (57, 124)       | −3.0 (−21, 13)                         |
| Week 48                    | 73 | 82 (56, 124)       | −6.0 (−20, 12)                         |
| Week 96                    | 72 | 79.0 (52, 118)     | −9.5 (−24, 14)                         |
| **Urine beta-2-microglobulin ratio (µg/g creatinine)** |   |                   |                                        |
| Baseline                   | 75 | 154.91 (40.71, 1636.09) |                                      |
| Week 4                     | 75 | 198.91 (25.72, 4779.66) | 45.02 (−317.50, 7143.57)             |
| Week 24                    | 74 | 185.88 (8.93, 9006.76) | 41.17 (−361.52, 7890.23)               |
| Week 48                    | 73 | 192.50 (13.02, 10,546.26) | 38.17 (−582.24, 9429.72)               |
| Week 96                    | 72 | 154.96 (11.20, 9052.18) | 15.19 (−166.80, 7935.65)               |
| **Serum phosphorus (mg/dL)** |   |                   |                                        |
| Baseline                   | 75 | 3.3 (1.9, 4.4)     |                                        |
| Week 4                     | 75 | 3.2 (2.0, 4.4)     | −0.10 (−1.0, 0.9)                      |
| Week 24                    | 74 | 3.3 (1.4, 4.5)     | −0.10 (−1.1, 1.4)                      |
| Week 48                    | 73 | 3.3 (2.0, 4.7)     | 0.00 (−1.3, 0.9)                       |
| Week 96                    | 72 | 3.4 (2.2, 4.5)     | 0.10 (−1.1, 1.2)                       |
| **%TRP**                   |   |                   |                                        |
| Baseline                   | 75 | 89.92 (77.91, 97.71) | −0.69 (−13.26, 6.75)                   |
| Week 4                     | 75 | 88.49 (77.04, 98.79) | −0.52 (−11.81, 9.75)                   |
| Week 24                    | 74 | 90.20 (78.96, 96.26) | 0.17 (−8.71, 8.27)                     |
| Week 48                    | 73 | 89.44 (81.51, 97.71) | −0.818 (−10.25, 15.47)                 |
| Week 96                    | 72 | 89.83 (81.07, 96.90) |                                        |
| **ALT (U/L)**              |   |                   |                                        |
| Baseline                   | 75 | 19.0 (7, 147)      |                                        |
| Week 4                     | 75 | 22.0 (8, 141)      | 4.0 (−14, 60)                         |
| Week 24                    | 74 | 22.5 (10, 130)     | 4.0 (−32, 61)                         |
| Week 48                    | 73 | 23.0 (12, 83)      | 4.0 (−79, 23)                         |
| Week 96                    | 72 | 21.5 (11, 220)     | 2.5 (−20, 73)                         |
was the only renal AE that was reported in more than one participant. An increase of mild intensity occurred in 15 participants (20%) and considered to be study drug-related.

In addition to the increase in median urine beta-2-microglobulin (median change from baseline 15.19 µg/g creatinine), a small increase in median serum creatinine (0.06 mg/dL) and a decrease in median estimated glomerular filtration rate (eGFR, −9.5 mL/min/1.73m²) were observed at week 96. Not all participants with an increase in urine beta-2-microglobulin experienced a decrease in eGFR, and there were no decreases in median percent renal tubular reabsorption of phosphate (%TRP) or serum phosphorus except for two participants who experienced hypophosphatemia, which were not considered to be study drug-related. Overall, there were three types of clinical courses observed amongst participants who experienced a change in renal parameters (Fig. 4): 1) participants with an increase in urine beta-2-microglobulin and decrease in eGFR (Fig. 4a), 2) those with an increase in urine beta-2-microglobulin and without change in eGFR (Fig. 4b), and 3) those without a change in urine beta-2-microglobulin and decrease in eGFR (Fig. 4c).

No participants had drug-related liver AEs. The only liver AESI was hepatic steatosis, not drug related, reported by one participant. No clinically significant elevation of ALT was observed (Table 3). At baseline, six participants (8%) had ALT levels above the upper limit of normal (≥40 U/L) compared with 10 participants (14%) at week 48 and five (7%) at week 96.

No participants had drug-related bone AEs or AESIs. The only bone AESI was jaw pain, not drug related, reported by one participant. A decrease in bone mineral density (BMD) (median change from baseline: femur, −2.09%; lumbar vertebra, −2.24%) was observed at 96 weeks.

**Discussion**

This study is the first prospective study to investigate the efficacy and safety of switching to TDF from ETV, focusing on patients with HBeAg-positive and HBV-DNA undetectable CHB in Japan. Because the results of our previous phase 3 study in treatment-naïve participants showed that reduction in HBsAg observed in the TDF group was predominantly in HBeAg-positive patients [13], we targeted patients with HBeAg-positive CHB (including five patients who were HBeAg-positive according to local in-hospital laboratory results; Table 1) treated with ETV for at least two years in this phase 4 study. To ensure an adequate sample size of HBeAg positive CHB patients with undetectable serum HBV-DNA, the study was conducted as a multi-center trial.

While our data showed the switch to TDF was still virologically suppressive, the proportion of patients achieving 0.25 log₁₀ HBsAg reduction from baseline at week 48 (4%) was lower than our original assumption (20%). This may be explained by the variation in duration of previous ETV treatment across the study population. Entecavir therapy for at least two years prior to screening was required, however, no upper limit was specified for duration of time on therapy and participant data were not collected in this regard. Previous studies have reported a greater rate of HBsAg reduction during the first year of NA therapy (ETV, TDF) compared with subsequent years [18]. Although we did not investigate the correlation between the duration of ETV therapy and HBsAg reduction in this study, it is possible that the duration of prior ETV therapy influenced the rate of patient achieving 0.25 log₁₀ HBsAg reduction from baseline at week 48.

Participants in this study with higher ALT levels (N=6, defined as ≥60 U/L at least once from baseline through week 48), including the three participants with 0.25 log₁₀ HBsAg reduction from baseline at week 48, had a numerically greater reduction from baseline in HBsAg level at both week 48 and week 96 versus those with lower
ALT levels (N=67, defined as < 60 U/L from baseline through week 48). This is similar to our previous study in naïve patients indicating that HBsAg reduction was predominant in patients with higher ALT levels (≥ 80 U/L) [13]. However, in terms of individual participants, there was no apparent pattern between ALT levels and HBsAg change from baseline for the nine participants in the current study who achieved > 0.25 log10 reduction at 96 weeks. It is possible that a combination of factors (e.g., high ALT together with HBeAg-positive status and some unknown factors) is associated with HBsAg reduction after switching to TDF. As previously reported, higher serum IFN λ3 levels were observed in patients treated with NAs (ADV and TDF), but not in those treated with NAs (lamivudine and ETV) [19]. Interferon-λ3 induction caused by the NAs further induces IFN-stimulated genes and results in a reduction of HBsAg production. Although we did not measure serum IFN λ3 levels in this study, greater HBsAg reduction in patients with higher ALT levels may be related to immunomodulatory effects associated with TDF therapy [20].

In this study, 25% of HBeAg-positive participants, with at least two years of prior ETV treatment, achieved HBeAg seroclearance up to 96 weeks after switching to TDF. The HBeAg seroclearance rate we observed was comparable to rates reported in previous studies in patients treated with ETV [21, 22]. In this limited number of participants, there was no apparent relationship between HBeAg status change and HBcrAg reduction. No new safety concerns were identified with TDF therapy (including bone, liver, or renal AESIs) compared with previous studies [5, 8, 11, 13, 14]. Among participants with a decrease in BMD, no apparent common trends were observed, such as a reduction in serum phosphate or %TRP, and there were no apparent associated common factors. Overall, decreases in BMD were consistent with historical data for participants with HBV and the known safety profile of TDF therapy [23]. There was no apparent link between changes in BMD and other renal parameters measured in the current study. Observed increases in serum creatinine and decreases in eGFR were also comparable to previous studies [8, 11, 14], and therefore, continuous monitoring for bone and renal events is necessary during treatment with TDF.

The percentage of participants with ALT above the upper limit of normal (> 40 U/L) increased to 14% at week 48 from 8% at baseline, although it is unclear if the lack of ALT normalization was due to TDF therapy [11]. We also carefully monitored urine beta-2-microglobulin to creatinine ratio and %TRP. Although urine beta-2-microglobulin to creatinine ratio and %TRP are considered sensitive and specific indicators of renal tubular dysfunction associated with long-term use of TDF [8, 16], no consistent trends were apparent in the current study between changes in these early indicators of kidney injury and other renal parameters such as eGFR. An increase in urine beta-2-microglobulin was not always accompanied by a change in eGFR and some participants had a decrease in eGFR with no change in urine beta-2-microglobulin. These observations suggest that urine beta-2-microglobulin may not be a sensitive marker for predicting renal dysfunction.
This study has several limitations that should be considered. In previous studies, HBsAg levels have been shown to decline with ETV treatment [21, 22, 24, 25]. Because there was no control arm in this study due to feasibility constraints, it was not clearly shown that the HBsAg decline (4% responder rate, mean 0.11 log10 IU/mL decline from baseline) observed at week 48 was attributed to TDF switch therapy. Another limitation is that most of the patients in the current study had genotype C, whereas HBsAg loss occurs more often and declines in HBsAg are greater in HBeAg+ patients with genotypes A or D infection [4]. Finally, patients with clinically significant renal impairment (creatinine clearance < 70 mL/min) were not assessed, which could have biased renal outcomes in this study.

Conclusions
Although switching from ETV to TDF remained virologically suppressive with no significant safety concerns, our analysis did not clearly show an additional clinical benefit of switching from ETV to TDF in HBeAg-positive and HBV-DNA undetectable CHB patients. Nevertheless, our analysis suggests that there are additional patient factors impacting HBsAg reduction. Evaluation of liver damage and its reversibility with CHB treatment are important clinical questions that were not addressed in the current study which was focused on virological response. Further investigation is needed to better understand the clinical impact of switching from ETV to TDF. Our study also indicates a need for further development of HBV therapies with a novel mechanism for HBsAg reduction and seroclearance.

Abbreviations
ADV, Adefovir dipivoxil; AE, Adverse event; AESI, Adverse event of special interest; ALT,Alanine aminotransferase; BMD, Bone mineral density; CHB, Chronic hepatitis B; eGFR, Estimated glomerular filtration rate; ETV, Entecavir; HBcrAg, Hepatitis B core-related antigen; HBsAg, Hepatitis B surface antigen; HBV, Hepatitis B virus; HCC, Hepatocellular carcinoma; IFN, Interferon; NA, Nucleos(t)ide analogue; TAF, Tenofovir alafenamide; TDF, Tenofovir disoproxil fumarate; TRP, Renal tubular reabsorption of phosphate.

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Study concept and design: FS, YT, MK1, HY, HI, YS2, WS. Acquisition of data: FS, YS1, YK1, YT, MK1, HY, TA, MA, TW, ME, NM, HK1, KJ, KM1, KM2, KT, TI, SF, TK, HI, HK2. Analysis and interpretation of data: FS, YS1, YK1, YT, MK1, HY, TA, MA, TW, ME, MK2, NM, HK1, KJ, KM1, KM2, KT, TI, SF, TK, HI, ST, YS2, YK2, WS, HK2. Drafting of the manuscript: All authors provided critical revision of the manuscript for important intellectual content. Statistical analysis: HI. Obtained funding: WS. Study supervision: HK2. All authors approved the submitted manuscript and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study can be requested for further research. Within 6 months of this publication, anonymized individual participant data, the annotated case report form, protocol, reporting and analysis plan, data set specifications, raw dataset, analysis-ready dataset, and clinical study report will be available for research proposals approved by an independent review committee. Proposals should be submitted to www.clinicalstudydatarequest.com. A data access agreement will be required.

Declarations
Ethics approval and consent to participate
The study was approved by the ethics committee at every participating institution (Ehime Prefectural Central Hospital IRB, Ehime, Japan; Kindai University Hospital IRB, Osaka, Japan; Kitakyushu Municipal Medical Center IRB, Fukuoka, Japan; KKR Clinical Trial Network Central Institutional Review Board, Tokyo, Japan; Kumamoto University General Hospital IRB, Kumamoto, Japan; Kurume University Hospital IRB, Fukuoka, Japan; Matsuyama Red Cross Hospital IRB, Ehime, Japan; Muashino Red Cross Hospital IRB, Tokyo, Japan; Nagoya City University Graduate School of Medical Sciences and Nagoya City University Hospital Institutional Review Board, Aichi, Japan; National Hospital Organization Kure Medical Center and Chugoku Cancer Center IRB, Hiroshima, Japan; National Hospital Organization Nagasaki Medical Center IRB, Nagasaki, Japan; Nippon Medical School Chiba Hokusho Hospital IRB, Chiba, Japan; Ohihiro-Kosei General Hospital IRB, Hokkaido, Japan; Osaka City University Hospital IRB, Osaka, Japan; Osan Rosai Hospital IRB, Tottori, Japan; Sapporo-Kosei General Hospital IRB, Hokkaido, Japan; St. Marianna University Group Institutional Review Board, Kanagawa, Japan) and was conducted according to the recommendations of Good Clinical Practice and the Declaration of Helsinki (2013). All participants provided written informed consent to participate in the study. This manuscript was developed following the CONSORT guidelines as much as possible even through this is not a randomized control trial

Consent for publication
Not Applicable.

Competing interests
This study (NCT03258710) was conducted with a research fund from GlaxoSmithKline (GSK), in which FS, YS1, YK1, TA, MA, MK1, TW, YT, ME, MK2, NM, HK1, KJ, KM1, KM2, KT, TI, HY, SF, and HK2 participated as investigators and their institutions received funding in relation to this work. FS has served on the advisory board for GSK, and has received personal fees from AbbVie Inc., Bristol Myers Squibb, and GSK; YT has served on several advisory boards for GSK, has received personal fees from Fujirebio Inc., GSK, and Sysmex Corporation, has received personal fees and grants from Gilead Sciences Inc. and grants from FUJIFILM Corporation and Janssen Pharmaceutical KK, and has served on the Board of Trustees of the Leland Stanford Junior University; MK1 and HY have served on the advisory board for GSK, and have received personal fees from AbbVie Inc.; MA has received grants and personal fees from AbbVie Inc.; JA has served on the Board of Trustees of the Leland Stanford Junior University; JM and NW have served on the advisory board for GSK, and have received personal fees from GSK; MM has served on the Board of Trustees of the Leland Stanford Junior University; MS has served on the advisory board for AbbVie Inc., and Merck Sharp & Dohme (MSD); KJ has received speaking fees from AbbVie Inc., Gilead Sciences Inc., MSD, and Otsuka Pharmaceutical Co., Ltd; TK, HI, YS2, and YK2 are employees of and own stock in GSK; ST is employee of GSK; WS is a former employee of GSK; HK2 has served as a speaker and trainer for AbbVie Inc., Eisai Co. Ltd; Gilead Sciences Inc., MSD KK, and Sumitomo Dainippon Pharma.

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