Efficacy and safety of tenofovir disoproxil fumarate rescue therapy for chronic hepatitis B patients who failed other nucleos(t)ide analogs

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Aim: Acquisition of nucleos(t)ide analog (NA) inhibitor resistance is critical in successful chronic hepatitis B treatment. As the pattern of tenofovir disoproxil fumarate (TDF) resistance mutations differs from that of other antiviral drugs, we sought to clarify the salvaging potential of TDF in patients with hepatitis B virus (HBV) infection who are poor responders or resistant to other NAs.

Methods: A prospective, multicenter, single-arm, open-label study was carried out from December 2011 to October 2014. Poor responders defined as subjects with serum HBV-DNA levels >4 log10 copies/mL were enrolled. Subjects receiving lamivudine (LAM) + adefovir pivoxil (ADV) before the initiation of the study were switched to LAM + TDF. Subjects on entecavir hydrate (ETV) with or without ADV were switched to ETV + TDF. The primary efficacy end-point was the proportion of subjects achieving HBV-DNA <2.1 log10 copies/mL (LLQ) at week 24. The secondary efficacy end-points were the proportion of subjects with LLQ at weeks 48 and 96, serum alanine aminotransferase normalization, hepatitis B envelope antigen/antibody and hepatitis B surface antigen/antibody seroconversion.

Results: Thirty-four subjects were enrolled, 21 subjects were switched to ETV + TDF, and 13 subjects were switched to LAM + TDF. Drug resistance mutations were determined in 85% of the subjects at the time of the enrolment. The proportion of subjects who achieved LLQ was 59%, 62%, and 71% at weeks 24, 48, and 96, respectively. No serious adverse event related to TDF was reported.

Conclusion: Our study clearly showed that TDF containing regimens were effective in salvaging poor responders and/or those who are drug-resistant to other NAs. This study is registered with ClinicalTrials.gov (NCT01475851) and the GSK Clinical Study Register (GSK LOC115912).

Key words: CHB, drug resistance, genotype C, HBsAg, tenofovir

INTRODUCTION

The treatment of chronic hepatitis B (CHB) infection aims primarily to reduce liver tissue necrosis and inflammation and prevent the progression of liver fibrosis.1 Specifically, nucleos(t)ide analogs (NAs) targeting polymerase–reverse transcriptase are used to inhibit hepatitis B virus (HBV) replication (thereby reducing serum HBV-DNA levels) and also to normalize serum alanine aminotransferase (ALT) levels, leading to remission of hepatitis.2–6 Today, four NAs are available, and due to their potent anti-HBV activities,7,8 entecavir hydrate (ETV) and tenofovir disoproxil fumarate (TDF)9 have been recommended as first-line therapy in HBV treatment guidelines. Although NA treatment has improved the prognosis of HBV significantly, acquisition of drug resistance10–14 has been the major barrier to successful treatment, and effective anti-HBV drugs to rescue patients with resistance are eagerly awaited. Tenofovir disoproxil fumarate, initially developed as an...
antiretroviral,\textsuperscript{15} was found to be effective against HBV and subsequently became available for CHB treatment. As TDF has no cross-resistance with the existing NAs and shows potent antiviral activity against multidrug-resistant (MDR) HBV,\textsuperscript{16,17} it is recommended as both first-line treatment for naïve patients and as salvage therapy for MDR patients. There is an urgent need for treatment to rescue poor NA responders as well as patients who are MDR, as failure to control CHB increases the risk of disease progression to hepatocellular carcinoma.\textsuperscript{18–20} We evaluated the efficacy and safety of TDF-containing regimens in Japanese CHB patients who failed in the treatment with other NAs.

**METHODS**

**Study design and subject enrolment**

This OPEN-LABEL, MULTICENTER, phase III study was carried out to evaluate the salvage potential and safety of adding TDF 300 mg/day to sub-optimal on-going NA regimens. The subjects enrolled in this study were: (i) 16–69 years of age with CHB; (ii) positive for serum hepatitis B surface antigen (HBsAg) for at least 6 months; and (iii) currently being treated with lamivudine (LAM) 100 mg/day + adefovir pivoxil (ADV) 10 mg/day or ETV 0.5 mg/day or ETV 0.5 mg/day + ADV 10 mg/day for >24 weeks. Further inclusion criteria were: (iv) high level of serum HBV-DNA \( \geq 4 \log_{10} \) copies/mL (HBV-DNA \( \geq 3 \log_{10} \) copies/mL in cirrhotic subjects); (v) ALT levels \( \leq 400 \) IU/L (10× the upper limit of normal [ULN]); and (vi) creatinine clearance \( \geq 70 \) mL/min, hemoglobin \( \geq 8 \) g/dL, and white blood cell count \( \geq 1000/\text{mm}^3 \) at screening. Subjects were excluded if they had protocol-defined decompensated CHB, co-infection with HIV-1 or hepatitis C virus, history or evidence of hepatocellular carcinoma at screening, or had received any interferon or HBV vaccine therapy within 24 weeks prior to initiation of the study drugs.

Subjects receiving the combination LAM + ADV were switched to a combination of LAM + TDF 300 mg/day; subjects on ETV with or without ADV were switched to a combination of ETV + TDF 300 mg/day. The enrolled subjects continued treatment with TDF until the drug became commercially available in 2014 (Fig. S1).

The study was carried out in accordance with good clinical practice and the latest amendments to the Declaration of Helsinki in 2008, and was approved by the local ethics committees. Written informed consent was obtained from each subject or their legal representative prior to the study.

**Virological and clinical evaluation**

The following parameters were evaluated during the study period: (i) serological markers of HBV infection and immunity, such as hepatitis B envelope antigen (HBeAg), hepatitis B envelope antibody (HBeAb), HBsAg, and hepatitis B surface antibody; (ii) HBV-DNA as measured by the COBAS AmpliPrep/COBAS TaqMan assay (Roche Molecular Systems, Inc., Branchburg, NJ, USA), which has a lower limit of quantitation (LLQ) of 2.1 \( \log_{10} \) copies/mL; (iii) HBV genotype (A–D) by EIA (Institute of Immunology Co., Tokyo, Japan) at screening; (iv) drug resistance-related mutations (DRMs) at the baseline to confirm possible resistance to LAM, ADV, and ETV, as measured by the INNOLIPA HBV DR version 2 or version 3 assays (Fujirebio Europe NV, Gent, Belgium) and by direct sequencing to detect virological breakthrough (an HBV-DNA increase from the nadir level of at least 1 \( \log_{10} \) copies/mL); (v) hematological and biochemical parameters; and (vi) drug adherence as monitored by recovering pill bottles to confirm any residual pills. Serum HBV-DNA and clinical laboratory data were assessed at screening, baseline, week 2 (only laboratory data), every 4 weeks (week 4 to week 48), and every 12 weeks after week 48. The HBeAg/Ab and HBsAg/Ab (including quantity) were assessed at screening, baseline, and every 12 weeks. The HBV-DNA and DRM genotyping were carried out at SRL, Inc. (Tokyo, Japan).

**Study end-points**

The primary efficacy end-point was the proportion of subjects achieving LLQ at week 24. The secondary efficacy end-points were the proportion of subjects with LLQ (complete suppressor [CS]) at weeks 48 and 96, serum ALT normalization, HBeAg/Ab and HBsAg/Ab seroconversion, the change from baseline in HBV-DNA and HBsAg level, and evaluations of DRM in subjects who failed to achieve LLQ (PS) and breakthrough subjects at weeks 24, 48, and 96. The safety analyses were carried out on all subjects who received at least one dose of the study drug and on all events that occurred during the treatment and follow-up periods.

**Statistical analyses**

Due to the exploratory nature of the study, the results are presented by treatment group. In the study, assuming an expected suppression rate of 35%,\textsuperscript{21} threshold suppression rate of 10%,\textsuperscript{12} and significance level (two-tailed) of 5%, the sample size needed to achieve at least 90% power was calculated to be 28 subjects. Allowing for a dropout rate of 10%, the sample size was set at 32 subjects.

Safety analyses were carried out in the safety population (SP), consisting of all subjects who received at least one dose of study drug. Verbatim terms of all adverse events were collected and coded using the Medical Dictionary for Regulatory Activities version 17.1. All adverse events (AEs) reported during the study were summarized as on-
treatment events and follow-up events. The Full Analysis Set (FAS) was used as the primary efficacy population in the study. The FAS was defined as all subjects who entered the study, received at least one dose of study drug, and had at least one efficacy assessment after treatment initiation. The population for the analysis of ALT normalization (biochemically evaluable population) was those in the SP with an ALT value >ULN at baseline. The rate of HBV-DNA suppression and its two-sided 95% confidence interval were calculated. The lower limit of viral detection (HBV-DNA below the assay LLQ) was set at the lower limit minus 0.1 (2.0 copies/mL) for calculation of actual HBV-DNA summary statistics and the changes from baseline values. For time-sequence data analysis, missing values observed during the treatment period were imputed by the last observation carried forward method. In addition, the difference in baseline characteristics between the CS and the PS subjects and the difference in HBsAg level, ALT level reduction, and HBeAg positivity were analyzed on an exploratory basis. All the analyses were carried out using SAS version 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Demographic and baseline characteristics

From December 2011 to June 2012, a total of 34 subjects from 11 hospitals were enrolled in the study

Table 1 Demographic and baseline characteristics of enrolled subjects with chronic hepatitis B who received tenofovir disoproxil fumarate (TDF) following failure of nucleos(t)ide analog treatment

|                          | LAM + TDF group (n = 13) | ETV + TDF group (n = 21) | Total (n = 34) |
|--------------------------|--------------------------|--------------------------|---------------|
| Age, years               |                          |                          |               |
| Mean (SD)                | 52.1 (7.57)              | 50.3 (9.18)              | 51.0 (8.52)   |
| Median (min., max.)      | 52.0 (38, 62)            | 53.0 (36, 66)            | 52.5 (36, 66) |
| Sex, n (%):              |                          |                          |               |
| Female                   | 5 (38)                   | 4 (19)                   | 9 (26)        |
| Male                     | 8 (62)                   | 17 (81)                  | 25 (74)       |
| HBV-DNA, log_{10} copies/mL |                        |                          |               |
| Mean (SD)                | 5.18 (1.513)             | 5.80 (1.861)             | 5.57 (1.739)  |
| Median (min., max.)      | 4.40 (3.5, 8.3)          | 4.90 (3.8, 9.0)          | 4.70 (3.5, 9.0) |
| HBV-DNA, n (%):          |                          |                          |               |
| ≥7 log_{10} copies/mL    | 2 (15)                   | 6 (29)                   | 8 (24)        |
| <7 log_{10} copies/mL    | 11 (85)                  | 15 (71)                  | 26 (76)       |
| ALT, IU/L                |                          |                          |               |
| Mean (SD)                | 48.1 (32.55)             | 91.0 (231.90)            | 74.6 (182.83) |
| Median (min., max.)      | 36.0 (16, 137)           | 39.0 (11, 1100)          | 36.0 (11, 1100) |
| HBeAg, n (%):            |                          |                          |               |
| Positive                 | 11 (85)                  | 17 (81)                  | 28 (82)       |
| Negative                 | 2 (15)                   | 4 (19)                   | 6 (18)        |
| Genotype (screening), n (%): |                  |                          |               |
| C                        | 13 (100)                 | 21 (100)                 | 34 (100)      |
| Genotypic analysis, n (%): |                        |                          |               |
| Any                      | 12 (92)                  | 17 (81)                  | 29 (85)       |
| LAM resistance-related mutation† | 11 (85) | 17 (81) | 28 (82) |
| ADV resistance-related mutation‡ | 4 (31) | 0 (0) | 4 (12) |
| ETV resistance-related mutation§ | 6 (46) | 16 (76) | 22 (65) |
| Prior treatment, n (%):  |                          |                          |               |
| LAM/ADV                  | 13 (100)                 | 0 (0)                    | 13 (38)       |
| ETV/ADV                  | 0 (0)                    | 11 (52)                  | 11 (32)       |
| ETV                      | 0 (0)                    | 10 (48)                  | 10 (29)       |

†Confirmed rtM204V/I and/or rtL180M in the hepatitis B virus (HBV) polymerase–reverse transcriptase (pol/RT).
‡Confirmed rtA181T/V and/or rtN236T in the HBV pol/RT.
§Confirmed rtT184I/L/F/M and/or rtS202I/G and/or rtM250V/L in the HBV pol/RT.
ADV, adefovir pivoxil; ALT, alanine aminotransferase; ETV, entecavir hydrate; HBeAg, hepatitis B envelope antigen; LAM, lamivudine; max., maximum; min., minimum; SD, standard deviation.

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and fulfilled the FAS population criteria. Ten subjects receiving ETV and 11 subjects receiving ETV + ADV were switched to ETV + TDF, and 13 subjects receiving LAM + ADV were switched to LAM + TDF. Adherence was 80% to less than 90% in one subject and 90% or greater in the other 33 subjects. One subject (ID#13, Table S1) in the LAM + TDF group was withdrawn from the study because of inability to visit the medical institution between weeks 24 and 48, which met protocol-defined withdrawal criteria.

As summarized in Table 1, all 34 subjects were genotype C and 82% of the subjects were HBeAg-positive. In addition, none of the subjects had liver cirrhosis at the time of enrolment. Regarding baseline DRMs, 85% of the subjects harbored DRMs at the time of enrolment (Table 1), and rtM204V/I and rtL180M were the two major mutations observed in 22 (65%) and 28 (82%) subjects, respectively. Entecavir hydrate DRM rtS202I/G was higher in the ETV group (2 subjects [15%]), and ADV-DRM rtA181V was detected only in the LAM + TDF group (4 subjects [31%]) (Fig. S2, Table S1). Thus, the patterns of baseline DRM clearly depended on their previous exposure to NAs. As the study enrolled subjects with poorly controlled viremia, 23 subjects depended on their previous exposure to NAs. As the study enrolled subjects with poorly controlled viremia, 23 subjects (68%) were found to be harboring multidrug-resistant virus at the baseline. Furthermore, all baseline characteristics were similar between the PS subjects and the CS subjects, except for the baseline HBV-DNA level, which was high in PS subjects in additional analysis (Table 2).

**Virological response**

All 34 subjects, except for one who was withdrawn between week 24 and week 48, completed the 96-week observation period. The proportion of CS subjects increased from 59% at week 24 to 71% at week 96 (Table 3a). The plots of HBV-DNA levels in each treatment group are shown in Figure 1. The reduction in HBV-DNA level from baseline also became larger with the continuation of the treatment (Table 3b). Nine subjects failed to achieve LLQ during the study period (Table S2).

**Biochemical and serological response**

Only 15 subjects had a baseline ALT >ULN. Of these, 60% (9/15) achieved ALT normalization by week 96 (Table 3c). Twenty-eight subjects (82%) were HBeAg-positive at baseline, and one subject achieved HBeAg loss at week 36. No subject achieved HBeAg/Ab seroconversion during the observation period. All 34 subjects were and HBsAg-positive at baseline. The serum HBsAg level decreased in 22 (65%) and 28 (82%) subjects, respectively. Complete suppressors and partial suppressors defined as subjects with and without lower limit of quantitation, respectively. One subject withdrawn between week 24 and 48 was excluded. Resistance, NC, P-value was not calculated for pretreatment, gender, or resistance.
HBsAg clearance potential, we classified the 34 subjects into two categories: subjects with ALT ≥ 60 IU/L at least once during the study, and subjects with ALT < 60 IU/L consistently throughout the study period. As summarized in Table 4, the reduction of HBsAg was significantly greater in the ALT ≥ 60 IU/L group than the ALT < 60 IU/L group at weeks 24, 36, 60, and 96 in the exploratory analysis, although the baseline data were comparable.

Virological breakthrough and drug resistance

To evaluate the effects of MDR on TDF efficacy, week 96 resistance analysis was carried out on the nine PS subjects (Table S1b). No changes in DRM patterns were determined in eight of the nine subjects. The remaining one subject (ID#8) had been treated with LAM + ADF previously and possessed five DRMs (V173 V/L, L180 L/M, M204 M/V, A181 A/T, and N236 N/T) at the baseline. The HBV-DNA was reduced from 7.3 to 3.3 log10 copies/mL by switching to LAM + TDF, but never achieved LLQ during the study period. The genotyping result at week 96 showed disappearance of rtV173 V/L and acquisition of rtT184T/I/L/F/M. However, the clinical significance of the changes remained unclear, as neither substitution is recognized as a TDF-DRM.

Subject ID#20 in the ETV + TDF group experienced a virological breakthrough at week 48. The HBV-DNA reached a nadir of 2.4 log10 copies/mL at week 40 but rebounded to 3.4 log10 copies/mL at week 48, and further relapsed to 5.3 log10 copies/mL at week 96. No exacerbation of hepatitis was observed and ALT levels remained at approximately 40 IU/L even after the rebound of HBV-DNA. This subject maintained over 90% adherence throughout the study. The direct sequencing of the pol/rt region was undertaken for baseline and week 96 samples to investigate the mechanism of virological breakthrough. Known ETV resistance mutations of rtL180M, S202G, and M204 V were determined at baseline and persisted through the observation period. Five additional substitutions were identified: rt109, 126, 223, 224, and 256. However, their virological functions are not understood.

Safety

All 34 enrolled subjects were included in the SP. The overall frequency of AEs was 85% (Table 5). There was no death reported during the treatment. Serious adverse events were reported in three subjects (appendicitis, lumbar spinal stenosis, and calculus ureteric obstruction, 9%). None of these events were considered related to the study drugs, and the subjects continued the study without any treatment modifications. Three subjects had renal AEs including increased blood creatinine, decreased blood phosphorus, and hypophosphatemia. According to
their histories, two of the three subjects had long-term treatment with ADV prior to the study. Blood creatinine peaked at week 20 (84.0 μmol/L) from baseline (49.5 μmol/L) and returned to the normal range at week 24 in one subject in the LAM + TDF group. Blood phosphorus decreased to 0.58 mmol/L (grade 3) at week 20 from baseline (0.97 mmol/L) and returned to the normal range at week 24 in one subject in the ETV + TDF group. The phosphorus level decreased to 0.65 mmol/L (grade 2) at week 36 again and resolved at week 48. Moderate hypophosphatemia was reported after week 48 in one subject in the ETV + TDF group but resolved after 78 days.

**DISCUSSION**

The salvage potential of TDF in 34 subjects for whom treatment with other NAs had failed was their histories, two of the three subjects had long-term treatment with ADV prior to the study. Blood creatinine peaked at week 20 (84.0 μmol/L) from baseline (49.5 μmol/L) and returned to the normal range at week 24 in one subject in the LAM + TDF group. Blood phosphorus decreased to 0.58 mmol/L (grade 3) at week 20 from baseline (0.97 mmol/L) and returned to the normal range at week 24 in one subject in the ETV + TDF group. The phosphorus level decreased to 0.65 mmol/L (grade 2) at week 36 again and resolved at week 48. Moderate hypophosphatemia was reported after week 48 in one subject in the ETV + TDF group but resolved after 78 days.
evaluated in this study. Serum HBV-DNA levels were undetectable in 59% of subjects at week 24, and increased to 62% and 71% at weeks 48 and 96, respectively, clearly showing the high salvage potential of TDF. Interestingly, this proportion is similar to that in other TDF rescue studies targeting subjects who had failed other NAs.21–23 The goal of our data analysis was to explore factors associated with TDF treatment responses. All nine PS subjects had significantly higher baseline viremia levels compared to CS subjects, suggesting active HBV replication and/or weaker host immunity to the virus may be risk factors of the failures. In contrast, baseline DRM patterns and frequencies were similar between PS and CS subjects. Apparently, DRM at baseline may not be critical in predicting subsequent TDF treatment responses. However, for a precise understanding of DRM data, we must consider that the resistance detected by INNO-LiPA assay is limited to

**Table 4** Comparison of hepatitis B surface antigen gross changes (mean ± SD log_{10} IU/mL) from the baseline in patients with chronic hepatitis B who received tenofovir disoproxil fumarate (TDF) rescue therapy following failure of nucleos(t)ide analogs, grouped according to alanine aminotransferase (ALT) level

|          | ALT ≥60 IU/L (n = 15) | ALT <60 IU/L (n = 19) | P-value |
|----------|-----------------------|-----------------------|---------|
| Baseline | 3.991 ± 0.693         | 3.740 ± 0.534         | –       |
| Week 12  | −0.367 ± 0.513        | −0.080 ± 0.089        | 0.0582  |
| Week 24  | −0.407 ± 0.501        | −0.102 ± 0.110        | 0.0352  |
| Week 36  | −0.425 ± 0.436        | −0.155 ± 0.152        | 0.0350  |
| Week 48  | −0.455 ± 0.449        | −0.200 ± 0.157        | 0.0510  |
| Week 60  | −0.476 ± 0.453        | −0.200 ± 0.162        | 0.0379  |
| Week 72  | −0.481 ± 0.431        | −0.241 ± 0.157        | 0.0555  |
| Week 84  | −0.496 ± 0.438        | −0.305 ± 0.188        | 0.1317  |
| Week 96  | −0.537 ± 0.414        | −0.228 ± 0.178        | 0.0147  |

Figure 3 Data for three subjects with chronic hepatitis B, treated with tenofovir disoproxil fumarate following failure of nucleos(t)ide analog treatment, with over 1 log reduction in serum hepatitis B surface antigen (HBsAg) level. Solid bar indicates serum alanine aminotransferase (ALT) level, solid circle with solid line indicates serum hepatitis B virus (HBV)-DNA level, and open circle with dotted line indicates HBsAg level. (a) Case ID #1, (b) ID #16, (c) ID #26.

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Table 5 Summary of adverse events (AEs), serious AEs (SAEs), and AEs of interest in patients with chronic hepatitis B who received tenofovir disoproxil fumarate rescue therapy following failure of nucleos(t)ide analogs

| Preferred term | LAM/ TDF | ETV/ TDF | TDF | Total |
|----------------|----------|----------|-----|-------|
| | n (%) | n (%) | n (%) | n (%) |
| Subjects with AE(s) | Any event | (n=13) | (n=21) | (n=34) |
| Nasopharyngitis | 7 (54) | 11 (52) | 18 (53) |
| Alanine aminotransferase increased | 1 (8) | 3 (14) | 4 (12) |
| Headache | 2 (15) | 2 (10) | 4 (12) |
| Hepatic steatosis | 1 (8) | 3 (14) | 4 (12) |
| Rash | 1 (8) | 3 (14) | 4 (12) |
| Aspartate aminotransferase increased | 1 (8) | 2 (10) | 3 (9) |
| Back pain | 1 (8) | 2 (10) | 3 (9) |
| Diarrhea | 0 (0) | 3 (14) | 3 (9) |
| Dyspepsia | 1 (8) | 2 (10) | 3 (9) |
| Abdominal discomfort | 0 (0) | 2 (10) | 2 (6) |
| Abdominal pain | 0 (0) | 2 (10) | 2 (6) |
| Blood creatine phosphokinase increased | 0 (0) | 2 (10) | 2 (6) |
| Contusion | 1 (8) | 1 (5) | 2 (6) |
| Fatigue | 0 (0) | 2 (10) | 2 (6) |
| Gallbladder polyp | 2 (15) | 0 (0) | 2 (6) |
| Hepatic enzyme increased | 1 (8) | 1 (5) | 2 (6) |
| Pruritus | 1 (8) | 1 (5) | 2 (6) |
| Subjects with SAEs | 1 (8) | 2 (10) | 3 (9) |
| Appendicitis | 0 (0) | 1 (5) | 1 (3) |
| Lumbar spinal stenosis | 1 (8) | 0 (0) | 1 (3) |
| Calculus ureteric | 0 (0) | 1 (5) | 1 (3) |
| Subjects with renal AEs | 1 (8) | 2 (10) | 3 (9) |
| Blood creatinine increased | 1 (8) | 0 (0) | 1 (3) |
| Blood phosphorus decreased | 0 (0) | 1 (5) | 1 (3) |
| Hypophosphatemia | 0 (0) | 1 (5) | 1 (3) |

DRM alleles covered by the assay, and also linkages among detected DRMs cannot be determined by this method. There were three subjects (ID#1, #6, and #8) with a combination of A181 A/T + N236 N/T, a known TDF and ADV resistance mutation,24 but the techniques used failed to show whether these two mutations were in the same viral genome. Two (ID#1 and #6) subjects were successfully treated with the TDF + ADF regimen, suggesting their resistance levels were lower than those expected from the combination. Furthermore, considering the characteristics of the enrolled subjects, it was interesting that baseline resistance genotyping detected more mixed mutant and wild-type results. The data suggest that these subjects may have had problems with adherence before enrolment in the study. Alternatively, the existence of a pharmacological sanctuary for wild-type viruses could be suspected and considered.25

Although HBV-DNA is currently the main clinical marker guiding CHB treatment, serum HBsAg level may alternatively be used to evaluate clinical status and pathogenesis of HBV.26 In addition to achieving LLQ, HBsAg reduction to <800 IU/mL is recognized as the long-term goal of treatment, as it may lower relapse risk following discontinuation of NAs.26 Therefore, it is notable that reduction in HBsAg level was observed in most of the subjects when the regimens were switched to TDF-containing regimens. As reported previously, high ALT level and HBsAg reduction are related.27,28 Indeed, the reduction in HBsAg level was greater in subjects with higher ALT levels (≥60 IU/L) than in those with lower ALT levels (<60 IU/L). Of note, in the three subjects with over 1 log reduction in HBsAg level, this was preceded by increased ALT level in our study. In terms of a correlation between HBeAg status and HBsAg reduction, a previous study has reported that HBeAg-positive subjects had a larger reduction of HBsAg.29 However, the exploratory analysis for this study found no statistical differences between HBeAg-positive (n = 28) and -negative (n = 6) subjects in the reduction of HBsAg.

The safety profile in this study was as previously reported,29 and no study-specific safety signals were recognized. Although increased creatinine, decreased phosphorus, and hypophosphatemia were reported in three subjects in this study, these changes were all transient and resolved during treatment with TDF. Consequently, no renal or bone toxicity signals were observed. However, further careful monitoring of renal function is advised.30,31

In conclusion, TDF was well tolerated and no specific safety signals were observed. The rate of virological response to rescue TDF-containing regimens was high in CHB subjects who had failed other NAs, and no DRMs related to TDF were detected during the study. Hepatitis B surface antigen reduction was observed in most subjects and the reduction in HBsAg level was preceded by increased ALT level. Because of the small number of subjects, who were all genotype C Japanese patients, and the short duration of follow-up, a further study is required to generalize our findings.

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found in the online version of this article at the publisher’s web site.

Table S1 Resistance mutation profile of the enrolled subjects.
Table S2 Profiles of the nine partial responders.
Table S3 Profiles of the three subjects achieved >1 log reductions of HBsAg.