CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208464Orig1s000

MICROBIOLOGY / VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 208-464 **SDN:** 031 **DATE REVIEWED:** 10/11/16

Date Submitted: 10/11/16 **Date Assigned:** 10/11/16

Date Received: 10/11/16

Sponsor: Gilead Science, Inc.
333 Lakeside Drive
Foster City, CA 94404
Sara Snow, Pharm.D.
650-425-8310

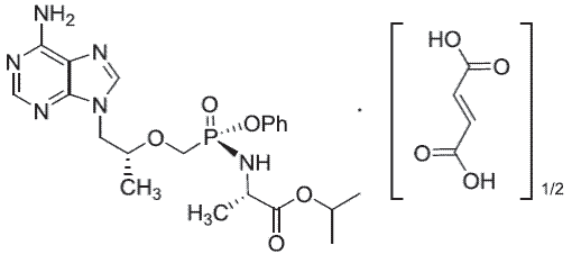
Reviewers Name: Sung S. Rhee, Ph.D.

Product Names: Tenofovir alafenamide (TAF, GS-7340); Tenofovir alafenamide fumarate (TAF fumarate, GS-7340-03); VEMLIDY™

Note: TAF refers to tenofovir alafenamide as the free base (GS-7340) and TAF fumarate refers to the hemifumarate form (GS-7340-03; 2:1 ratio of GS-7340 to fumarate) which is the drug substance.

Chemical Name: (TAF fumarate) L-Alanine, N-[(S)-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methyl ethoxy]methyl]phenoxyphosphinyl]-, 1-methylethyl ester, (2E)-2-butenedioate (2:1)

Structural Formula:



Tenofovir alafenamide fumarate (TAF fumarate)

Molecular Formula: (TAF fumarate) $C_{21}H_{29}O_5N_6P \bullet ½(C_4H_4O_4)$; (TAF) $C_{21}H_{29}O_5N_6P$

Molecular Mass: (TAF fumarate) 534.50; (TAF) 476.5

Drug Category: Antiviral

Indication: Treatment of chronic hepatitis B virus infection

Dosage Form/Route of Administration: Film-coated tablet (25 mg)/Oral

Note: TAF 25-mg tablets contain 28 mg of TAF fumarate.

Supporting Documents: IND 115561

Abbreviations: HBeAg, HBV e antigen; HBV, hepatitis B virus; NDA, new drug application; PMC, postmarketing commitment; PMR, postmarketing requirement; sNDA, supplemental new drug application; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate

BACKGROUND/SUMMARY

This NDA submission contains the applicant's responses to the Division's proposed postmarketing requirement (PMR)/postmarketing commitment (PMC) comments sent on October 06, 2016, regarding the original NDA for VAMLIDY™ (TAF) tablets for the proposed indication for the treatment of chronic HBV infection in adult (b) (4) with compensated liver disease.

RESPONSES FROM APPLICANT

Post Marketing Requirements (PMRs)

Virology PMR Comment 1: Perform genotypic (also phenotypic if qualified) resistance analysis of baseline virus samples from all HBeAg-positive nucleos(t)ide reverse transcriptase inhibitor experienced subjects and of Week-48 virus samples from all evaluable subjects, regardless of their Week 96 virologic outcome.

Study Completion: (Please propose the date)

Final Report Submission: March, 2017

Applicant Response: As this PMR is proposed (b) (4), (b) (4) Gilead agrees to include genotypic and qualified phenotypic resistance analysis of baseline virus samples from all HBeAg-positive nucleos(t)ide reverse transcriptase inhibitor experienced subjects and of Week-48 virus samples from all evaluable subjects regardless of their Week 96m virologic outcome (b) (4) (b) (4) (b) (4) Gilead proposes a final report submission date of June, 2017. Gilead's proposed revisions are below:

(b) (4)

Final Report Submission: (b) (4) June, 2017

Virology Response: (b) (4) (b) (4)

Virology PMR Comment 2: Evaluate the anti-HBV activity of TAF in combination with sofosbuvir.

Study Completion: (Please propose the date)

Final Report Submission: June, 2017

Applicant Response: Gilead agrees to conduct a study to evaluate the anti-HBV activity of TAF in combination with sofosbuvir with a final report submission date of June, 2017. (b) (4) (b) (4)

Virology Response: (b) (4) (b) (4).

Post Marketing Commitment (PMC)

Virology PMC Comment 1: Phenotype Week-48 virus samples from Subjects 4296-5147 and 8758-5188 in the TAF group and Subjects 1507-4546 and 9035-4845 in the TDF group in Study GS-US- 320-0110.

Study Completion: (Please propose the date)

Final Report Submission: March, 2017

Applicant Response: (b) (4) (b) (4)

Virology Response: (b) (4) (b) (4)

_____
Sung S. Rhee, Ph.D.
Clinical Virology Reviewer

CONCURRENCE

_____ **Date:** _____
HFD-530/VirologyTL/J O'Rear

cc:
HFD-530/NDA # 208464
HFD-530/Division File
HFD-530/RPM/M. Hong

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

SUNG S RHEE
10/13/2016

JULIAN J O REAR
10/13/2016

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 208-464 **SDN:** 001 **DATE REVIEWED:** 10/05/16

Applicant Name and Address: Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404

Reviewer Name(s): Sung S. Rhee, Ph.D.

Initial Submission Dates:

Correspondence Date: January 11, 2016

CDER Receipt Date: January 11, 2016

Reviewer Assignment Date: January 11, 2016

Review Complete Date: October 05, 2016

PDUFA Goal Date: November 10, 2016

Subsequent Submissions:

- Response to Information Request to submit a resistance dataset for the baseline INNO LiPA results summarized in Study Report PC-320-2009 (SDN 003): January 25, 2016
- Response to Information Request to identify virology assays utilized in the Phase 3 studies (SDN 004): February 24, 2016
- Submission of the next generation sequencing data (SDN 009): March 29, 2016
- Submission of the phenotypic resistance data (SDN 012): April 21, 2016
- Response to Nonclinical Virology Information Request (SDN 026): September 06, 2016
- Response to Clinical Virology Information Request (SDN 027): September 20, 2016
- Amendment to the Proposed Prescribing Information (SDN 028): September 22, 2016

Related/Supporting Documents: IND 115561

Product Name(s):

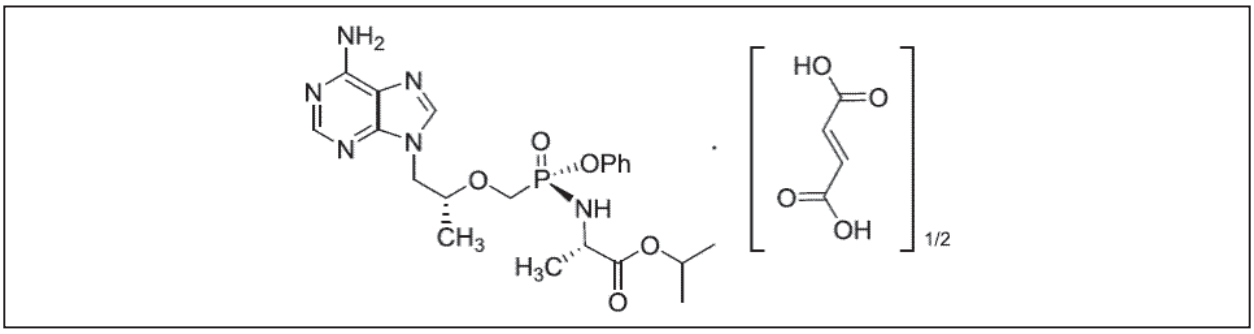
Proprietary: VEMLIDY™

Non-Proprietary/USAN: Tenofovir alafenamide fumarate

Code Name/Number: GS-7340-03

Chemical Name: L-Alanine, N-[(S)-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl] phenoxyphosphinyl]-, 1-methylethyl ester, (2E)-2-butenedioate (2:1)

Structural Formula:



Tenofovir alafenamide fumarate (TAF fumarate)

Molecular Formula: $C_{21}H_{29}O_5N_6P \bullet ½(C_4H_4O_4)$

Molecular Weight: 534.50 g/mol

Dosage Form(s): Tablet containing 25 mg of TAF (equivalent to 28 mg of TAF fumarate)

Route(s) of Administration: Oral

Indication(s): Treatment of chronic HBV infection in adults with compensated liver disease

Recommended Dosage: One tablet (25 mg) taken orally once daily, with (b) (4) food

Dispensed: Rx _X_ OTC ___ (Discipline relevant)

Abbreviations: ADV, adefovir dipivoxil; ADV-R, adefovir resistance; AFV, adefovir; ALT, alanine aminotransferase; BNPP, bis(p-nitrophenyl)phosphate; CatA, cathepsin A; CC_{50}, 50% cytotoxic concentration; CES1, carboxylesterase 1; CHB, chronic hepatitis B virus infection; CI, confidence interval; CLV or L-FMAU, clevudine; C_{max}, maximum or peak concentration of a drug observed after its administration; CMV, cytomegalovirus; COBI, cobicistat; dATP, deoxyadenosine triphosphate; ddC, zalcitabine; EC_{50}, effective concentration inhibiting viral replication by 50%; ETV, entecavir; ETV-R, entecavir resistance; EVG, elvitegravir; FBS, fetal bovine serum; FDC, fixed-dose combination; FTC, emtricitabine; HBeAb, antibody against HBV e antigen; HBeAg, HBV e antigen; HBsAg, HBV surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; HTLV-1, human T-lymphotropic virus type 1; IC_{50}, half maximal inhibitory concentration; IFN, interferon; IU, international unit; LAM, lamivudine; LAM-R, lamivudine resistance; LC-MS/MS, liquid chromatography/tandem mass spectrometry; LdT, telbivudine (TYZEKA® or SEBIVO®); LLOQ, lower limit of quantification; mtDNA, mitochondrial DNA; M=F, missing=failure (all missing data including true failures are treated as representing failure); NDA, new drug application; NGS, next generation sequencing; NRTI, HIV-1 nucleoside/nucleotide analog reverse transcriptase inhibitor; NrtI, HBV nucleoside/nucleotide analog reverse transcriptase inhibitor; NrtI-R, HBV NrtI resistance; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PI, protease inhibitor; QD, once daily; RPV, rilpivirine; rt, HBV reverse transcriptase; SD, standard deviation; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TE, treatment-experienced; TFV, tenofovir; TFV-DP, tenofovir diphosphate; TFV-MP, tenofovir monophosphate; TN, treatment-naïve; TVR, telaprevir; ULN, upper limit of normal; VF, virologic failure; VL, viral load

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EXECUTIVE SUMMARY

This application was submitted in support of a new drug application (NDA) for VAMLIDY™ tablets that contain 25 mg of tenofovir alafenamide (TAF) as TAF fumarate (28 mg, drug substance) for the proposed indication for the treatment of chronic hepatitis B virus infection in adult (b) (4) with compensated liver disease. VAMLIDY (TAF) is a phosphonamidate prodrug of the nucleotide analogue reverse transcriptase inhibitor (HBV NrtI) tenofovir (TFV) that inhibits HBV reverse transcriptase. VIREAD (TDF) is another prodrug of TFV that has been marketed for the treatment of chronic HBV infection in adult patients since 2008 and in pediatric patients (12 years of age and older) since 2012. To date, TAF is approved as a component of several fixed-dose combination (FDC) products indicated for the treatment of HIV-1 infection, DESCOVY® (TAF/FTC [10/200 mg and 25/200 mg]), GENVOYA® (TAF/FTC/EVG/COBI [10/200/150/150 mg]), and ODEFSEY® (TAF/FTC/RPV [25/200/25 mg]).

Currently, 8 therapeutic agents have received regulatory approval for the treatment of chronic hepatitis B virus (HBV) infection: the therapeutic biologics recombinant human interferon-alpha-2b (IFNα-2b, INTRON® A, approved on June 04, 1986 [BLA 103132]; and its pegylated form, PEGINTRON®, approved on January 19, 2001 [BLA 103949]) and pegylated recombinant human IFNα-2a (PEGASYS®, approved on October 16, 2002 [BLA 103964]), and the HBV nucleoside/nucleotide analog reverse transcriptase inhibitors (HBV NrtIs) adefovir dipivoxil (HEPSERA®, approved on September 20, 2002 [NDA 21449]), entecavir (BARACLUDE®, approved on March 29, 2005 [NDA 21797]), lamivudine (EPIVIR-HBV®, approved on December 8, 1998 [NDA 21003]), telbivudine (TYZEKA®, approved on October 25, 2006 [NDA 22011]), and tenofovir disoproxil fumarate (TDF; VIREAD®, approved on August 11, 2008 [NDA 21356]). Each produces therapeutic responses in patients chronically infected with HBV but suboptimally, largely due to suboptimal efficacy, poor tolerability, and/or the emergence of viral resistance to the agents. None of these treatments clears the virus. The interferons are administered for one year and can lead to serum HBV DNA and HBsAg loss in 20-30% of patients. HBV DNA and HBsAg loss is associated with improved clinical outlook and reduced development of hepatocellular carcinoma. However, interferons are poorly tolerated. NrtIs reduce HBV DNA, improve clinical outlook, and are better tolerated. However, resistance can develop to these, they do not lead to HBsAg loss and they have to be taken chronically.

TAF was designed to overcome the cell membrane permeability limitations of TFV and increase the plasma stability of the prodrug compared to TDF. In target cells, TAF is converted to TFV through hydrolysis primarily by carboxylesterase 1 (CES1) in primary hepatocytes, and by cathepsin A in PBMCs and other HIV target cells, which then undergoes sequential phosphorylation by cellular kinases to the pharmacologically active metabolite tenofovir diphosphate (TFV-DP). TFV-DP inhibits the activity of HBV and HIV reverse transcriptases by competing with the natural substrate deoxyadenosine triphosphate (dATP), causing the termination of viral DNA chain elongation. TAF provides higher intracellular levels of TFV-DP and >90% lower circulating levels of TFV following administration of TAF relative to TDF.

This NDA package includes clinical data from 2 on-going randomized, active-controlled, Phase 3 trials conducted in HBeAg-negative (Study GS-US-320-0108) and HBeAg-positive (Study GS-US-320-0110) subjects with chronic hepatitis B (CHB), treatment-naïve or treatment-experienced, including a subset with compensated cirrhosis at study entry. In both of these similarly designed noninferiority studies, subjects are randomized 2:1 to receive either TAF (25 mg QD) or TDF (VIREAD, 300 mg QD) for 144 weeks. The primary endpoints for efficacy and

safety are at Week 48 of treatment.

Based on the Week-48 virology data submitted, antiviral efficacy of TAF was noninferior to TDF at Week 48 in HBeAg-negative and HBeAg-positive subjects, and no specific amino acid substitutions in the HBV rt domain that emerged in treatment failure virus samples while on TAF during the first year of the study were identified to be associated with TAF genotypic resistance. In subgroups of subjects with high baseline HBV DNA levels (≥7 $\log_{10}$ IU/mL for HBeAg-negative subjects and ≥8 $\log_{10}$ IU/mL for HBeAg-positive subjects), virologic suppression (HBV DNA levels to <29 IU/mL) was achieved at Week 48 by a numerically lower proportion of TAF-treated subjects than of TDF-treated subjects. Negative correlation between baseline HBV DNA levels and antiviral efficacy of TAF and TDF monotherapy was noted, independent of prior NrtI treatment history. Similarly, prior HBV NrtI exposure (regardless of treatment duration or detection of NrtI resistance substitutions) appeared to have a negative impact on Week-48 virological response to TAF and TDF monotherapy, independent of baseline viral load. These independent FDA analysis results are in agreement with similar results obtained by the applicant.

1. Recommendations

1.1. Recommendation and Conclusion on Approvability: Approval of this original NDA for VEMLIDY™ 25 mg QD is recommended with respect to Clinical Virology for the treatment of chronic hepatitis B virus infection in adults with compensated liver disease.

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable:

Post-Marketing Requirement 1: Perform genotypic (also phenotypic if qualified) resistance analysis of baseline virus samples from all HBeAg-positive NrtI-experienced subjects and of Week-48 virus samples from all evaluable subjects, regardless of their Week-96 virologic outcome.

Post-Marketing Requirement 2: Phenotype Week-48 virus samples from Subjects 4296-5147 and 8758-5188 in the TAF group and Subjects 1507-4546 and 9035-4845 in the TDF group in Study GS-US-320-0110.

Post-Marketing Requirement 3: Evaluate the anti-HBV activity of TAF in combination with sofosbuvir.

2. Summary of OND Virology Assessments

2.1. Nonclinical Virology

TAF is a phosphonamidate prodrug of nucleotide analogue reverse transcriptase inhibitor tenofovir (TFV) that has activity against HBV reverse transcriptase as well as HIV-1 reverse transcriptase. TAF inhibited clinical isolates of HBV representing genotypes A to H with EC_{50} values of 34.7 to 134.4 nM and an overall mean EC_{50} value of 86.6 nM in transiently transfected human hepatoblastoma cells HepG2. TAF had no observed cellular toxicity up to the highest tested concentration in proliferating HepG2 cells with a CC_{50} value of >44.4 μM following 5 days of continuous exposure, as observed with TFV and TDF. In addition, no specific depletion of

mitochondrial DNA was observed in HepG2 cells by TAF at up to 1.0 μM.

TAF contains a lipophilic group masking the charged phosphonate moiety that allows for high permeability and efficient delivery of TFV into target cells and tissues. In addition, hepatic uptake transporters OATP1B1 and OATP1B3 make a small contribution to TAF uptake into primary human hepatocytes. After TAF penetrates cells, the prodrug carboxylester bond is cleaved by a hydrolase, releasing an intermediate metabolite TFV-alanine, which is further hydrolyzed to parent TFV. In primary human hepatocytes, TAF is hydrolyzed primarily by carboxylesterase 1 (CES1), whereas in immortalized cell lines of hepatic origin, HepG2 and HepAD38 cells, cathepsin A is the major hydrolyzing enzyme of TAF. Intracellular TFV, following its release from TAF, is subsequently phosphorylated by cellular kinases to the pharmacologically active diphosphate metabolite (TFV-DP) that inhibits HBV reverse transcriptase by competing with the natural substrate deoxyadenosine triphosphate (dATP), causing the termination of viral DNA chain elongation. TFV-DP is a weak inhibitor of mammalian DNA polymerases that include mitochondrial DNA polymerase γ.

In a cell culture 2-drug combination study, no antagonistic effects on the anti-HBV activity of TAF were observed in combination with HCV PIs boceprevir, simeprevir, and telaprevir, and host serine PIs, apixaban and rivaroxaban (inhibitors of factor Xa), argatroban and dabigatran (inhibitors of thrombin), and sitagliptin (dipeptidyl peptidase 4 [DPP-4] inhibitor), as well as the ritonavir-derived pharmacokinetic enhancing agent cobicistat. The anti-HBV activity of TAF in combination with the HCV nucleotide analog NS5B polymerase inhibitor sofosbuvir, which is also metabolized by CES1, was not evaluated. In a cell-based combination antiviral activity study in Huh-7-derived cells stably expressing HCV genotype 1a subgenomic replicons, the presence of TAF (1 μM) did not impact the anti-HCV activity of sofosbuvir.

TAF was moderately stable in human plasma with a half-life of 74.7 minutes, and bound to human plasma with the percent of unbound TAF being 46.8% when determined by equilibrium dialysis at the final TAF concentration of 2 μM.

Cross-resistance studies carried out in HepG2 cells expressing transiently HBV variants resistant to adefovir, entecavir, and lamivudine (generated by site-directed mutagenesis) demonstrated some degree of cross-resistance between TAF and adefovir. Compared to the wild-type isolate, HBV isolates expressing the lamivudine resistance-associated substitutions rtM204V/I (±rtL180M±rtV173L) and expressing the entecavir resistance-associated substitutions rtT184G, rtS202G, or rtM250V in the presence of rtL180M and rtM204V showed <2-fold reduced susceptibility (within the inter-assay variability) to TAF. In addition, HBV isolates expressing the rtA181T, rtA181V, or rtN236T single substitutions associated with resistance to adefovir also remained largely susceptible to TAF with <2-fold changes in EC_{50} values. However, the HBV isolate expressing the rtA181V plus rtN236T double substitutions exhibited reduced susceptibility (3.7-fold) to TAF.

2.2. Clinical Virology

In 2 ongoing Phase 3 studies, GS-US-320-0108 (Study 108) and GS-US-320-0110 (Study 110), antiviral efficacy of TAF (25 mg QD) was compared at Week 48 to TDF (300 mg QD) separately in the HBeAg-negative and HBeAg-positive CHB subject populations, respectively. The primary efficacy endpoint in these 2 studies was the proportion of subjects with plasma HBV DNA <29 IU/mL (approximately 169 copies/mL) at Week 48.

When analyzed using the Week-48 censored, as-treated subject populations, comparable proportions of subjects in the TAF and TDF treatment groups achieved HBV DNA <29 IU/mL after 48 weeks of monotherapy in each study: 97.5% versus 94.9% of subjects with HBeAg-negative CHB (Study 108) and 64.7% versus 67.9% of subjects with HBeAg-positive CHB (Study 110), respectively. Median plasma HBV DNA levels (log_{10} IU/mL) at Baseline were well matched between the treatment groups, TAF versus TDF, in each study population with much higher baseline HBV DNA levels in the HBeAg-positive population than the HBeAg-negative population: 5.59 (ranging from 2.58 to 9.89) versus 5.73 (ranging from 1.79 to 8.20) for the HBeAg-negative subjects and 7.92 (ranging from 2.54 to 9.92) versus 7.98 (ranging from 2.60 to 9.90) for the HBeAg-positive subjects. In each study (full-analysis population), the kinetics of HBV DNA decline over 48 weeks (as assessed by the proportion of subjects with HBV DNA <29 IU/mL by visit) and mean changes in HBV DNA levels from Baseline by study visit were also similar between the 2 treatment groups through Week 48.

In both studies, a numerically lower proportion of subjects with high baseline HBV DNA levels (≥7 log_{10} IU/mL for HBeAg negative subjects in Study 108 and ≥8 log_{10} IU/mL for HBeAg positive subjects in Study 110) achieved HBV DNA <29 IU/mL at Week 48 in the TAF group compared with the TDF group: in HBeAg negative subjects, 85.5% versus 95.8% with the difference between the 2 proportions of -10.4% (95% CI: -25.5% to 4.5%) and in HBeAg positive subjects, 43.0% versus 50.7% with the difference of -7.6% (95% CI: -17.8% to 2.5%). However, in both studies, the applicant claimed the subgroup of subjects with high baseline HBV DNA levels was not identified as statistically significant for the primary endpoint. Additional analyses revealed numerically greater differences in HBV DNA suppression (HBV DNA <29 IU/mL) between the TAF and TDF groups were observed in subjects having intermediate baseline HBV DNA levels (7 to <8 log_{10} IU/mL in HBeAg negative subjects and 8 to <9 log_{10} IU/mL in HBeAg positive subjects) compared to those having low or very high baseline HBV DNA levels (<7 or ≥8 log_{10} IU/mL in Study 108 and <8 or ≥9 log_{10} IU/mL in Study 110, respectively). In HBeAg negative subjects, the differences between the 2 proportions were +3.9%, -11.9%, and +1.9% for subgroups of subjects with baseline HBV DNA levels <7, 7 to <8, and ≥8 log_{10} IU/mL and in HBeAg positive subjects, the differences were +0.2%, -10.9%, and -1% for those with baseline HBV DNA levels <8, 8 to <9, and ≥9 log_{10} IU/mL, respectively. When the early HBV suppression rate and kinetics of HBV DNA decline during the first 24 weeks of treatment in subjects with intermediate baseline HBV DNA levels were evaluated, no significant differences between TAF and TDF were observed.

By Week 48, 0.7% of HBeAg-positive subjects in the TAF group and 0.3% of those in the TDF group experienced HBsAg loss, while no HBeAg-negative subjects in either treatment group experienced HBsAg loss. Among those with HBsAg loss (4 TAF and one TDF), 3 subjects in the TAF group (overall 0.5%) and none in the TDF group (overall 0%) also experienced HBsAg seroconversion at Week 48. This rate of HBsAg loss is similar to the spontaneous rate. In HBeAg-positive subjects (Study 110), 13.8% and 10.3% of TAF subjects, and 11.9% and 8.1% of TDF subjects experienced HBeAg loss and HBeAg seroconversion, respectively.

In subgroup analyses to identify baseline factors that may affect antiviral response to TAF, negative correlation between baseline HBV DNA levels and antiviral efficacy of TAF and TDF monotherapy, independent of prior NrtI treatment history was noted. Similarly, prior HBV NrtI exposure (regardless of treatment duration or detection of NrtI resistance substitutions) appeared to have a negative impact on Week-48 virological response to TAF and TDF monotherapy, independent of baseline viral load. In addition, there were significant observed

differences in the TAF response rate (HBV DNA <29 IU/mL) at Week 48, independent of prior NrtI treatment history, favoring subjects with baseline wild-type like HBV (absence of known NrtI-R substitutions [rt substitutions associated with resistance to HBV NrtIs] detectable by LiPA probes) over those with baseline virus expressing NrtI-R substitutions and also favoring subjects with baseline HBV non-D genotype over those with baseline genotype D. However, the lower rates of viral suppression at Week 48 observed in those subgroups of subjects with baseline virus expressing NrtI-R substitutions or with baseline genotype D may be impacted by their higher baseline viral load (based on the median baseline HBV DNA level and the proportion of subjects with HBV DNA ≥8 $\log_{10}$ IU/mL) compared with their respective counterpart subject groups. In HBeAg-positive TAF-treated subjects, the baseline median (mean) viral loads ($\log_{10}$ IU/mL) were 7.87 (7.54) versus 8.21 (7.98) for those with baseline wild-type like virus and HBV variants expressing NrtI-R substitutions, respectively, and 7.82 (7.48) versus 8.34 (7.92) for those with baseline HBV genotype non-D and genotype D. It should be noted that, regardless of baseline virus (with or without detectable known NrtI-R substitutions) or HBV genotype, prior treatment with HBV NrtI(s) was confirmed to have a negative impact on Week-48 virological response to TAF monotherapy. Among HBeAg-positive subjects in the TAF group with baseline wild-type like virus, the difference between HBV NrtI-naïve subjects and those with prior NrtI exposure in the Week-48 response rate was 11.3%, and among those with baseline HBV variants expressing NrtI-R substitutions, the difference was 6.8%. Within each subgroup of subjects, grouped by baseline HBV genotype non-D or D, 16.6% and 6.7% lower virologic response rates to TAF, respectively, were observed in subjects with prior NrtI exposure compared to NrtI-naïve subjects. Similar trends of reduced responses were also observed in prior NrtI-exposed subjects treated with TDF.

A total of 1,270 subjects with CHB received TAF (n=850) or TDF (n=420) for ≥24 weeks in Studies 108 and 110, and after up to 48 weeks of treatment, 230 of them (158 TAF and 72 TDF; 18.6% and 17.1%, respectively) had HBV DNA ≥69 IU/mL. Among these Week-48 virologic failures with HBV DNA ≥69 IU/mL, 41 (27 TAF and 14 TDF) were identified to be eligible for Week-48 resistance testing per Virology Analysis Plan: 33 subjects who experienced virologic breakthrough by Week 48 (20 TAF and 13 TDF; 74.1% and 92.9%, respectively) and 8 subjects who never suppressed HBV DNA levels to <69 IU/mL without evidence of virologic breakthrough at the time of early discontinuation (7 TAF and 1 TDF; 25.9% and 7.1%, respectively). The HBV rt amino acid sequences of on-treatment failure isolates, in addition to their baseline isolates, were obtained from 27 subjects (17 TAF and 10 TDF) including 24 subjects who experienced virologic breakthrough (14 TAF and 10 TDF). In addition, genotypic data of 3 subjects' virus isolates collected one day after the last dose (follow-up visit isolates) were also compared to their respective baseline sample genotypic data: all 3 subjects received TAF and discontinued study drug without experiencing virologic breakthrough. No evidence of rt genotypic changes was observed in the majority (70%) of the 30 subjects with evaluable data (15 TAF and 6 TDF), including 5 of the 6 subjects who discontinued from the study before Week 48 without experiencing virologic breakthrough and had evaluable genotypic data.

During TAF or TDF monotherapy, emergence of rt substitutions was observed in 9 subjects' failure isolates occurring at 2 conserved amino acid positions (Q67H and Q288stop; both were seen in the TDF treatment-failure isolates) and at 16 polymorphic amino acid positions (S13N**,** I91L, S117P, D134E, R153Q, S256C, L267Q, L269I, E271A, and M309K were seen in the TAF treatment-failure isolates; and R110G, N118T, N123D, D134E, M207V, V214A, L269I, A317S were seen in the TDF treatment-failure isolate).

Overall, no TAF-treatment failure subjects evaluated were found to develop HBV variants with conserved site substitutions during the first year of the study; and there were no polymorphic site substitutions detected in more than one subject's failure isolate. Two TDF-treatment failure subjects developed HBV variants with a unique conserved site substitution but there were no conserved or polymorphic site substitutions detected in more than one subject's failure isolate during the first year of TDF monotherapy. Thus, with no specific amino acid substitutions in the HBV rt domain being commonly detectable in TAF or TDF treatment failure isolates during the first year of the study, no genotypic resistance pathways were identified for tenofovir.

When TAF and TDF treatment-emergent rt substitutions observed in these 2 studies were pooled together, only 2 substitution (rtD134E and rtL269I) was detected in more than one subject (each substitution in 2 subjects). The rtD134E substitution was previously evaluated in phenotypic assays and retained sensitivity to TFV. In addition, among the evaluated 412 TDF recipients' baseline isolates in previous TDF clinical trials (GS-US-174-0102 and GS-US-174-0103), a glutamic acid reside at HBV rt amino acid position 134 (rtE134) was detected in 10 subjects and 9 of them responded to TDF treatment by achieving HBV DNA <400 copies/mL (~69 IU/mL) at Week 48. The rtL269I substitution is a reversion towards the consensus amino acid: an isoleucine residue at position 269 (rtI269) was present in 95.1% (392/412; including those with a viral mixture) of the baseline viruses examined in the TDF studies.

Among the remaining observed emerging rt substitutions (S13N**,** Q67H, I91L, R110G, S117P, N118T, N123D, R153Q, M207V, V214A, S256C, L267Q, E271A, Q288stop, M309K, and A317S; each observed once; conserved-site substitutions are underlined), substitutions rtQ67H, rtS117P, rtM207V, rtL267Q, rtL269I, rtQ288stop, and rtA317S were never observed in the previously analyzed 126 TDF-treatment failure isolates: no substitutions occurring at these amino acid positions while on TDF were noted. All 3 subject-derived Week-48 pooled viruses containing these substitutions except A317S appeared to remain sensitive to their respective study drugs with observed fold changes of <2 which were within assay variability, compared to wild-type control HBV. Furthermore, when clones expressing the conserved-site substitutions rtQ67H or rtQ288stop were isolated from the subjects' Week-48 virus pool and phenotyped, the rtQ67H clone also appeared to be sensitive to TDF (1.5-fold change), while the rtQ288stop clone was unable to replicate in cell culture. Substitutions rtS13N, rtD134E, rtV214A, and rtE271A were also never detected in those previously analyzed TDF-treatment failure isolates but different amino acid substitutions were observed occurring at residues 13 (R13H, R13L, L13H), 134 (D134N, N134S), 214 (A214V), and 271 (E271D and M271L). The Week-48 pooled viruses containing rtS13N showed 0.7-fold reduced susceptibility to TAF, compared to wild-type control HBV. The remaining rt substitutions emerged during TAF or TDF monotherapy, I91L, R110G, N118T, N123D, R153Q, S256C, and M309K were previously observed once or twice in TDF-treatment failure isolates. A Week-48 subject-derived failure isolate containing rtN118T and/or N123D displayed 1.5-fold reduced susceptibility to TFV. Previously, TDF-treated subject-derived failure isolates in TDF trials expressing rtR110G or rtR153Q showed 1- to 1.3-fold reductions in TFV susceptibility.

Overall, based on the population-based nucleotide sequencing results, no failure isolates (from TAF or TDF treated subjects) developed during therapy well-characterized rt substitutions that have been reported to be associated with HBV NrtI resistance (L80V/I, V84M, I169T, V173L, L180M, A181S/T/V, T184A/F/G/I/L/S, A194T, S202G/I, M204I/S/V, Q215S, L229V/W, I233V, N236T, and M250V; substitutions reported to be associated with tenofovir resistance are underlined). Among other additional rt substitutions that were also implicated in HBV NrtI

resistance (including L82M, S85A, A200V, V214A, P237H, and N238T/D; substitutions reported to be associated with tenofovir resistance are underlined), emergence of the rtV214A substitution was noted in one TDF-treated subject's Week-48 virologic failure isolate (with 2 other emerging rt substitutions D134D/E and A317A/S).

3. Administrative

3.1. Reviewer's Signature(s)

_____
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Clinical Virology Reviewer

3.2. Concurrence

_____
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HFD-530/Division File
HFD-530/PM/M. Hong

OND VIROLOGY REVIEW

1. Introduction and Background

Chronic hepatitis B virus (HBV) infection causes necroinflammatory liver disease, cirrhosis, and hepatocellular carcinoma. Chronic HBV infection, detected as chronic seropositivity for HBV surface antigen (HBsAg), remains a widely prevalent global health problem with an estimated 350-400 million carriers worldwide with some 600,000 deaths per year attributed to the virus (El-Serag and Rudolph, 2007; Kanwal *et al*., 2015). The World Health Organization estimates that more than 4 million new cases of chronic HBV infection occur every year and approximately 15-25% of chronic HBV carriers, if left untreated, would develop liver cirrhosis or primary liver cancer (Block *et al*., 2003; 2007). Approximately 45% of the world population lives in areas of high endemicity, including many African and Asian countries (The EASL Jury, 2003), with about 75% of infected persons in Asia and 12% in Africa (Gust, 1996). Up to 2.2 million persons with CHB live in the United States, with a particularly high prevalence (3.45%) among the foreign-born (Kowdley *et al*., 2012). The development of an effective vaccine and assays for the detection of HBV in the blood supply has reduced the incidence in the United States.

In patients with chronic HBV infection, persistent and potentially progressive liver inflammation tends to correlate with serologic evidence of ongoing HBV replication, in addition to the presence of chronically detectable serum HBsAg. Patients who are positive for HBV replication markers such as HBV "e" antigen (HBeAg) or HBV DNA are at greatest risk for progressive liver injury (Imperial, 1999; Lee *et al*., 2002). Conversely, reversion to a low-replicative state through HBeAg seroconversion (spontaneous or treatment-induced) or long-lasting suppression of HBV replication in the absence of HBeAg seroconversion with effective antiviral therapy is thought to reduce the risk of further disease progression (Liaw, 2005). Some patients with chronic HBV infection achieve spontaneous remissions during early disease stages, but the spontaneous resolution rate is low in patients with advanced disease (Chisari and Ferrari, 1995; Fattovich *et al*., 1986; Hoofnagle *et al*., 1981; Liaw *et al*., 1983; Margolis *et al*., 1995; Rehermann *et al*., 1996). Therefore, the goal of antiviral therapy for chronic HBV infection is to reduce patients' risks for progressive liver disease through prolonged suppression of HBV replication or clearance of the virus.

There are currently five FDA-approved direct-acting antivirals for CHB in the US, all of which are nucleos(t)ide analogues: adefovir dipivoxil (HEPSERA®), entecavir (BARACLUDE®), lamivudine (EPIVIR-HBV®), telbivudine (TYZEKA®), and tenofovir disoproxil fumarate (TDF; VIREAD®). All of them inhibit the reverse transcriptase activity of the HBV polymerase and therefore interfere with the synthesis of viral DNA from pregenomic RNA, resulting in a decrease in viral replication as measured by reductions in serum HBV DNA.

Tenofovir (TFV) is a nucleotide analog that is intracellularly phosphorylated by cellular kinases to the active triphosphate form (TFV-DP). TFV-DP can be then incorporated into DNA by competing with the natural substrate, dATP (deoxyadenosine triphosphate). Since TFV-DP lacks a 3' hydroxyl group, incorporation of TFV-DP causes DNA chain termination, resulting in inhibition of DNA synthesis mediated by HBV reverse transcriptase (rt) and also by HIV-1 reverse transcriptase (RT). TFV has low oral bioavailability in preclinical models, presumably resulting from the presence of two negative charges on the phosphonyl group (Cundy *et al*., 1998). Tenofovir disoproxil fumarate (TDF; VIREAD) is an ester prodrug of TFV that was developed to improve the poor oral absorption of TFV (Lyseng-Williamson *et al*., 2005). TDF

undergoes rapid metabolism (highly susceptible to serum esterases) to the parent drug, TFV, in the systemic circulation after oral administration (Robbins *et al*., 1998). Oral dosing of TDF results in approximately 25 - 30% bioavailability measured as circulating TFV concentrations. TDF has been marketed for the treatment of HIV-1 infection in combination with other antiretroviral agents since 2001 and for the treatment of chronic HBV infection since 2008.

Approval of TDF for the treatment of chronic HBV infection in adults in the United States was based primarily on Week-48 safety and efficacy data from two Phase 3 clinical studies, GS-US-174-0102 and GS-US-174-0103. These 2 studies were conducted separately in the predominantly treatment-naïve, $HBeAg^-$ ($HBeAb^+$) and $HBeAg^+$ subject populations, respectively, to determine whether TDF 300 mg QD is noninferior to adefovir (HEPSERA) 10 mg QD for the treatment of chronic HBV infection in the both subject groups with compensated liver disease. The 2 studies were similarly designed, involving 48 weeks of double-blind therapy either with TDF or adefovir, followed by open-label TDF treatment through Week 480 (Year 10). Efficacy of TDF 300 mg QD at Week 48 was superior to adefovir 10 mg QD in both subject groups as measured by the proportion of subjects with complete response (plasma HBV DNA levels <400 copies/mL or 69 IU/mL, and histologic improvement indicated by at least a 2-point reduction in Knodell necroinflammatory score without worsening in Knodell fibrosis score). Complete response was achieved in 71% versus 49% of $HBeAg^-$ TDF and adefovir recipients, and 67% versus 12% of $HBeAg^+$ TDF and adefovir recipients, respectively. The Week-384 (Year 8) analyses currently completed revealed a sustained anti-HBV treatment effect of TDF in both studies, continuing to support the use of TDF for the treatment of chronic HBV infection in adults with compensated liver disease (Virology review N021356.943). TDF genotypic resistance testing on virus samples from subjects with evidence of virologic failure (HBV DNA ≥400 copies/mL) through Week 384 identified no specific amino acid substitutions in the HBV rt domain that occurred at sufficient frequency to establish an association with genotypic TDF resistance.

The second-generation oral prodrug of TFV, tenofovir alafenamide (TAF; VEMLIDT™), was designed to overcome the permeability limitations of TFV by masking the di-anion with a neutral promoiety, and to circulate systemically as the prodrug and thus to undergo selective conversion to TFV inside cells (Lee *et al*., 2005). Both TAF and the first generation prodrug TDF become active through the pharmacologically active metabolite tenofovir diphosphate (TFV-DP). Compared to TDF, TAF is more stable in plasma and thus >90% lower circulating levels of TFV following administration of TAF relative to TDF (Birkus *et al*., 2008; Lee *et al*., 2005). TAF as a lipophilic cell-permeant compound enters cells primarily by passive diffusion and then the carboxyl ester promoiety of the prodrug is hydrolyzed primarily by carboxylesterase 1 (CES1) in primary hepatocytes and by cathepsin A in PBMCs and other HIV target cells to yield the alaninyl-TFV intermediate which is then chemically and/or enzymatically converted to TFV (Birkus *et al*., 2007 and 2008; Chou *et al*., 2007; Furman *et al*., 2011; Murakami *et al*., 2010 and 2015). Recently, TAF has gained approval by FDA for the treatment of HIV-1 infection in adult and pediatric patients (≥12 years of age) as a component of FDC products GENVOYA® (TAF/FTC/EVG/COBI [10/200/150/150 mg]) on November 5, 2015, of ODEFSEY® (TAF/FTC/RPV [25/200/25 mg]) on March 1, 2016, and of DESCOVY® (TAF/FTC [10/200 mg and 25/200 mg]) on April 4, 2016.

This original new drug application (NDA) is submitted for U.S. marketing approval of TAF 25 mg tablets as a single agent for once daily use for the treatment of chronic HBV infection in adult patients with compensated liver disease. The NDA package includes nonclinical and clinical

study data, supportive of TAF tablets for the proposed indication, from 2 pivotal Phase 3 trials: GS-US-320-0108 and GS-US-320-0110 are being conducted in HBeAg-negative and HBeAg-positive subjects, respectively, with CHB, treatment-naïve or treatment-experienced, including a subset with compensated cirrhosis at study entry. These 2 on-going studies are randomized, double-blind, noninferiority trials of TAF 25 mg QD compared to TDF 300 mg QD for a double-blind treatment period of 144 weeks in HBV mono-infected subjects, followed by open-label treatment with TAF through Week 384. The primary endpoints for efficacy and safety are at Week 48 of treatment. These studies were conducted at multiple study centers (105 sites for Study GS-US-320-0108 and 161 sites for GS-US-320-0110), both within the U.S. and at international sites.

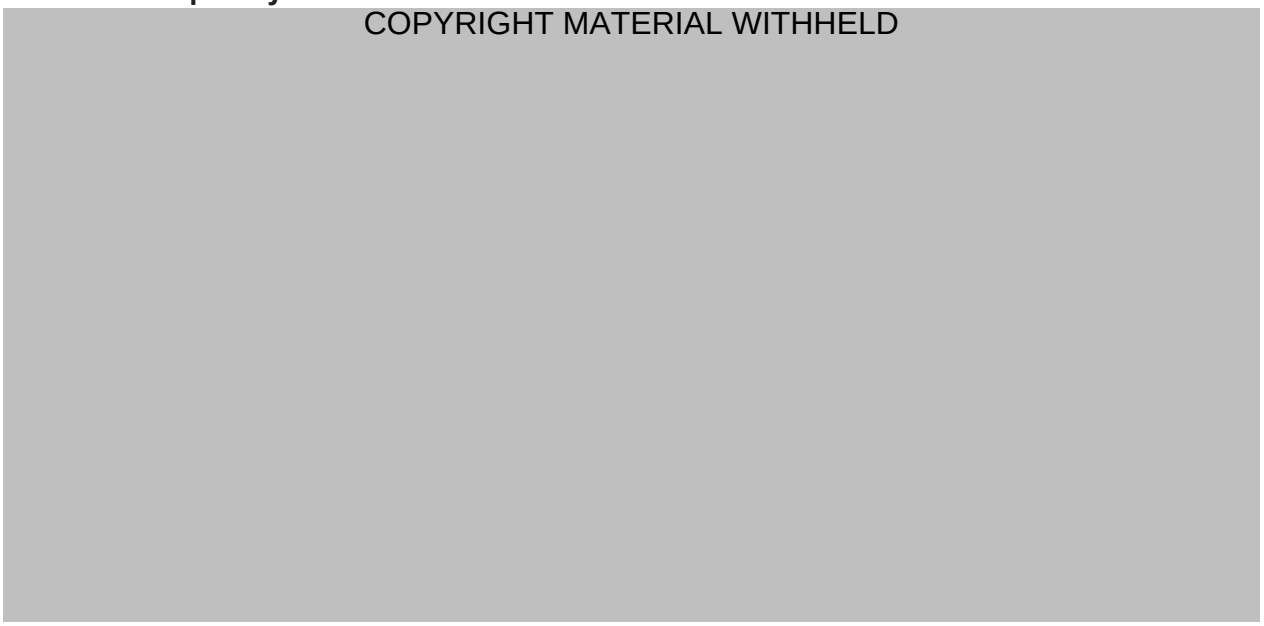
2. Nonclinical Virology

Numerous nonclinical virology study results reviewed below were included in this original NDA package to support the proposed use of single-agent TAF 25 mg tablets for the treatment of CHB.

2.1. Mechanism of Action

Hepadnaviruses utilize a reverse transcription-mediated replication cycle to convert a pregenomic RNA template into a partially double-stranded circular DNA genome that is characteristic of HBV (Ganem *et al*. 1994). HBV polymerase-mediated genome replication reaction begins with an initial priming step, followed by first-strand or minus (-) DNA strand synthesis (reverse transcription of the pregenomic RNA), then second-strand or plus (+) DNA strand synthesis (DNA-dependent DNA synthesis). TAF is a second-generation oral prodrug of nucleotide analogue reverse transcriptase inhibitor TFV. The proposed mechanism of action for TAF in primary hepatocytes is shown in Figure 1 (from Murakami *et al*., 2015, Figure 6).

Figure 1: Proposed Mechanism of Metabolic Activation of TAF in Human Primary Hepatocytes



Source: Murakami *et al*., 2015, Figure 6.

Results from several nonclinical studies that support the proposed mechanism of action of TAF in hepatocytes are summarized below. Briefly, TAF as a lipophilic cell-permeant compound enters primary hepatocytes by passive diffusion and by the hepatic uptake transporters OATP1B1 and OATP1B3, and then the carboxyl ester promoiety of the prodrug is hydrolyzed primarily by carboxylesterase 1 (CES1) to yield the alaninyl-TFV intermediate which is then chemically and/or enzymatically converted to TFV. The authors reasoned a cellular histidine triad nucleotide binding protein 1 (HINT1) phosphoramidase may be responsible for the removal of the amino acid moiety, as the P-N bonds in other phophoramidate prodrugs such as sofosbuvir were shown to be cleaved by this enzyme (Chou *et al*., 2007; Furman *et al*., 2011; Murakami *et al*., 2010). Intracellular TFV, following its release from TAF, is subsequently phosphorylated by cellular kinases to the pharmacologically active diphosphate metabolite (TFV-DP) that inhibits HBV reverse transcriptase (rt) by competing with the natural substrate deoxyadenosine triphosphate (dATP), causing the termination of viral DNA chain elongation (reviewed by De Clercq, 2003).

According to the applicant, while TAF was found to be a substrate for hepatic transporters organic anion transporting polypeptide 1B1 and 1B3 (OATP1B1 and OATP1B3, respectively), the major route of TAF uptake into hepatocytes appears to be passive permeability with only a small contribution of OATP-mediated active transport (Study Reports AD-120-2022 and AD-120-2042; Murakami *et al*., 2015). TAF contains a lipophilic group masking the charged phosphonate moiety that allows for high permeability and efficient delivery of TFV into target cells and tissues.

In Study AD-120-2042, the effect of an inhibitor (rifampicin) of OATP1B1 and OATP1B3 on TAF uptake into primary human hepatocytes was assessed by measuring the intracellular accumulation of TFV-DP. A mean of 13% inhibition of TAF uptake by rifampicin was observed in hepatocytes from four different donors (b) (4), while rifampicin inhibited accumulation of cell-associated levels of bosentan (a known OATP substrate, positive control) by approximately 38%.

In Study AD-120-2022, the contribution of the 2 hepatic transporters OATP1B1 and OATP1B3 to TAF uptake was determined in Chinese hamster ovary cells transiently expressing human OATP1B1 (CHO-OATP1B1) or OATP1B3 (CHO-OATP1B3). The rate of TAF uptake increased only by 30% and 168%, respectively, in CHO-OATP1B1 and CHO-OATP1B3 cells when compared to untransfected CHO cells (CHO-WT): rates were 9.0 pmol/min/10^6 cells, 12.0 pmol/min/10^6 cells, and 24.1 pmol/min/10^6 cells in CHO-WT, CHO-OATP1B1, and CHO-OATP1B3 cells, respectively. The uptake rate was decreased by 48% in CHO-OATP1B1 cells and 76% in CHO-OATP1B3 cells in the presence of rifampicin. Positive control atorvastatin (known OATP substrate) showed a significantly increased uptake rate of 4.6 pmol/min/10^6 cells in CHO-OATP1B1 and of 5.5 pmol/min/10^6 cells in CHO-OATP1B3, compared to that of 0.38 pmol/min/10^6 cells in CHO-WT cells (ratios of 11.9 and 14,5, respectively), while in the presence of rifampin atorvastatin uptake rates in CHO-OATP1B1 and CHO-OATP1B3 were similar to that in CHO-WT (ratios of 1.3 and 1.7, respectively). Passive permeability control antipyrin was also included in this study to show uptake rates of antipyrin in CHO-OATP1B1 and CHO-OATP1B3 similar to that in CHO-WT (ratio of 1.0 each), which were not affected by rifampin.

Together, these results indicated that OATP1B1 and OATP1B3 only make a small contribution to TAF uptake into primary human hepatocytes due to the high passive permeability of the prodrug. Of note, TAF was not a substrate for renal organic anion transporters, OAT1 and

OAT3 (Study Report PC-120-2018; Bam *et al*., 2014) which were reported to mediate the active uptake of TFV into renal proximal tubule cells for its active tubular secretion (Cihlar *et al*., 2001; Kearney *et al*., 2004). Bam *et al*. (2014) observed no increased intracellular accumulation of TAF in human epithelial kidney cells (HEK293T) transiently expressing human OAT1 or OAT3 transporters, while OAT expression significantly increased TFV intracellular accumulation (>70-fold and 8.2-fold in OAT1- and OAT3-expressing cells, respectively).

TAF was moderately stable in human plasma with a plasma half-life of 74.7 minutes (Study Report AD-120-2025). Lee *et al*. (2004) also estimated the half-life of 90 ± 12 minutes in human plasma at 37°C for TAF, >200-fold more stable compared to TDF (half-life of 0.41 ± 0.2 minutes).

Protein binding of TAF was moderate in human plasma with the percent of unbound TAF being 46.8% when determined by equilibrium dialysis at the final TAF concentration of 2 μM (Study Report AD-120-2026). According to the applicant, this value was higher than those observed in multiple human *ex vivo* studies with the mean percent of unbound TAF ranging from 14% to 23% in all subjects (GS-US-120-0108 and GS-US-120-0114). Of note, binding of TFV to plasma and serum protein was low, less than 0.7% and 7.2%, respectively, over the TFV concentration range 0.01 to 25 μg/mL (Virology review N021356.SE1-025).

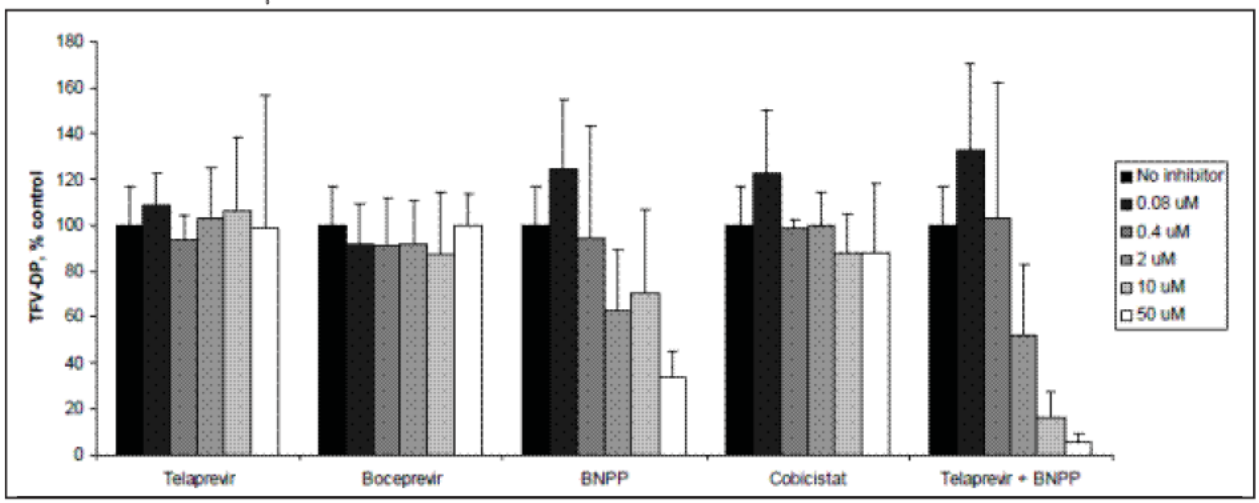
After TAF penetrates cells, the prodrug carboxylester bond is cleaved by a hydrolase, releasing an intermediate metabolite TFV-alanine, which is further hydrolyzed to parent TFV and sequentially phosphorylated to the active metabolite TFV-DP (Figure 1; Birkus *et al*., 2007 and 2008; Eisenberg *et al*., 2001; Murakami *et al*., 2015). In primary human hepatocytes, TAF is primarily hydrolyzed by carboxylesterase 1 (CES1) with carboxypeptidase known as cathepsin A (CatA; Birkus *et al*., 2007 and 2008) making a minor contribution (Study Report AD-120-2031; Murakami *et al*., 2015). In PBMCs and other lymphatic tissues, the carboxylester bond of TAF is cleaved by a lysosomal serine protease and CatA.

As shown in Figure 2, when primary human hepatocytes were incubated with 0.5 μM TAF for 24 hours in the presence of a known CES1 inhibitor, bis(p-nitrophenyl)phosphate (BNPP), TAF metabolism (by measuring formation of its pharmacologically active metabolite TFV-DP) was inhibited in a dose-dependent manner: relative to no inhibitor control, approximately 30% and 65% reductions in TFV-DP formation were observed at BNPP concentrations of 10 μM and 50 μM, respectively. In contrast, two approved HCV protease inhibitors boceprevir and telaprevir that were found to inhibit CatA-mediated TAF activation with IC_{50} values of 0.3 and 0.16 μM, respectively, in a biochemical assay (Study Report PC-120-2001) did not affect TAF metabolism significantly at up to 50 μM (highest concentration tested). However, when BNPP were combined with telaprevir, a greater inhibition was seen at higher concentrations of the inhibitors (10 μM and 50 μM): approximately 84% and 95% reductions in TFV-DP formation were observed, respectively, relative to BNPP alone. Together, these results indicated that CES1 is likely to be the major enzyme involved in TAF activation in primary human hepatocytes and CatA also makes a minor contribution.

CatA is expressed in multiple cell types, and its levels are high in lymphoid cells and tissues (Satake *et al*., 1994), while CES1 has a restricted tissue expression with the highest levels being present in the liver cells (Imai, 2006; Murakami *et al*., 2010 and 2015). CES1 was also reported as the major enzyme that hydrolyzes the carboxyl ester moiety of an approved anti-

HCV nucleotide phosphoramidate prodrug, sofosbuvir (Murakami *et al.*, 2010).

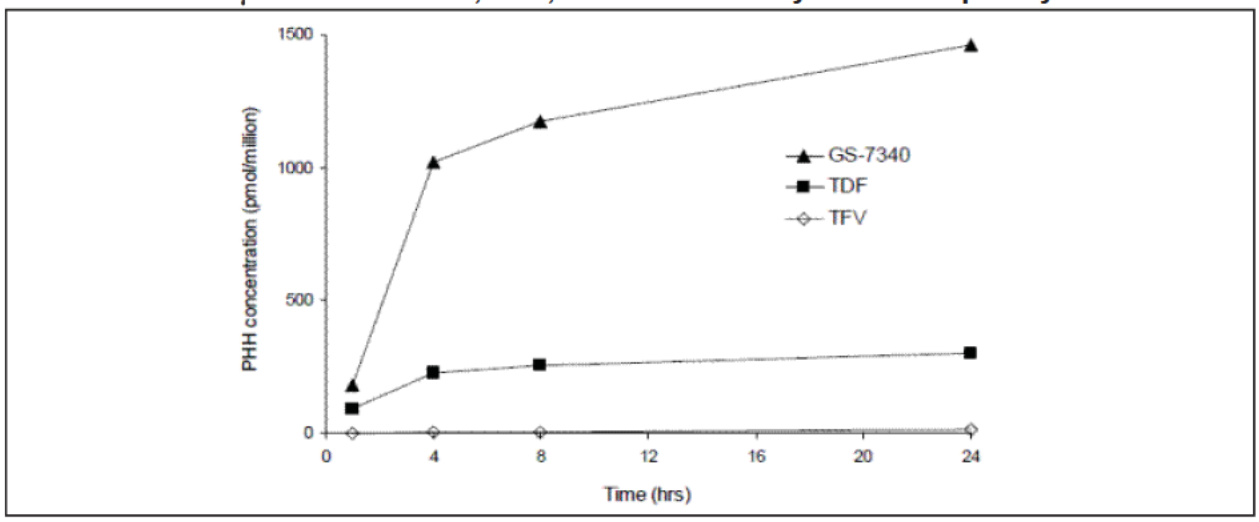
Figure 2: Intracellular Formation of TFV-DP in Primary Human Hepatocytes Incubated with 0.5 μM TAF for 24 Hours in the Presence of an Inhibitor of CatA and CES1



Source: Study Report AD-120-2031, Figure 2, page 11.
Note: BNPP, bis(p-nitrophenyl)phosphate, is a specific CES1 inh bitor (Block and Arndt, 1978; Imai, 2006).

In primary human hepatocytes (from a single donor, purchased from [redacted] (b) (4)) incubated with 5 μM of either TAF, TDF, or TFV for 24 hours, levels of active triphosphate analog TFV-DP persistently increased during the continuous incubation period, reaching a maximum of 1,470, 302, and 12.1 pmol/10^6 cells, respectively, when quantified at the selected time points (1, 4, 8, and 24 hours) using the LC-MS/MS method (Figure 3; Study Report AD-120-2017; Murakami *et al.*, 2015): incubation with TAF resulted in approximately 120- and 5-fold higher levels of intracellular TFV-DP compared to TFV and TDF, respectively.

Figure 3: Intracellular Concentrations of TFV-DP Following a 24h Continuous Incubation with 5 μM of Either TAF, TDF, or TFV in Primary Human Hepatocytes



Source: Study Report AD-120-2017, Figure 2, page 10.
GS-7340=TAF
Intracellular concentration in μM can be estimated assuming an intracellular volume of 1 μL/10^6 cells.

Of note, Delaney *et al*. (2006) reported TFV-DP has a long intracellular half-life of 95 ± 6 hours in primary human hepatocytes, compared to 75 ± 1 hours for adefovir diphosphate when quantified by LC/MS/MS. Previously, the effect of TFV-DP on the HBV polymerase activity was determined in a biochemical assay using the baculovirus-expressed, purified recombinant enzyme (Delaney *et al*., 2006): TFV-DP inhibited the HBV polymerase activity in a dose-dependent manner with the kinetic inhibition constant (K_i) of 0.18 μM, 2.1-fold lower than that of dATP (0.38 μM). These data confirmed that TFV-DP is a competitive inhibitor of HBV rt. TFV-DP was previously reported to be a weak inhibitor of human cellular DNA polymerases α, β, and mitochondrial DNA polymerase γ (Cherrington *et al*., 1995).

In Study PC-320-2006, the roles of CES1 and CatA in TAF metabolism were assessed in immortalized cell lines of hepatic origin, HepG2 cells and HepAD38 cells (derived from HepG2 cells expressing HBV under the control of an inducible tetracycline promoter; Ladner *et al*., 1997), that are commonly used in HBV antiviral phenotyping assays. Quantitative Western blot analysis of cell extracts revealed both CatA and CES1 are expressed at similar levels in HepG2 and HepAD38 cells (Table 1). The expression levels of CatA in HepG2 and HepAD38 cells were greater than those in pooled human liver S9 fractions (2.4- and 4.8-fold, respectively), whereas CES1 levels in both cells were less than those in pooled human liver S9 fractions (43- and 17-fold, respectively).

Table 1: Expression of CatA and CES1 in Hepatic Cell Lines

	Relative Concentration (ng/μg Total Protein)[a]	
Cell Type	**CatA**	**CES1**
HepG2	2.9 ± 0.4	0.9 ± 0.2
HepAD38	5.7 ± 0.2	2.3 ± 0.4
Pooled human liver S9 fraction	1.2 ± 0.1	39.0 ± 0.2
Pooled human intestinal S9 fraction	1.4 ± 0.2	< 0.1

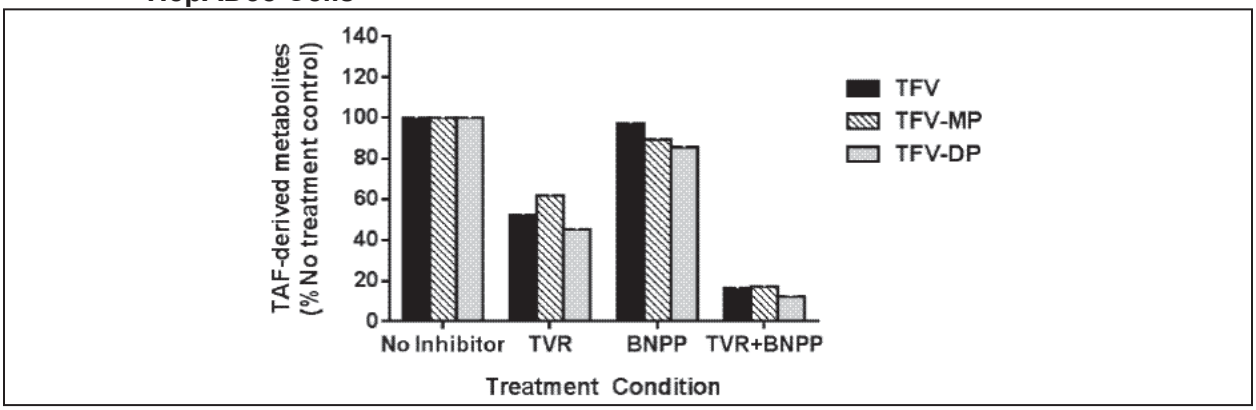
Source: Study Report PC-320-2006, Table 2, page 10.
[a] Data shown represent the mean and standard error values of three independent experiments.

In a cell-free assay, while the expression levels of CatA and CES1 were similar in these two hepatic cell lines, their relative contributions to TAF metabolism differed significantly. The CatA inhibitor telaprevir was a potent inhibitor of TAF hydrolysis to TAF-alanine in both HepG2 and HepAD38 cell extracts with similar IC_{50} values of 0.2 and 0.1 μM, respectively, but the CES1 inhibitor BNPP showed no impact on TAF hydrolysis in either cell extracts up to the highest concentration tested (50 μM), indicating minimal contribution of CES1 in these hepatic cell lines.

In addition, intracellular metabolism of TAF was evaluated by measuring the formation of TFV, TFV-MP, and TFV-DP following continuous incubation with 0.5 μM TAF in the presence of 50 μM CatA (telaprevir) and/or CES1 (BNPP) inhibitors in HepAD38 cells. When cells were treated with TAF in the absence of any inhibitors, 2.7- and 2.8-fold higher levels of TFV-DP (126.0 ± 9.9 pmol/10^6 cells) were formed relative to TFV (46.2 ± 6.9 pmol/10^6 cells) and TFV-MP (44.9 ± 5.1 pmol/10^6 cells), respectively. As shown in Figure 4, telaprevir (TVR) significantly inhibited formation of TFV-DP by 2.2-fold, while the effect of BNPP was negligible (1.2-fold). When both telaprevir and BNPP (TVR+BNPP) were combined, a 3.7-fold greater inhibition of TFV-DP formation was observed, relative to telaprevir alone. These results agreed with observations

made in the cell-free TAF hydrolysis study showing CatA is the major hydrolyzing enzyme of TAF in HepAD38 and HepG2 cells, with a minor contribution coming from CES1. Together, these results differ from those obtained in primary human hepatocytes where CES1 is commonly expressed at greater levels and appears to play a more dominant role than CatA in TAF metabolism (Murakami *et al*., 2010 and 2015). One possible explanation for this difference may be that CES1 expression levels were significantly reduced in hepatic cell lines compared to pooled human liver S9 fractions (Table 1; Murakami *et al*., 2010; Yang *et al*., 2007).

Figure 4: Effects of CatA and/or CES1 Inhibitors on Intracellular TAF Metabolites in HepAD38 Cells



Source: Study Report PC-320-2006, Figure 2, page 12

Cell extracts were prepared from HepAD38 cells following incubation with 0.5 μM TAF and 50 μM CatA (telaprevir) and/or CES1 (BNPP) inhibitors. Each TAF metabolite eluted was quantified by LC-MS/MS and expressed as a percentage of the response of the same metabolite quantified in the absence of inh bitor, set to 100%. Data shown represent mean from a single experiment performed in duplicate.

2.2. Anti-HBV Activity in Cell Culture

TAF exhibited similar antiviral activity across HBV genotypes when 11 wild-type clinical HBV isolates representing genotypes A to H were evaluated in human hepatoblastoma cells HepG2 (growing in the presence of 10% FBS): mean EC_{50} values ranged from 34.7 to 134.4 nM with an overall mean EC_{50} value of 86.6 nM (Table 2; Study Report PC-320-2003). Compared to the TAF EC_{50} value for the pHY92 genotype A control laboratory strain of 102.3 ± 30.0 nM, the mean fold-changes ranged from 0.3 to 1.3 for the different genotypes (Table 2). These results support the on-going global TAF Phase 3 clinical trial program in subjects infected with diverse HBV genotypes. In this study, full-length HBV genomes (genotypes A-E) or the rt region (genotypes F-H) were amplified from treatment-naïve subjects with HBV infection enrolled in Gilead clinical trials and cloned into the CMV promoter-driven HBV expression vectors pHY106 (Yang *et al*., 2004) and pRTAN (Zhu *et al*., 2011), respectively, and the resulting plasmids were then utilized to transiently transfect HepG2 cells. The parental plasmid vector, pHY92, containing a full-length HBV genome of wild-type genotype A laboratory strain was used as a positive control. Cells were then cultured in medium containing serial dilutions of TAF for 7 days. On Day 7, HBV replication was measured by quantifying intracellular HBV DNA by real-time PCR as described by Zhu *et al*. (2011). According to the applicant, all transfected isolates produced similar levels of intracellular HBV DNA with no significant difference observed between any isolate and the pHY92 control (p value of ≥0.06). Regression analyses of antiviral data were performed with TableCurve2D software and best fit equations were used to calculate EC_{50} values. Statistical analyses were performed using a paired Wilcoxon exact test to account

for small sample sizes using SAS 9.2 (SAS Institute Inc.). Results are summarized in Table 2. Of note, the CC_{50} value of >44,400 nM was reported for TAF in in HepG2 cells (Study Report PC-120-2007).

Table 2: Anti-HBV Activity of TAF against Wild-Type HBV Clinical Isolates in HepG2 Cells

Subject Number	Genotype	TAF EC_{50} (nM)[a,b]	Fold Change[c]
001	A	112.0±51.1	1.1
002	B	109.3±56.7	1.1
003	C	107.5±36.1	1.1
004	C	64.6±23.9	0.6
005	D	70.5±11.3	0.7
006	D	62.8±11.3	0.6
007	E	134.4±16.0	1.3
008	F	92.5±45.2	0.9
009	G	120.4±16.5	1.2
010	G	43.8±26.6	0.4
011	H	34.7±3.0	0.3
pHY92	A	102.3±30.0	1.0

Source: Study Report PC-320-2003, Table 4, page 11.
[a] Each isolate was tested a minimum of two times.
[b] Mean EC_{50} value (± standard deviation)
[c] Fold change was calculated as a ratio of the subject's mean EC_{50} value to the mean EC_{50} value of pHY92.

TAF exhibited minimal antiviral activity with EC_{50} values >1,000 nM (<50% inhibition at the highest concentration tested 1,000 nM) against one or several isolates of the following human viruses (Study Report PC-120-2003): adenovirus type 40 (in HeLa cells), dengue type 2 (New Guinea-C strain in Huh7 cells), influenza A virus (strain A/PR/8/34 [H1N1] in A549 cells), respiratory syncytial virus (Long strain in A549 cells), coxsackie B3 virus (in Vero cells), rhinovirus 1B (in MRC-5 cells), herpes simplex virus type 1 (strain HF in MRC-5 cells), cytomegalovirus (strain AD169 in MRC-5 cells), varicella zoster virus (Ellen strain in MRC-5 cells), vaccinia virus (strain Western Reserve in Vero E6 cells), and HCV (genotype 1b Con1 replicon in Huh-Luc/Neo ET cells). Among the human viruses evaluated in this study, in addition to HIV-1 with EC_{50} values of 2.04 nM (R5 strain BaL) and 5.89 nM (X4 strain NL4-3) in PBMCs, TAF weakly inhibited both herpes simplex virus type 2 strains (KW [clinical isolate] and MS [laboratory isolate] in MAGI R5 cells) with EC_{50} value ranges of 424 to 697 nM and 146 to 278 nM, respectively. In addition, TAF also exhibited activity against parainfluenza virus type 3 (strain C243 in Vero cells) with an EC_{50} value of 843 nM. No cytotoxicity up to 1,000 nM (highest concentration tested) was observed for these evaluations. Of note, TAF has activity against HIV-1 but TAF treatment of patients co-infected with HBV and HIV-1 in the absence of antiretroviral therapy is not recommended due to the potential development of HIV-1 resistance.

2.3. Cytotoxicity

In Study PC-120-2007, the cytotoxicity of TAF was evaluated in HepG2 cells (hepatic-derived)

and in two T-lymphoblastoid cell lines (MT-2 and MT-4 cells, both $CD4^+$ human T-cell lines that has been transformed with human HTLV-1) following 5 days of exposure. According to the applicant, HepG2 cells utilized in this study were adapted to grow in glucose-free galactose-containing medium so that the cells have a relatively higher sensitivity to inhibitors of mitochondrial oxidative phosphorylation compared to the same cells maintained in standard glucose-containing culture medium (Marroquin *et al*., 2007). Cell viability was measured using the CellTiter-Glo® luminescent cell viability assay (Promega): CC_{50} values were determined as the compound concentration that caused a 50% decrease in the luminescence signal, a measure of toxicity, and calculated by non-linear regression using Pipeline Pilot software (Accelrys).

Results are summarized in Table 3. TAF had no observed cellular toxicity up to the highest tested concentration in HepG2 cells with a CC_{50} value of >44.4 μM, as observed with TFV and TDF (Table 3; Cihlar *et al*., 2002; Schinazi *et al*., 2012). Puromycin (PUR), a protein synthesis inhibitor, was used as the positive control for cytotoxicity in this assay and yielded the CC_{50} value of 1 μM, consistent with historical values of 0.8 - 1.1 μM. In T cells, CC_{50} values for TAF ranged from 23.2 (MT-4) to >53.0 μM (MT-2), comparable to TDF (22.9 to 37.1 μM) but significantly lower than the parent drug TFV (6,264 to 7,605 μM).

Table 3: Cytotoxicity Evaluation of TAF

			Cytotoxicity CC_{50}, μM (MSD)[a]		
			Hepatic	T-Cell	
Compound	Class	Abbrev.	HepG2	MT-2	MT-4
GS-007340	NtRTI	TAF	>44.4 (1)	>53.0 (1)	23.2 (1.13)
GS-004331	NtRTI	TDF	>44.4 (1)	37.1 (1.02)	22.9 (1.04)
GS-001278	NtRTI	TFV	>44.4 (1)	7605 (1.06)	6264 (1.13)
GS-009019	NRTI	FTC	>44.4 (1)	>53.0 (1)	>53.0 (1)
GS-281429	NRTI	3TC	>44.4 (1)	>53.0 (1)	>53.0 (1)
GS-281430	NRTI	ABC	>44.4 (1)	40.7 (1.02)	>53.0 (1)
GS-278805	NRTI	AZT	>44.4 (1)	>53.0 (1)	>53.0 (1)
GS-278801	NRTI	ddI	>44.4 (1)	>53.0 (1)	>53.0 (1)
GS-278802	NRTI	ddC	>44.4 (1)	>53.0 (1)	>53.0 (1)
GS-017437	NNRTI	EFV	10.1 (1.05)	25.4 (1.03)	26.4 (1.04)
GS-339793	INSTI	RAL	>44.4 (1)	>53.0 (1)	>53.0 (1)
GS-277980	PI	ATV	>44.4 (1)	>53.0 (1)	>53.0 (1)
GS-444198	Control	PUR[b]	1.0 (1.12)	0.4 (1.05)	0.2 (1.06)

Source: Study Report PC-120-2007, Table 1, page 11
[a] All cells were treated for 5 days. Cytotoxicity CC_{50} values represent the geometric mean of three independent experiments generated using 384-well assays. Multiplicative standard deviations (MSD) are shown in parenthesis.
[b] Puromycin (PUR) was used as a positive control in cytotoxicity assays.

Of note, in the similar experiment, 2 TAF metabolites, M18 (isopropylalaninyl TFV) and M28 (alaninyl TFV), also had no observed cellular toxicity up to the highest tested concentration (57 μM) in MT-2 and MT-4 cells (no data available for HepG2 cells; Study Report PC-120-2021).

These 2 evaluated TAF metabolites have been identified as intermediate metabolites during intracellular conversion of TAF to TFV (Pharmacology review by C. Wrzesinski, Ph.D. for NDA 207-561, 2014) (b) (4)

The cytotoxicity potential of TAF was also evaluated using the MTT assay in primary cultures of cryopreserved hepatocytes that were initially prepared from human livers (2 female and one male Caucasians; (b) (4)) by collagenase perfusion (Study Report AD-120-2032). Following 3 days of exposure to 1 μM TAF, the MTT assay showed no decrease of hepatocyte viability in all three hepatocyte lots, relative to vehicle control. However, decreases of >20% in viability were observed in the cells from one of the 3 lots cultured at TAF 10 μM and in those from all three lots at 100 μM: mean cell viabilities at 1, 10 and 100 μM TAF were 109, 76, and 58%, respectively. The more marked decrease in viability at 100 μM was consistent with the observed morphological changes at this concentration for all 3 lots of hepatocytes. Tamoxifen (50 μM), the positive control cytotoxic agent yielded the expected results with reduced viability of 1 to 3% across the lots.

In additional cell culture studies, TAF demonstrated a favorable toxicity profile. In the continuous incubation with TAF for 5 days of dividing and resting human PBMCs obtained from normal healthy volunteers (b) (4) average CC_{50} values were 6.8 μM (range: 3.5 - 9.3) to 25.1 μM (range: 6.8 - 40.1), respectively (Study Report PC-120-2009). In the continuous incubation with TAF for 14 days of normal human bone marrow light density cells obtained from 3 different pre-qualified frozen bone marrow lots (b) (4) (b) (4)), the extrapolated EC_{50} values were 3.3 μM for erythroid progenitor cell proliferation and >3 μM for myeloid progenitor cell proliferation (Study Report PC-120-2016). In the continuous incubation with TAF for 3 days of human proliferating osteoblast cells (b) (4), the CC_{50} value was 10.4 μM (Promega CellTiter-Glo® Luminescent Cell Viability assay; Study Report PC-120-2008).

HIV-1 patients treated with NRTIs have exhibited a range of clinical side effects including myopathy, sensory neuropathy, lactic acidosis, and hepatic steatosis (reviews by Apostolova *et al*., 2011; Maagaard and Kvale, 2009; and Gardner *et al*., 2014). It is believed that NRTI-induced mitochondrial toxicity plays a major role in these adverse symptoms. Incorporation of NRTIs into mitochondrial DNA (mtDNA) by the mitochondrial DNA polymerase γ and subsequent inhibition of mtDNA synthesis were suggested as the primary cause of NRTI-induced mitochondrial toxicity (Lee *et al*., 2003). The authors further observed that the toxic side effects of NRTIs are correlated with the kinetics of its incorporation by the mitochondrial DNA polymerase γ, varying over 6 orders of magnitude in the sequence zalcitabine (ddC) > didanosine (ddI) > stavudine (d4T) >> lamivudine (3TC) > tenofovir (PMPA) > zidovudine (AZT) > abacavir (ABC).

In Study PC-120-2006, the potential of TAF to affect mtDNA replication was evaluated in HepG2 cells. Cells were treated with TAF (0.1, 0.3, or 1.0 μM) for 10 days, and then the total DNA was extracted from the cells to quantify mtDNA by real-time PCR. As a positive control, cells were treated with ddC (0.2, 2.0, or 20.0 μM): previously, in HepG2 cells ddC at 1 μM decreased mtDNA levels by 85%, when treated for 14 days (Pan-Zhou *et al*., 2000). As summarized in Table 4, treatment of HepG2 cells with 0.1, 0.3, and 1.0 μM TAF for 10 days resulted in no significant reduction in mtDNA content, compared to the untreated DMSO control cells (DMSO

normalized to 1.0% were used for all treatments). In contrast, a parallel treatment with 0.2, 2.0, and 20.0 μM ddC resulted in a marked dose-dependent reduction in mtDNA levels: 17.6%, 88.5%, and 93.4% reductions in mtDNA content were observed, respectively, compared to the untreated DMSO control cells. Thus, these findings of no specific depletion of mtDNA observed in HepG2 cells by TAF at up to 1.0 μM (a level exceeding the maximum clinical systemic exposure of the 25 mg dose of TAF by 2-fold [C_{max} = 0.48 μM; Study Report GS-US-120-0104]), the applicant concluded that TAF has a low potential for inhibiting mtDNA synthesis and inducing NRTI-related mitochondrial toxicities. Previously, the low potential to interfere with mitochondrial functions was also reported for TFV, the parent drug of TAF (Virology review N021356.SE1-025; Birkus *et al*., 2002). In HepG2 cells following a 9-day treatment with TFV (up to 300 μM, greatly exceeding EC_{50} values for TFV ranging from 0.14 to 1.5 μM in HepG2 cells), no effects were observed on the synthesis of mtDNA. Similar effects including no depletion of mtDNA by TFV were observed in skeletal muscle cells, both proliferating and quiescent non-proliferating, following 9-day incubation with up to 300 μM and a 3-week treatment, respectively.

Table 4: Effects of TAF on Mitochondrial DNA (mtDNA) Content in HepG2 Cells

Compound	Concentration (μM)	Relative Amount of mtDNA (% mtDNA)[a]	*p*-value compared to DMSO (Control)[b]
DMSO (control)	-	100.0 ± 15.3	-
GS-7340	0.1	86.4 ± 30.5	0.190
	0.3	88.1 ± 35.5	0.294
	1.0	94.6 ± 17.3	0.318
ddC	0.2	86.7 ± 24.2	0.127
	2.0	11.5 ± 6.2	**< 0.0001**
	20.0	6.6 ± 1.5	**< 0.0001**

Source: Study Report PC-120-2006, Table 3, page 8.
[a] The data represent the mean ± SD of 3 independent experiments performed in triplicate.
[b] Paired, two-tailed Student's t-test.

2.4. Resistance and Cross-Resistance in Cell Culture

In the absence of a robust tissue culture system that allows for sustained HBV propagation, the applicant did not perform cell-based resistance selection experiments against TAF. Typically, the nonclinical resistance profile of TAF (or TDF and parent TFV) would be studied in a series of dose-escalation resistance selection experiments in HBV-infected cells. The effect of increasing selective drug pressure on the wild-type HBV would be assessed by analyzing the genotypic and phenotypic changes in the propagating virus over time. However, despite the advent of various model systems to characterize the HBV replication cycle, primary human hepatocytes retain susceptibility for infection for only a short time and hepatoma cell lines are not susceptible to infection due to lack of receptor expression (reviewed by Zeisel *et al*., 2015).

Since the antiviral activity of TAF and TDF are driven by the same active entity, TFV-DP, TAF is expected to have a similar resistance profile to that of TDF. For HIV-1, TAF's cell-based HIV-1

resistance profile is comparable to the resistance profile of the parent nucleotide TFV (Margot *et al.*, 2015). In two Phase 3 pivotal clinical studies, GS-US-174-0102 and GS-US-174-0103, of TDF (VIREAD®), TDF genotypic resistance testing on virus samples from subjects with evidence of virologic failure (HBV DNA ≥400 copies/mL) through Week 384 identified no specific amino acid substitutions in the HBV rt domain that occurred at sufficient frequency to establish an association with genotypic TDF resistance (Virology review N021356.943; Corsa *et al.*, 2014; Marcellin *et al.*, 2014).

Cross-resistance studies for TAF were carried out in HepG2 cells against a panel of HBV variants bearing amino acid substitutions in the HBV rt domain that confer clinically-relevant resistance to adefovir, entecavir, and lamivudine (Study Report PC-320-2007). In this study, 11 drug resistant variants were created in a treatment-naïve subject-derived full-length HBV genotype D clone (under the transcriptional control of a CMV immediate-early promoter; Zhu *et al.*, 2007) by site-directed mutagenesis (QuikChange™ site-directed mutagenesis kit, Stratagene). All variants along with the wild-type parental isolate were tested for susceptibility to TAF and TFV (in addition, some for susceptibility to adefovir [active moiety of the diester prodrug adefovir disoproxil], entecavir, or lamivudine) in a transient transfection assay: on Day 7 post-transfection, the intracellular HBV DNA intermediates were extracted from transfected HepG2 cells that had been maintained in media containing serial dilutions of each drug and then quantified by real-time PCR as described by Zhu *et al.* (2011). The inter-assay variability observed in EC_{50} values was within 2-fold in this antiviral phenotypic assay. Results are summarized in Table 5.

Table 5: Antiviral Susceptibility of HBV Variants Expressing rt Substitutions Associated with Resistance to HBV NrtIs

	Mutations	TAF EC_{50} (nM)	TAF Fold Change	TFV EC_{50} (μM)	TFV Fold Change
wild-type	5508-WT	99.1 ± 32.7	1.0	14.4 ± 5.1	1.0
ADV-R	rtA181T/sW172*[a]	166.8 ± 32.1	1.7	13.1 ± 5.7	0.9
	rtA181T/sW172L	107.3 ± 38.3	1.1	7.8 ± 1.6	0.6
	rtA181V	118.8 ± 46.5	1.2	13.7 ± 3.9	1.0
	rtN236T	142.8 ± 70.4	1.4	8.8 ± 2.6	0.6
	rtA181V+rtN236T	364.7 ± 45.8	3.7	40.5 ± 4.9	2.8
LAM-R	rtM204I	161.9 ± 15.4	1.6	25.3 ± 0.8	1.8
	rtL180M+rtM204V	176.4 ± 35.5	1.8	21.3 ± 1.3	1.5
	rtV173L+rtL180M+rtM204V	85.4 ± 36.5	0.9	23.7 ± 0.5	1.6
ETV-R	rtL180M+rtM204V+rtT184G	164.4 ± 70.7	1.7	17.7 ± 1.5	1.2
	rtL180M+rtM204V+rtS202G	152.0 ± 17.7	1.5	13.1 ± 0.4	0.9
	rtL180M+rtM204V+rtM250V	114.7 ± 42.0	1.2	20.9 ± 0.6	1.5

Source: Study Report PC-120-2007, Table 5, page 10.

[a] Two rtA181T variants were created, reflecting two possible changes to the overlapping surface gene (HBsAg). One mutation that encoded rtA181T encoded a stop codon at HBsAg amino acid position 172 (rtA181T/sW172*). The other mutation that also encoded rtA181T resulted in a substitution at HBsAg amino acid position 172 (rtA181T/sW172L).

All tested lamivudine- and entecavir-resistant variants (n=3 each) showed <2-fold reduced susceptibility to TAF, compared to the wild-type isolate. In addition, HBV isolates expressing

the rtA181T, rtA181V, or rtN236T single substitutions associated with resistance to adefovir also remained largely susceptible to TAF with <2-fold changes in EC_{50} values; however, the HBV isolate expressing the rtA181V plus rtN236T double substitutions exhibited reduced susceptibility (3.7-fold) to TAF, indicating some degree of cross-resistance between TAF and adefovir. Similar results were obtained against TFV with all but one isolate (expressing rtA181V plus rtN236T) demonstrating <2-fold changes in TFV susceptibility. As expected, all tested lamivudine- and entecavir-resistant variants exhibited significantly reduced susceptibility to lamivudine (>48.8-fold) and entecavir (>28.6-fold), respectively

2.5. Anti-HBV Activity in Drug Combination

Previous 2-drug combination studies in HepAD38 cells expressing stably HBV under the control of a tetracycline-inducible promoter (Lander *et al*., 1997) demonstrated TFV had additive anti-HBV activity when combined with other NrtIs, adefovir, emtricitabine, entecavir, lamivudine, and telbivudine (Virology review N021356.SE1-025). No cytotoxic or antagonistic effects were observed with any of the drug combinations tested. Similarly, using different HBV-expressing stable cell lines, an additive effect of TFV in combination with adefovir (in HepG2 49-29 cells; Delaney *et al*., 2004) or emtricitabine (in HepG2-CMV1.1HBV cells) was demonstrated.

The HCV NS3 PI telaprevir has been demonstrated to inhibit CatA activity in enzymatic assays (Murakami *et al*., 2010). Additionally, covalent HCV PIs boceprevir and telaprevir were shown to inhibit CatA-mediated TAF activation (IC_{50} values of 0.27 and 0.16 μM, respectively) in enzymatic assays, and also reduced the anti-HIV activity of TAF in primary human $CD4^+$ T cells (21- and 3-fold, respectively) at pharmacologically relevant concentrations (Birkus *et al*., 2016). In contrast, the authors noted little-to-no inhibition of CatA-mediated activation of TAF or no significant effect on the anti-HIV activity of TAF when combined with cobicistat, noncovalent HIV PIs (atazanavir, darunavir) and an HCV PI (simeprevir), or various prescribed inhibitors of host serine proteases (such as apixaban, argatroban, dabigatran, and sitagliptin).

In Study PC-320-2004, the potential effect of PIs on the anti-HBV activity of TAF was evaluated in 2-drug combination studies utilizing HepAD38 cells. The PIs evaluated in combination with TAF were the HIV PI darunavir, the HCV PIs boceprevir, simeprevir, and telaprevir, and the host serine PIs, apixaban and rivaroxaban (inhibitors of factor Xa), argatroban and dabigatran (inhibitors of thrombin), and sitagliptin (dipeptidyl peptidase 4 [DPP-4] inhibitor), as well as the ritonavir-derived pharmacokinetic enhancing agent cobicistat. Cells were exposed to compounds for 4 days and then used to quantify intracellular HBV DNA replicative intermediates by real-time PCR. TAF was tested at concentrations of 0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1,000, and 2,000 nM, while PIs were tested at their clinical C_{max} except for simeprevir, which was evaluated at 25% of the C_{max} due to observed cytotoxicity at C_{max} (consistent with previously observed CC_{50} values; Lin *et al*., 2009). Regression analyses of antiviral data were performed with TableCurve2D software and best fit equations were used to calculate EC_{50} values. At least 2 independent experiments in quadruplicate were performed to obtain an average TAF EC_{50} value. Fold change (FC) in TAF susceptibility was calculated as a ratio of the TAF EC_{50} value in the presence and absence of tested compounds. A Student t-test was used to determine if there were any significant changes in the TAF EC_{50} value in the presence of PIs when compared to TAF alone.

As summarized in Table 6, no significant differences (p values of >0.05) were observed in mean TAF EC_{50} values in presence of any PI compared to that in the absence of PIs (TAF only). In

addition, no significant differences (p values of >0.05) were observed for all tested compounds when comparing the percent cell viability in drug treated cells to untreated cells (CellTiter-Glo® Luminescent Cell Viability assay, Promega). Thus, at the concentrations tested, PIs appeared to have no antagonistic effects on the anti-HBV activity of TAF.

Table 6: Effect of Various Protease Inhibitors and Cobicistat on the Anti-HBV Activity of TAF in HepAD38 Cells

Inhibitor Class	Compound	C_{max}, μM	TAF EC_{50}, nM[a]	TAF EC_{50} FC in the Presence of PI[b]	EC_{50} p-Value[c]
TAF only			138.2 ± 24.4	1.00	1.000
HIV PI	Darunavir	8.90	126.1 ± 3.0	0.91 ± 0.02	0.560
HCV PI	Telaprevir	5.20	163.3 ± 27.0	1.18 ± 0.20	0.432
HCV PI	Boceprevir	3.30	172.5 ± 62.4	1.25 ± 0.45	0.544
HCV PI	Simeprevir	3.33[d]	100.7 ± 48.8	0.73 ± 0.35	0.434
Booster, CYP3A inhibitor	Cobicistat	2.20	117.3 ± 5.4	0.85 ± 0.04	0.359
Factor Xa inhibitor	Apixaban	0.17	116.2 ± 3.8	0.84 ± 0.03	0.336
Factor Xa inhibitor	Rivaroxaban	0.29	123.85 ± 7.8	0.90 ± 0.06	0.513
Thrombin inhibitor	Argatroban	0.39	131.0 ± 16.3	0.95 ± 0.12	0.762
Thrombin inhibitor	Dabigatran	0.40	141.2 ± 94.6	1.02 ± 0.68	0.969
DPP4 inhibitor	Sitagliptin	0.95	100.2 ± 3.3	0.73 ± 0.02	0.161

Source: Study Report PC-120-2004, Table 4, page 11.
[a] TAF EC_{50} value was determined from 2 independent experiments.
[b] Mean fold change in the TAF EC_{50} value was expressed as a ratio of the activity in the presence versus the absence of the tested PI.
[c] TAF EC_{50} value in presence of PI compared to TAF EC_{50} value in absence of PI, Student t-test.
[d] 25% of clinical C_{max}

The main enzyme, CES1, responsible for the initial step of TAF intracellular metabolic activation in human primary hepatocytes was reported to be also involved in sofosbuvir activation in the liver (Murakami *et al*., 2010). Sofosbuvir (SOVALDI®) is a marketed HCV nucleotide analog NS5B polymerase inhibitor. In a cell-based combination antiviral activity study (Study Report PC-320-2001) in Huh-7-derived cells stably expressing HCV genotype 1a subgenomic replicons (Robinson *et al*., 2010), the presence of TAF (1 μM) did not impact the anti-HCV activity of sofosbuvir. Approximately 1.3-fold increase in the mean EC_{50} value of sofosbuvir was observed compared to that of sofosbuvir alone; CC_{50} values were not impacted by the presence of TAF (Anaspec Calcein AM cell viability assay). The combination effect of TAF and sofosbuvir on the TAF anti-HBV activity was not evaluated.

3. Clinical Virology

Clinical virology analyses were conducted to evaluate the development of TAF resistance and the antiviral potency of TAF monotherapy (25 mg QD) in adult subjects with CHB, treatment-naïve (TN) or treatment-experienced (TE), in two active-controlled Phase 3 studies of HBeAg-negative (GS-US-320-0108) and HBeAg-positive (GS-US-320-0110) subjects (referred to Studies 108 and 110, respectively, in this review). In these two similarly designed, noninferiority studies, the comparator groups received TDF 300 mg QD (VIREAD®). A brief description of the

trial design of each study is provided in Table 7. Assays for HBV DNA quantification (Roche COBAS® TaqMan® HBV Test for Use with the High Pure System) and for resistance testing that were utilized in these studies are described in Appendix 1 A1.1 and A1.3, respectively.
In Studies 108 and 110, the primary efficacy endpoint was the proportion of subjects with plasma HBV DNA <29 IU/mL (approximately 169 copies/mL) at Week 48. A missing=failure (M=F) approach was used. A plasma HBV DNA level of 29 IU/mL represents the lower limit of quantification [LLOQ] of the polymerase chain reaction (PCR) assay employed (Roche COBAS Taqman HBV Test for Use with the High Pure System; Allice *et al*., 2007). The Week-48 analysis was conducted after the last subject completed the Week-48 visit (or prematurely discontinued study drugs before their Week-48 visit). All data collected by the Week-48 data cutoff date (September 18, 2015 for Study 108 and November 07, 2015 for Study 110) except dual-energy x-ray absorptiometry and laboratory data collected up to the data finalization date (October 01, 2015 for Study 108 and November 17, 2015 for Study 110) were included in this original NDA submission.

Table 7: Overview of Study Designs of Studies 108 and 110

Common study design features		
Study population	- Documented evidence of chronic HBV infection (e.g., HBsAg positive for >6 months) - Screening HBV DNA ≥2 x 10^4 IU/mL - TN subjects (defined as <12 weeks of oral antiviral treatment with any nucleos(t)ide analogue including TDF or TAF) or TE subjects (defined as ≥12 weeks of previous treatment with ADV and/or nucleoside analogues CLV, ETV, LAM, or LdT) - Previous IFN treatment (pegylated or non-pegylated) that had ended at least 6 months prior to the baseline visit - No co-infection with HCV, HIV or HDV	
Active comparator	TDF 300 mg	
Stratified randomization	- 2:1 ratio to receive either TAF 25 mg QD or TDF 300 mg QD - Stratified by plasma HBV DNA level (<7, ≥7 to <8, ≥8 $\log_{10}$ IU/mL for Study 108; <8 and ≥8 $\log_{10}$ IU/mL for Study 110) and oral antiviral treatment status (TN versus TE)	
Duration of treatment	- Double-blind phase to Week 144 - Open-label extension phase to Week 384	
Primary efficacy endpoint	Proportion of subjects with HBV DNA <29 IU/mL (approximately 169 copies/mL) at Week 48	
Study-specific design features		
Study	108	110
HBeAg status at screening	Negative	Positive
Number of subjects treated	425 (285 TAF and 140 TDF)	873 (581 TAF and 292 TDF)

3.1. Development of TAF Resistance

To identify treatment-emergent genotypic changes in the HBV polymerase rt domain (344 amino acids) that are potentially associated with virologic resistance to TAF, Studies 108 and 110 are designed to perform annual drug resistance genotypic testing (viral population-based nucleotide sequencing assay) on paired virus samples (pre-treatment and on-treatment) collected from resistance testing eligible subjects. Resistance testing eligible subjects are those who remain viremic with HBV DNA ≥69 IU/mL (approximately 400 copies/mL) (1) at the end of each study year (e.g., Weeks 48 and 96) or (2) at the last on-treatment visit (only for those who

discontinued treatment at/after Week 24 but before the end of each study year). Of note, cumulative genotypic resistance analyses (up to 8 years) of virus samples from viremic subjects in several TDF clinical studies (including pivotal Phase 3 studies of HBeAg-negative [GS-US-174-0102] and HBeAg-positive [GS-US-174-0103] subjects) identified no specific amino acid substitutions in the HBV rt domain that occurred at sufficient frequency to establish an association with genotypic TDF resistance (US Prescribing Information for VIREAD, revised in February, 2016; Virology review N021356.943).

In this Week-48 genotypic resistance analysis, as proposed in the draft Virology Analysis Plan (PC-320-2002; Virology review I115561.055.059), subjects having HBV DNA ≥69 IU/mL at Week 48 in the absence of virologic breakthrough or subjects experiencing virologic breakthrough at Week 48 (last study visit prior to the data finalization cutoff date for the Week-48 analysis; the Week-48 virologic breakthrough was thus not confirmed) were not included. The Week-48 samples from these subjects will be included in the Week-96 resistance analysis if they remain viremic at Week 96. Virologic breakthrough is defined as having HBV DNA ≥69 IU/mL after HBV DNA levels <69 IU/mL being achieved or having HBV DNA increase by ≥1 $\log_{10}$ IU/mL from nadir. Two consecutive visits that meet the definition were required for a subject to be classified as having virologic breakthrough.

The rationale to delay resistance analysis of viremic subjects with HBV DNA ≥69 IU/mL at Week 48 in the absence of virologic breakthrough until Week 96 is based on the virology results noted in phase 3 studies evaluating TDF in subjects with CHB (Gordon *et al*., 2013). The authors observed in Studies GS-US-174-0102 and GS-US-174-0103 subjects with high baseline viral load (HBV DNA ≥9 $\log_{10}$ copies/mL) were found to require a longer time course to suppress HBV DNA to <400 copies/mL (using the Roche COBAS® TaqMan® HBV Test for Use with the High Pure System; LLOQ of 169 copies/mL [29 IU/mL]), compared to those with low baseline viral load (HBV DNA <9 $\log_{10}$ copies/mL); however, the percentage of subjects achieving HBV DNA <400 copies/mL by Week 96 was similar between the two groups. Similar observations were made in a long-term study evaluating entecavir in CHB subjects with a median baseline viral load of approximately 10^{10} IU/mL, where the proportion of subjects achieving HBV DNA <300 copies/mL (using the Roche COBAS® Amplicor PCR assay, version 2.0; LLOQ of 300 copies/mL [57 IU/mL]) continued to increase through 240 weeks of treatment (Chang *et al*., 2010).

As summarized in Table 8 (FDA analysis), a total of 1,270 subjects received TAF or TDF for ≥24 weeks (Resistance Analysis Population: 850 TAF and 420 TDF), and 230 of them (158 TAF and 72 TDF) had HBV DNA ≥69 IU/mL at Week 48 (during the Week-48 evaluation window, Treatment Days between 322 and 363, inclusive) or at the last evaluable visit before Week 48. Among these 230 Week-48 virologic failures (VFs) with HBV DNA ≥69 IU/mL, 41 subjects (27 TAF and 14 TDF) were identified to be eligible for Week-48 resistance testing per virology analysis plan (Appendix 2 Table A2 summarizes available genotypic and phenotypic resistance data from 41 subjects and Appendix 3 describes briefly their virological outcomes during 48 weeks of treatment).

Of those 41 Week-48 resistance testing eligible subjects, 33 experienced virologic breakthrough by Week 48 (20 TAF and 13 TDF) and 8 never suppressed HBV DNA levels to <69 IU/mL without evidence of virologic breakthrough at the time of early discontinuation (7 TAF and 1 TDF). The remaining 189 Week-48 VFs with HBV DNA ≥69 IU/mL (131 TAF and 58 TDF; not eligible for Week-48 resistance analysis per virology analysis plan) include 170 subjects who

never achieved HBV DNA levels of <69 IU/mL with no observed episodes of virologic breakthrough (117 TAF and 53 TDF), 8 subjects with unconfirmed virologic breakthrough observed at Week 48 (last evaluable visit; 7 TAF and 1 TDF), and 11 subjects with a transient viral increase at Week 48 to ≥69 IU/mL (HBV DNA <69 IU/mL was observed at Weeks 44 and 56; 7 TAF and 4 TDF). The Week 48 genotype of these subjects will be assessed when the Week 96 efficacy results are available as subjects with reduced response may suppress with longer treatment. Of particular interest are subjects with prior NrtI experience as the Week-48 virologic response indicates some degree of cross-resistance.

Table 8: Number of Subjects Evaluated for Week-48 Genotypic Resistance Analysis

Study	108		110	
Study treatment	TAF	TDF	TAF	TDF
As-treated population[1]	285	140	581	292
Resistance analysis population[2]	**281**	**136**	**569**	**284**
Week-48 VFs with HBV DNA ≥69 IU/mL[3]	**7** (2.5%)	**3** (2.2%)	**151** (26.5%)	**69** (24.3%)
Week-48 resistance testing eligible VFs with HBV DNA ≥69 IU/mL (per virology analysis plan)[4]	**2**	**2**	**25**	**12**
• experienced virologic breakthrough[5]	2	1	18	12
• discontinued early (before Week 48) without experiencing virologic breakthrough[5]	0	1	7	0
Week-48 resistance testing eligible VFs (HBV DNA ≥69 IU/mL) with paired genotypic data	**2**	**0**	**18**	**10**
• detectable treatment-emergent rt substitutions	0	-	5	4
• no detectable treatment-emergent rt substitutions	2	-	13	6
Week-48 VFs with HBV DNA ≥69 IU/mL who were excluded from Week-48 resistance analysis (per virology analysis plan)[4]	**5**	**1**	**126**	**57**
• without experiencing virologic breakthrough[5]	4	0	113	53
• unconfirmed virologic breakthrough[5] observed at Week 48	1	0	6	1
• had a transient viral increase at Week 48[6]	0	1	7	3

[1] As-treated population includes all subjects who were randomized into the study and received at least one dose of study drugs.

[2] Resistance analysis population includes subjects who received ≥24 weeks of study treatment.

[3] Week-48 virologic failures (VFs) with HBV DNA ≥69 IU/mL are those who were included in the resistance analysis population and had HBV DNA ≥69 IU/mL at Week 48 (during the Week-48 evaluation window, Treatment Days between 322 and 363, inclusive) or at the last evaluable visit before Week 48.

[4] Week-48 resistance testing eligible virologic failures with HBV DNA ≥69 IU/mL are those who were eligible for Week-48 resistance testing. As proposed in the draft virology analysis plan, Week-48 resistance testing was conducted for those, among the Week-48 VFs with HBV DNA ≥69 IU/mL, who experienced confirmed virologic breakthrough by Week 48 or who never suppressed HBV DNA levels to <69 IU/mL without evidence of virologic breakthrough at the time of early discontinuation (before the Week-48 visit).

[5] Virologic breakthrough is defined as having HBV DNA ≥69 IU/mL after HBV DNA levels <69 IU/mL being achieved or having HBV DNA increase by ≥1 $\log_{10}$ IU/mL from nadir. Two consecutive visits that meet the definition were required for a subject to be classified as having virologic breakthrough.

[6] Transient viral increase at Week 48 is defined as a transiently observed viral load increase to ≥69 IU/mL at Week 48 after attaining <69 IU/mL, which returns to <69 IU/mL at Week 56 without change in therapy.

Out of the 41 virologic failure subjects with HBV DNA ≥69 IU/mL who were identified to be eligible for Week-48 resistance testing (Week-48 resistance testing eligible VFs with HBV DNA ≥69 IU/mL, Table 8; 27 TAF and 14 TDF), the HBV rt amino acid sequences of treatment-failure isolates, in addition to their baseline isolates, were obtained from 30 subjects (20/27 TAF and 10/14 TDF; Table 8) including 24/33 subjects who experienced virologic breakthrough (14/20 TAF and 10/13 TDF). The rt deduced amino acid sequences of virus samples collected at Week 48 (or at the last on-treatment visit, if discontinued prior to Week 48) from these 30 subjects were compared to the sequences present at their baseline isolates to identify treatment-emergent genotypic changes in HBV rt. This analysis employed direct nucleotide sequence analysis of the HBV rt domain (344 amino acids) that was amplified from the subject sera by PCR as described in Appendix 1 A1.3. This HBV rt population-based sequencing assay can detect nucleotide mixtures when present in a population at approximately 25%.

When paired baseline and on-treatment samples from the 30 Week-48 resistance testing eligible VFs with HBV DNA ≥69 IU/mL were compared, treatment-emergent amino acid substitutions were observed in 9 subjects' failure isolates (30% [9/30]; 5 TAF and 4 TDF, all are HBeAg positive; Table 8). No evidence of rt genotypic changes was observed in the majority (70% [21/30]) of evaluated subjects (15 TAF and 6 TDF), including 5 of the 6 subjects who discontinued from the study before Week 48 without experiencing virologic breakthrough and had evaluable genotypic data. It should be noted that TAF or TDF treatment-emergent substitutions in the HBV rt domain were observed in 23.5% (4/17) of HBV NrtI-naïve subjects evaluated and 38.5% (5/13) of evaluated subjects with prior NrtI exposure. Brief virology histories (HBV DNA and resistance profiles) of those 9 VF subjects who had HBV variants harboring genotypic changes in HBV rt emerged while on TAF or TDF are described below (Figures 5 and 6; see also Appendix 2 Table A2):

- Subject 2826-4527 (Figure 5), previously exposed to TAF with genotype C infection, was randomized to the TAF group, and had baseline viruses (8.24 $\log_{10}$ IU/mL) showing mixed HBV rt sequences, wild-type and rtA194T detectable by INNO-LiPA (positive signals for A194 and T194). This subject had a continual decline in HBV DNA until Week 36 (203 IU/mL) when she discontinued the study (withdrew consent). Overall, the subject achieved a >5 $\log_{10}$ IU/mL decline during 36 weeks of treatment. Sequence analysis of the Week 36 sample identified 2 polymorphic site rt substitutions (rtD134E and rtM309K). The rtA194T substitution detected at baseline by INNO-LiPA was not detectable by population-based sequencing at baseline or Week 36. This subject's baseline sample was not subjected to Next Generation Sequence analysis to confirm the presence of an rtA194T substitution. The subject was 100% adherent to the study drug regimen through early discontinuation based on pill count.

- Subject 4296-5147 (Figure 5), previously exposed to TDF with genotype C infection, was randomized to the TAF group, and had baseline viruses (4.62 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA through Week 16 (2.51 $\log_{10}$ IU/mL), and then experienced virologic breakthrough at Week 24 (3.77 $\log_{10}$ IU/mL) which was maintained through Week 48 (4.68 $\log_{10}$ IU/mL). Sanger sequence analysis of the baseline and Week 48 samples identified one polymorphic site substitution (rtS256S/C). This subject was 99% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 8017-4565 (Figure 5), previously exposed to lamivudine, telbivudine, and IFN with genotype D infection, was randomized to the TAF group, and had baseline viruses (8.21 $\log_{10}$

IU/mL) showing mixed HBV rt sequences, wild-type and rtL80I and rtM204I detectable by INNO-LiPA (positive signals for L80, I80, M204, and I204). This subject achieved virologic suppression with HBV DNA to <29 IU/mL by Week 32. At the next visit (Week 36), the subject experienced virologic breakthrough with HBV DNA 3.58 $\log_{10}$ IU/mL and remained >69 IU/mL through Week 48 (411 IU/mL; declining HBV DNA levels from Weeks 36 to 48 were observed). Sequence analysis of the Week 48 sample identified 2 polymorphic site substitutions (rtI91I/L and rtE271A/E). Those lamivudine-R substitutions rtL80I and rtM204I detected at baseline by INNO-LiPA persisted through TAF treatment; with population-based sequencing, wild-type viruses with rtL80 and/or rtM204 were not seen (only detecting rtI80 and rtI204) at baseline but at Week 48, mixed populations of wild-type and lamivudine-R variants were detected (detecting mixtures L/I80 and M/I204), possibly due to the outgrowth of or reversion to wild-type virus in the absence of lamivudine (received prior to TAF monotherapy). This subject was 99.7% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 8758-5188 (Figure 5), treatment-naïve with genotype D infection, was randomized to the TAF group, and had baseline viruses (4.74 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved HBV DNA levels <69 IU/mL by Week 24, and experienced virologic breakthrough at Week 36 (3.12 $\log_{10}$ IU/mL) which was maintained through Week 48 (91 IU/mL). Sanger sequence analysis of the baseline and Week 48 samples identified one polymorphic site substitution (rtR153Q). This subject was 97% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 9035-5187 (Figure 5), previously exposed to entecavir with genotype C infection, was randomized to the TAF group, and had baseline viruses (4.94 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved HBV DNA <69 IU/mL by Week 24, which was maintained until Week 40 (38 IU/mL). The subject then experienced virologic breakthrough at Weeks 44 - 48 (ranging from 148 to 161 IU/mL). Sanger sequence analysis of the baseline and Week 48 samples identified 4 polymorphic site substitutions (rtS13S/N, rtS117S/P, rtL267L/Q, and rtL269L/I). This subject was 99.9% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

Figure 5: HBV DNA and ALT Profiles for 5 TAF Recipients Having HBV Variants with Detectable Treatment-Emergent rt Substitutions

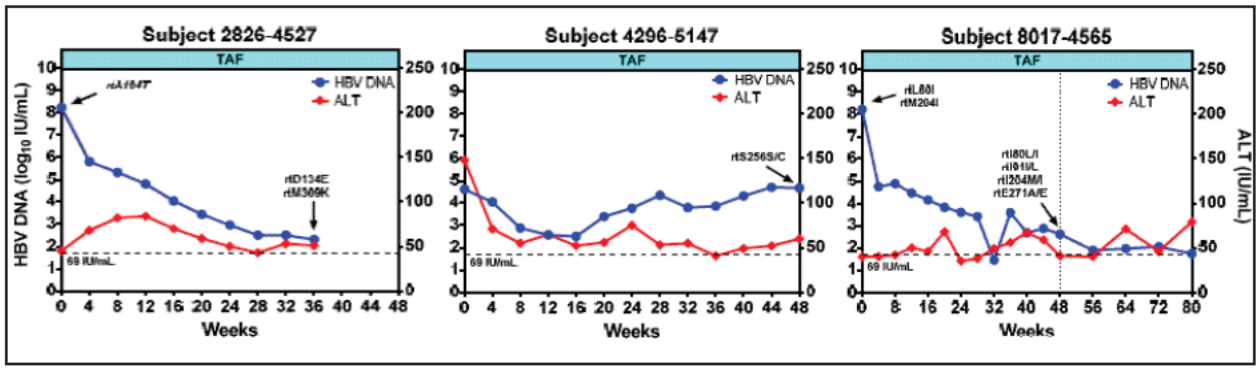
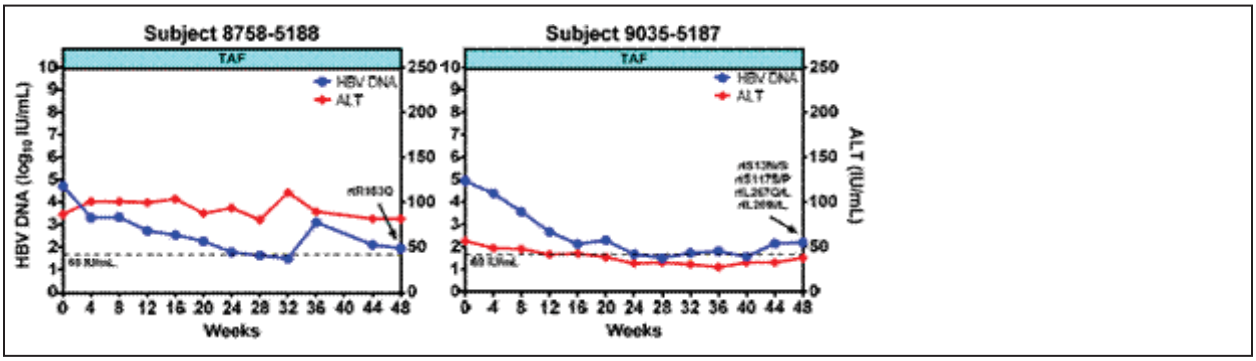


Figure 5-continued



Source: Study report PC-320-2009, Figure 3, pages 32-34.

- Subject 1507-4546 (Figure 6), treatment-naïve with genotype C infection, was randomized to the TDF group, and had baseline viruses (8.07 $\log_{10}$ IU/mL) showing mixed HBV rt sequences, wild-type and rtT184I/L/F/M detectable by INNO-LiPA (positive signals for T184 and I/L/F/M184). This subject had a continual decline in HBV DNA through Week 40 (87 IU/mL) and then experienced virologic breakthrough at Week 44 (4.71 $\log_{10}$ IU/mL) which was maintained through Week 48 (3.0 $\log_{10}$ IU/mL). Sanger sequence analysis of the baseline and Week 48 samples identified 3 polymorphic site substitutions (rtD134D/E, rtV214V/A, rtA317A/S). The rtT184I/L/F/M entecavir-R substitution detected at baseline by INNO-LiPA was not detectable by population-based sequencing at baseline or Week 48. The subject was 98.8% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

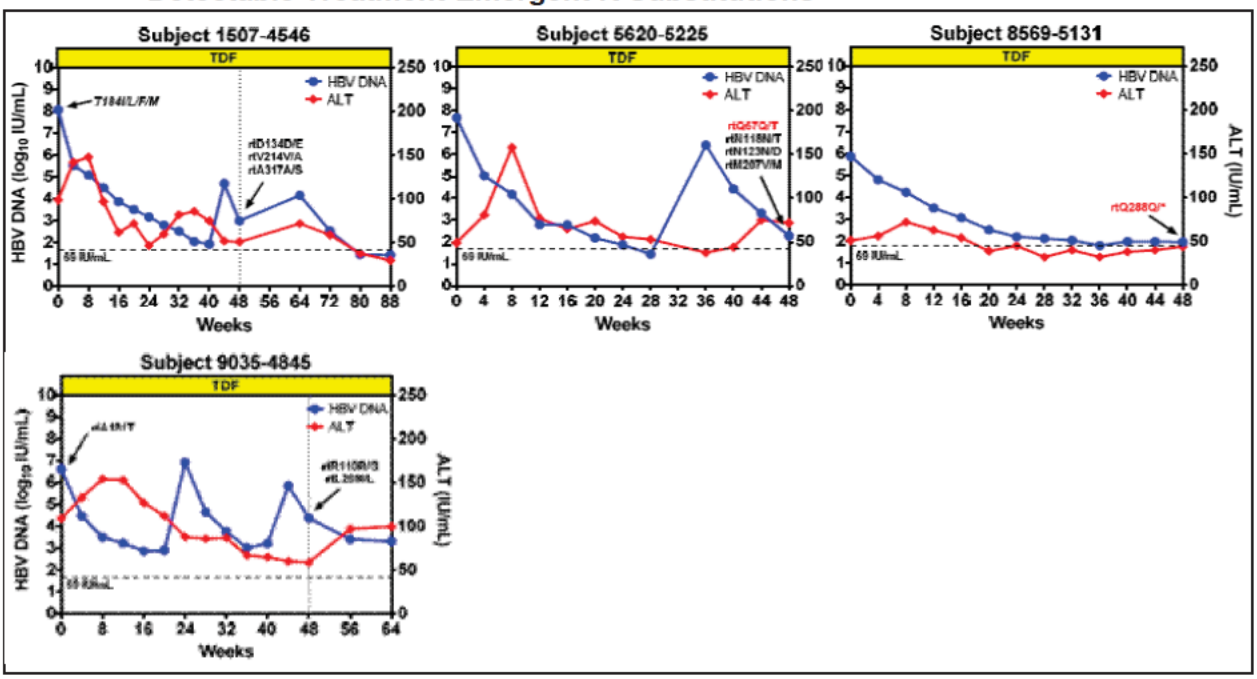
- Subject 5620-5225 (Figure 6), treatment-naïve with genotype B infection, was randomized to the TDF group, and had baseline viruses (7.68 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved virologic suppression (HBV DNA <29 IU/mL) by Week 28. The subject missed the Week 32 visit and experienced virologic breakthrough at Week 36 (6.43 $\log_{10}$ IU/mL), which was maintained with declining HBV DNA levels through Week 48 (193 IU/mL). Sanger sequence analysis of the baseline and Week 48 samples identified one conserved site substitution (rtQ67Q/H) and 3 polymorphic site substitutions (rtN118N/T, rtN123N/D, rtM207M/V). This subject was 99.8% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 8569-5131 (Figure 6), treatment-naïve with genotype D infection, was randomized to the TDF group, and had baseline viruses (5.87 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA levels and achieved HBV DNA <69 IU/mL at Week 36. The subject experienced virologic breakthrough at Week 40 (98 IU/mL) which was maintained through Week 48 (92 IU/mL). Sanger sequence analysis of the baseline and Week 48 samples identified one conserved site substitution (rtQ288Q/stop). The applicant reasoned the introduction of a stop codon at position 288 in the HBV rt domain is unlikely to be enzymatically active and may represent a sequencing artifact. This subject was 99.7% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 9035-4845 (Figure 6), previously exposed to adefovir, entecavir, and lamivudine with

genotype C infection, was randomized to the TDF group, and had baseline viruses (6.62 log_{10} IU/mL) showing mixed HBV rt sequences, wild-type and rtA181T detectable by INNO-LiPA (positive signals for A181 and T181). This subject had a continual decline in HBV DNA through Week 20 (2.91 log_{10} IU/mL), and then experienced 2 episodes of virologic breakthrough at Weeks 24 - 28 (ranging from 4.66 to 6.92 log_{10} IU/mL) and at Weeks 44 - 48 (ranging from 4.38 to 5.86 log_{10} IU/mL). Sanger sequence analysis of the baseline and Week 48 samples identified 2 polymorphic site substitutions, rtR110R/G and rtL269I/L. The adefovir-R substitution rtA181T detected at baseline (also by population-based sequencing) persisted through TDF treatment. This subject was 99.7% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

Figure 6: HBV DNA and ALT Profiles for 4 TDF Recipients Having HBV Variants with Detectable Treatment-Emergent rt Substitutions



Source: Study report PC-320-2009, Figure 4, pages 39-40.
HBV rt conserved-site substitutions are written in red.

As summarized Table 9, during TAF or TDF monotherapy, emergence of rt substitutions were observed occurring at 2 conserved amino acid positions (Q67H and Q288stop, written in red; both were seen in the TDF treatment-failure isolates) and at 16 polymorphic amino acid positions (S13N, I91L, S117P, D134E, R153Q, S256C, L267Q, L269I, E271A, and M309K were seen in the TAF treatment-failure isolates; and R110G, N118T, N123D, D134E, M207V, V214A, L269I, A317S were seen in the TDF treatment-failure isolate). Overall, no TAF-treatment failure subjects evaluated were found to develop HBV variants with conserved site substitutions during the first year of the study; and there were no polymorphic site substitutions detected in more than one subject's failure isolate. Two TDF-treatment failure subjects developed HBV variants with a unique conserved site substitution but there were no conserved or polymorphic site substitutions detected in more than one subject's failure isolate during the first year of TDF monotherapy. The location of polymorphic and conserved sites in the HBV rt domain was identified by comparing amino acid sequences from 628 subjects with available baseline data in

TDF clinical studies GS-US-174-0102 and GS-US-174-0103. Conserved sites were defined as those positions where only one amino acid was found, or two amino acids was present and the prevalence of the minority amino acid was <1%; all other positions within the HBV rt domain were considered polymorphic sites (Kitrinos *et al*., 2014). Overall, 64% (220/344) of amino acid positions were classified as conserved with the remaining 36% (124/344) classified as polymorphic.

It should be noted that, based on the population-based nucleotide sequencing results, no failure isolates (from TAF or TDF treated subjects) developed during therapy well-characterized rt substitutions that have been reported to be associated with HBV NrtI resistance (L80V/I, V84M, I169T, V173L, L180M, A181S/T/V, T184A/F/G/I/L/S, A194T, S202G/I, M204I/S/V, Q215S, L229V/W, I233V, N236T, and M250V; reviews by Gupta *et al*., 2014; Keeffe *et al*., 2008; Lok *et al*., 2007; Michailidis *et al*., 2012; and Rhee *et al*., 2010; substitutions reported to be associated with tenofovir resistance are underlined). Among other additional rt substitutions that were also implicated in HBV NrtI resistance (including L82M, S85A, A200V, V214A, P237H, and N238T/D; substitutions reported to be associated with tenofovir resistance are underlined), emergence of the rtV214A substitution was noted in one TDF-treated subject's Week-48 virologic failure isolate (with 2 other emerging rt substitutions D134D/E and A317A/S; Subject 1507-4546, Figure 6 and Table 9). No phenotypic data for the Week-48 isolate are available. Subject 1507-4546, genotype C-infected treatment-naïve, remains on study in the TDF treatment group with the last evaluable viral load data from the Week-88 visit (last study visit prior to the data finalization cutoff date for the Week-48 analysis) and achieved virologic suppression (HBV DNA <29 IU/mL) at Week 88 (Figure 6). The rtV214A substitution was considered as a secondary rt substitution associated with adefovir and TDF resistance (reviews by Ghany and Liang, 2007; and Michailidis *et al*., 2012). In addition to the valine to alanine substitution at 214 (rtV214A), emergence of several different amino acid substitutions at this residue (rtV214E/I/L/P) were also observed in entecavir-treatment failure subjects' virus samples (review by Virology Reviewer Takashi E. Komatsu, Ph.D. for NDA 21797 SDN 869 for details).

Table 9: Summary of Genotypic and Phenotypic Resistance Data

Subject ID	HBV genotype	Prior treatment[1]	Study treatment	Baseline HBV DNA[2]	Resistance testing		
					Isolate (HBV DNA[2])	Treatment-emergent rt substitution[3]	Drug susceptibility[4] (fold-change[5])
2826-4527	C	TAF	TAF	8.24	Week 36 (2.31)	D134E, M309K	nd
4296-5147	C	TDF	TAF	4.62	Week 48 (4.68)	S256S/C	nd
8017-4565	D	LAM, LdT	TAF	8.21	Week 48 (2.61)	I91I/L, E271A/E (I80I/L), (I204I/M)	nd
8758-5188	D	NrtI-naïve	TAF	4.74	Week 48 (1.96)	R153Q	nd
9035-5187	C	ETV	TAF	4.94	Week 48 (2.21)	S13S/N, S117S/P, L267L/Q, L269L/I	0.7 (0.8)
1507-4546	C	NrtI-naïve	TDF	8.07	Week 48 (3.00)	D134D/E, V214V/A, A317A/S	nd
5620-5225	B	NrtI-naïve	TDF	7.68	Week 48 (2.29)	**Q67Q/H**, N118N/T, N123N/D, M207M/V	1.5 (1.0)
						Week-48 clone with Q67H[6]	1.5 (1.0)
8569-5131	D	NrtI-naïve	TDF	5.87	Week 48 (1.96)	**Q288Q/stop**	1.1 (0.7)

						Week-48 clone with Q288stop[7]	unable to replicate
9035-4845	C	ADV, ETV, LAM	TDF	6.62	Week 48 (4.38)	R110R/G, L269I/L (A/T181A/T)	nd

nd, not determined

Note: Amino acid substitutions in parentheses associated with resistance to HBV NrtIs were detected in the subjects' baseline isolates and persistently detectable in their Week-48 failure samples.

[1] Prior treatment with HBV NrtI(s) regardless of treatment duration.

[2] HBV DNA expressed as $\log_{10}$ IU/mL.

[3] Amino acid substitutions that occurred at HBV rt conserved amino acid positions are written in red.

[4] Per virology analysis plan described in Appendix 1 A1.3, 5 subjects from the TAF group and 4 subjects from the TDF group who were identified to be eligible for Week-48 genotypic resistance testing qualified for cell culture phenotypic analyses of TAF or TFV susceptibility. Additionally, phenotypic analysis of one subject from the TAF group (Subject 6958-1318) was completed prior to pharmacokinetic analysis of plasma tenofovir levels. Thus, among the 9 subjects' failure isolates with evidence of treatment-emergent rt substitutions, 6 subjects' samples were not phenotyped. Subject 2826-4527 in the TAF group discontinued from the study early with no virologic breakthrough and thus did not qualify for phenotypic analysis. Subjects 4296-5147 and 8758-5188 in the TAF group, as well as Subjects 1507-4546 and 9035-4845 in the TDF group, experienced virologic breakthrough that was coincident with TFV plasma levels below the limit of quantification, indicating nonadherence to study medication and were not included in phenotypic analysis. Subject 8017-4565 in the TAF group was excluded from the analysis since the subject experienced after breakthrough a continual decline in HBV DNA levels through Week 80 (last study visit prior to the data finalization cutoff date for the Week-48 analysis; 55 IU/mL).

[5] Fold-change in drug susceptibility of virologic failure isolates (subject-derived pooled and clonal virus sample), compared to wild-type reference HBV (pHY92 genotype A laboratory strain; Yang *et al*., 2004) and to their respective baseline isolates (in parentheses). TAF susceptibilities were determined for paired isolates (baseline and treatment-failure) collected from TAF recipients and TFV susceptibilities were for those collected from TDF recipients. Of note, the EC_{50} values of TAF and TFV were approximately 33.5 nM and 3.20 μM, respectively, for the wild-type reference HBV pHY92. A value <2-fold is within assay variability.

[6] Since the conserved-site substitution was a mixture (rtQ67Q/H), a clone containing the full rtQ67H substitution was isolated and its susceptibility to TFV was also determined in cell culture.

[7] Since the conserved-site substitution was a mixture (rtQ288A/stop), a clone containing the full rtQ288stop change was isolated and its susceptibility to TFV was also determined in cell culture. The clone containing the full rtQ288stop substitution was unable to replicate in cell culture.

Previously, TDF treatment-emergent rt substitutions detected in TDF-treatment failure isolates (n=126 with evaluable data from 96 resistance testing eligible VFs treated for up to 384 weeks) were analyzed collectively from 6 TDF trials (listed in Appendix 4 Table A3). This integrated analysis concluded no specific amino acid substitutions in the HBV rt domain were commonly detectable in TDF-treatment failure isolates, and thus no genotypic resistance pathways were identified for TDF. Moreover, substitutions that have been reported to be associated with TDF resistance, rtA181T/V, rtA194T, rtV214A, or rtQ215S (Ghany and Liang, 2007; Locarnini, 2006; Locarnini and Mason, 2006; Michailidis *et al*., 2012) did not emerged in these evaluated TDF-treatment failure isolates. Of note, the rtA181T, rtA181V, rtV214A, and rtQ215S substitutions conferred 1-1.5-, 2.9-10-, >10-, and >10-fold resistance to TFV, respectively (reviewed by Gupta *et al*., 2014). Cell culture studies evaluating the effect of the rtA194T substitution on TDF susceptibility demonstrated conflicting results. The rtA194 substitution was initially observed by Sheldon *et al*. (2005) in 2 lamivudine-refractory patients after 14-17 months of therapy with TDF and lamivudine. These patients developed this substitution in the background of the lamivudine-resistance rt substitutions, L180M+M204V; however, its development was not clearly associated with HBV viral load rebound (defined as 2 consecutive viral load measurements with >1 $\log_{10}$ increase from treatment nadir). The authors reported 7.6-fold reduced susceptibility by the rtA194T substitution alone to TDF (over 10-fold by rtA194T in combination with rtL180M and rtM204V) compared to wild type reference HBV. In addition, Amini-Bavil-Olyaee *et al*. (2009) also reported the rtA194 substitution, alone or in conjunction with L180M+M204V, conferred partial resistance to TFV (5.4- and 6.9-fold, respectively). In contrast, based on the applicant's phenotypic assay previously conducted in a TDF nonclinical study (Delaney *et al*., 2006; Virology review NDA21356.SE1-025), the rtA194T substitution

alone resulted in a 1.5-fold increase in the EC_{50} value compared to that of wild-type reference HBV, while the rtA194T change in combination with the rtL180M+rtM204V substitutions led to a 2.4-fold increase which was not significantly different than the 2.1-fold increase observed with just the rtL180M+rtM204V substitutions.

In Studies GS-US-174-0102 and GS-US-174-0103 (pivotal Phase 3 studies of TDF), when baseline rt polymorphisms were evaluated for their effects on the TDF response in 412 TDF recipients, only one subject (HBeAg-positive, genotype D with HBV DNA 9.21 log_{10} copies/mL at Baseline) had baseline HBV expressing the adefovir resistance-associated substitution (rtA181A/T) and the subject achieved HBV DNA <400 copies/mL by Week 96 (HBV DNA 3.2 log_{10} copies/mL at Week 48). Six subjects had baseline HBV variants expressing the rtV214A substitution (all 6 with no detectable rtQ215S) and all achieved HBV DNA <400 copies/mL at Week 48. The rtQ215S substitution was detected in the baseline viruses of 22 subjects (all 22 with no detectable rtV214A) and all also achieved HBV DNA <400 copies/mL at Week 48. There were no subjects entered the studies with baseline variants expressing the rtA194T substitution.

As described in Section 2.4, since the antiviral activity of TAF and TDF are driven by the same pharmacologically active form of the drug, TFV-DP, TAF is expected to have a similar resistance profile to that of TDF. Thus, TAF and TDF treatment-emergent rt substitutions observed in these current 2 TAF studies were pooled together: only 2 substitution (rtD134E and rtL269I) was detected in more than one subject (each substitution in 2 subjects [one in each treatment group]). According to the applicant, the rtD134E substitution was previously evaluated in phenotypic assays and retained sensitivity to TFV. In addition, among the evaluated 412 TDF recipients' baseline isolates in TDF studies GS-US-174-0102 and GS-US-174-0103, a glutamic acid reside at HBV rt amino acid position 134 (rtE134) was detected in 10 subjects and 9 of them responded to TDF treatment by achieving HBV DNA <400 copies/mL at Week 48. The rtL269I substitution is a reversion towards the consensus amino acid: an isoleucine residue at position 269 (rtI269) was present in 95.1% (392/412; including those with a viral mixture) of the baseline viruses examined in the TDF studies.

Among the observed emerging rt substitutions in the TAF studies (S13N**,** Q67H, I91L, R110G, S117P, N118T, N123D, D134E, R153Q, M207V, V214A, S256C, L267Q, L269I, E271A, Q288stop, M309K, and A317S; conserved-site substitutions are underlined), substitutions rtQ67H, rtS117P, rtM207V, rtL267Q, rtL269I, rtQ288stop, and rtA317S were never observed in those previously analyzed 126 failure isolates on TDF (Appendix 4 Table A3; no substitutions occurring at these amino acid positions while on TDF were noted). All 3 subject-derived Week-48 pooled viruses containing these substitutions except A317S appeared to remain sensitive to their respective study drugs with observed fold changes of <2 which were within assay variability, compared to wild-type control HBV (Table 9, data from a TAF-treated subject [9035-5187] and 2 TDF-treated subjects [5620-5225 and 8569-5131]). Furthermore, when clones expressing the conserved-site substitutions rtQ67H or rtQ288stop were isolated from the subjects' Week-48 virus pool and phenotyped, the rtQ67H clone also appeared to be sensitive to TDF (1.5-fold change), while the rtQ288stop clone was unable to replicate in cell culture. Substitutions rtS13N, rtD134E, rtV214A, and rtE271A were also never detected in those previously analyzed isolates on TDF (Appendix 4 Table A3) but different amino acid substitutions were observed occurring at the residues 13 (R13H, R13L, L13H), 134 (D134N, N134S), 214 (A214V), and 271 (E271D and M271L). The Week-48 pooled viruses containing rtS13N (from Subject 9035-5187) showed 0.7-fold reduced susceptibility to TAF, compared to

wild-type control HBV. The remaining rt substitutions emerged during TAF or TDF monotherapy, I91L, R110G, N118T, N123D, R153Q, S256C, and M309K were previously observed in TDF treatment failure isolates but once or twice (Appendix 4 Table A3). A Week-48 subject-derived failure isolate containing rtN118T and/or N123D (TDF-treated Subject 5620-5225; Table 9) displayed 1.5-fold reduced susceptibility to TFV. Previously, TDF-treated subject-derived failure isolates in TDF trials expressing rtR110G or rtR153Q showed 1- to 1.3-fold reductions in TFV susceptibility (Virology reviews N021356.511 and N021356.843).

In summary, a total of 1,270 subjects with CHB received TAF (n=850) or TDF (n=420) for ≥24 weeks (resistance analysis population) in Studies GS-US-320-0108 and GS-US-320-0110. After up to 48 weeks of treatment, 230 of them (158 TAF and 72 TDF) had HBV DNA ≥69 IU/mL, and 41 (27 TAF and 14 TDF) were eligible for Week-48 resistance testing who experienced virologic breakthrough by Week 48 (20 TAF and 13 TDF) or who never suppressed HBV DNA levels to <69 IU/mL at the time of early discontinuation (7 TAF and 1 TDF). Among the 27 resistance testing eligible subjects in the TAF treatment group, 15 had no changes detected in the HBV rt sequence from baseline, 5 had polymorphic site substitutions, and 7 were unable to be genotyped. Among the 14 subjects in the TDF treatment group who qualified for resistance analysis, 6 had no changes detected in the rt sequence from baseline, 2 had conserved site substitutions, 2 had polymorphic site substitutions, and 4 were unable to be genotyped. No conserved site substitutions were observed in the TAF treatment group; and no polymorphic site substitutions were observed in more than one subject in both the TAF or TDF treatment groups. With no specific amino acid substitutions in the HBV rt domain being commonly detectable in TAF or TDF treatment failure isolates during the first year of the study, no substitutions could be associated with resistance to tenofovir.

Of the 41 subjects who qualified for genotypic resistance testing through Week 48, baseline and virologic failure isolates of 6 subjects from the TAF group (including 2 subjects with polymorphic site substitutions and 4 subjects with no detectable treatment-emergent substitutions) and of 4 subjects from the TDF group (including 2 subjects with conserved site substitutions and 2 subjects with no detectable treatment-emergent substitutions) were phenotyped. All 10 subjects had failure isolates exhibiting <2-fold reduced susceptibility (within assay variability) to TAF or tenofovir, compared to wild-type control HBV. In addition, of the 41 subjects who qualified for genotypic resistance testing through Week 48, the applicant performed next generation sequencing (NGS) of the HBV rt domain of paired virus samples (baseline and post-baseline with HBV DNA >159 IU/mL) from 8 TAF recipients (including 7 subjects with no treatment-emergent substitutions by population sequencing) and from 8 TDF recipients (including 5 subjects with no treatment-emergent substitutions by population sequencing). NGS results are reviewed by Virology Reviewer Eric F. Donaldson, Ph.D. (see Virology review of NDA208464 SDNs 001 and 009): there were no robust (detected by multiple variant detectors and in multiple subjects) resistance-associated substitutions in either the TAF or TDF group among the 16 subjects who were analyzed for development of resistance using NGS.

3.2. Antiviral Efficacy of TAF

3.2.1. Week-48 Antiviral Response to TAF

In 2 ongoing Phase 3 studies 108 and 110, the antiviral efficacy analyses were conducted separately, due to expected differences in antiviral responses to study drugs in the 2 patient populations, HBeAg-negative (Study 108) and HBeAg-positive (Study 110), to determine

whether TAF (25 mg QD) is noninferior (noninferiority margin of 10%) to TDF (300 mg QD) for the treatment of chronic HBV infection in the both patient groups. The primary efficacy endpoint in these 2 studies was the proportion of subjects with plasma HBV DNA <29 IU/mL (approximately 169 copies/mL) at Week 48.

In this review, antiviral efficacy analyses were conducted using the Week-48 censored, as-treated subject population (411 and 860 subjects in Studies 108 and 110, respectively) including all randomized subjects who received at least one dose of study medication with at least one virologic observation after Baseline but excluding subjects who discontinued their assigned treatment before the primary efficacy assessment at Week 48 while they had a suppressed viral load (HBV DNA <29 IU/mL, LLOQ of the PCR assay employed in these studies, Roche COBAS® TaqMan® HBV Test for Use With the High Pure System; Appendix 1 A1.1). A total of 27 subjects (14 and 13 subjects from Studies 108 and 110, respectively) who were included in the full-analysis population for overall efficacy analysis (425 and 873 subjects in Studies 108 and 110) were censored: (1) 11 subjects (3 and 8 in Studies 108 and 110) who had no reported post-baseline viral load data, (2) 13 subjects (8 and 5 in Studies 108 and 110) who discontinued early from the study while suppressed (HBV DNA <29 IU/mL), (3) 2 subjects in Study 108 who had missing Week-48 viral load data (HBV DNA <29 IU/mL at Week 44), and (4) one subject in Study 108 who had HBV DNA <29 IU/mL at Baseline.

As summarized in Table 10 (FDA analysis), comparable proportions of subjects on TAF and TDF achieved HBV DNA <29 IU/mL after 48 weeks of monotherapy when analyzed using the Week-48 censored, as-treated subject populations: 97.5% versus 94.9% of subjects with HBeAg-negative HBV infection and 64.7% versus 67.9% of subjects with HBeAg-positive HBV infection, respectively.

Table 10: Proportion of Subjects with HBV DNA <29 IU/mL at Week 48

Outcome	Study 108: HBeAg-negative		Study 110: HBeAg-positive	
	TAF	TDF	TAF	TDF
Number of subjects in antiviral efficacy analysis[1]	**275**	**136**	**573**	**287**
By prior treatment history				
• HBV NrtI-naïve	216 (78.5%)	106 (77.9%)	425 (74.2%)	210 (73.2%)
• Prior treatment with HBV NrtI(s)[2]	59 (21.5%)	30 (22.1%)	148 (25.8%)	77 (26.8%)
Median plasma HBV DNA at Baseline (range)[3]	**5.59 (2.58 - 9.89)**	**5.73 (1.79 - 8.20)**	**7.92 (2.54 - 9.92)**	**7.98 (2.60 - 9.90)**
By prior treatment history				
• HBV NrtI-naïve	5.67	5.72	7.88	7.96
• Prior treatment with HBV NrtI(s)[2]	5.46	6.25	8.01	8.15
By Week-48 virologic response				
• HBV DNA <29 IU/mL	5.57	5.72	7.52	7.78
• HBV DNA ≥29 IU/mL	7.96	6.07	8.52	8.58
Rate of HBV DNA <29 IU/mL at Week 48	**97.5%** (268/275)	**94.9%** (129/136)	**64.7%** (371/573)	**67.9%** (195/287)
By prior treatment history				
• HBV NrtI-naïve	98.1% (212/216)	95.3% (101/106)	68.7% (292/425)	72.9% (153/210)
• Prior treatment with HBV NrtI(s)[2]	94.9% (56/59)	93.3% (28/30)	53.4% (79/148)	54.5% (42/77)
By baseline HBV DNA levels				
• <7	99.6% (221/222)	94.6% (106/112)	89.2% (132/148)	81.1% (60/74)

• 7 to <8	92.5% (37/40)	100% (20/20)	78.2% (122/156)	86.3% (63/73)
• 8 to <9	75.0% (9/12)	75.0% (3/4)	49.8% (111/223)	61.3% (68/111)
• ≥9	100% (1/1)	-[4]	13.0% (6/46)	13.8% (4/29)

[1] The Week-48 antiviral efficacy analysis was conducted using the Week-48 censored, as-treated subject population that includes all randomized subjects who received at least one dose of study medication with at least one virologic observation after Baseline but excludes subjects who discontinued their assigned treatment before Week 48 while they had a suppressed viral load (HBV DNA <29 IU/mL).

[2] Prior treatment with HBV NrtI(s) regardless of treatment duration.

[3] HBV DNA expressed as $\log_{10}$ IU/mL.

[4] At Baseline, no subjects with HBeAg-negative HBV infection in the TDF treatment group (Study 108) had HBV DNA ≥9 $\log_{10}$ IU/mL.

Of note, median plasma HBV DNA levels ($\log_{10}$ IU/mL) at Baseline were well matched between the treatment groups, TAF versus TDF, in each study population with much higher pretreatment HBV DNA levels in the HBeAg-positive population than the HBeAg-negative population (Table 10): 5.59 (ranging from 2.58 to 9.89) versus 5.73 (ranging from 1.79 to 8.20) for the HBeAg-negative subjects and 7.92 (ranging from 2.54 to 9.92) versus 7.98 (ranging from 2.60 to 9.90) for the HBeAg-positive subjects. Among the HBeAg-positive subjects, the median baseline HBV DNA level was higher for subjects who failed to achieve HBV DNA <29 IU/mL at Week 48, compared with those who suppressed their viral loads to <29 IU/mL, across both treatment groups, reflective of decreased rates of virologic suppression (HBV DNA <29 IU/mL) at Week 48 in the subgroup of subjects with higher baseline HBV DNA levels (Table 10). Similar observations of negative correlation between baseline HBV DNA levels and antiviral efficacy were made previously at Week 48 with entecavir and TDF (Gordon *et al*., 2013; Zoutendijk *et al*., 2011). In contrast, significantly different rates of HBV DNA suppression at Week 48 were noted between subjects who previously received HBV NrtI therapy (regardless of treatment duration) and those with no prior HBV NrtI therapy (NrtI-naïve) across both treatment groups but their median baseline viral loads were comparable (Table 10). Antiviral efficacy analyses performed in the subgroup of subjects to identify baseline characteristics that may affect antiviral response to TAF will be discussed further below (Section 3.2.2).

In the overall efficacy analysis using the full-analysis population, as observed in the Week-48 censored, as-treated subject population, the TAF group met the primary endpoint of noninferiority to the TDF group in both studies (please refer to the review by Statistical Reviewer Frazer Smith, Ph.D. for details). Briefly, at Week 48, in Study 108 (HBeAg-negative subjects), 94.0% (268/285) of subjects in the TAF treatment group and 92.9% (130/140) of subjects in the TDF treatment group achieved virologic success with HBV DNA <29 copies/mL. The stratum-adjusted difference in the virologic success rate between TAF and TDF treatment groups was 1.7%, and the 95% CI was -3.6% to 7.0%. In Study 110 (HBeAg-positive subjects), 63.9% (371/581) and 66.8% (195/292) of subjects in the TAF and TDF treatment groups, respectively, had virologic success with the TAF versus TDF stratum-adjusted difference of -3.5% (95% CI: -9.7% to 2.6%). Furthermore, in subgroup analyses predefined by demographic or baseline disease characteristics, the applicant calculated the rates of HBV DNA <29 IU/mL, which did not differ statistically for the TAF group compared with the TDF group in both studies, for subgroups according to age (<50 years versus ≥50 years), sex, race (Asian versus non-Asian), baseline HBV DNA level (<7 $\log_{10}$ IU/mL versus ≥7 $\log_{10}$ IU/mL for HBeAg-negative subjects in Study 108 and <8 $\log_{10}$ IU/mL versus ≥8 $\log_{10}$ IU/mL for HBeAg-positive subjects in Study 110), treatment status (treatment-experienced versus treatment-naïve), region (East Asia, Europe, North America, other), study drug adherence, HBV genotype, baseline ALT (≤ ULN versus >ULN by central laboratory normal range), and baseline FibroTest score (<0.75 or ≥ 0.75).

In both studies a numerically lower proportion of subjects with high baseline HBV DNA levels

(≥7 log_{10} IU/mL for HBeAg-negative subjects in Study 108 and ≥8 log_{10} IU/mL for HBeAg-positive subjects in Study 110) achieved HBV DNA <29 IU/mL at Week 48 in the TAF group compared with the TDF group: in Study 108, 85.5% [47/55] versus 95.8% [23/24] with the difference between the 2 proportions of -10.4% (95% CI: -25.5% to 4.5%) and in Study 110, 43.0% [117/272] versus 50.7% [72/142] with the difference of -7.6% (95% CI: -17.8% to 2.5%). However, in both studies, the applicant claimed the subgroup of subjects with high baseline HBV DNA levels was not identified as statistically significant for the primary endpoint (homogeneity of odds ratio based on the Wald test of the interaction between treatment and subgroup [p values of 0.08 and 0.34 for Studies 108 and 110, respectively]). However, when subjects were grouped into 2 baseline viral load subgroups (<7 versus ≥7 log_{10} IU/mL), FDA analysis by Statistician Fraser Smith, Ph.D. found a statistically significant risk difference in HBeAg-positive subjects (p value for Zelen's test=0.017; see Statistical Review and Evaluation for NDA 208-464 for details): the differences in the rate of virologic suppression (HBV DNA <29 IU/mL) at Week 48 between the TAF and TDF groups (negative difference favoring TDF) were 10.1% (95% CI: 0.1% to 21.6%, p value of 0.05) for subjects with baseline HBV DNA <7 log_{10} IU/mL and -7.3% (95% CI: -15.3% to 0.8%, p value of 0.08) for those with baseline HBV DNA ≥7 log_{10} IU/mL. In additional analyses, the applicant observed numerically greater differences in HBV DNA suppression between the TAF and TDF groups at intermediate baseline HBV DNA levels (7 to <8 log_{10} IU/mL in Study 108 and 8 to <9 log_{10} IU/mL in Study 110) compared to differences observed at the low or very high baseline HBV DNA levels (<7 or ≥8 log_{10} IU/mL in Study 108 and <8 or ≥9 log_{10} IU/mL in Study 110, respectively). In Study 108, the differences between the 2 proportions were +3.9%, -11.9%, and +1.9% for subgroups of subjects with baseline HBV DNA levels <7, 7 to <8, and ≥8 log_{10} IU/mL and in Study 110, the differences were +0.2%, -10.9%, and -1% for those with baseline HBV DNA levels <8, 8 to <9, and ≥9 log_{10} IU/mL, respectively. Thus, the applicant reasoned that the similar rates of virologic suppression in subjects treated with TAF or TDF in the very high baseline HBV DNA subgroup may indicate a lack of biological rationale for the lower rates for TAF of HBV DNA suppression observed in the intermediate baseline HBV DNA subgroup.

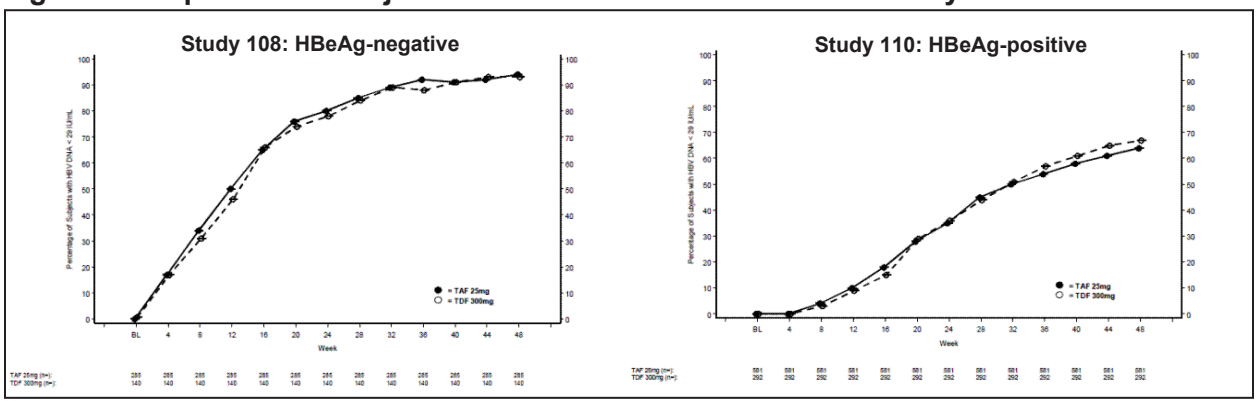
These observations made by the applicant were confirmed by this reviewer's virology analysis performed using the Week-48 censored as-treated subject population. As summarized in Table 10, at Week 48, subjects with intermediate baseline HBV DNA levels (7 to <8 log_{10} IU/mL for HBeAg-negative subjects in Study 108, and 7 to <8 and 8 to <9 log_{10} IU/mL for HBeAg-positive subjects in Study 110) in the TAF group achieved virologic suppression with HBV DNA <29 IU/mL at lower rates compared to those in the TDF group, while numerically comparable or higher proportions of subjects with low or very high baseline viral load (<7 or ≥8 log_{10} IU/mL in Study 108 and <7 or ≥9 log_{10} IU/mL in Study 110, respectively) in the TAF treatment group achieved virologic suppression. However, among the subjects with intermediate baseline HBV DNA levels, virologic breakthrough rates through Week 48 in Study 110 were similar between the 2 treatment groups: in subjects with baseline HBV DNA 7 to <8 log_{10} IU/mL, TAF 3.2% (5/156) versus TDF 2.7% (2/73) and in subjects with baseline HBV DNA 8 to <9 log_{10} IU/mL, TAF, 5.4% (12/223) versus TDF 6.3% (7/111). This analysis was not performed for subjects in Study 108 as a total of 3 subjects experienced confirmed virologic breakthrough through Week 48. Of note, virologic breakthrough (defined as having confirmed HBV DNA ≥69 IU/mL after HBV DNA levels <69 IU/mL being achieved or having confirmed HBV DNA increase by ≥1 log_{10} IU/mL from nadir) was not the primary cause of the virologic failure having HBV DNA ≥29 IU/mL at Weeks 48 (or at the time of early study drug discontinuation). Among the 308 Week-48 virologic failures (14 in Study 108 and 294 in Study 110), a total of 42 subjects (25 TAF and 17 TDF) experienced confirmed virologic breakthrough: overall rates of confirmed virologic

breakthrough in the TAF and TDF groups are 0.7% (2/275) versus 0.7% (1/136) in Study 108 and 4.0% (23/573) versus 5.6% (16/287) in Study 110, respectively.

According to the applicant, in both studies (full-analysis population), the kinetics of HBV DNA decline over 48 weeks, as assessed by the proportion of subjects with HBV DNA <29 IU/mL by visit using the M=F method, were similar between treatment groups (Figure 7). When the change from Baseline in HBV DNA levels was evaluated by visit based on observed data (i.e., missing data were excluded), the applicant noted the decline in HBV DNA levels occurred rapidly during the first 4 weeks of treatment and continued to decline out to Week 48 (Figure 8). Overall, the decreases in HBV DNA levels (log_{10} IU/mL) were similar between the 2 treatment groups through Week 48. Mean (SD) changes (log_{10} IU/mL) in HBV DNA from baseline at Weeks 4, 24, and 48 were as follows:

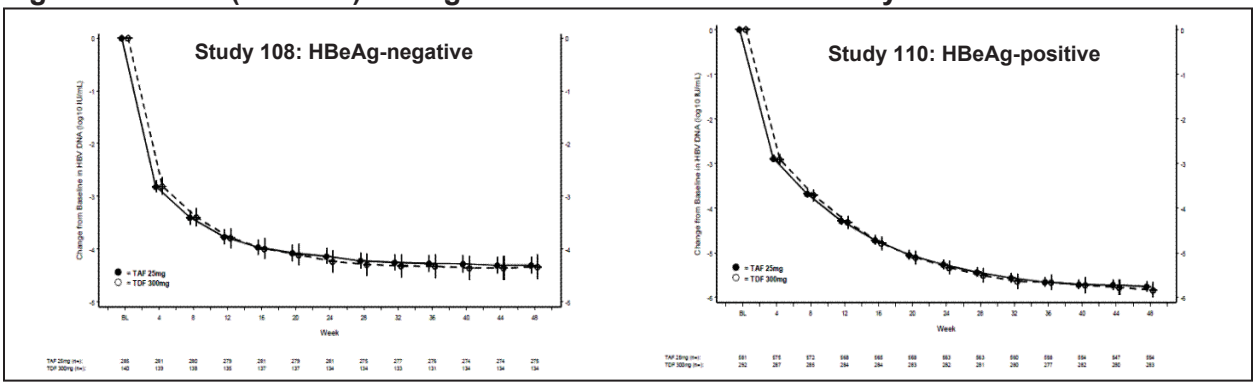
- In Study 108 (HBeAg-negative subjects)
  - TAF group: -2.81 (0.945) at Week 4; -4.13 (1.250) at Week 24; -4.30 (1.295) at Week 48
  - TDF group: -2.80 (0.940) at Week 4; -4.23 (1.193) at Week 24; -4.33 (1.316) at Week 48
- In Study 110 (HBeAg-positive subjects)
  - TAF group: -2.88 (0.872) at Week 4; -5.26 (1.238) at Week 24; -5.75 (1.310) at Week 48
  - TDF group: -2.90 (0.953) at Week 4; -5.33 (1.290) at Week 24; -5.83 (1.427) at Week 48

Figure 7: Proportion of Subjects with Plasma HBV DNA <29 IU/mL by Visit



Source: Summary of Clinical Efficacy, Figures 1 and 2, pages 27-28.

Figure 8: Mean (95% CIs) Change from Baseline in HBV DNA by Visit

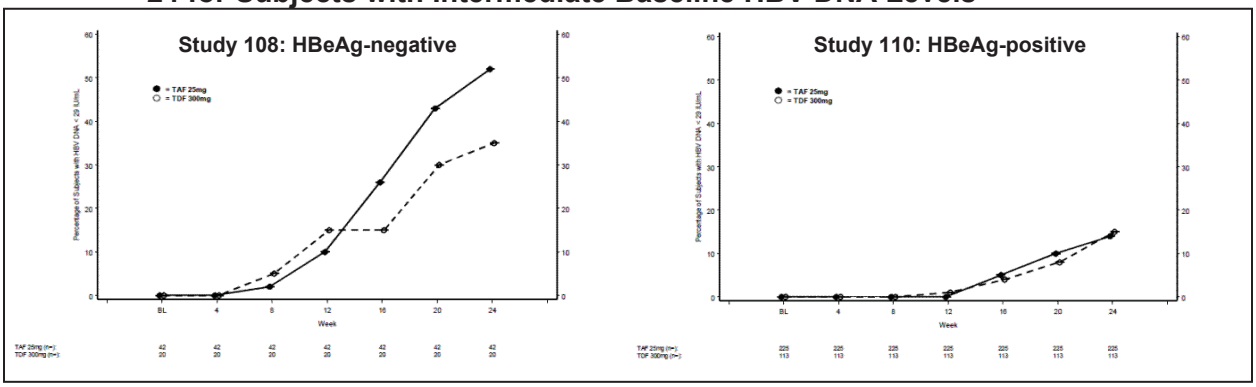


Source: Summary of Clinical Efficacy, Figures 3 and 4, pages 30-31.

The applicant also assessed the early HBV suppression rate (HBV DNA to <29 IU/mL) and kinetics of HBV DNA decline during the first 24 weeks of treatment in subjects with intermediate baseline HBV DNA levels (7 to <8 log_{10} IU/mL for HBeAg-negative subjects in Study 108 and 8 to <9 log_{10} IU/mL for HBeAg-positive subjects in Study 110). Results showed no significant difference in antiviral potency between TAF and TDF in these subgroups of subjects (Figures 9 and 10). In Study 108, 52.4% of subjects (22/42) with intermediate baseline HBV DNA levels achieved viral suppression at Week 24 in the TAF group compared with 35.0% (7/20) in the same subgroup receiving TDF. In Study 110, viral suppression in subjects with baseline intermediate HBV DNA levels at Week 24 was 13.8% (31/225) in the TAF group and 15.0% (17/113) in the TDF groups. The following mean (SD) changes (log_{10} IU/mL) in HBV DNA from baseline were observed at Weeks 12 and 24:

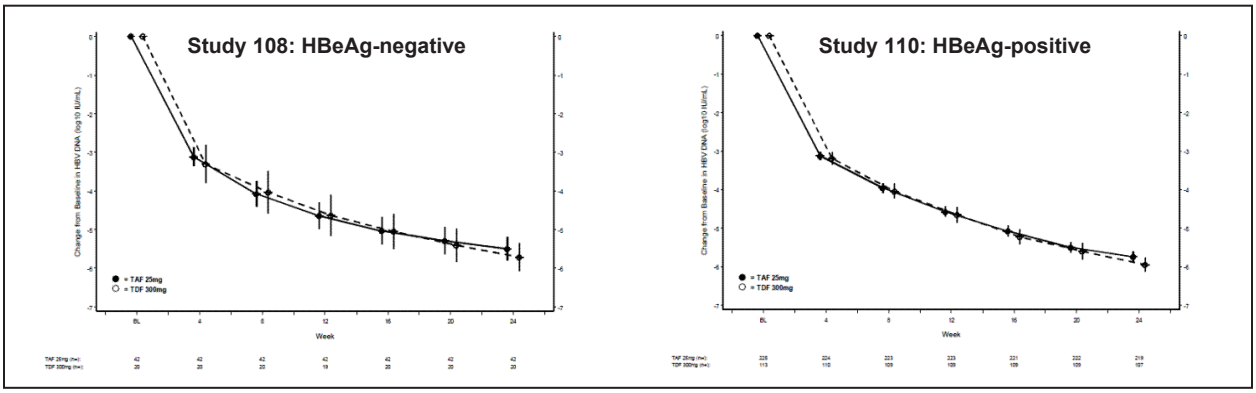
- In Study 108 (HBeAg-negative subjects with baseline HBV DNA 7 to <8 log_{10} IU/mL)
  - TAF group: −4.64 (1.10) at Week 12; −5.50 (0.95) at Week 24
  - TDF group: −4.63 (1.10) at Week 12; −5.71 (0.74) at Week 24
- In Study 110 (HBeAg-positive subjects with baseline HBV DNA 8 to <9 log_{10} IU/mL)
  - TAF group: −4.57 (0.99) at Week 12; −5.74 (1.04) at Week 24
  - TDF group: −4.65 (1.04) at Week 12; −5.95 (0.91) at Week 24

Figure 9: Proportion of Subjects with Plasma HBV DNA <29 IU/mL by Visit Up to Week 24 for Subjects with Intermediate Baseline HBV DNA Levels



Source: Summary of Clinical Efficacy, Figures 11 and 12, pages 53-54.

Figure 10: Mean (95% CIs) HBV DNA Decline from Baseline by Visit Up to Week 24 for Subjects with Intermediate Baseline HBV DNA Levels



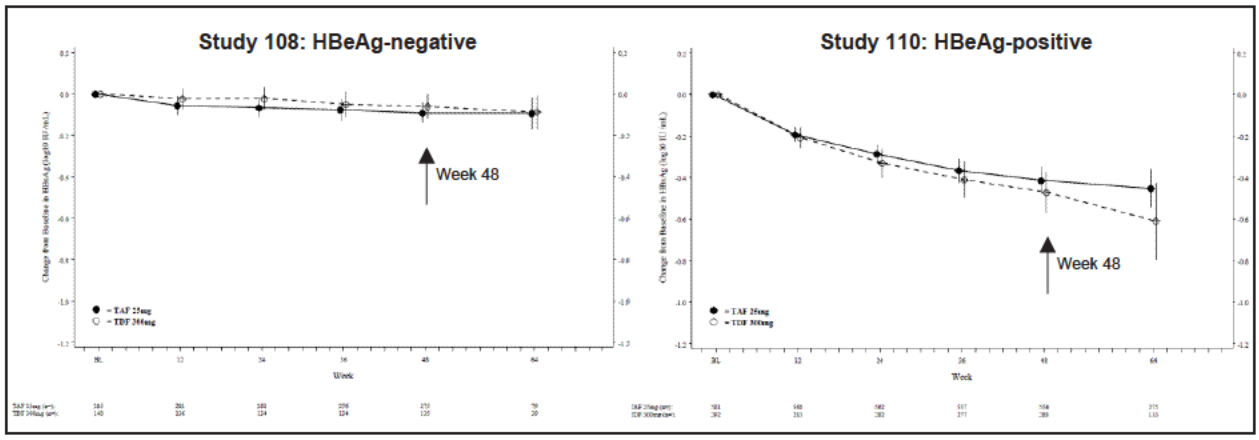
Source: Summary of Clinical Efficacy, Figures 9 and 10, pages 51-52.

By Week 48, in Study 108, no HBeAg-negative subjects in either treatment group experienced HBsAg loss with only small mean changes from baseline in serum HBsAg observed (Figure 11): -0.09 ± 0.401 log_{10} IU/mL (95% CI: -0.14 to -0.04) for the TAF group and -0.06 ± 0.329 log_{10} IU/mL (95% CI: -0.12 to 0.00) for the TDF group. Mean levels (log_{10} IU/mL) of baseline serum HBsAg were 3.38 ± 0.662 (95% CI: 3.31 to 3.46) and 3.39 ± 0.726 (95% CI: 3.27 to 3.51), respectively. In Study 110, 4 of 576 subjects (0.7%) in the TAF group and one of 288 subjects (0.3%) in the TDF group experienced HBsAg loss through Week 48. Among these subjects with HBsAg loss, 3 in the TAF group (0.5%, 3/576) and none in the TDF group (0%, 0/288) also experienced HBsAg seroconversion at Week 48. All 4 TAF subjects with HBsAg loss were treatment-naïve, male, and 30-43 years old; 3 were Asian (all from India) and one was white; and 3 had HBV genotype D infection and one had genotype A infection. The one subject in the TDF group with HBsAg loss was a 22-year old Asian male (from India) and had HBV genotype A infection. Previously, HBsAg loss was observed in HBeAg-positive subjects treated with TDF (Study GS-US-174-0103) with cumulative probabilities (estimated by Kaplan–Meier analysis) of 3, 6, and 8% after 1, 2, and 3 years of therapy, respectively (Heathcote *et al*., 2011; Marcellin *et al*., 2014); 2 of the 5 subjects who lost HBsAg at Week 48 also had seroconversion to anti-HBs (1.3% [2/158]; Marcellin *et al*., 2008). The authors observed no HBsAg loss in HBeAg-negative subjects by Week 144 (Study GS-US-174-0102). In an entecavir clinical trial conducted in treatment-naïve subjects with HBeAg-positive CHB (Chang *et al*., 2006), at Week 48, HBsAg loss occurred in 1.7% of 354 subjects in the entecavir treatment group (0.5 mg QD) and in 1.1% of 355 subjects in the lamivudine group (100 mg QD).

At Baseline, HBeAg-positive subjects in the TAF and TDF treatment groups (Study 110) had mean serum HBsAg levels (log_{10} IU/mL) of 4.03 ± 0.794 (95% CI: 3.97 to 4.10) and 4.13 ± 0.682 (95% CI: 4.05 to 4.21), respectively, and mean (SD) changes (log_{10} IU/mL) from baseline were similar between the 2 treatment groups through Week 48 (Figure 11):

- TAF group: -0.19 (0.411) at Week 12; -0.29 (0.538) at Week 24; -0.41 (0.790) at Week 48
- TDF group: -0.21 (0.418) at Week 12; -0.33 (0.576) at Week 24; -0.47 (0.844) at Week 48

Figure 11: Mean (95% CIs) Change from Baseline in HBsAg by Visit



Source: Study GS-US-320-0108 Interim Week 48 Clinical Study Report, Figure 7, page 648; and Study GS-US-320-0110 Interim Week 48 Clinical Study Report, Figure 7, page 725.

Of note, levels of HBsAg in serum were quantified every 12 weeks by the Abbott ARCHITECT® assay with a lower limit of quantification of ≤0.05 IU/mL (Lou *et al*., 2011). HBsAg loss was

defined as HBsAg test result (Abbott ARCHITECT HBsAg qualitative assay) changes from HBsAg positive at baseline to HBsAg negative at a post-baseline visit with baseline anti-HBs negative or missing. HBsAg seroconversion was defined as HBsAg loss and anti-HBs test result (Siemens ADVIA Centaur® Anti-HBs2 ReadyPack™) changes from anti-HBs negative or missing at baseline to anti-HBs positive at a post-baseline visit.

In Study 110, the proportion of subjects with HBeAg loss or seroconversion to anti-HBe at Week 48 was also evaluated in the serologically evaluable full-analysis population (including subjects with HBeAg positive and HBeAb negative/missing at Baseline). As summarized in Table 11, at Week 48, 78 (13.8%) of 565 subjects in the TAF group and 34 (11.9%) of 285 subjects in the TDF groups experienced HBeAg loss (M=F analysis; p=0.47); 58 (10.3%, 58/565) and 23 (8.1%, 23/285) of those with HBeAg loss in the TAF and TDF groups, respectively, achieved HBeAg seroconversion (M=F analysis; p=0.32). In the previous TDF study GS-US-174-0103, HBeAg loss and HBeAg seroconversion were reported to occur at Week 48 in 22.2% (n=34) and 20.9% (n=32), respectively, of 153 subjects with evaluable data (Marcellin *et al*., 2008). In the entecavir clinical trial described above (Chang *et al*., 2006), at Week 48, HBeAg loss and seroconversion occurred in 22.9% (n=78) and 20.9% (n=74), respectively, of 354 subjects in the entecavir group, similar to those in the lamivudine group (19.7% [70/355] and 18.0% [64/355]).

HBeAg loss rates in Study 110 were numerically (1) higher for both treatment groups in subjects with low baseline HBV DNA levels (<8 $\log_{10}$ IU/mL) compared with subjects with high baseline HBV DNA levels (≥8 $\log_{10}$ IU/mL), and (2) higher in subjects in the TAF group compared with the TDF group having low and high baseline HBV DNA levels: in subjects with HBV DNA <8 $\log_{10}$ IU/mL, TAF 17.4% (51/293) versus TDF 16.0% (23/144) and in subjects with HBV DNA ≥8 $\log_{10}$ IU/mL, TAF 9.9% (27/272) versus TDF 7.8% (11/141). Of note, HBeAg loss was defined as changes from HBeAg positive at Baseline (with HBeAb negative/missing) to HBeAg negative at a post-baseline, and HBeAg seroconversion was defined as HBeAg loss and HBeAb changes from negative/missing at baseline to positive at a post-baseline visit. DiaSorin ETI-EBK PLUS and ETI-AB-EBK PLUS assays were utilized to detect qualitatively HBeAg and HBeAb, respectively.

Table 11: Proportion of Subjects with HBeAg Loss/Seroconversion by Visit

			TAF 25 mg vs TDF 300 mg	
	TAF 25 mg	TDF 300 mg	P-Value[a]	Prop Diff (95% CI)[b]
HBeAg Loss				
Week 48	78/565 (13.8%)	34/285 (11.9%)	0.47	1.8% (−3.0% to 6.5%)
HBeAg Seroconversion				
Week 48	58/565 (10.3%)	23/285 (8.1%)	0.32	2.1% (−2.0% to 6.3%)

Source: Study GS-US-320-0110 Interim Week 48 Clinical Study Report, Table 9-5, page 97.
Prop Diff = difference in proportions.
[a] P value was from the Cochran-Mantel-Haenszel tests stratified by baseline HBV DNA categories and oral antiviral treatment status strata.
[b] Difference in the proportion between treatment groups and its 95% CI were calculated based on the Mantel-Haenszel proportions adjusted by baseline HBV DNA categories and oral antiviral treatment status strata.

3.2.2. Baseline Factors Affecting Antiviral Response to TAF

As summarized in Table 10 above, at Week 48 in both studies, subgroups of TAF recipients in the Week-48 censored, as-treated subject population with high baseline HBV DNA levels or with a history of prior HBV NrtI exposure achieved HBV DNA levels <29 IU/mL at lower rates, compared to those with low baseline HBV DNA levels or to those who had never received HBV NrtIs for the treatment of HBV infection (NrtI-naïve), respectively. In an attempt to identify baseline factors that may affect antiviral response to TAF, antiviral efficacy analyses were performed in subgroups of subjects categorized by baseline characteristics in the Week-48 censored, as-treated subject population (Table 12; FDA analysis).

As noted with other HBV NrtIs (Gordon *et al*., 2013; Chang *et al*., 2010), decreased rates of virologic suppression achieving HBV DNA <29 IU/mL at Week 48 were generally observed in the subgroups of TAF-treated subjects with higher baseline HBV DNA levels, regardless of prior NrtI treatment history (Table 12). In previous TDF studies GS-US-174-0102 (HBeAg negative) and GS-US-174-0103 (HBeAg positive), subjects with high baseline viral load (HBV DNA ≥9 $\log_{10}$ copies/mL) were found to require a longer time course to achieve HBV DNA <400 copies/mL, compared to those with low baseline viral load (HBV DNA <9 $\log_{10}$ copies/mL); however, the percentage of subjects who achieved HBV DNA <400 copies/mL by Week 96 was similar between the subjects with high and low baseline viral load (Gordon *et al*., 2013). Furthermore, regardless of the additional time needed for high baseline viral load subjects to achieve HBV DNA <400 copies/mL, the authors observed no resistance to TDF through Week 240. Similar results were observed in a long term study evaluating entecavir in CHB subjects with a median baseline viral load of approximately 10^{10} IU/mL, where the proportion of subjects achieving HBV DNA <300 copies/mL continued to increase through 240 weeks of treatment, with no resistance observed through Week 96 (Chang *et al*., 2010; Zoutendijk *et al*., 2011).

Table 12: Proportion of Subjects with HBV DNA <29 IU/mL at Week 48 by Baseline HBV DNA Level and Prior HBV NrtI Exposure History

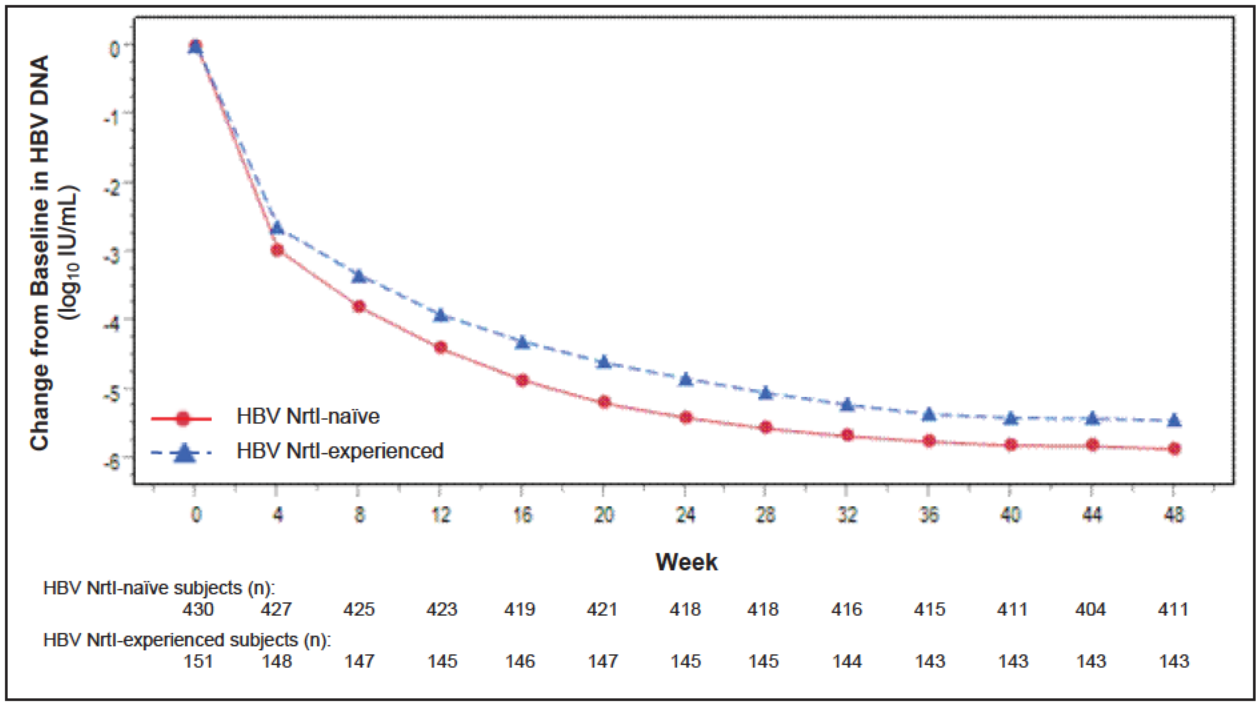
Treatment		**TAF**				TDF			
HBV DNA ($\log_{10}$ IU/mL) at Baseline		**<7**	**7 to <8**	**8 to <9**	**≥9[1]**	<7	7 to <8	8 to <9	≥9[1]
Study 108	HBV NrtI-naïve	**99.4%** (178/179)	**96.3%** (26/27)	**77.8%** (7/9)	**100%** (1/1)	95.7% (88/92)	100% (13/13)	0% (0/1)	-[1] (0/0)
	with prior NrtI experience[2]	**100%** (43/43)	**84.6%** (11/13)	**66.7%** (2/3)	-[1] (0/0)	90.0% (18/20)	100% (7/7)	100% (3/3)	-[1] (0/0)
Study 110	HBV NrtI-naïve	**91.1%** (102/112)	**84.0%** (100/119)	**54.2%** (84/155)	**15.4%** (6/39)	90.6% (48/53)	86.2% (50/58)	64.3% (54/84)	6.7% (1/15)
	with prior NrtI experience[2]	**83.3%** (30/36)	**59.5%** (22/37)	**39.7%** (27/68)	**0%** (0/7)	57.1% (12/21)	86.7% (13/15)	51.9% (14/27)	21.4% (3/14)

[1] All subjects had baseline HBV DNA ≥9 but <10 $\log_{10}$ IU/mL. At Baseline, in Study 108, only one subject (NrtI-naïve in the TAF treatment group) had baseline HBV DNA ≥9 $\log_{10}$ IU/mL.
[2] Prior treatment with HBV NrtI(s) regardless of treatment duration.

In HBeAg positive subjects (Study 110), statistically significant differences in the TAF response rate (HBV DNA <29 IU/mL) were seen at Week 48 favoring HBV NrtI-naïve subjects over those who previously received HBV NrtI therapy (regardless of treatment duration; 68.7% [292/425] versus 53.4% [79/148], p value of <0.001; Table 10). HBV DNA reduction from Baseline was

greater in the NrtI-naïve group than in the NrtI-experienced group at all periods over 48 weeks (Figure 12): the NrtI-naïve group showed a mean $\log_{10}$ HBV DNA reduction (IU/mL) of 2.96, 4.40, 5.40, and 5.86, respectively, at Weeks 4, 12, 24, and 48 compared with 2.64, 3.91, 4.83, and 5.44 at each time point in the NrtI-experienced group. These persistent differences in TAF antiviral activity (0.32 - 0.59 $\log_{10}$ IU/mL) observed in these two subject populations were further analyzed in subgroups of subjects grouped by baseline viral load level (<7, 7 to <8, 8 to <9, and ≥9 $\log_{10}$ IU/mL; Table 12). At each level of baseline viral load, subjects with prior HBV NrtI exposure were less responsive to TAF treatment, particularly in those in the intermediated-to-high baseline HBV DNA level groups (Table 12): among HBeAg-positive subjects (Study 110) with baseline viral load 7 to <8 and 8 to <9 $\log_{10}$ IU/mL, the difference between HBV NrtI-naïve subjects and those with prior NrtI exposure in the Week-48 response rate was statistically significant at 24.5% (84.0%-59.5%, p value of <0.005) and 14.5% (54.2%-39.7%, p value of <0.05), respectively. Due to small sample sizes, differences for HBeAg-negative subjects (Study 108, TAF-treated) in these intermediated-to-high baseline viral load groups (7 to <8 and 8 to <9 $\log_{10}$ IU/mL**)** could not be reliably calculated; however, numerically higher (>10%) rates of viral suppression were observed for NrtI-naïve subjects, compared to those with a history of prior HBV NrtI exposure. Thus, prior HBV NrtI exposure appeared to have a negative impact on Week-48 virological response to TAF monotherapy, independent of baseline viral load. It is possible that prior treatment with HBV NrtI(s) may select for HBV variants that are less sensitive to TAF treatment. Similar trends were also observed for TDF-treated subjects in Study 110 (excluding some groups with small samples). Interestingly, in both the TAF and TDF treatment groups, greater proportions of subjects previously exposed to HBV NrtIs had baseline HBV variants expressing rt substitutions associated with HBV NrtI resistance (primary and/or compensatory substitution detected by LiPA probes) in Studies 108 and 110 than NrtI-naïve subjects (Table 13).

Figure 12: Mean Change in HBV DNA from Baseline by Prior HBV NrtI Treatment History for TAF-Treated Subjects in Study 110



Baseline (or screening) virus samples from all subjects treated in both studies (full-analysis population) were assessed for the presence of pre-existing rt substitutions known to be associated with resistance to HBV NrtIs using the INNO-LiPA Multi DR v2/v3 assay (Innogenetics; Appendix 1 A1.2). Overall, 7.8% (101/1298) of subjects harbored pre-treatment HBV variants (mostly detected as a mix of mutant and wild-type virus) with clinically relevant NrtI resistance-associated substitutions occurring at the tested 10 amino acid positions in the HBV rt region of the polymerase gene: L80I/V, G/V173L, L180M, A181T/V, T184A/C/F/G/I/L/M/S, A194T, S202C/G/I, M204I/S/V, N236T, and M250I/L/V.

The percentage of subjects with mutant variants detectable by LiPA probes was significantly higher for prior NrtI-exposed subjects (20.7% [66/319]) than for NrtI-naïve subjects (3.6% [35/979]). The distribution of subjects with mutant variants was similar between the treatment groups among NrtI-naïve subjects, regardless of HBeAg status (2.8% to 4.0% of subjects; Table 13), while in prior NrtI-exposed subjects, mutant variants were detected more frequently in HBeAg-positive subjects than in HBeAg-negative subjects, 22.5% versus 10% of TAF subjects and 28.6% versus 12.9% of TDF subjects. Similar distribution patterns were observed for baseline mutant variants with primary NrtI-resistance rt substitutions (listed in Table 13, Footnote 3). Primary resistance rt substitutions were observed in 5.4% of subjects overall (70/1298), with a higher percentage observed in prior NrtI-exposed subjects (18.2% [58/319]) compared to NrtI-naïve subjects (1.2% [12/979]). Lamivudine-R variants (expressing rtM204V/I/S) were predominant, observed in 3.9% of subjects overall (51/1298), followed by adefovir-R (expressing rtA181T/V and/or rtN236T; 1.9% [25/1298]) and entecavir-R (expressing rtT184A/C/F/G/I/L/M/S, rtS202C/G/I, and/or rtM250I/L/V in the presence of rtM204V/I/S; 1.0% [13/1298]) variants. In addition, similar percentages of NrtI-naïve (1.4% [14/979]) and prior NrtI-exposed (1.3% [4/319]) subjects harbored variants expressing entecavir resistance-associated substitutions (rtT184A/C/F/G/I/L/M/S, rtS202C/G/I, and/or rtM250I/L/V) in the absence of detectable rtM204V/I/S (Table 13; FDA analysis).

A total of 8 subjects (6 TAF and 2 TDF) had baseline variants expressing the rtA194T substitution (possibly associated with TDF resistance), 2 of which had virus populations also expressing rtM204I (n=1; Table 13, Footnote 4), or T184F/I/L/M and S202S/C/I (n=1; Table 13, Footnote 5). Of note, in these two Phase 3 studies, pre-treatment samples (baseline or screening) from 72 subjects also had sequencing data (population-based) that were compared with results generated by the INNO-LiPA assay: full concordance between the 2 assays for all 10 positions covered by LiPA probes was observed in 91.7% of the tested subjects (66/72). Discrepant results (partial concordance) were observed in the remaining 8.3% [6/72] of the tested subjects: LiPA probes detected mixed wild-type and mutant virus populations in all 6 subjects' samples, but sequencing detected only wild-type sequences in 4 subjects and only mutant sequences in 2 subjects. The current version of the INNO-LiPA assay is designed to detect specific substitutions occurring at 10 different amino acid positions when present in ≥5% of the virus population (Degertekin *et al*., 2009), whereas the HBV rt population-based sequencing assay can detect nucleotide mixtures when present in a population at approximately 25%.

Table 13: Number of Subjects with Baseline HBV Expressing rt Substitutions Detected by INNO-LiPA Probes

Treatment	**TAF**	TDF

Study	**108 (HBeAg-negative)**		**110 (HBeAg-positive)**		108 (HBeAg-negative)		110 (HBeAg-positive)	
Prior NrtI treatment[1]	**No (n=225)**	**Yes (n=60)**	**No (n=430)**	**Yes (n=151)**	No (n=109)	Yes (n=31)	No (n=215)	Yes (n=77)
Detection of rt substitutions[2]	**7 (3.1%)**	**6 (10.0%)**	**17 (4.0%)**	**34 (22.5%)**	3 (2.8%)	4 (12.9%)	8 (3.7%)	22 (28.6%)
Primary NrtI-R substitutions[3]	**3 (1.3%)**	**5 (8.3%)**	**4 (0.9%)**	**29 (19.2%)**	1 (0.9%)	4 (12.9%)	4 (1.9%)	20 (26.0%)
• LAM-R	**2**	**3**	**1**	**16**	0	3	0	6
• ADV-R	**1**	**0**	**3**	**6**	0	0	4	5
• LAM-R + ADV-R	**0**	**1**	**0**	**2**	1	0	0	2
• LAM-R + ETV-R	**0**	**1**	**0**	**4**	0	1	0	7
• LAM-R + A194T[4]	**0**	**0**	**0**	**1**	0	0	0	0
A194T (M204I/V undetectable)	**2**	**0**	**2**	**1**	1	0	1[5]	0
ETV-R substitutions[6] (M204I/V undetectable)	**2**	**0**	**8**	**3**	0	0	4[5]	1
Other substitutions only[7]	**0**	**1**	**3**	**1**	1	0	0	1

[1] Prior treatment with HBV NrtI(s) regardless of treatment duration.

[2] Detection of mutant codons at any of the 10 amino acid positions included in the INNO-LiPA Multi DR v2/v3 assay.

[3] Detection of rt substitutions primarily associated with HBV NrtI resistance included in the INNO-LiPA Multi DR v2/v3 assay:
- ADV-R: rtA181T/V, rtN236T
- ETV-R: rtM204V/I/S + (rtT184A/C/F/G/I/L/M/S, rtS202C/G/I, and/or rtM250I/L/V)
- LAM-R: rtM204V/I/S
- Possible TDF-R: LAM-R + rtA194T

[4] Detection of rtA194A/T, rtM204I, and rtL80I/V in one subject's baseline sample (Subject 8001-4691). This subjects had HBV DNA 8.9 $\log_{10}$ IU/mL at Baseline and achieved virologic suppression (HBV DNA <29 IU/mL) at Week 44. The subject maintained viral suppression through Week 72 (last available data).

[5] Three rt substitutions, A194A/T, T184T/F/I/L/M, and S202S/C/I were detected in the absence of detectable rtM204V/I/S in one subject's baseline sample (Subject 2826-4532). This subject had HBV DNA 8.7 $\log_{10}$ IU/mL at Baseline and 43 IU/mL at Week 48. This subject had persistent viremia through Week 88 (42 IU/mL).

[6] Detection of rtT184A/C/F/G/I/L/M/S, rtS202C/G/I, and/or rtM250I/L/V in the absence of detectable rtM204V/I/S.

[7] Detection of rtL80I/V, G/V173L, or L180M in the absence of detectable rtA181T/V, rtT184A/C/F/G/I/L/M/S, rtA194T, rtS202C/G/I, rtM204V/I/S, rtN236T, and/or rtM250I/L/V primary NrtI-R substitutions.

As summarized in Table 14, at Week 48, lower rates of HBV DNA suppression (<29 IU/mL) were obtained in TAF recipients who had HBV variants at Baseline expressing rt substitutions associated with NrtI resistance detectable by LiPA (NrtI-R substitutions), regardless of prior NrtI treatment history, compared to those with baseline wild-type like virus (no detection of mutant codons at any of the 10 amino acid positions included in the INNO-LiPA Multi DR v2/v3 assay): in Study 108, 80.0% versus 98.6% of subjects with prior NrtI-exposure and 83.3% versus 96.2% of NrtI-naïve subjects, respectively, and in Study 110, 41.2% versus 69.9% of subjects with prior NrtI-exposure and 34.4% versus 58.6% of NrtI-naïve subjects. The lower proportion of subjects with NrtI-R substitutions (any detectable by LiPA probes) achieving HBV DNA <29 IU/mL in the TAF treatment group may be impacted by the greater proportion of subjects with high baseline viral load (Table 14), when compared with those with baseline wild-type like virus: baseline HBV DNA levels of ≥8 $\log_{10}$ IU/mL were observed in 20.0% versus 4.3% of prior NrtI-exposed subjects and 33.3% versus 1.9% of NrtI-naïve subjects in Study 108, and in 70.6% versus 44.6% of prior NrtI-exposed subjects and 62.5% versus 47.4% of NrtI-naïve subjects in Study 110. Interestingly, in the TDF treatment group (subgroup of subjects with prior HBV NrtI treatment in Study 110; Table 14), between the two subgroups of subjects (having baseline

variants with NrtI-R substitutions or having baseline wild-type like virus), similar proportions of subjects had baseline HBV DNA ≥8 log_{10} IU/mL (50% versus 54.5%, respectively) and also achieved virology suppression at Week 48 (54.5% for both subgroups). Together, these limited data indicate subjects with baseline HBV variants expressing NrtI-R substitutions having a lower virologic response to TAF at Week 48 may be at least partly due to their higher baseline viral load. However, regardless of detectable known NrtI-R substitutions, prior treatment with HBV NrtI(s) was confirmed to have a negative impact on Week-48 virological response to TAF monotherapy, independent of baseline viral load. Even among subjects (TAF-treated in Study 110) with baseline wild-type like virus (absence of detectable known NrtI-R substitutions), the difference between HBV NrtI-naïve subjects and those with prior NrtI exposure in the Week-48 response rate was 11.3% (69.9%-58.6%, respectively, Table 14; p value of <0.05). It should be noted median baseline HBV DNA levels and the proportions of subjects with high baseline HBV DNA (≥8 log_{10} IU/mL) were comparable between HBV NrtI-naïve subjects and those with prior NrtI-exposure in subjects with baseline wild-type like virus (Table 14; FDA analysis): median baseline HBV DNA levels were 7.86 versus 7.92 log_{10} IU/mL, and 44.6% versus 47.4% of subjects had high baseline HBV DNA, respectively.

Table 14: Impact of Baseline HBV Variants Expressing NrtI Resistance-Associated Substitutions on Virologic Response at Week 48[1]

Treatment	**TAF**				TDF			
Study	**108 (HBeAg-negative)**		**110 (HBeAg-positive)**		108 (HBeAg-negative)		110 (HBeAg-positive)	
Prior NrtI treatment[2]	**No** (n=216)	**Yes** (n=59)	**No** (n=425)	**Yes** (n=148)	No (n=106)	Yes (n=30)	No (n=210)	Yes (n=77)
Overall[1]	**98.1%** (212/216)	**94.5%** (56/59)	**68.7%** (292/425)	**53.4%** (79/148)	95.3% (101/106)	93.3% (28/30)	72.9% (153/210)	54.5% (42/77)
- Median VL at Baseline[3] - % baseline VL ≥8[4]	**5.67** **4.6%** (10/216)	**5.46** **5.1%** (3/59)	**7.88** **45.6%** (194/425)	**8.01** **50.7%** (75/148)	5.72 0.9% (1/106)	6.25 10.0% (3/30)	7.96 47.1% (99/210)	8.15 53.2% (41/77)
No detection of rt substitutions[5]	**98.6%** (208/211)	**96.2%** (51/53)	**69.9%** (285/408)	**58.6%** (68/116)	96.1% (99/103)	92.6% (25/27)	72.8% (147/202)	54.5% (30/55)
- Median VL at Baseline[3] - % baseline VL ≥8[4]	**5.68** **4.3%** (9/211)	**5.15** **1.9%** (1/53)	**7.86** **44.6%** (182/408)	**7.92** **47.4%** (55/116)	5.71 1.0% (1/103)	6.15 11.1% (3/27)	7.97 48.0% (97/202)	8.26 54.5% (30/55)
Detection of rt substitutions[6]	**80.0%** (4/5)	**83.3%** (5/6)	**41.2%** (7/17)	**34.4%** (11/32)	66.7% (2/3)	100% (3/3)	75.0% (6/8)	54.5% (12/22)
- Median VL at Baseline[3] - % baseline VL ≥8[4]	**5.63** **20.0%** (1/5)	**7.36** **33.3%** (2/6)	**8.53** **70.6%** (12/17)	**8.15** **62.5%** (20/32)	6.47 0% (0/3)	7.82 0% (0/3)	6.43 25.0% (6/8)	8.02 50.0% (12/22)
Primary NrtI-R substitutions[7]	**66.7%** (2/3)	**80.0%** (4/5)	**50.0%** (2/4)	**40.7%** (11/27)	100% (1/1)	100% (3/3)	100% (4/4)	50.0% (10/20)

[1] The Week-48 antiviral efficacy analysis was conducted using the Week-48 censored, as-treated subject population that includes all randomized subjects who received at least one dose of study medication with at least one virologic observation after Baseline but excludes subjects who discontinued their assigned treatment before Week 48 while they had a suppressed viral load (HBV DNA <29 IU/mL). The overall rate was calculated using the Week-48 censored, as-treated subject population.

[2] Prior treatment with HBV NrtI(s) regardless of treatment duration.

[3] Median plasma viral load (VL) at Baseline, expressed as HBV DNA log_{10} IU/mL.

[4] Proportion of subjects with high baseline viral load (HBV DNA ≥8 log_{10} IU/mL).

[5] No detection of mutant codons at any of the 10 amino acid positions included in the INNO-LiPA Multi DR v2/v3 assay.

[6] Detection of mutant codons at any of the 10 amino acid positions included in the INNO-LiPA Multi DR v2/v3 assay.

[7] Detection of rt substitutions primarily associated with HBV NrtI resistance included in the INNO-LiPA Multi DR v2/v3 assay:
- ADV-R: rtA181T/V, rtN236T
- ETV-R: rtM204V/I/S + (rtT184A/C/F/G/I/L/M/S, rtS202C/G/I, and/or rtM250I/L/V)
- LAM-R: rtM204V/I/S
- Possible TDF-R: LAM-R + rtA194T

Three groups of rt substitutions were reported to be associated with TDF resistance (Ghany and Liang, 2007; Locarnini, 2006; Locarnini and Mason, 2006; Michailidis *et al*., 2012): Group 1, rt180M+rtA194T+rtM204V; Group 2, rtV214A, rtQ215S; and Group 3, rtA181V+rtM204I. Based on the LiPA assay results (for rt180M, rtA181V, rtA194T, and rtM204V/I substitutions) and population-based sequencing results (for rtV214A and rtQ215S substitutions from 72 subjects' data available), only 2 subjects (both receiving TAF) had baseline variants with rt substitutions identified as a TDF resistance group:

- Subject 8001-4691 with genotype D infection in Study 110, previously exposed to telbivudine: This subject had baseline viruses (8.92 $\log_{10}$ IU/mL) showing mixed HBV rt sequences, wild-type, and rtA194T detectable by INNO-LiPA (positive signals for A194 and T194) in addition to rtM204I + rtL80V/I (TDF resistance group 1). This subject suppressed his viral load to <29 IU/mL at Week 44 and remained suppressed through Week 72 (last available data).

- Subject 8602-1354 with genotype D infection in Study 108, previously exposed to lamivudine: This subject had baseline viruses (8.51 $\log_{10}$ IU/mL) showing mixed HBV rt sequences, wild-type, and rtA181T/V detectable by INNO-LiPA (positive signals for A181, T181, and V181) in addition to rtM204M/V/I + rtL180L/M (TDF resistance group 3). The subject had a continual decline in HBV DNA through Week 48 (254 IU/mL; last available data) without experiencing confirmed virologic breakthrough (thus, not included in the Week-48 resistance testing eligible population).

The HBV genotype was implied to have no substantial impact on the response to HBV NrtIs including adefovir, entecavir, and lamivudine (Liu *et al*. 2005). However, Palumbo (2007) noted that the rate of resistance to lamivudine was higher in patients with genotype A infection than in those infected by genotype D, whereas no difference in the risk of lamivudine resistance is found between patients infected with genotype B and with genotype C. The author did not observe evidence of any difference in virologic response to adefovir among the different HBV genotypes. A meta-analysis of published treatment results by Wiegand *et al*. (2008) also showed that treatment response to HBV NrtIs (adefovir, entecavir, and lamivudine) is not significantly influenced by HBV genotype in HBeAg-positive or HBeAg-negative individuals. In previous TDF studies (Studies GS-US-174-0102 and GS-US-174-0103), genotype D-infected HBeAg-positive subjects had a lower virologic response to TDF (Virology review N021356.SE1-025). Compared to the mean rate of virologic failure with HBV DNA ≥400 copies/mL (~69 IU/mL) of 21.3% in the overall HBeAg-positive subject population, a higher failure rate of 38.9% was observed in HBeAg-positive subjects infected with HBV genotype D, whereas those for genotypes A, B, and C (10%, 8%, and 18%) were lower. These limited data indicated that the response to TDF may be affected by HBV genotype, genotype D in particular. However, these differences in virologic response to TDF were not observed in HBeAg-negative infected with different HBV (Study GS-US-174-0102), possibly due to the small sample size. It should be noted that genotype D-infected HBeAg-positive subjects had a slightly higher median baseline viral load of 9.2 $\log_{10}$ copies/mL (ranging from 7 to 10.9 $\log_{10}$ copies/mL), compared to that of HBeAg-positive subjects infected with genotypes A, B, or C (pooled; median=8.8 $\log_{10}$ copies/mL, ranging from 6.3 to 9.9 $\log_{10}$ copies/mL).

In this review, the Week-48 antiviral response to TAF was evaluated based on the viral genotype at Baseline. Of note, in cell culture studies, TAF exhibited similar antiviral activity across HBV genotypes (Table 2): the mean fold-changes in EC_{50} values of 0.6 and 0.7 for genotype D, relative to that for the pHY92 genotype A control laboratory strain. The distribution of HBV isolates by genotypes at Baseline (using the INNO-LiPA® HBV Genotyping assay) is shown in Table 15 by HBeAg-status and prior NrtI treatment history (full-analysis population). To date, 8 major genotypes (A to H) of HBV have been recognized by a sequence divergence in the entire HBV genome of >8% in the order of discovery (Arauz-Ruiz *et al*., 2002; Norder *et al*., 1994; Stuyver *et al*., 2000); and 2 additional ones, I and J, were proposed as the ninth and tenth human HBV genotype, respectively (Olinger *et al*., 2008; Tatematsu *et al*., 2009). The most common genotypes in the US are A and C, followed by genotypes B and D; genotypes E, F, and G combined were only present in 2% of the study population (Chu *et al*., 2003). The prevalence of HBV genotypes in the US study population was as follows: A, 34.7%; B, 22.0%; C, 30.8%; D, 10.4%; E, 0.4%; F, 0.6%; G, 1.1%.

In subjects who received TAF in Studies 108 and 110 (full analysis population), 5 (genotypes A, B, C, D, and E) and 6 (genotypes A, B, C, D, E, and F) HBV genotypes were identified at Baseline with genotype C being the most common in both studies. In the TAF treatment group, Genotypes A, B, C, and D were noted in 5.3%, 21.1%, 40.4%, and 31.6% of HBeAg-negative subjects, and in 6.7%, 17.2%, 52.2%, and 23.1% of HBeAg-positive subjects, respectively. The proportions of subjects infected with HBV genotypes E or F were minimal with no one having genotypes G and H: genotype E in 1.8% and 0.3% of HBeAg-negative and HBeAg-positive subjects, respectively, and genotype F only in 0.5% of HBeAg-positive subjects. The percentage of subjects with each HBV genotype was comparable between prior NrtI-exposed and NrtI-naïve subjects (Table 15; FDA analysis).

Table 15: Distribution of HBV Genotype

Treatment		**TAF**				TDF			
Study		**108 (HBeAg-negative)**		**110 (HBeAg-positive)**		108 (HBeAg-negative)		110 (HBeAg-positive)	
Prior NrtI treatment[1]		**No** (n=225)	**Yes** (n=60)	**No** (n=430)	**Yes** (n=151)	No (n=109)	Yes (n=31)	No (n=215)	Yes (n=77)
HBV genotype	A	**5.3%** (n=12)	**5.0%** (n=3)	**6.3%** (n=27)	**7.9%** (n=12)	2.8% (n=3)	9.7% (n=3)	8.4% (n=18)	9.1% (n=7)
	B	**21.3%** (n=48)	**20.0%** (n=12)	**19.1%** (n=82)	**11.9%** (n=18)	33.0% (n=36)	12.9% (n=4)	17.7% (n=38)	13.0% (n=10)
	C	**38.2%** (n=86)	**48.3%** (n=29)	**51.9%** (n=223)	**53.0%** (n=80)	30.3% (n=33)	45.2% (n=14)	54.4% (n=117)	45.5% (n=35)
	D	**32.9%** (n=74)	**26.7%** (n=16)	**21.9%** (n=94)	**26.5%** (n=40)	29.4% (n=32)	32.3% (n=10)	18.1% (n=39)	31.2% (n=24)
	Others[2]	**2.2%** (n=5)	**0%** (n=0)	**0.9%** (n=4)	**0.7%** (n=1)	4.6% (n=5)	0% (n=0)	1.4% (n=3)	1.3% (n=1)

[1] Prior treatment with HBV NrtI(s) regardless of treatment duration.
[2] Other genotypes include genotypes E (n=7) and F (n=3) in the TAF treatment group; and genotypes E (n=3), F (n=2), and H (n=2), and unknown (n=2) in the TDF treatment group.

The proportion of subjects achieving HBV DNA <29 IU/mL at Week 48 was evaluated based on the viral genotype at Baseline in the Week-48 censored, as-treated subject population. As

summarized in Table 16 (FDA analysis), in the TAF treatment group, the proportion of subjects achieving virologic suppression of HBV DNA <29 IU/ml at Week 48 was significantly lower for subjects with genotype D within each of the baseline subgroups (grouped by HBeAg status and prior NrtI treatment history) as compared to the corresponding subgroup of subjects having genotypes A, B, or C. Similar trends of reduced responses were also observed in genotype D subjects treated with TDF, particularly in HBeAg-positive subjects (Study 110; Table 16). Based on these initial observations, an additional subgroup analysis was conducted where subjects were grouped into 2 HBV genotype populations, genotype D versus genotype non-D (including subjects with genotypes A, B, C, E, F, and H but excluding those of 'unknown' genotype). Results are summarized in Table 17. In general, lower virologic response rates to TAF were observed, within each of the baseline subgroups (by HBeAg status and prior NrtI treatment history), for subjects with genotype D, compared to those with genotype non-D: among HBeAg-positive subjects in the TAF group, the difference between genotype D subjects and those with genotype non-D was statistically significant at -30.1% (45.2% versus 75.3%, p value of <0.001) in NrtI-naïve subjects and -20.2% (38.5% versus 58.7%, p value of <0.05) in prior NrtI-exposed subjects. In the TDF group, HBeAg-positive subjects also showed significant differences in the virologic response rate of 32.7% (p=<0.001) and 13.8% (not reaching statistical significance because of the small sample size) favoring subjects with genotype non-D over those with genotype D, respectively. As noted with HBeAg-positive subjects in the TDF trial (GS-US-174-0102; Virology review N021356.SE1-025), in these TAF studies (108 and 110), genotype D subjects also had higher baseline viral load (based on the median baseline HBV DNA level and the proportion of subjects with HBV DNA ≥8 $\log_{10}$ IU/mL), compared to those with genotype non-D (Table 17; FDA analysis). Thus, subjects (HBeAg-positive subjects in particular) infected with HBV genotype D having a lower virologic response to TAF and TDF at Week 48 may be at least partly due to their higher baseline viral load.

Table 16: Week-48 Virologic Response to TAF Treatment by HBV Genotype[1]

Treatment		**TAF**				TDF			
Study		**108 (HBeAg-negative)**		**110 (HBeAg-positive)**		108 (HBeAg-negative)		110 (HBeAg-positive)	
Prior NrtI treatment[2]		**No** (n=216)	**Yes** (n=59)	**No** (n=425)	**Yes** (n=148)	No (n=106)	Yes (n=30)	No (n=210)	Yes (n=77)
Overall[1]		**98.1%** (212/216)	**94.9%** (56/59)	**68.7%** (292/425)	**53.4%** (79/148)	95.3% (101/106)	93.3% (28/30)	72.9% (153/210)	54.5% (42/77)
HBV genotype	A	**100%** (12/12)	**100%** (3/3)	**74.1%** (20/27)	**83.3%** (10/12)	100% (3/3)	100% (3/3)	76.5% (13/17)	57.1% (4/7)
	B	**97.7%** (43/44)	**100%** (11/11)	**64.6%** (53/82)	**61.1%** (11/18)	97.0% (32/33)	100% (4/4)	73.0% (27/37)	60.0% (6/10)
	C	**98.8%** (83/84)	**96.6%** (28/29)	**79.5%** (174/219)	**55.1%** (43/78)	93.9% (31/33)	84.6% (11/13)	81.6% (93/114)	60.0% (21/35)
	D	**97.2%** (69/71)	**87.5%** (14/16)	**45.2%** (42/93)	**38.5%** (15/39)	93.8% (30/32)	100% (10/10)	46.2% (18/39)	45.8% (11/24)
	Others[2]	**100%** (5/5)	**0%** (n=0)	**75.0%** (3/4)	**0%** (0/1)	100% (5/5)	0% (n=0)	66.7% (2/3)	1.3% (n=1)

[1] The Week-48 antiviral efficacy analysis was conducted using the Week-48 censored, as-treated subject population that includes all randomized subjects who received at least one dose of study medication with at least one virologic observation after Baseline but excludes subjects who discontinued their assigned treatment before Week 48 while they had a suppressed viral load (HBV DNA <29 IU/mL). The overall rate was calculated using the Week-48 censored, as-treated subject population.

[2] Prior treatment with HBV NrtI(s) regardless of treatment duration.

[3] Other genotype includes genotypes E (n=7) and F (n=3) in the TAF treatment group; and genotypes E (n=3), F (n=2), and H (n=2),

and unknown (n=2) in the TDF treatment group.

Table 17: Week-48 Virologic Response to TAF Treatment by HBV Genotypes D versus Non-D[1]

Treatment	**TAF**				TDF			
Study	**108 (HBeAg-negative)**		**110 (HBeAg-positive)**		108 (HBeAg-negative)		110 (HBeAg-positive)	
Prior NrtI treatment[2]	**No** (n=216)	**Yes** (n=59)	**No** (n=425)	**Yes** (n=148)	No (n=106)	Yes (n=30)	No (n=210)	Yes (n=77)
Overall[1]	**98.1%** (212/216)	**94.5%** (56/59)	**68.7%** (292/425)	**53.4%** (79/148)	95.3% (101/106)	93.3% (28/30)	72.9% (153/210)	54.5% (42/77)
- Median VL at Baseline[3]	**5.67**	**5.46**	**7.88**	**8.01**	5.72	6.25	7.96	8.15
- % baseline VL ≥8[4]	**4.6%** (10/216)	5.1% (3/59)	**45.6%** (194/425)	**50.7%** (75/148)	0.9% (1/106)	10% (3/30)	47.1% (99/210)	53.2% (41/77)
Genotype Non-D[5]	**98.6%** (143/145)	**97.7%** (42/43)	**75.3%** (250/332)	**58.7%** (64/109)	95.9% (70/73)	90.0% (18/20)	78.9% (135/171)	59.6% (31/52)
- Median VL at Baseline[3]	**5.72**	**5.14**	**7.81**	**7.87**	5.58	6.14	7.95	7.80
- % baseline VL ≥8[4]	**3.4%** (5/145)	**2.3%** (1/43)	**40.1%** (133/332)	**45.8%** (50/109)	0% (0/73)	10.0% (2/20)	46.2% (79/171)	42.3% (22/52)
Genotype D	**97.2%** (69/71)	**87.5%** (14/16)	**45.2%** (42/93)	**38.5%** (15/39)	93.8% (30/32)	100% (10/10)	46.2% (18/39)	45.8% (11/24)
- Median VL at Baseline[3]	**5.59**	**6.73**	**8.41**	**8.22**	5.87	7.08	8.29	8.69
- % baseline VL ≥8[4]	**7.0%** (5/71)	**12.5%** (2/16)	**65.6%** (61/93)	**64.1%** (25/39)	3.1% (1/32)	10.0% (1/10)	51.3% (20/39)	75.0% (18/24)

[1] The Week-48 antiviral efficacy analysis was conducted using the Week-48 censored, as-treated subject population that includes all randomized subjects who received at least one dose of study medication with at least one virologic observation after Baseline but excludes subjects who discontinued their assigned treatment before Week 48 while they had a suppressed viral load (HBV DNA <29 IU/mL). The overall rate was calculated using the Week-48 censored, as-treated subject population.

[2] Prior treatment with HBV NrtI(s) regardless of treatment duration.

[3] Median plasma viral load (VL) at Baseline, expressed as HBV DNA $\log_{10}$ IU/mL.

[4] Proportion of subjects with high baseline viral load (HBV DNA ≥8 $\log_{10}$ IU/mL).

[5] Genotype non-D subject population includes subjects with genotypes A, B, C, E, F, and H but excludes those of 'unknown' genotype.

In summary, the antiviral efficacy of TAF was noninferior to TDF, based on the Week-48 virology data. The rates of virologic success of HBV DNA suppression <29 IU/mL (169 copies/mL) through Week 48 were comparable between the two TAF and TDF treatment groups in censored, as-treated snapshot analysis: 97.5% versus 94.9% in Study 108 (HBeAg-negative subjects) and 64.7% versus 67.9% in Study 110 (HBeAg-positive subjects). By Week 48, 0.7% of HBeAg-positive subjects in the TAF group and 0.3% of those in the TDF group experienced HBsAg loss, while no HBeAg-negative subjects in either treatment group experienced HBsAg loss. Among those with HBsAg loss (4 TAF and one TDF), 3 subjects in the TAF group (overall 0.5%) and none in the TDF group (overall 0%) also experienced HBsAg seroconversion at Week 48. In HBeAg-positive subjects, 13.8% and 10.3% of TAF subjects, and 11.9% and 8.1% of TDF subjects experienced HBeAg loss and HBeAg seroconversion, respectively. Subgroup analyses conducted to identify baseline factors that may affect antiviral response to TAF showed negative correlation between baseline HBV DNA levels and antiviral efficacy of TAF, independent of prior NrtI treatment history. Similarly, prior HBV NrtI exposure appeared to have a negative impact on Week-48 virological response to TAF monotherapy, independent of baseline viral load. In addition, there were significant observed differences in the TAF response

rate (HBV DNA <29 IU/mL) at Week 48, favoring subjects with baseline wild-type like HBV (absence of known NrtI-R substitutions detected by LiPA probes) over those with baseline virus expressing NrtI-R substitutions and also favoring subjects with baseline HBV non-D genotype over those with baseline genotype D. However, the lower rates of viral suppression at Week 48 observed in those subgroups of subjects with baseline virus expressing NrtI-R substitutions or with baseline genotype D may be impacted by their higher baseline viral load (based on the median baseline HBV DNA level and the proportion of subjects with HBV DNA ≥8 $\log_{10}$ IU/mL) compared with their respective counterpart subject groups.

4. Conclusion

This original NDA for VEMLIDY™ 25 mg QD is approvable for the treatment of chronic HBV infection in adult patients with compensated liver disease with respect to Clinical Virology, based on Week-48 data from 2 Phase 3 studies, GS-US-320-0108 (HBeAg-negative) and GS-US-320-0110 (HBeAg-positive). Based on the Week-48 virology data, antiviral efficacy of VEMLIDY (tenofovir alafenamide; TAF) was noninferior to TDF (VIREAD® 300 mg QD), and no specific amino acid substitutions in the HBV rt domain were commonly detected in TAF-treatment failure isolates during the first year of the study. Thus, the association of those observed rt substitutions was not established with TAF genotypic resistance. In subgroup analyses, negative correlation between baseline HBV DNA levels and antiviral efficacy of TAF, independent of prior NrtI treatment history, was observed. Similarly, prior HBV NrtI exposure appeared to have a negative impact on Week-48 virological response to TAF monotherapy, independent of baseline viral load.

TAF is a second-generation, phosphonamidate prodrug of nucleotide analogue reverse transcriptase inhibitor tenofovir (TFV; 2'-deoxyadenosine monophosphate analogue) that has activity against HBV reverse transcriptase (rt) as well as HIV-1 reverse transcriptase (RT). In target cells, TAF is converted to TFV through hydrolysis primarily by carboxylesterase 1 (CES1) in primary hepatocytes, and by cathepsin A in PBMCs and other HIV target cells. TAF inhibited clinical isolates of HBV with EC_{50} values of 34.7 to 134.4 nM with an overall mean EC_{50} value of 86.6 nM in transiently transfected human hepatoblastoma cells. CES1 was reported to be also involved in sofosbuvir activation in the liver. In a cell-based combination antiviral activity study, the presence of TAF did not impact the anti-HCV activity of sofosbuvir. The combination effect of TAF and sofosbuvir on the TAF anti-HBV activity was not evaluated.

The first generation prodrug of TFV, VIREAD® (tenofovir disoproxil fumarate; TDF), has been marketed for the treatment of chronic HBV infection in adult patients since 2008 and in pediatric patients (12 years of age and older) since 2012. TAF was designed to overcome the permeability limitations of TFV and increase the plasma stability of the prodrug compared to TDF. Recently, TAF has gained approval by FDA for the treatment of HIV-1 infection in adult and pediatric patients (≥12 years of age) as a component of GENVOYA® (TAF/FTC/EVG/COBI [10/200/150/150 mg]) on November 5, 2015, of ODEFSEY® (TAF/FTC/RPV [25/200/25 mg]) on March 1, 2016, and of DESCOVY® (TAF/FTC [10/200 mg and 25/200 mg]) on April 4, 2016.

PACKAGE INSERT: Sections 12.1 and 12.4

The words with double strikethroughs are the text the applicant was requested to delete and the words written in red are recommended insertions.

Section 12.1 Mechanism of Action

Tenofovir alafenamide is an antiviral drug against the hepatitis B virus [(b) (4) see *Microbiology (12.4)*].

Section 12.4 Microbiology

Mechanism of Action

Tenofovir alafenamide is a phosphonamidate prodrug of tenofovir (2'-deoxyadenosine monophosphate analogue). Tenofovir alafenamide as a lipophilic cell-permeant compound enters primary hepatocytes by passive diffusion and by the hepatic uptake transporters OATP1B1 and OATP1B3. Tenofovir alafenamide is then converted to tenofovir through (b) (4) hydroly (b) (4) sis primarily by carboxylesterase 1 (CES1) in primary hepatocytes, (b) (4) (b) (4). Intracellular tenofovir is subsequently phosphorylated by cellular kinases to the pharmacologically active metabolite tenofovir diphosphate. Tenofovir diphosphate inhibits HBV replication through incorporation into viral DNA by the HBV reverse transcriptase, which results in DNA chain-termination. (b) (4) (b) (4) (b) (4) Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases that include mitochondrial DNA polymerase γ and there is no evidence of (b) (4) toxicity to mitochondria in cell culture (b) (4) (b) (4).

Antiviral Activity in Cell Culture

The antiviral activity of tenofovir alafenamide was assessed in a transient transfection assay using HepG2 cells against a panel of HBV clinical isolates representing genotypes A-H. The EC_{50} (50% effective concentration) values for tenofovir alafenamide ranged from 34.7 to 134.4 nM, with an overall mean EC_{50} value of 86.6 nM. The CC_{50} (50% cytotoxicity concentration) values in HepG2 cells (b) (4) were (b) (4) greater than 44,400 nM. In cell culture combination antiviral activity studies of tenofovir with the HBV nucleoside reverse transcriptase inhibitors (b) (4) (b) (4), entecavir, lamivudine, and telbivudine, no antagonistic activity was observed.

Resistance in Clinical Trials

In a pooled analysis of treatment-naïve and treatment-experienced subjects receiving VEMLIDY in Stud (b) (4) es 108 and (b) (4) 110, (b) (4) genotypic resistance analysis was performed on pai ed baseline and on-treatment HBV isolates for subjects who either experienced virologic breakthrough (2 consecutive visits with HBV DNA (b) (4) greater than or equal to 69 IU/mL [400 copies/mL] after having been (b) (4) less than 69 IU/mL, or 1.0-log_{10} or greater increase in HBV DNA from nadir) through Week 48, or had HBV DNA ≥ greater than or equal to 69 IU/mL at early discontinuation at or after Week 24. (b) (4) (b) (4) Treatment-emergent amino acid substitutions in the HBV reverse transcriptase

domain, all occurring at polymorphic positions, were observed in some HBV isolates evaluated (5/20); however, (b) (4) no specific (b) (4) substitutions occurred at a sufficient frequency to be associated with resistance to VEMLIDY (b) (4).

Cross-Resistance

The antiviral activity of tenofovir alafenamide was evaluated against a panel of isolates containing (b) (4) substitutions associated with HBV NrtI resistance in a transient transfection assay using HepG2 cells. HBV isolates expressing the lamivudine resistance-associated substitutions (b) (4) rtM204V/I (±rtL180M±rtV173L) (b) (4) and expressing the entecavir resistance-associated substitutions (b) (4) rtT184G, rtS202G, or rtM250V in the presence of rtL180M and rtM204V (b) (4) (b) (4) showed less than 2-fold reduced susceptibility (within the inter-assay variability) to tenofovir alafenamide. HBV isolates expressing the rtA181T, rtA181V, or rtN236T single substitutions associated with resistance to adefovir (b) (4) (b) (4) also had less than 2-fold changes in EC_{50} values; however, the HBV isolate expressing the rtA181V plus rtN236T double substitutions exhibited reduced susceptibility (3.7-fold) to tenofovir alafenamide (b) (4) The clinical relevance of these substitutions is not known.

APPENDICES

Appendix 1: Materials and Methods

A1.1. Quantification of Plasma HBV DNA Levels

Plasma HBV DNA was quantified using the Roche COBAS® TaqMan® HBV Test for Use with the High Pure System (FDA-approved in September, 2008 [P050028]), which has a lower limit of quantification (LLOQ) of 29 IU/mL (169 copies/mL) and a limit of detection (LOD) of 10-12 IU/mL (58-70 copies/mL; Allice *et al*., 2007). The assay kit includes primer pairs and probes specific for both HBV DNA and HBV Quantification Standard DNA, which gives equivalent results for HBV from genotypes A through G (Weiss *et al*., 2004). According to the manufacturer, the HPS/COBAS TaqMan HBV assay has a linear range of 29 to $1.1x10^8$ IU/mL (169 to $6.4x10^8$ copies/mL), which was confirmed by the applicant (Report PC-174-2001). For specimens that contain high levels of HBV DNA (above the upper limit of quantification), diluted samples were requantified. When HBV DNA levels were below the LLOQ, the applicant reported viral load results as "<29 IU/mL, HBV DNA detected" or "<29 IU/mL, no HBV DNA detected" as recommended by the Division (Virology review I115561.016).

Of note, the HBV DNA levels are expressed in International Units (IU)/mL. The conversion factor between HBV copies/mL and HBV IU/mL is 5.82 copies/IU based on linkage to the WHO International Standard for HBV DNA for nucleic acid amplification technology assays (NIBSC code 97/746).

A1.2. Baseline Resistance Analysis of HBV Reverse Transcriptase (rt)

Baseline serum samples from all subjects enrolled in Studies GS-US-320-0108 and GS-US-320-0110 were assessed for the presence of rt substitutions known to be associated with resistance to HBV nucleos(t)ide analog reverse transcriptase inhibitors (NrtIs), including adefovir, entecavir, lamivudine, telbivudine, and TDF using the INNO-LiPA Multi DR v2/v3 assay (Innogenetics). The assay is a biochemical reverse hybridization line probe assay for use on human serum or plasma samples that can simultaneously detect wild-type and clinically relevant NrtI resistance-associated mutant sequences at the following 10 amino acid positions in the HBV rt region of the polymerase gene: 80 (L or I/V), 173 (G/V or L), 180 (L or M), 181 (A or T/V), 184 (T or A/C/F/G/I/L/M/S), 194 (A or T), 202 (S or C/G/I), 204 (M or I/S/V), 236 (N or T), and 250 (M or I/L/V). The current version of the assay is designed to detect these specific substitutions when present in ≥5% of the virus population (Degertekin *et al*., 2009). Substitutions observed at any of these amino acid positions were then assigned to a resistance category as defined by the INNO-LiPA kit (Table A1). Of note, if no hybridization signals at an amino acid of interest were observed, the result for that amino acid position was recorded as an unknown variant.

Subjects' baseline serum samples were accessed from the specimen management system maintained by the central laboratory and

shipped to (b) (4) for analysis. The assay has a lower limit of detection of 170 IU/mL (approximately 990 copies/mL). The assay procedure comprises the following steps: (1) HBV DNA isolation from clinical serum specimens and PCR amplification using biotinylated primers (867-bp long fragment of the HBV rt region) and (2) reverse hybridization of the amplified DNA product onto 2 strips coated with probes that can differentiate wild-type versus mutant sequences at the positions listed above.

Table A1: Resistance Substitution Categories Defined by the INNO-LiPA Assay

Mutation Category	Mutations
Adefovir Resistance	rtN236T
Lamivudine Resistance	rtM204V/I/S
Lamivudine/Adefovir Resistance	rtL180M, rtA181T/V
Entecavir Resistance	rtT184S/C/G/A/I/L/F/M, rtS202G/C/I, rtM250V/I/L
Tenofovir Predisposition	rtA194T
Compensatory Mutations	rtL80V/I, rtV173G/L

Source: Integrated Virology Study Report PC-320-2009, Table 2, page 13

A1.3. HBV rt Resistance Analysis of Samples Collected from Resistance Testing Eligible Subjects

Subjects eligible for resistance testing were identified based on the following inclusion and exclusion criteria:

- Viremic subjects with HBV DNA ≥69 IU/mL at the end of each study year (e.g., Weeks 48 and 96).
- Viremic subjects with HBV DNA ≥69 IU/mL at the time of study discontinue who received ≥24 weeks of treatment and discontinued early from the study.
- In the Week-48 resistance analysis, subjects having HBV DNA ≥69 IU/mL at Week 48 in the absence of virologic breakthrough were not included. The Week-48 samples from these subjects will be included in the Week-96 resistance analysis if they remain viremic at Week 96. Virologic breakthrough is defined as having HBV DNA ≥69 IU/mL after HBV DNA levels <69 IU/mL being achieved, or having HBV DNA increase by ≥1 $\log_{10}$ from nadir. Two consecutive visits that meet the definition were required for a subject to be classified as having virologic breakthrough. If subjects experienced virologic breakthrough at Week 48, the subject's

Week-56 viral load data (if available) were used to determine if the subject met the definition of virologic breakthrough. For those whose Week-56 viral load data were unavailable for the Week-48 analysis, resistance analysis will be conducted during the Week-96 analysis if the subject is later confirmed to meet the definition of virologic breakthrough.

Genotypic Resistance Analysis by Population-Based Sequencing of the rt Domain of the HBV Polymerase Gene

HBV polymerase is a large protein with three distinct functional domains: the terminal protein domain, the rt domain, and the RNase H domain (Nassal and Schaller, 1993). The entire rt domain (amino acids 1-344) were amplified and sequenced for subjects' samples qualified for resistance testing in a treatment-blinded fashion. Subjects' serum samples (baseline and qualified post-baseline with HBV DNA ≥69 IU/mL) were accessed from the specimen management system maintained by the central laboratory and shipped to (b) (4) for analysis. The genotypic analysis procedure comprises the following steps (Report PC-320-2008): (1) DNA isolation from 200 μL of serum using the Roche automated MagNA Pure System; (2) PCR and nested PCR amplification of the HBV rt domain using the Roche Expand High-Fidelity PCR System; (3) Standard population dideoxy sequencing of the amplified product using the ABI Big Dye® Terminator version 3.1 Cycle Sequencing Kit employing a selection of forward and reverse primers; and (4) Analysis of the raw sequence data using the ABI SeqScape® Software version 2.7. This HBV rt population-based sequencing assay can detect nucleotide mixtures when present in a population at approximately 25%. The lower limit for amplification of the HBV rt domain is 69 IU/mL.

Post-baseline rt sequences for resistance testing qualified subjects were aligned to their respective baseline sequences. For subjects who switch to open-label treatment, the last sequence obtained in the blinded study arm will serve as the baseline sequence for comparison to results obtained after the switch to open-label treatment. Differences between the baseline and post-baseline samples were categorized as polymorphic or conserved site changes based on the definition provided below. Changes from baseline by amino acid position (full change and mixtures) observed at the post-baseline (or post-switch visit) were reported: mixtures of amino acids present at conserved positions in the baseline sample which resolved to a single amino acid were reported, while mixtures of amino acids present at polymorphic positions in the baseline sample which resolved to a single amino acid were not reported. If the post-baseline sample contained a mixture of amino acids at a polymorphic or conserved site not present in the baseline sample, the development of a change was reported.

The location of polymorphic and conserved sites in the HBV rt domain were identified by comparing baseline amino acid sequences collected from the two VIREAD Phase 3 studies GS-US-174-0102 and GS-US-174-0103 (n=628). Conserved sites were defined as those positions with only one amino acid present, or at which two amino acids were present but the prevalence of the minority amino acid was <1%. All other positions within the HBV rt domain were considered polymorphic sites. Overall, 64% of amino acid positions were classified as conserved with the remaining 36% classified as polymorphic (Kitrinos *et al*., 2014).

Phenotypic Resistance Analysis for antiviral susceptibility to TAF

Cell culture phenotypic analyses of TAF or TFV susceptibility were performed on paired baseline and post-baseline samples from subjects whose post-baseline samples were genotyped and who (1) experienced virologic breakthrough while on treatment regardless of treatment-emergent rt genotypic changes observed, (2) developed an emerging amino acid substitution at conserved sites of the HBV rt domain, or (3) developed an emerging amino acid substitution at polymorphic sites of the HBV rt domain if the change was observed in more than one subject. However, the following samples were not phenotyped collected from subjects (1) who did not experience virologic breakthrough and did not develop treatment-emergent rt substitutions, (2) who developed treatment-emergent rt substitutions but subsequently suppressed their HBV DNA to <69 IU/mL for at least 2 consecutive visits, or (3) who experienced virologic breakthrough associated with plasma TFV levels below the limit of quantification or other measures that documented nonadherence to study drug unless a conserved site change was detected. Nonadherence to study drug was measured by evaluation of TFV levels in plasma using liquid chromatography/mass spectroscopy. Nonadherence was defined as TFV levels below the limit of quantification or TFV trough levels >10 times below the average (<1 ng/mL).

A cell culture model of HBV replication, utilizing HepG2 cells transiently transfected with a pool of recombinant HBV plasmid DNA derived from subject serum HBV quasi-species harboring various amino acid substitutions in HBV rt, was used as the standard drug susceptibility assay (Nonclinical Reports PC-320-2002 and PC-320-2008). Briefly, the HBV rt region was amplified by PCR using HBV DNA extracted from subject serum samples as template and then cloned into pRTAN (Zhu *et al*., 2011), a plasmid vector containing the full genome wild-type HBV laboratory strain as in the parent vector pHY92 (genotype A, subtype adw2; Yang *et al*., 2004), except missing the majority sequences of rt (from amino acid 17 to 311). Of note, the extracted HBV DNA and/or PCR products which were generated for genotypic resistance testing were used to generate the recombinant HBV genome containing the rt region from clinical isolates. Cell culture susceptibility to TAF of samples derived from TAF recipients and to TFV of those derived from TDF recipients (since TDF is unstable in cell culture [Callebaut *et al*., 2015]) was then assessed by transient transfection with plasmid DNA of recombinant HBV quasi-species pools into HepG2 hepatoma cells and culturing cells in the presence of serial dilutions of the drug. After 3 days of incubation, cells were cultured for 4 more days in fresh medium containing the drug and culture supernatant was harvested. Extracellular HBV DNA in the supernatant was then quantified using the Affymetrix QuantiGene® 2.0 assay that utilizes branched DNA technology to amplify the HBV DNA signal. Phenotyping assays were performed at the Gilead Sciences Clinical Virology laboratory (Foster City, CA).

Typically the phenotypic testing of each recombinant construct is performed in 3 independent experiments and the average EC_{50} value along with standard deviation is reported. The average EC_{50} value of the subject's post-baseline isolate is compared to that of the corresponding baseline isolate (or last on blinded treatment for subjects who switched to open-label treatment) to calculate the fold-changes in susceptibility to the tested drug. With the established cutoff fold-change value for TAF of 2, post-baseline isolates displaying >2-fold reductions in TAF susceptibility were considered to have a reduced susceptibility to TAF, and these isolates were also characterized for cross resistance against approved HBV NrtIs.

Appendix 2: Week-48 Resistance Testing Eligible Virologic Failures' Genotypic and Phenotypic Data

Forty-one subjects (4 HBeAg$^-$ and 37 HBeAg$^+$ subjects; 27 TAF- and 14 TDF-treated subjects) were identified to be eligible for Week-48 resistance testing: 33 subjects who experienced virologic breakthrough by Week 48 (20 TAF- and 13 TDF- treated subjects) and 8 subjects who never suppressed HBV DNA levels to <69 IU/mL without evidence of virologic breakthrough at the time of early discontinuation (7 TAF- and 1 TDF- treated subjects).

Table A2: Week-48 Resistance Testing Eligible Virologic Failures[1] and Their Genotypic and Phenotypic Resistance Data

Subject ID	HBV genotype	Prior treatment[2]	Study treatment	Baseline		Resistance testing		
				HBV DNA[3]	HBV Nrtl resistance[4]	Isolate (HBV DNA[3])	Treatment-emergent rt substitution[5]	Drug susceptibility (fold-change[6])
STUDY 108 (HBeAg-negative)								
5613-1163	C	ETV	TAF	7.53	wild-type	Week 48 (2.15)	None detectable	1.01 (1.27)
6958-1318	D	LdT, TDF	TAF	7.81	wild-type	Week 48 (3.19)	None detectable	1.21 (1.42)
6963-1339	D	Nrtl-naïve	TDF	5.58	wild-type	Week 24[7] (2.20)	No data	nd
8519-1176	C	Nrtl-naïve	TDF	6.07	wild-type	Week 48 (2.33)	No data	nd
STUDY 110 (HBeAg-positive)								
0481-4873	A	Nrtl-naïve	TDF	8.84	wild-type	Week 48 (1.97)	None detectable	0.72 (0.75)
1507-4546	C	Nrtl-naïve	TDF	8.07	T/ILFM184	Week 48 (3.00)	D134D/E, V214V/A, A317A/S	nd
1659-4721	D	ETV	TDF	8.98	wild-type	Week 48 (7.91)	None detectable	nd
1659-4788	D	ETV	TAF	8.03	wild-type	Week 48 (4.00)	None detectable	0.65 (1.42)
2826-4527	C	TAF	TAF	8.24	A/T194	Week 36 (2.31)	D134E, M309K	nd
3912-5084	C	TDF	TDF	8.48	wild-type	Week 48 (2.33)	None detectable	0.96 (1.02)
4037-5358	D	TDF	TAF	7.50	A/V181	Week 48 (3.54)	No data	nd
4074-4571	C	TDF	TAF	4.28	wild-type	Week 48 (3.43)	No data	nd
4164-4604	B	LAM	TDF	7.72	wild-type	Week 48 (2.58)	No data	nd
4164-5252	D	Nrtl-naïve	TAF	8.72	wild-type	Week 48 (1.94)	No data	nd

4296-4510	B	Nrtl-naïve	TAF	7.86	wild-type	Week 48 (2.05)	None detectable	1.33 (1.35)
4296-4759	C	LAM, TDF	TAF	8.05	wild-type	Week 48 (4.42)	No data	nd
4296-5147	C	TDF	TAF	4.62	wild-type	Week 48 (4.68)	S256S/C	nd
5606-5140	B	Nrtl-naïve	TAF	8.40	wild-type	Week 48 (5.04)	None detectable	nd
5617-4835	C	ETV	TAF	7.80	wild-type	Week 48 (2.12)	No data	nd
5617-4966	C	Nrtl-naïve	TAF	8.55	wild-type	Week 48[8] (3.07)	None detectable	nd
5620-5225	B	Nrtl-naïve	TDF	7.68	wild-type	Week 48 (2.29)	**Q67Q/H**, N118N/T, N123N/D. M207M/V	1.50 (1.05)
							Week-48 clone with Q67H[9]	1.46 (1.01)
5691-4594	C	Nrtl-naïve	TAF	8.27	wild-type	Week 32[8] (2.23)	No data	nd
5691-4944	C	Nrtl-naïve	TAF	8.67	wild-type	Week 48 (5.99)	None detectable	nd
6336-5050	C	ADV, ETV, LAM, TDF	TAF	4.63	L/I80, M/I204	Week 24[8] (4.11)	None detectable	nd
6338-4707	C	Nrtl-naïve	TDF	6.54	Wild-type	Week 48 (2.15)	No data	nd
6958-5201	D	TDF	TAF	8.69	Wild-type	Week 48 (5.22)	None detectable	nd
6965-5170	A	Nrtl-naïve	TAF	7.22	Wild-type	Week 36 (2.36)	None detectable	nd
7515-5071	D	Nrtl-naïve	TAF	8.52	Wild-type	Week 28 (5.10)	None detectable	nd
8006-5282	D	Nrtl-naïve	TAF	9.09	Wild-type	Week 48 (3.16)	None detectable	nd
8017-4565	D	LAM, LdT	TAF	8.21	L/I80, M/I204	Week 48 (2.61)	I91I/L, E271A/E (I80I/L), (I204I/M)	nd
8312-4689	C	ADV, ETV	TAF	6.79	M/I204	Week 28[8] (6.97)	None detectable	nd
8569-5131	D	Nrtl-naïve	TDF	5.87	wild-type	Week 48 (1.96)	**Q288Q/stop**	1.07 (0.68)
							Week-48 clone with Q288stop[10]	unable to replicate
8569-5132	D	Nrtl-naïve	TAF	8.41	wild-type	Week 48 (1.96)	No data	nd
8599-4712	D	Nrtl-naïve	TDF	9.56	wild-type	Week 48 (7.94)	None detectable	nd
8599-4790	D	Nrtl-naïve	TDF	9.48	wild-type	Week 48 (5.90)	None detectable	nd

8599-5374	D	NrtI-naïve	TDF	5.34	wild-type	Week 48 (2.66)	None detectable	nd
8600-4558	D	NrtI-naïve	TAF	8.58	wild-type	Week 48 (2.42)	None detectable	nd
8758-5188	D	NrtI-naïve	TAF	4.74	wild-type	Week 48 (1.96)	R153Q	nd
9035-4845	C	ADV, ETV, LAM	TDF	6.62	A/T181	Week 48 (4.38)	R110R/G,L269I/L (A/T181A/T)	nd
9035-5187	C	ETV	TAF	4.94	wild-type	Week 48 (2.21)	S13S/N, S117S/P, L267L/Q, L269L/I	0.66 (0.80)
9695-5283	D	NrtI-naïve	TAF	8.80	wild-type	Week 24 (5.70)	None detectable	1.05 (0.77)

nd, not determined

Note: Amino acid substitutions in parentheses associated with resistance to HBV NrtIs were detected in the subjects' baseline isolates and persistently detectable in their Week-48 failure samples.

[1] Week-48 resistance testing eligible virologic failures (VFs) are those who were eligible for Week-48 resistance testing. Week-48 resistance testing was conducted for those, among the Week-48 VFs with HBV DNA ≥69 IU/mL, who experienced confirmed virologic breakthrough by Week 48 or who never suppressed HBV DNA levels to <69 IU/mL without evidence of virologic breakthrough at the time of early discontinuation (before the Week-48 visit).

[2] Prior treatment with HBV NrtI(s) regardless of treatment duration.

[3] HBV DNA expressed as $\log_{10}$ IU/mL.

[4] Presence of rt substitutions associated with resistance to HBV NrtIs in baseline samples were assessed using the INNO-LiPA Multi DR v2/v3 assay (Innogenetics). This assay is designed to differentiate wild-type versus substitutions at 10 amino acid positions of the rt domain of HBV polymerase (Degertekin *et al*., 2009; Niesters *et al*., 2010): L80I/V, G/V173L, L180M, A181T/V, T184A/C/F/G/I/L/M/S, A194T, S202C/G/I, M204I/S/V, N236T, and M250I/L/V.

[5] Amino acid substitutions that occurred at HBV rt conserved amino acid positions are written in red.

[6] Fold-change in drug suscept bility of virologic failure isolates (subject-derived pooled and clonal virus sample), compared to wild-type reference HBV (pHY92 genotype A laboratory strain; Yang *et al*., 2004) and to their respective baseline isolates (in parentheses). TAF susceptibilities were determined for paired isolates (baseline and treatment-failure) collected from TAF recipients and TFV susceptibilities were for those collected from TDF recipients. Of note, the EC_{50} values of TAF and TFV were approximately 33.5 nM and 3.20 μM, respectively, for the wild-type reference HBV pHY92. A value <2-fold is within assay variability.

[7] The isolate was collected 2 days after the last dose.

[8] The isolate was collected one day after the last dose.

[9] Since the conserved-site substitution was a mixture (rtQ67Q/H), a clone containing the full rtQ67H substitution was isolated and its susceptibility to TFV was also determined in cell culture.

[10] Since the conserved-site substitution was a mixture (rtQ288A/stop), a clone containing the full rtQ288stop change was isolated and its susceptibility to TFV was also determined in cell culture. The clone containing the full rtQ288stop substitution was unable to replicate in cell culture.

Appendix 3: Summary of Virologic Responses during 48 Weeks of Treatment for the 41 Week-48 Resistance Testing Eligible Virologic Failures

A3.1. Study 108 (HBeAg-negative)

- Subject 5613-1163, previously exposed to entecavir and IFN with genotype C infection, was randomized to the TAF group, and had baseline viruses (7.53 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. The subject achieved HBV DNA <69 IU/mL by Week 36 and experienced a transient viral increase to >69 IU/mL at Week 40 (100 IU/mL). The subject

experienced virologic breakthrough at Week 48 (140 IU/mL) which was confirmed at Week 56 with HBV DNA 350 IU/mL. Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 99.7% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 6958-1318, previously exposed to telbivudine and TDF with genotype D infection, was randomized to the TAF group, and had baseline viruses (7.81 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved treatment nadir of 2.82 $\log_{10}$ IU/mL at Week 12 and then experienced two episodes of virologic breakthrough at Weeks 16 - 24 (ranging from 5.48 to 6.30 $\log_{10}$ IU/mL) and Weeks 32 - 40 (ranging from 5.98 to 8.40 $\log_{10}$ IU/mL). The subject remained viremic at Week 48 (3.19 $\log_{10}$ IU/mL), and sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 98.9% adherent to study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 6963-1339, treatment-naïve with genotype D infection, was randomized to the TDF group, and had baseline viruses (5.58 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA when she discontinued the study with HBV DNA 2.20 $\log_{10}$ IU/mL at Week 24 (2 days after last dose; follow-up Week 4 visit). The subject discontinued due to an adverse event of hepatocellular carcinoma development. Overall, HBV DNA levels declined >3 $\log_{10}$ IU/mL over 24 weeks of treatment. The follow-up Week 4 sample was unable to be genotyped. This subject was 99.1% adherent to the study drug regimen through early discontinuation based on pill count.

- Subject 8519-1176, treatment-naïve with genotype C infection, was randomized to the TDF group, and had baseline viruses (6.07 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. The subject achieved virologic suppression (HBV DNA <29 IU/mL) at Week 12, which was maintained until he experienced virologic breakthrough at Week 44 (98 IU/mL, confirmed at Week 48 with HBV DNA 213 IU/mL). The Week 48 sample was unable to be genotyped. This subject was 89.5% adherent to the study drug regimen through Week 48 based on pill count and discontinued from the study prior to Week 56 due to death (attributed to hepatocellular carcinoma).

A3.2. Study 110 (HBeAg-positive)

- Subject 0481-4873, treatment-naïve with genotype A infection, was randomized to the TDF group, and had baseline viruses (8.84 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA through Week 44 (57 IU/mL), and then experienced virologic breakthrough at Week 48 (94 IU/mL, confirmed at Week 56 with HBV DNA 86 IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. The subject was 100% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 1507-4546, treatment-naïve with genotype C infection, was randomized to the TDF group, and had baseline viruses (8.07 $\log_{10}$ IU/mL) showing mixed HBV rt sequences, wild-type and rtT184I/L/F/M detectable by INNO-LiPA (positive signals for T184 and I/L/F/M184). This subject had a continual decline in HBV DNA through Week 40 (87 IU/mL) and then experienced virologic breakthrough at Week 44 (4.71 $\log_{10}$ IU/mL) which was maintained through Week 48 (3.0 $\log_{10}$ IU/mL). Sequence analysis of the Week 48 sample identified 3 polymorphic site substitutions (rtD134D/E, rtV214V/A, rtA317A/S). The rtT184I/L/F/M entecavir-R substitution detected at baseline by INNO-LiPA was not detectable by population-based sequencing at baseline or Week 48. The subject was 98.8% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 1659-4721, previously exposed to entecavir with genotype D infection, was randomized to the TDF group, and had baseline viruses (8.98 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject experienced approximately a 3 $\log_{10}$ IU/mL decline in HBV DNA through Week 16 (6.12 $\log_{10}$ IU/mL), and then had a transient viral increase back to the baseline level at Week 20 (9.04 $\log_{10}$ IU/mL). HBV DNA declined again from Weeks 24 to 36 (ranging from 5.75 to 5.98 $\log_{10}$ IU/mL), followed by virologic breakthrough at Week 40 (7.55 $\log_{10}$ IU/mL) which was maintained through Week 48 (7.91 $\log_{10}$ IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. The subject was 99.9% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 1659-4788, previously exposed to entecavir and IFN with genotype D infection, was randomized to the TAF group, and had baseline viruses (8.03 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved HBV DNA <69 IU/mL by Week 40 (57 IU/mL). At Week 48, the subject experienced virologic breakthrough (4.0 $\log_{10}$ IU/mL, confirmed at Week 56 with HBV DNA 5.38 $\log_{10}$ IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 100% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 2826-4527, previously exposed to TAF with genotype C infection, was randomized to the TAF group, and had baseline viruses (8.24 $\log_{10}$ IU/mL) showing mixed HBV rt sequences, wild-type and rtA194T detectable by INNO-LiPA (positive signals for A194 and T194). This subject had a continual decline in HBV DNA until Week 36 (203 IU/mL) when she discontinued the study (withdrew consent). Overall, the subject achieved a >5 $\log_{10}$ IU/mL decline during 36 weeks of treatment. Sequence analysis of the Week 36 sample identified 2 polymorphic site rt substitutions (rtD134E and rtM309K). The rtA194T substitution detected at baseline by INNO-LiPA was not detectable by population-based sequencing at baseline or Week 36. The subject was 100% adherent to the study drug regimen through early discontinuation based on pill count.

- Subject 3912-5084, previously exposed to TDF with genotype C infection, was randomized to the TDF group, and had baseline viruses (8.48 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject reached HBV DNA <69

IU/mL by Week 40 (54 IU/mL) and then experienced virologic breakthrough at Week 48 (216 IU/mL, confirmed at Week 56 with HBV DNA 119 IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. The subject was 99.4% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 4037-5358, previously exposed to TDF and IFN with genotype D infection, was randomized to the TAF group, and had baseline viruses (7.50 $\log_{10}$ IU/mL) showing mixed HBV rt sequences, wild-type and rtA181V detectable by INNO-LiPA (positive signals for A181 and V181). This subject experienced approximately a 3 $\log_{10}$ IU/mL decline in HBV DNA through Week 16 (4.18 $\log_{10}$ IU/mL), and then had confirmed but transient virologic breakthrough at Weeks 20 - 28 (ranging from 5.31 to 8.08 $\log_{10}$ IU/mL). HBV DNA then declined again through Week 48 (3.54 $\log_{10}$ IU/mL). The subject's virus samples were not genotyped. The subject was 95.3% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 4074-4571, previously exposed to TDF with genotype C infection, was randomized to the TAF group, and had baseline viruses (4.28 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved virologic suppression with HBV DNA <29 IU/mL by Week 12, which was maintained through Week 24. The subject experienced virologic breakthrough at Week 28 (3.29 $\log_{10}$ IU/mL), which was maintained through Week 48 (3.43 $\log_{10}$ IU/mL). The Week 48 sample was unable to be genotyped. This subject was 98.1% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 4164-4604, previously exposed to lamivudine with genotype B infection, was randomized to the TDF group, and had baseline viruses (7.72 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved virologic suppression (HBV DNA <29 IU/mL) by Week 32, and then experienced virologic breakthrough at Week 40 (115 IU/mL) which was maintained through Week 48 (384 IU/mL). The Week 48 sample was unable to be genotyped. This subject was 97.5% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 4164-5252, treatment-naïve with genotype D infection, was randomized to the TAF group, and had baseline viruses (8.72 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved HBV DNA <69 IU/mL by Week 36, and then experienced virologic breakthrough at Week 44 (78 IU/mL, confirmed at Week 48 with HBV DNA 88 IU/mL). The Week 48 sample was unable to be genotyped. This subject was 99% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 4296-4510, treatment-naïve with genotype B infection, was randomized to the TAF group, and had baseline viruses (7.86 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved virologic suppression with HBV

DNA <29 IU/mL by Week 16, which was maintained through Week 44, with the exception of a virologic blip at Week 32 (139 IU/mL). The subject experienced virologic breakthrough at Week 48 (111 IU/mL, confirmed at Week 56 with HBV DNA 2,870 IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 96.7% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subjects 4296-4759, previously exposed to lamivudine and TDF with genotype C infection, was randomized to the TAF group, and had baseline viruses (8.05 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved treatment nadir of 3.08 $\log_{10}$ IU/mL at Week 16, and then experienced virologic breakthrough at Weeks 28 - 36 (ranging from 4.28 to 5.87 $\log_{10}$ IU/mL). The subject remained viremic at Week 48 (4.42 $\log_{10}$ IU/mL). The subject's virus samples were not genotyped. This subject was 97.8% adherent to study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 4296-5147, previously exposed to TDF with genotype C infection, was randomized to the TAF group, and had baseline viruses (4.62 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA through Week 16 (2.51 $\log_{10}$ IU/mL), and then experienced virologic breakthrough at Week 24 (3.77 $\log_{10}$ IU/mL) which was maintained through Week 48 (4.68 $\log_{10}$ IU/mL). Sequence analysis of the Week 48 sample identified one polymorphic site substitution (rtS256S/C). This subject was 99% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 5606-5140, treatment-naïve with genotype B infection, was randomized to the TAF group, and had baseline viruses (8.40 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved virologic suppression (HBV DNA <29 IU/mL) at Week 24 and remained virologically suppressed through Week 36. The subject then experienced virologic breakthrough at Week 40 (4.54 $\log_{10}$ IU/mL) which was maintained through Week 48 (5.04 $\log_{10}$ IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 99.1% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 5617-4835, previously exposed to entecavir with genotype C infection, was randomized to the TAF group, and had baseline viruses (7.80 $\log_{10}$ IU/mL). The subject's screening viruses showed wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA until Week 12 (3.47 $\log_{10}$ IU/mL), and then had confirmed but transient virologic breakthrough at Weeks 16 - 20 (ranging from 4.71 to 8.08 $\log_{10}$ IU/mL). HBV DNA then declined again through Week 48 (132 IU/mL). The subject's virus samples were not genotyped. The subject was 90.9% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 5617-4966, treatment-naïve with genotype C infection, was randomized to the TAF group, and had baseline viruses (8.55

log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA (overall >5 log_{10} IU/mL over 48 weeks of treatment) when he discontinued the study (withdrew consent) with HBV DNA 3.07 log_{10} IU/mL at Week 48 (one day after last dose; follow-up Week 4 visit). Sequence analysis of the follow-up Week 4 sample found no changes compared to the baseline isolate. This subject was 96.1% adherent to the study drug regimen through Week 48 based on pill count.

- Subject 5620-5225, treatment-naïve with genotype B infection, was randomized to the TDF group, and had baseline viruses (7.68 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved virologic suppression (HBV DNA <29 IU/mL) by Week 28. The subject missed the Week 32 visit and experienced virologic breakthrough at Week 36 (6.43 log_{10} IU/mL), which was maintained with declining HBV DNA levels through Week 48 (193 IU/mL). Sequence analysis of the Week 48 sample identified one conserved site substitution (rtQ67Q/H) and 3 polymorphic site substitutions (rtN118N/T, rtN123N/D, rtM207M/V). This subject was 99.8% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 5691-4594, treatment-naïve with genotype C infection, was randomized to the TAF group, and had baseline viruses (8.27 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA when he discontinued the study (withdrew consent) with HBV DNA 2.23 log_{10} IU/mL at Week 32 (one day after last dose; follow-up Week 4 visit). Overall, HBV DNA declined >6 log_{10} IU/mL over 32 weeks of treatment. The follow-up Week 4 sample was unable to be genotyped. This subject was 99.1% adherent to the study drug regimen through early discontinuation based on pill count.

- Subject 5691-4944, treatment-naïve with genotype C infection, was randomized to the TAF group, and had baseline viruses (8.67 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA through Week 36 (88 IU/mL), and then experienced virologic breakthrough at Week 40 (3.91 log_{10} IU/mL) which was maintained through Week 48 (5.99 log_{10} IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 95.3% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 6336-5050, previously exposed to adefovir, entecavir, lamivudine, and TDF with genotype C infection, was randomized to the TAF group, and had baseline viruses (4.63 log_{10} IU/mL) showing mixed HBV rt sequences, wild-type and rtL80I and rtM204I detectable by INNO-LiPA (positive signals for L80, I80, M204, and I204). This subject had a decline in HBV DNA of approximately 1.5 log_{10} IU/mL through Week 20 (3.19 log_{10} IU/mL). At week 24 (one day after last dose; follow-up Week 4 visit), HBV DNA increased to 4.11 log_{10} IU/mL. The subject met protocol specified criteria for withdrawal, and discontinued from the study. Sequence analysis of the follow-up Week 4 sample found no changes compared to the baseline isolate. The rtL80I and rtM204I lamivudine-R substitutions detected at baseline by INNO-LiPA were not detectable by population-based sequencing at baseline or follow-up Week 4. This subject was 97% adherent to the study drug regimen based on pill count through early discontinuation.

- Subject 6338-4707, treatment-naïve with genotype C infection, was randomized to the TDF group, and had baseline viruses (6.54 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved virologic suppression (HBV DNA <29 IU/mL) by Week 20, and then experienced virologic breakthrough at Week 28 (4.93 log_{10} IU/mL) which was maintained through Week 48 (2.15 log_{10} IU/mL; declining HBV DNA levels from Weeks 36 to 48 were observed). The subject resuppressed her viral load to <29 IU/mL at Week 56. The Week 48 sample was unable to be genotyped. This subject was 96.4% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 6958-5201, previously exposed to TDF with genotype D infection, was randomized to the TAF group, and had baseline viruses (8.69 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject experienced approximately a 6 log_{10} IU/mL decline in HBV DNA through Week 20 (2.56 log_{10} IU/mL), and then had virologic breakthrough from Weeks 24 - 28 (ranging from 3.66 - 5.21 log_{10} IU/mL). HBV DNA declined again from Weeks 32 - 36 with HBV DNA <69 IU/mL at Week 36, followed by virologic breakthrough at Weeks 40 - 48 (ranging from 5.22 to 8.31 log_{10} IU/mL). Sequence analysis of the Week 48 sample (5.22 log_{10} IU/mL) found no changes compared to the baseline isolate. This subject was 99.6% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 6965-5170, treatment-naïve with genotype A infection, was randomized to the TAF group, and had baseline viruses (7.22 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA until Week 28 (2.11 log_{10} IU/mL), followed by a slight increase at Weeks 32 - 36 (ranging from 2.36 to 2.48 log_{10} IU/mL). At Week 36, the subject withdrew consent and discontinued the study with HBV DNA of 2.36 log_{10} IU/mL. Overall, HBV DNA levels declined >4 log_{10} IU/mL over 36 weeks of treatment. Sequence analysis of the Week 36 sample found no changes compared to the baseline isolate. This subject was 99.8% adherent to the study drug regimen based on pill count through early discontinuation.

- Subject 7515-5071, treatment-naïve with genotype D infection, was randomized to the TAF group, and had baseline viruses (8.52 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a decline in HBV DNA of >3 log_{10} IU/mL through Week 20 (5.31 log_{10} IU/mL), followed by a transient viral increase at Week 24 (7.76 log_{10} IU/mL). At Week 28, Subject discontinued the study due to being lost to follow up with HBV DNA of 5.10 log_{10} IU/mL. Sequence analysis of the Week 28 sample found no changes compared to the baseline isolate. This subject was 97.1% adherent to the study drug regimen based on pill count through early discontinuation.

- Subject 8006-5282, treatment-naïve with genotype D infection, was randomized to the TAF group, and had baseline viruses (9.09 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA through Week 20 (2.61 log_{10} IU/mL), and then experienced virologic breakthrough at Weeks 24 - 32 (ranging from 3.69 to 3.99 log_{10} IU/mL). The subject remained viremic through Week 48 (3.16 log_{10} IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 100% adherent to the study drug regimen through Week 48 based on

pill count and remains on study in the TAF treatment group.

- Subject 8017-4565, previously exposed to lamivudine, telbivudine, and IFN with genotype D infection, was randomized to the TAF group, and had baseline viruses (8.21 $\log_{10}$ IU/mL) showing mixed HBV rt sequences, wild-type and rtL80I and rtM204I detectable by INNO-LiPA (positive signals for L80, I80, M204, and I204). This subject achieved virologic suppression with HBV DNA to <29 IU/mL by Week 32. At the next visit (Week 36), the subject experienced virologic breakthrough with HBV DNA 3.58 $\log_{10}$ IU/mL and remained >69 IU/mL through Week 48 (411 IU/mL; declining HBV DNA levels from Weeks 36 to 48 were observed). Sequence analysis of the Week 48 sample identified 2 polymorphic site substitutions (rtI91I/L and rtE271A/E). Those lamivudine-R substitutions rtL80I and rtM204I detected at baseline by INNO-LiPA persisted through TAF treatment; with population-based sequencing, wild-type viruses with rtL80 and/or rtM204 were not seen (only detecting rtI80 and rtI204) at baseline but at Week 48, mixed populations of wild-type and lamivudine-R variants were detected (detecting mixtures L/I80 and M/I204), possibly due to the outgrowth of or reversion to wild-type virus in the absence of lamivudine (received prior to TAF monotherapy). This subject was 99.7% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 8312-4689, previously exposed to adefovir, entecavir, and IFN with genotype C infection, was randomized to the TAF group, and had baseline viruses (6.79 $\log_{10}$ IU/mL) showing mixed HBV rt sequences, wild-type and rtM204I detectable by INNO-LiPA (positive signals for M204 and I204). There was minimal change in HBV DNA, ranging from 0.08 $\log_{10}$ IU/mL decrease to 0.67 $\log_{10}$ IU/mL increase from baseline during 28 weeks of TAF. The subject discontinued the study due to lack of efficacy with HBV DNA of 6.97 $\log_{10}$ IU/mL (one day after last dose; follow-up Week 4). Sequence analysis of the follow-up Week 4 sample found no changes compared to the baseline isolate. The rtM204I lamivudine-R substitution detected at baseline by INNO-LiPA was not detectable by population-based sequencing at baseline or follow-up Week 4. This subject was 100% adherent to the study drug regimen based on pill count through early discontinuation.

- Subject 8569-5131, treatment-naïve with genotype D infection, was randomized to the TDF group, and had baseline viruses (5.87 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA levels and achieved HBV DNA <69 IU/mL at Week 36. The subject experienced virologic breakthrough at Week 40 (98 IU/mL) which was maintained through Week 48 (92 IU/mL). Sequence analysis of the Week 48 sample identified one conserved site substitution (rtQ288Q/stop). The applicant reasoned the introduction of a stop codon at position 288 in the HBV rt domain is unlikely to be enzymatically active and may represent a sequencing artifact. This subject was 99.7% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 8569-5132, treatment-naïve with genotype D infection, was randomized to the TAF group, and had baseline viruses (8.41 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA levels and achieved HBV DNA <69 IU/mL at Week 36. The subject experienced virologic breakthrough at Weeks 44 - 48 (ranging from 91 to 106 IU/mL). The Week 48 sample was unable to be genotyped. This subject was 100% adherent to the study drug

regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 8599-4712, treatment-naïve with genotype D infection, was randomized to the TDF group, and had baseline viruses (9.56 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved HBV DNA <69 IU/mL by Week 24. The subject experienced virologic breakthrough at Week 28 (109 IU/mL), which was maintained through Week 48 (7.94 log_{10} IU/mL) with HBV DNA levels continually increasing. Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 98.5% adherent to the study drug regimen through Week 48 based on pill count and discontinued from the study prior to Week 64 due to protocol violation (last evaluable viral load of 8.28 log_{10} IU/mL at Week 56).

- Subject 8599-4790, treatment-naïve with genotype D infection, was randomized to the TDF group, and had baseline viruses (9.48 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a continual decline in HBV DNA until Week 24 (4.61 log_{10} IU/mL) and then a transient viral increase at Week 28 (7.27 log_{10} IU/mL). The subjects eventually experienced virologic breakthrough from Weeks 40 through 48 (ranging from 5.90 to 9.62 log_{10} IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 98.7% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 8599-5374, treatment-naïve with genotype D infection, was randomized to the TDF group, and had baseline viruses (5.34 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved HBV DNA <69 IU/mL by Week 28, and then experienced virologic breakthrough at Week 36 (5.03 log_{10} IU/mL) which was maintained through Week 48 (458 IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 99.4% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 8600-4558, treatment-naïve with genotype D infection, was randomized to the TAF group, and had baseline viruses (8.58 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved HBV DNA <69 IU/mL by Week 24, and then experienced virologic breakthrough at Week 28 (3.42 log_{10} IU/mL) which was maintained through Week 48 (263 IU/mL). Sequence analysis of the Week 48 sample found no changes compared to the baseline isolate. This subject was 100% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 8758-5188, treatment-naïve with genotype D infection, was randomized to the TAF group, and had baseline viruses (4.74 log_{10} IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved HBV DNA levels <69 IU/mL by Week 24, and experienced virologic breakthrough at Week 36 (3.12 log_{10} IU/mL) which was maintained through Week 48 (91 IU/mL). Sequence analysis of the Week 48 sample identified one polymorphic site substitution (rtR153Q). This subject was 97% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 9035-4845, previously exposed to adefovir, entecavir, and lamivudine with genotype C infection, was randomized to the TDF group, and had baseline viruses (6.62 $\log_{10}$ IU/mL) showing mixed HBV rt sequences, wild-type and rtA181T detectable by INNO-LiPA (positive signals for A181 and T181). This subject had a continual decline in HBV DNA through Week 20 (2.91 $\log_{10}$ IU/mL), and then experienced 2 episodes of virologic breakthrough at Weeks 24 - 28 (ranging from 4.66 to 6.92 $\log_{10}$ IU/mL) and at Weeks 44 - 48 (ranging from 4.38 to 5.86 $\log_{10}$ IU/mL). Sequence analysis of the Week 48 sample identified 2 polymorphic site substitutions, rtR110R/G and rtL269I/L. The adefovir-R substitution rtA181T detected at baseline (also by population-based sequencing) persisted through TDF treatment. This subject was 99.7% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TDF treatment group.

- Subject 9035-5187, previously exposed to entecavir with genotype C infection, was randomized to the TAF group, and had baseline viruses (4.94 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject achieved HBV DNA <69 IU/mL by Week 24, which was maintained until Week 40 (38 IU/mL). The subject then experienced virologic breakthrough at Weeks 44 - 48 (ranging from 148 to 161 IU/mL). Sequence analysis of the Week 48 sample identified 4 polymorphic site substitutions (rtS13S/N, rtS117S/P, rtL267L/Q, and rtL269L/I). This subject was 99.9% adherent to the study drug regimen through Week 48 based on pill count and remains on study in the TAF treatment group.

- Subject 9695-5283, treatment-naïve with genotype D infection, was randomized to the TAF group, and had baseline viruses (8.80 $\log_{10}$ IU/mL) showing wild-type HBV rt sequences detectable by INNO-LiPA. This subject had a decline in HBV DNA of >4 $\log_{10}$ IU/mL through Week 16 (4.15 $\log_{10}$ IU/mL), followed by virologic breakthrough at Week 24 (8.01 $\log_{10}$ IU/mL at Treatment Day 162). At Week 24 (Treatment Day 173), the subject withdrew consent and discontinued the study with HBV DNA of 5.70 $\log_{10}$ IU/mL. Sequence analysis of the Week 24 sample found no changes compared to the baseline isolate. This subject was 95.3% adherent to the study drug regimen based on pill count through early discontinuation.

Appendix 4: List of TDF Treatment-Emergent HBV rt Substitutions Detected in Virologic Failure Subjects' Samples

Table A3: Cumulative Frequency and Position of Individual Amino Acid Changes Emerged during TDF Treatment (Up To 384 Weeks) in Resistance Testing Eligible Virologic Failure Samples[1] Collected from 6 Clinical Trials of TDF[2]

Occurrence	Treatment-emergent substitutions at amino acid position in HBV rt	
	Polymorphic positions[3]	Conserved positions[3]
1	7, 11, 18, 27, 38, 76, 91, 113, 115, 118, 120, 122, 132, 139, 142, 145, 164, 190, 191, 214, 219, 220, 222, 231, 234, 248, 253, 256, 266, 309, 313, 319, 336 (n=33)	44, 51, 63, 74, 85, 101, 152, 156, 167, 177, 180, 184, 194, 204, 233, 302, 307 (n=17)
2	16, 78, 123, 124, 126, 128, 134, 153, 212, 215, 221, 270, 271, 325, 333 (n=15)	173 (n=1)

3	13, 110, 131, 213, 238, 332 (n=6)	-
4	53 (n=1)	-
Substitutions in HBV rt	G7D, E11K, [R13H, R13L, L13H], [T16I (n=2)], R18K, V27A, A38V, [V53S, K53N, S53I, N53T], N76D, [S78T (n=2)], I91L, [R110G (n=2), G110R], A113G, L115V, A118T, R120G, S122F, [N123T, N123D], [Y124H (n=2)], [H126R, R126H], [T128N, N128T], [N131D, D131E, N131T], L132M, [D134N, N134S], N139T, V142E, L145M, [R153Q (n=2)], M164L, V190G, V191I, [K212T (n=2)], [T213S (n=2), S213T], A214V, [P215S, Q215H], A219S, I220L, [F221Y, H221Y], A222T, L231V, H234R, [N238H (n=2), N238D], H248Q, V253I, S256C, V266E/I/K, [K270R, K270T], [E271D, M271L], M309K, A313V, R319Q, [P325Q, S325P], [R332S (n=2), C332G], [K333Q, H333K], I336M	V44A, R51K, I63V, S74P, S85P, L101F, G152E, H156R, R167G, [V173L (n=2)], P177S, L180M, T184I/M, A194P, M204V, I233T, Q302R, A307T

[1] Resistance-testing eligible virologic failure samples were collected from subjects who received at least 24 weeks of TDF monotherapy and were viremic with HBV DNA levels ≥400 copies/mL at the end of each study year or at discontinuation of TDF monotherapy. Genotypic data were available for 96 resistance testing eligible virologic failure subjects (126 isolates) from 6 TDF clinical trials (see below Footnote 2).

[2] Six clinical trials of TDF:

- Studies 102 and 103 (GS-US-174-0102 and -0103, respectively) are ongoing Phase 3 studies conducted separately in the predominantly treatment-naïve, $HBeAg^-$ ($HBeAb^+$) and $HBeAg^+$ subject populations, respectively, to determine whether TDF 300 mg QD is noninferior to ADV 10 mg QD for the treatment of chronic HBV infection in the both subject groups with compensated liver disease. The two studies were similarly designed, involving 48 weeks of double-blind therapy either with TDF or ADV, followed by open-label TDF treatment through Week 480 (Year 10). Resistance data from subjects receiving up to 384 weeks of TDF treatment were analyzed and included in this list (see Virology Review N021356.943 for resistance analysis results).
- Study 106 (GS-US-174-0106) is a Phase 2, randomized, double-blind trial for 168 weeks to assess the efficacy, safety, and tolerability of TDF 300 mg QD monotherapy versus FTC 200 mg QD+TDF 300 mg QD combination therapy in subjects who were being treated with ADV for 24 to 96 weeks for chronic HBV infection. The study has been completed (see Virology Review N021356.511 for resistance analysis results).
- Study 108 (GS-US-174-0108) is a Phase 2, active-controlled study of TDF for up to 168 weeks, compared to ETV or to FTC+TDF, in subjects with chronic HBV infection and decompensated liver disease. The study has been completed (see Virology Reviews N021356.450 and N021356.844 for resistance analysis results).
- Study 115 (GS-US-174-0115) is a Phase 3, placebo-controlled study of TDF in HBV-infected adolescents. This study has completed 72 weeks of randomized, double-blind treatment (see Virology Review N021356.808 for resistance analysis results).
- Study 121 (GS-US-174-0121) is a Phase 3b, randomized, double-blind, active-controlled 240-week trial to compare the antiviral efficacy, safety, and tolerability of TDF monotherapy (300 mg QD) versus FTC/TDF combination therapy (FTC 200 mg/TDF 300 mg QD) in HBV-infected subjects who developed genotypic resistance to LAM with evidence of HBV rt substitutions rtM204I/V. Resistance data from subjects receiving up to 96 weeks of TDF treatment after switching from LAM were analyzed and included in this list (see Virology Review N021356.843 for resistance analysis results). The study has been completed (Virology review I071576.266).

[3] The location of polymorphic and conserved sites in the HBV rt domain was identified by comparing amino acid sequences from 628 subjects with available baseline data in TDF clinical studies GS-US-174-0102 and GS-US-174-0103. Conserved sites were defined as those positions where only one amino acid was found, or two amino acids was present and the prevalence of the minority amino acid was <1%; all other positions within the HBV rt domain were considered polymorphic sites (Kitrinos *et al*., 2014). Overall, 64% (220/344) of amino acid positions were classified as conserved with the remaining 36% (124/344) classified as polymorphic.

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/s/

SUNG S RHEE
10/06/2016

JULIAN J O REAR
10/06/2016

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208464 **SDN** 000 0009 **DATE REVIEWED:** 06/08/2016

Reviewer: Eric F. Donaldson, Ph.D.
Date Submitted: 03/29/16 **Date Assigned:** 03/30/16
Date Received: 03/29/16

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Amendments:

SDN	Date Submitted	Date Received	Date Assigned
000 0009	03/29/2016	03/29/2016	03/30/2016

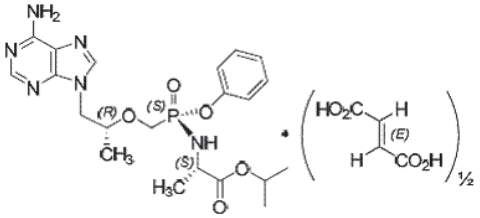
Related/Supporting Documents: IND115561, IND063737

Product Names: Tenofovir alafenamide (TAF, GS-7340); Tenofovir alafenamide fumarate (TAF fumarate, GS-7340-03)

Note: TAF refers to tenofovir alafenamide as the free base (GS-7340) and TAF fumarate refers to the hemifumarate form (GS-7340-03; 2:1 ratio of GS-7340 to fumarate) which is the drug substance.

Chemical Name: (TAF fumarate) L-Alanine, N-[(S)-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methyl ethoxy]methyl]phenoxyphosphinyl]-, 1-methylethyl ester, (2E)-2-butenedioate (2:1)

Structure:



Tenofovir Alafenamide Fumarate (GS-7340-03)

Molecular formula: (TAF fumarate) $C_{23}H_{31}O_7N_6P$; (TAF) $C_{21}H_{29}O_5N_6P$
Molecular mass: (TAF fumarate) 534.50; (TAF) 476.5

Drug category: Antiviral

Indication: Treatment of chronic hepatitis B virus infection

Dosage Form/Route of administration: Film-coated tablet (25 mg)/Oral
Note: TAF 25-mg tablets contain 28 mg of TAF fumarate.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208464 **SDN** 000 0009 **DATE REVIEWED:** 06/08/2016

Abbreviations: ADV, adefovir; CHB, chronic hepatitis B virus infection; EC_{50}, mean 50% effective concentration; ETV, entecavir; FDC, fixed-dose combination; GSI, Gilead Sciences, Inc.; HBeAg, HBV e antigen; HBV, hepatitis B virus; HIV-1, human immunodeficiency virus 1; LAM, lamivudine; LFVD, Low Frequency Variant Detector; NDA, new drug application; NGS, next generation sequencing; NrtI, nucleos(t)ide reverse transcriptase inhibitor; Pol, polymerase; PVD75, Probabilistic Variant Detector set at 75%; QbVD, Quality-based Variant Detector; QD, once daily; rt, reverse transcriptase; TAF, tenofovir alafenamide; TBV, telbivudine, TDF, tenofovir disoproxil fumarate; TFV, tenofovir;

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DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 208464 **SDN** 000 0009 **DATE REVIEWED:** 06/08/2016

I. EXECUTIVE SUMMARY

This review specifically focuses on the regulatory review of Next Generation Sequencing data generated during resistance analysis that was submitted in support of NDA 208464, which is seeking an indication for treatment of adults chronically infected with hepatitis B virus (HBV) with tenofovir alafenamide (TAF). TAF is an oral prodrug of tenofovir (TFV), previously approved for the treatment of chronic infection with HBV as tenofovir disoproxil fumarate (TDF; marketed as Viread® and approved for HBV by FDA in 2008). TAF purportedly has several properties that could potentially improve its clinical profile compared with TDF, including 1) TAF is more stable in serum than TDF facilitating transit across cellular membranes; 2) TAF provides higher intracellular levels of TFV-DP, the active nucleotide; and 3) TAF results in approximately 90% lower circulating levels of TFV relative to TDF. The sponsor compared the efficacy of TAF versus TDF in two pivotal Phase 3 clinical trials: GS-US-320-0108, which enrolled adult subjects chronically infected with HBV who were hepatitis B virus e-antigen (HBeAg)-negative, and GS-US-320-0110, which enrolled adult subjects with chronic HBV infection who were HBeAg-positive. The results from these trials indicate that TAF was non-inferior to TDF, and the sponsor is seeking an indication for adults with chronic HBV who are HBeAg-negative or HBeAg-positive. Of note, TAF appeared to be less effective than TDF in subjects infected with HBV who had viral loads >$1x10^7$ or were treatment-experienced (see the primary virology review NDA208464 SDN 000).

The sponsor performed resistance analysis for 38 subjects (24 from the TAF groups and 14 from the TDF groups) who failed to meet the primary endpoint of HBV DNA below the limit of detection at Week 48, but qualified for resistance testing. Next Generation Sequencing (NGS) was used to determine if any baseline or treatment-emergent substitutions could be associated with treatment failure among 16 subjects (8 from TAF groups and 8 from TDF groups) who failed treatment and for whom population sequencing data did not detect Pol/rt substitutions associated with drug resistance. The sponsor concluded that no resistance-associated substitutions could be identified in their Phase 3 program. The division requested that the sponsor submit the raw NGS data for an independent analysis. This review describes the results obtained in the independent analysis of the NGS resistance data for GS-US-320-0108 and GS-US-320-0110. Overall, the frequencies of amino acid substitutions in the reverse transcriptase of the HBV for subjects who failed treatment reported by the sponsor were in agreement with the independent analysis performed by the division. The division agrees with the sponsor that no clear resistance pathways were identified for TAF or TDF in these clinical trials; however, we recommend that the sponsor genotype subjects who failed to reach the primary endpoint (<29 IU/mL and were evaluable (>69 IU/mL) at Week 48 as part of their postmarketing evaluations. Subjects failing to meet the primary endpoint at Week 48 may help identify resistance substitutions.

II. BACKGROUND AND SUMMARY

Tenofovir (TFV) is a nucleotide analog that inhibits DNA synthesis of both the hepatitis B virus (HBV) and human immunodeficiency virus 1 (HIV-1) reverse transcriptase. Tenofovir disoproxil fumarate (TDF) is an oral prodrug of TFV, which is rapidly converted extracellularly to TFV and which is metabolized intracellularly to the active phosphorylated metabolite, TFV diphosphate, which acts as a competitive inhibitor of HBV and HIV-1 reverse transcriptases leading to DNA chain termination. TDF, which is marketed under Viread®, was approved for the treatment of HIV-1 infection in adults in 2001 and for chronic hepatitis B virus infection in adults in 2008.

Tenofovir alafenamide (TAF) is a phosphonoamidate oral prodrug of TFV that according to the sponsor has several properties that could potentially improve its clinical profile compared with TDF, including 1) TAF is more stable in serum than TDF facilitating transit across cellular membranes; 2) TAF provides higher intracellular levels of TFV-DP; and 3) TAF results in approximately 90% lower circulating levels of TFV relative to TDF. The stability of TAF in serum is important because the negative charge of tenofovir impedes cellular uptake. The sponsor reported that TAF demonstrated an improved clinical profile among HIV-1 infected subjects participating in 2 large non-inferiority studies wherein bone and renal effects were significantly reduced at 48 weeks in those randomized to the fixed dose combination (FDC) of elvitegravir, cobicistat,

emtricitabine, and TAF (Genvoya®) compared with the FDC of elvitegravir, cobicistat, emtricitabine, and TDF (Stribild®)(Post et al., 2016).

The sponsor reported that TAF is a potent and selective inhibitor of HBV activity in cell culture where TAF exhibited comparable anti-HBV activity in HepG2 cells against wild type clinical HBV isolates representing genotypes A to H, with an overall mean 50% effective concentration (EC_{50} value) of 86.6 nM. TAF showed no observed cytotoxicity up to the highest concentration tested (44.4 µM), and provided >513-fold selectivity index, relative to anti-HBV activity in HepG2 cells.

TAF antiviral activity was also assessed against a panel of HBV nucleos[t]ide reverse transcriptase inhibitor (NrtI) substitutions that have been associated with resistance. HBV NrtI resistance-associated substitutions have been identified for adefovir (ADV), lamivudine (LAM), entecavir (ETV), and telbivudine (TBV) (Figure 1). None of the substitutions at 72 positions previously found in the HBV of individuals failing TDF therapy have occurred with sufficient frequency to be associated with tenofovir resistance (see Clinical Virology review of NDA208464 SDN 000 by Sung Rhee, Ph.D.). The sponsor reported that TAF maintained anti-HBV activity in HepG2 cells against ETV, LAM, and most ADV NrtI-resistance-associated substitutions created in a laboratory strain of HBV. The activity of TAF was reduced by 3.7-fold in cell culture when tested against clinical isolates of HBV containing the rtA181V+rtN236T ADV-resistance-associated substitutions.

Molecule	Structure	Brand name	Company	Year of FDA approval	*In vitro* IC_{50} (µmol/L)[23]	Resistant mutations	5 yr cumulative resistance rate[25-31]

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Figure 1. NrtI resistance-associated substitutions (copied from Kim et al., 2014). Additional ETV resistance-associated substitutions have been identified at positions rtL80I/V/F, rtR18K/S, rtA186T/S, rtC188L, rtA200V, rtV214A/P/I/L/E, rtL247F, and rtF201Y (see NDA 021797 SDN 869).

The sponsor, Gilead Sciences, Inc. (GSI), has an ongoing Phase 3 program to evaluate TAF for the treatment of chronic HBV infection. Two prospective, randomized, active controlled trials in hepatitis e-antigen (HBeAg)-negative (Study GS-US-320-0108) and HBeAg-positive (Study GS-US-320-0110) subjects with chronic HBV infection are being conducted. In both studies, subjects were randomized to receive TAF or TDF for 96 weeks and then will be switched to open-label TAF through Week 144, with the primary efficacy endpoint at Week 48. The NDA submission presented individual and integrated analyses of the Week 48 virology resistance data from Studies GS-US-320-0108 and GS-US-320-0110.

One thousand two hundred and ninety-eight adult HBeAg-negative or HBeAg-positive subjects with chronic HBV infection were enrolled in Studies GS-US-320-0108 and GS-US-320-0110 where these subjects were randomized 2:1 to receive TAF (N = 866, 197 TE and 669 TN) or TDF (N = 432, 99 TE and 333 TN) through 48 weeks of treatment. Seven HBV genotypes were identified at baseline (A-F, H) with genotype C being the most commonly observed across both treatment arms (47.5%). The primary review for this NDA is being conducted by Dr. Sung Rhee and her review can be located under NDA 208464 SDN 000. The following excerpt pertaining to the efficacy of TAF compared to TDF was copied and modified from Dr. Rhee's review (NDA208464 SDN 000):

> Clinical virology analyses were conducted using the censored, as-treated subject population (n=1271, pooled from the two studies) including all randomized subjects who received at least one dose of study medication with at least one virologic observation after Baseline but excludes subjects who discontinued their assigned treatment before the primary efficacy assessment (Week 48) while they had a suppressed viral load (HBV DNA <29 IU/mL). As observed in the overall efficacy analysis using the ITT population, TAF was non-inferior to TDF in both studies: in Study 108, 97.5% (268/275) of subjects in the TAF treatment group and 94.9% (129/136) of subjects in the TDF treatment group had virologic success (HBV DNA <29 IU/mL at Week 48), while in Study 110, 64.7% (371/573) and 67.9% (195/287) of subjects in the TAF and TDF treatment groups, respectively, had virologic success. Of note, for these analyses the Clinical Virology reviewer (Sung Rhee, Ph.D.) defined TE as those subjects who were previously exposed to NrtIs (regardless of treatment duration). Based on this definition, Study GS-US-320-0108 had 216 NrtI-naïve subjects in the TAF arm and 106 NrtI-naïve subjects in the TDF arm, compared to 59 HBV NrtI treatment-experienced subjects in the TAF arm and 30 NrtI treatment-experienced subjects in the TDF arm. In Study GS-US-320-110, there were 425 NrtI-naïve subjects in the TAF arm and 210 subjects in the TDF arm compared to 148 HBV NrtI treatment-experienced subjects in the TAF arm and 77 in the TDF arm (see NDA208464 SDN 000 for more details). For the overall efficacy of TAF, please refer to the review by Statistical Reviewer Frazer Smith, Ph.D. (NDA208464 SDN 000).

III. RESISTANCE ANALYSIS OVERVIEW

Blood samples were collected from all subjects at screening, baseline, during on-treatment study visits (every 4 to 12 weeks), and at any early on-treatment termination visits. A nucleotide fragment (1032 base pairs) encoding amino acids 1-344 of the Pol/rt domain of HBV polymerase was sequenced by standard population di-deoxy sequencing. Alternatively, samples were analyzed via the INNO-LiPA Multi-DR v2/v3 assay (Innogenetics) to identify the presence of known resistance substitutions.

A. Sequencing Analysis Inclusion and Exclusion Criteria

Sequencing analysis was performed for all subjects in a treatment-blinded fashion. Samples identified through resistance surveillance were analyzed based on the following inclusion criteria:

- Baseline samples for all enrolled subjects were assessed for the presence of known resistance-associated substitutions in the Pol/rt using the HBV INNO-LiPA Multi-DR v2/3 assay.

- Population di-deoxy sequencing of the Pol/rt was conducted for the following samples:
    - Baseline and Week 48 samples from subjects who experienced virologic breakthrough, defined as HBV DNA ≥69 IU/mL after having been <69 IU/mL, or a 1.0 $\log_{10}$ or greater increase in HBV DNA from nadir. Two consecutive visits that meet the definition were required for a subject to be classified as having virologic breakthrough. A subject with one visit that met the definition was classified as having a virologic blip.
    - Baseline and on-treatment samples from viremic subjects (HBV DNA ≥69 IU/mL) with at least 24 weeks of treatment who discontinued early from the study.

The following exclusion, exception, and/or substitution criteria for sequencing analysis were employed:

- Samples from subjects with HBV DNA <69 IU/mL were not sequenced due to current assay limitations.
- Samples from subjects with viremia in the absence of virologic breakthrough were not evaluated until Week 96 to allow additional time to achieve virologic suppression as previously observed in TDF trials in subjects with chronic HBV infection.
- For any subject who experienced a virologic blip at Week 48 and required a confirmatory visit to determine if they should be classified as a virologic breakthrough or a virologic blip, the Week 56 HBV DNA (when available at the Week 48 analysis) was used to determine if the subject met the definition of virologic breakthrough or virologic blip. For subjects where Week 56 HBV DNA was not available at the Week 48 analysis, sequence analysis will be conducted during the Week 96 analysis if they are later confirmed to meet the definition of virologic breakthrough.
- If a sample was not available, the earliest available on-treatment sample was substituted.

Note: There were several issues noted with the subjects listed above regarding which should have been included in the resistance analysis. The issues noted were: 1) all subjects with viremia in the absence of virologic breakthrough were not evaluated until Week 96 to allow additional time to achieve virologic suppression but the HBV from these subjects should have been sequenced at the Week 48 timepont as responses in individuals with high viral loads were less in subjects receiving TAF; 2) the HBV from subjects with a blip at Week 48 who missed Week 56 should have been sequenced at the Week 48 timepoint; and 3) If a subject missed a timepoint, the sponsor sequenced the earliest available on-treatment sample but this should have been the last evaluable sample prior to Week 48. These issues were communicated to the primary virology reviewer, Sung Rhee, Ph.D. In addition, the treatment-experienced population in Study GS-US-320-0110 had a lower response rate than the treatment-naïve population indicating cross-resistance. Importantly, the sponsor did not provide this information in the PreNDA meeting package.

Overall, the number of subjects who qualified for HBV sequence analysis through Week 48 due to virologic breakthrough or early discontinuation at or after Week 24 with HBV DNA ≥69 IU/mL was low, 24 of 866 subjects in the TAF group (2.8%) and 14 of 432 subjects in the TDF group (3.2%) (see Table 1 for a breakdown based on study and previous treatment status). Of the 24 subjects in the TAF group who qualified for sequence analysis, 15 had no change from baseline, 4 were unable to be sequenced, and 5 had unique polymorphic site substitutions. There were no conserved site substitutions detected and no polymorphic site substitutions detected in more than one subject in the TAF group. Of the 14 subjects in the TDF group who qualified for sequence analysis, 6 had no change from baseline, 4 were unable to be sequenced, 2 had unique polymorphic site substitutions, and 2 had a unique conserved site substitution. There were no conserved or polymorphic site substitutions detected in more than 1 subject in the TDF group. Overall, the types of sequence changes observed were similar in the TAF and TDF groups. No subject was found to develop resistance-associated substitutions to tenofovir during the first year of the study.

Table 1. Breakdown of the 38 subjects who qualified for HBV sequence analysis through Week 48 due to virologic breakthrough or early discontinuation (DAVP analysis). Percentages based on censored, as-treated subject population (n=1271).

Trial	TAF		TDF		Total Subjects
	TE	TN	TE	TN	
GS-US-320-0108	3.4% (**2**/59)	0% (**0**/216)	0% (**0**/30)	1.9% (**2**/106)	**4**
GS-US-320-0110	5.4% (**8**/148)	3.3% (**14**/425)	5.2% (**4**/77)	3.8% (**8**/210)	**0**
Totals Subjects	**10**	**14**	**4**	**10**	**38**

TN, treatment-naïve; TE, treatment-experienced

B. Analysis of NGS Data

The sponsor stated in the Virology Analysis Plan that deep sequencing of the Pol/rt would be conducted if the population sequencing data did not detect Pol/rt substitutions associated with drug resistance and the subject demonstrated persistent viremia and/or virologic breakthrough on treatment.

GSI used Illumina-MiSeq as the deep sequencing platform for analysis of all HBV clinical samples and the deep sequencing was performed by [redacted] (b) (4) Serum or plasma samples were processed for production of amplicons encoding sample-derived HBV Pol/rt coding sequences and the resulting amplicons were processed by [redacted] (b) (4) through a series of steps including, library preparation, multiplexing, and deep sequencing. The HBV Pol/rt deep sequencing protocols utilized by [redacted] (b) (4) were presented in Appendix 1 of the NGS Analysis Report. This protocol was nearly identical to the procedures used for performing hepatitis C virus deep sequencing; however, the HBV Pol/rt deep sequencing protocol omitted the cDNA synthesis step.

The deep sequencing data were received by the sponsor from [redacted] (b) (4) as FASTQ files that were split into sets of 2 FASTQ files (R1 and R2) per sample using only 100% matched barcodes to bin the reads and then the barcodes were removed. An overview of GSI's analysis pipeline is presented in Table 2. In general, this pipeline was similar to the pipeline used for HCV drug approvals (**Donaldson et al., 2014**; **Donaldson et al., 2015**).

Table 2. Overview of pipeline from raw to mapped reads and tabulation of amino acid substitutions (Table 1, page 6, NGS Analysis Report).

Step	Description
1	Paired end reads that are overlapping are merged using PEAR {Zhang et al 2014}; paired end reads that do not have sufficient overlap are treated as individual reads and are combined into a single FASTQ file
2	Split FASTQ files into FASTA and QUAL
3	Chop sequences based on low quality scores: starting from 5′ end, look for score < 15 and chop 3′ when found
4	Filter FASTA file for any sequences < 50 base pairs
5	Execute MOSAIK alignment tool on filtered reads and using the subject's baseline population sequence as the reference sequence
6	If read contains indel(s): 1) If number of indels found in the read is in multiples of 3, the read is translated to AA in the proper frame and aligned with the reference AA sequence to identify the optimal AA based alignment and the locations of the insertions and deletions are recorded in the respective locations in the nucleotide sequence. 2) If number of indels is not in a multiple of 3, only the longest fragment not containing an insertion or deletion is recorded.
7	Mutations tabulated at the amino acid level. Only mutations with more than one hit are included in the final analysis. In addition, the first and last 15 bases of an aligned read are not included in the tabulation to ensure the quality of results. An exception to this is made for the first 15 bases and last 200 bases of the amplicon in order to ensure coverage over these regions.
8	In order to compare homologous positions across genotypes, subject-specific coordinates are converted to both subject-genotype specific reference coordinates and the genotype A reference coordinates. The reference sequences are listed in Table 2.

AA = amino acid; Indel = insertion or deletion of DNA bases

The reference sequences that were used for mapping short reads and comparing baseline sequences to later timepoints to identify polymorphisms and treatment-emergent substitutions for each HBV genotype are shown in Table 3.

Table 3. HBV genotype references and accession numbers (Table 2, page 6, NGS Analysis Report).

Genotype	Reference Sequence GenBank Accession Number[a]
A	X02763
B	AB219428
C	GQ924620
D	AF121240
E	AB106564
F	AY090458
G	AB064313
H	AY090454

a HBV viruses identified as reference genomes {Hayer et al 2013}

Validation of NGS Methodology. The sponsor performed a number of validation steps. First, a 2% cutoff for Pol/rt variant analysis was selected based on variability observed in plasmid sequencing and plasmid mixture analyses. Next, the reproducibility of viral variant detection in serum samples across a range of HBV viral loads was assessed and it was determined that deep sequencing of HBV Pol/rt from subject serum with HBV DNA down to 159 IU/mL (922 copies/mL or ~20 copies/PCR reaction) would be attempted. Additionally, alternative primers sets were assessed and found to produce concordant results.

Assessment Resistance Using NGS. The sponsor used the pipeline described above to sequence 32 samples from 16 subjects from the two pivotal phase 3 clinical trials. The sponsor used the terminology in Table 4 for this assessment.

Table 4. Description of sequence changes (Table 6, page 12, NGS Analysis Report).

Sequence Change	Description
Emerged	Not detected at baseline visit, detected at > 2% post-baseline visit
Enriched	Detected in baseline and post-baseline visits, but enriched by ≥ 2-fold in post-baseline visit versus baseline visit
Lost	Detected at baseline visit, not detected at post-baseline visit (≤ 2%)
Depleted	Detected in baseline and post-baseline visits, but decreased by ≥ 2-fold in post-baseline visit versus baseline visit
Net	(Emerged + Enriched) − (Lost + Depleted)

This report describes the procedures for Illumina-MiSeq deep sequencing of HBV Pol/rt from subject serum or plasma samples for identification of the potential development of sequence variants associated with resistance to TAF. The sponsor concluded that validation experiments supported their use of a 2% cutoff for variant analysis and a viral load cutoff of 159 IU/mL for deep sequencing of HBV Pol/rt from subject serum or plasma samples.

Independent Assessment of NGS Data. The sponsor provided the NGS data for two clinical trials on a hard drive and the dataset included: 1) coverage information, file map, and frequency tables showing amino acid variation that occurred at each position in the Pol/rt for each treatment failure sample that was successfully sequenced using Illumina and met the criteria for next generation sequencing; 2) raw sequence data in fastq format for all samples that were deep sequenced; 3) summary resistance data for each study; and 4) cross study comparisons of resistance data.

Given that next generation sequencing is an emerging technology with no current standards for analysis, the division requested raw data so that an independent analysis could be performed on the NGS data. The sponsor's summary NGS data were compared to the results generated by DAVP following these criteria:

- The sponsor's frequency tables were used to generate a summary and do a direct comparison of the results reported by the sponsor;
- Frequency tables were generated by DAVP using an independent mapping of reads to a reference for each sample and using two independent variant detection algorithms and the results were compared with those reported by the sponsor and those generated using the sponsor's frequency table; and
- The conclusions from the NGS data were compared to the results reported by the sponsor using Sanger population sequence analysis when applicable.

An overview of the NGS analysis pipeline used by the division has been published and is publicly available (**Donaldson et al., 2014**; **Donaldson et al., 2015**).

IV. CLINICAL STUDIES – Resistance Analysis of NGS Data

The sponsor performed an integrated virology analysis for two Phase 3 studies (Studies GS-US-320-0108 and GS-US-320-0110) that were performed with treatment-naive and treatment-experienced adults with CHB who received either TAF or TDF. A total of 1298 adult subjects infected with HBeAg-negative or HBeAg-positive CHB from Studies GS-US-320-0108 and GS-US-320-0110, respectively, were randomized 2:1 to receive TAF (N = 866) or TDF (N = 432) through 48 weeks of treatment. Integrated baseline and Week 48 virology resistance analyses with population sequencing have been previously described and are reviewed in detail elsewhere (NDA 208464 SDN 000). This section describes the results and review of the NGS analyses of the HBV Pol/rt.

Of the 38 subjects who qualified for population sequencing analysis through Week 48, there were a total of 16 subjects who qualified for deep sequencing analyses. Of the 16 subjects, one treatment-experienced subject was from clinical trial GS-US-320-0108 and of the remaining 15 subjects from clinicial trial GS-US-320-0110, 7 were treatment-experienced and 8 were treatment-naïve (Table 5). The sponsor stated in the Virology Analysis Plan that deep sequencing of the Pol/rt would only be conducted if the population sequencing data did not detect Pol/rt substitutions associated with drug resistance and the subject demonstrated persistent viremia and/or virologic breakthrough on treatment. Of the 16 subjects who qualified for NGS analysis, the sponsor reported that 4 subjects had no emerged, enriched, lost, or depleted substitutions detected at ≥2% detection or a ≥2 fold change from baseline. For the remaining 12 subjects, the sponsor reported that deep sequencing detected 21 emerged substitutions (among 9 subjects), 9 enriched substitutions (among 5 subjects), 32 lost substitutions (among 8 subjects) and 4 depleted substitutions (among 3 subjects). The definitions of the various sequence changes are listed in Table 4.

The sponsor reported that overall, one ADV resistance-associated substitution was detected in a subject from the TDF group (rtN236T) and two polymorphic substitutions were detected in two subjects each (rtN123D, TAF n=1, TDF n=1; rtH124D, TAF n = 2). All of the subjects with these substitutions who remained on study through Week 48 achieved HBV DNA <69 IU/mL with continued treatment. Thus, the sponsor concluded that no TAF-associated resistance substitutions associated with virologic breakthrough were identified through 48 weeks of these studies. Of note, rtN123D is a possible ETV resistance-associated substitution (NDA 021797 SDN 869).

Of the 38 subjects who qualified for population sequencing analysis, 8 subjects from the TAF group and 8 subjects from the TDF group qualified for deep sequencing analyses and NGS data were obtained for all 16 subjects at baseline and at the timepoint close to failure (qualifying visit). The remaining 22 subjects did not qualify for deep sequencing analysis for a number of reasons:

- According to the sponsor, subjects with HBV DNA ≤159 IU/mL did not qualify for deep sequencing due to assay limitations (TDF: Subjects 0481-4873 (107 IU/mL) and 8569-5131 (91 IU/mL); TAF: Subjects 4296-4510 (162 IU/mL), 5613-1163 (350 IU/mL), 8758-5188 (91 IU/mL), and 9035-5187 (161 IU/mL)).
- The PCR amplification step during population sequencing analysis failed for the on-treatment samples from 4 subjects from the TDF group (Subjects 4164-4604, 6338-4707, 6963-1339, and 8519-1176) and 4 subjects from the TAF group (Subjects 4074-4571, 4164-5252, 5691-4594, and 8569-5132) and thus samples were unable to be subjected to deep sequencing.
- Five subjects from the TAF group discontinued from the study early on or after Week 24 with no virologic breakthrough and did not qualify for deep sequencing (Subjects 2826-4527, 5617-4966, 6336-5050, 6965-5170, and 7515-5071). According to the sponsor, these subjects did not meet the criteria for deep sequencing analysis because they did not demonstrate persistent viremia and/or virologic breakthrough on treatment. However, these samples should have been sequenced as part of the resistance analysis plan.
- Three subjects in the TAF group experienced virologic breakthrough at Week 48 due to potential sample mishandling, with a significant decrease in HBV DNA levels of >2 $\log_{10}$ IU/mL or to <69 IU/mL for one visit immediately preceding an increase to previous HBV DNA levels at the next visit (Subjects 8006-5282 (1445 IU/mL at Week 48 but undetected at Week 56), 8600-4558 (380 IU/mL at Week 48 but 1600 IU/mL at Week 56), and 8017-4565 (55 IU/mL at Week 48 but 76 IU/mL at Week 56). These subjects experienced an overall decline in HBV DNA levels compared to baseline.

Comment: Subjects 4296-4510, 5613-1163, and 9035-5187 had HBV DNA titers at the last PCR assessment that were >159 IU/mL, qualifying them for deep sequencing analysis. Please submit the fastq files and analyses for these subjects. In addition, despite to potential mishandling of samples, subjects 8006-5282 and 8600-4558 had HBV DNA titers at the last PCR assessment that were >159 IU/mL, qualifying them for deep sequencing analysis. Please submit the fastq files and analyses for these subjects.

There were a total of 32 NGS files for the two clinical trials for 16 subjects, with one subject from GS-US-320-0108 and 15 subjects from GS-US-320-0110 (Table 5). Three different HBV genotypes were represented, including GTs B, C, and D (Table 5). References sequences for each genotype were downloaded and used for mapping reads to (GTs B, C, D were AB219428, GQ924620, and AF121240, respectively).

Table 5. Subjects for whom NGS data were submitted (DAVP analysis).

Trial	Subject ID	TREATMENT STATUS	ARM	GENOTYPE
GS-US-320-0108	06958-1318	Experienced	TAF	D
GS-US-320-0110	05606-5140	Naïve	TAF	B
	05620-5225	Naïve	TDF	B
	08312-4689	Experienced	TAF	C
	05691-4944	Naïve	TAF	C
	04296-5147	Experienced	TAF	C
	01507-4546	Naïve	TDF	C
	09035-4845	Experienced	TDF	C
	03912-5084	Experienced	TDF	C
	01659-4788	Experienced	TAF	D
	06958-5201	Experienced	TAF	D
	09695-5283	Naïve	TAF	D
	08599-4712	Naïve	TDF	D
	01659-4721	Experienced	TDF	D
	08599-4790	Naïve	TDF	D
	08599-5374	Naïve	TDF	D

DAVP performed an independent analysis of the NGS data using the NGS Analysis Pipeline referred to above to analyze the raw sequence data (fastq sequences) submitted by the sponsor for the 16 subjects from the 2 clinical trials. Briefly, the reads for each sample were aligned to the appropriate HBV reference sequence for the Pol/rt gene. Variants were called using three algorithms, including the Low Frequency Variant detector

(LFVD), the Probabilistic Variant detector (PVD75) and the Quality based Variant detector QbVD, and the variant tables were combined to generate frequency tables showing all amino acid substitutions that occurred above a frequency ≥1% for a given position within a given sample for each subject. Resistance analysis tables were generated for each frequency table for each variant detection algorithm (the sponsor's, GSI, and the three used by DAVP, including LFVD, PVD75, and QbVD) using a threshold of 0.05, and the four resistance analysis tables were compared to determine how closely the four variant call algorithms agreed with one another. To further assess the overall population-based results generated using the resistance analysis tables, DAVP used the SUBS10 filtering threshold to look at individual changes occurring at each position in the frequency tables (**Donaldson et al., 2014**; **Donaldson et al., 2015**).

Using this approach, the resistance analysis tables were used to compare algorithms and identify population based positions of interest and the SUBS10 threshold for analyzing the frequency tables provided the treatment-emergent substitutions of each individual subject who met this threshold. This provided a more robust prediction of substitutions that were likely to be associated with resistance. DAVP considered all substitutions to be potential emerging resistance-associated substitutions if they met these criteria: 1) met the SUBS10 threshold, 2) were detected by 2-of-4 variant detection methods (LFVD, QbVD, PVD75, or variant calls made by the sponsor designated G or GSI), and 3) occurred in 2 or more subjects.

A. GSI Resistance Analysis Summary

The sponsor reported that there were 9 subjects who had failure isolates with treatment-emergent Pol/rt substitutions detected by Sanger, NGS or both, out of the 30 resistance testing eligible subjects with evaluable genotypic data. The summary table below was copied from the primary virology reviewer's (Sung Rhee, Ph.D.) review (Table 6).

Table 6. Summary of genotypic and phenotypic resistance data (copied from NDA 21356 SDN 843).

Subject ID	HBV genotype	Prior treatment[1]	Study treatment	Baseline HBV DNA[2]	Resistance testing		
					Isolate (HBV DNA[2])	Treatment-emergent rt substitution[3]	Drug susceptibility (fold-change[4])
2826-4527	C	TAF	TAF	8 24	Week 36 (2 31)	D134E, M309K	nd
4296-5147	C	TDF	TAF	4 62	Week 48 (4 68)	S256S/C	nd
8017-4565	D	LAM, LdT	TAF	8 21	Week 48 (2 61)	I91I/L, E271A/E (I80I/L), (I204I/M)	nd
8758-5188	D	treatment-naïve	TAF	4 74	Week 48 (1 96)	R153Q	nd
9035-5187	C	ETV	TAF	4 94	Week 48 (2 21)	S13S/N, S117S/P, L267L/Q, L269L/I	0 7 (0 8)
1507-4546	C	treatment-naïve	TDF	8 07	Week 48 (3 00)	D134D/E, V214V/A, A317A/S	nd
5620-5225	B	treatment-naïve	TDF	7 68	Week 48 (2 29)	Q67Q/H, N118N/T, N123N/D, M207M/V	1 5 (1 0)
						Week-48 clone with Q67H[5]	1 5 (1 0)
8569-5131	D	treatment-naïve	TDF	5 87	Week 48 (1 96)	Q288Q/stop	1 1 (0 7)
						Week-48 clone with Q288stop[6]	unable to replicate
9035-4845	C	ADV, ETV, LAM	TDF	6 62	Week 48 (4 38)	R110R/G, L269I/L (A/T181A/T)	nd

nd, not determined

Note: Amino acid substitutions in parentheses associated with resistance to HBV NrtIs were detected in the subjects baseline isolates and persistently detectable in their Week-48 failure samples.

[1]Prior treatment with HBV NrtI(s) regardless of treatment duration.

[2]HBV DNA expressed as $\log_{10}$ IU/mL.

[3]Amino acid substitutions that occurred at HBV rt conserved amino acid positions are written in red.

[4]Fold-change in drug susceptibility of virologic failure isolates (subject-derived pooled and clonal virus sample), compared to wild-type reference HBV (pHY92 genotype A laboratory strain; Yang et al., 2004) and to their respective baseline isolates (in parentheses). TAF susceptibilities were determined for paired isolates (baseline and treatment-failure) collected from TAF recipients and TFV susceptibilities were for those collected from TDF recipients. Of note, the EC_{50} values of TAF and TFV were approximately 33.5 nM and 3.20 mM, respectively, for the wild-type reference HBV pHY92. A value <2-fold is within assay variability.

[5]Since the conserved-site substitution was a mixture (rtQ67Q/H), a clone containing the full rtQ67H substitution was isolated and its susceptibility to TFV was also determined in cell culture.

[6]Since the conserved-site substitution was a mixture (rtQ288A/stop), a clone containing the full rtQ288stop change was isolated and its susceptibility to TFV was also determined in cell culture. The clone containing the full rtQ288stop substitution was unable to replicate in cell culture.

Note: Subject 1507-4546 had rtT184I/L/F/M as detected by INNO-LiPA but was negative by Sanger and by NGS analysis.

Note: Two subjects had D134 substitutions and it's not clear why the HBV rt from these subjects weren't phenotyped. In addition, one of these had a V214V/A substitution that has been associated with ADV and ETV resistance.

B. GSI NGS Resistance Analysis Results

Overall, the sponsor reported that 16 subjects were analyzed by deep sequencing, with 4 subjects having no emerged, enriched, lost, or depleted substitutions detected at ≥2% detection or ≥2 fold change from baseline. GSI detected 21 emerged substitutions (among 9 subjects), 9 enriched substitutions (among 5 subjects), 32 lost substitutions (among 8 subjects) and 4 depleted substitutions (among 3 subjects) among the remaining subject samples analyzed by NGS.

The sponsor further analyzed the NGS results to determine if any amino acid substitutions detected in the Pol/rt gene were associated with known resistance substitutions or were observed in more than one subject who qualified for sequence analysis in the TAF or TDF treatment groups (Table 7). The sponsor reached the conclusion that no amino acid substitutions in the Pol/rt gene were observed in more than one subject in the TAF or TDF treatment groups by previous population sequencing analyses.

Table 7. Summary of treatment-emergent HBV Pol/rt substitutions at week 48 by deep sequencing (Table 3, page 13, NGS Integrated Virology Report).

Substitution	Net, n (%)[a]	Emerged Subjects[b]	Enriched Subjects[b]	Lost Subjects[b]	Depleted Subjects[b]
rtN123D	2 (12.5)	5606-5140 (TAF)	5620-5225 (TDF)		
rtH124D	1 (6.25)	8312-4689 (TAF) 5606-5140 (TAF)		8599-5374 (TDF)	
rtN236T	1 (6.25)	1507-4546 (TDF)			

a Net number of subjects = (Emerged + Enriched) – (Lost + Depleted); percent of subjects is out of N = 16 total subjects subjected to deep sequencing
b Treatment group is noted in parentheses after subject number

The sponsor's NGS analysis identified two substitutions that developed in more than one subject. GSI reported that the rtN123D substitution emerged on treatment in one subject in the TAF group and was enriched in one subject in the TDF group, which, the sponsor noted, was also observed by population sequencing in the latter case. The sponsor stated that amino acid rtN123 is polymorphic, and rtD123 was present in 2.7% of treatment-naive subjects enrolled in TDF Phase 3 studies, where 16 of the 17 subjects with rtD123 at baseline achieved HBV DNA <69 IU/mL on treatment.

Subject 5606-5140 in the TAF group had rtN123D emerge at low frequency (2.84%) during an episode that the sponsor described as virologic breakthrough associated with non-adherence to study medication. In addition, the sponsor reported that the virus from subject 5620-5225 in the TDF group had an rtN123D enriched from 4.29% at baseline to 34.12% at Week 48, when HBV DNA was declining during a transient episode of virologic breakthrough. Subjects 5606-5140 and 5620-5225 remained on study through Week 48 and achieved HBV DNA <69 IU/mL by Week 64 and Week 56, respectively.

The sponsor reported that the rtH124D substitution emerged on treatment in two subjects in the TAF group and was lost in one subject in the TDF group. According to the sponsor, amino acid rtH124 is polymorphic and rtD124 was present in the virus of 2.9% of treatment-naive subjects enrolled in TDF Phase 3 studies. The virus from subject 5606-5140 in the TAF group had an rtN124D emerge at a low frequency (2.33%) during an episode described by the sponsor as a virologic breakthrough associated with non-adherence to study medication. This subject also had rtN123D emerge at low level as described above. Subject 8312-

4689 in the TAF group had rtH123D emerge at a low level (2.13%) at follow-up Week 4 when this subject was discontinued from the study due to lack of efficacy, which, according to the sponsor, was associated with non-adherence to study medication throughout the dosing period.

The sponsor stated that the virus from subject 8599-5374 in the TDF group had rtY124D at a frequency of 7.97% at baseline, which was then lost at Week 48 during a transient episode of virologic breakthrough associated with non-adherence to study medication. In addition the sponsor reported that subject 8312-4689 was discontinued from the study for lack of efficacy at the time of resistance analysis; however, Subjects 5606-5140 and 8599-5374 remained on study through Week 48 and achieved HBV DNA <69 IU/mL by Week 56. The rtN124D substitution was observed at baseline in the virus of Subject 2009 in Study GS-US-174-0108.

The sponsor reported that deep sequencing of the Week 48 sample from Subject 1507-4546 identified the ADV-associated substitution rtN236T present in the viral population at a frequency of 14.7% that was not observed at baseline by deep sequencing or at either timepoint by population sequencing. According to the sponsor, subject 1507-4546 was randomized to the TDF group and was reportedly oral antiviral treatment-naive at baseline. This subject harbored the rtT184I/L/F/M substitution at baseline by INNO-LiPA and had a baseline HBV DNA of 8.07 $\log_{10}$ IU/mL. This subject had a continual decline in HBV DNA through Week 40 and then experienced virologic breakthrough at Week 44, which was maintained through Week 48 and was associated with non-adherence as measured by plasma tenofovir levels below the limit of detection. At Week 48, deep sequencing analysis identified 3 polymorphic site substitutions that emerged or were enriched (rtA317S, rtS109Q, rtV214A), 2 conserved site substitutions that emerged or were enriched (rtN236T, rtK154Q), and 2 polymorphic site substitutions that were lost (rtR138K, rtN139K), indicating that a minor quasispecies may have been amplified at the Week 48 timepoint.

The rtT184I/L/F/M substitution detected at baseline by INNO-LiPA was not detected by population or deep sequencing at baseline or Week 48. Additional deep sequencing analyses were conducted for this subject at Weeks 12, 24, 44, 56, and 64; rtN236T was not detected in ≥2% of the population at all other timepoints (data not shown). Subject 1507-4546 achieved HBV DNA <69 IU/mL by Week 80, and remains on study in the TDF treatment group with HBV DNA <69 IU/mL through Week 96. The suppression of rtN236T with continued TDF treatment is consistent with previous reports showing that rtN236T and wild type populations decline similarly in subjects on TDF treatment.

> ***Note:*** rtV214A and rtN236T are ADV resistance-associated substitutions and according to Sung Rhee, Ph.D. (the primary reviewer of this NDA) rtN236T has not been associated with resistance to TAF/TDF; however, the emergence of the rtV214A substitution was noted in one TDF-treated subject's Week-48 virologic failure isolate (with 2 other emerging rt substitutions D134D/E and A317A/S; Subject 1507-4546). Of note, rtV214A has been associated with entecatvir resistance and was determined to be a conserved position in entecavir studies by DAVP (NDA021797 SDN 869). It has also been reported as an accessory TDF resistance substitution (Ghany and Liang, 2007; and Michailidis et al., 2012).

C. Review of GS-US-320-0108

In study GS-US-320-0108, four subjects were assessed for resistance, including two in the TAF group (Table 8) and two in the TDF group (Table 9). Only one subject in this clinical trial qualified for resistance-analysis by deep sequencing, and that was Subject 06958-1318.

1. GS-US-320-0108 Overall Resistance Analyses

Below are the sponsor's tables for resistance analysis performed for the four subjects in study GS-US-320-0108 who were assessed for treatment-emergent resistance-associated substitutions.

Table 8. Changes in amino acids from Baseline to Post-Baseline for subjects in the TAF group (Study GS-US-320-0108 Virology Listing 2, page 317, Integrated Virology Study Report).

SUBJID	ACCSNNUM	Genotype	Country	Oral Antiviral Treatment Status	ARM	VISIT	Visit Date	HBV DNA (log_{10} IU/mL)	Ref POL	Coverage POL	POL Change From BL
1163	D707934	C	TWN	Treatment Experienced	TAF	Baseline	7/7/2014	7.53	HBV_GTA_X02763_POLRT	1-344 (344)	NA
1163	6600110487	C	TWN	Treatment Experienced	TAF	Week 48	6/8/2015	2.15	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
1318	F346784	D	IND	Treatment Experienced	TAF	Baseline	9/23/2014	7.81	HBV_GTA_X02763_POLRT	1-344 (344)	NA
1318	6600211694	D	IND	Treatment Experienced	TAF	Week 48	8/21/2015	3.19	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline

IND = India; NA = not applicable; TAF = tenofovir alafenamide; TWN = Taiwan

Table 9. Changes in amino acids from Baseline to Post-Baseline for subjects in the TDF group (Study GS-US-320-0108 Virology Listing 3, page 318, Integrated Virology Study Report).

SUBJID	ACCSNNUM	Genotype	Country	Oral Antiviral Treatment Status	ARM	VISIT	Visit Date	HBV DNA (log_{10} IU/mL)	Ref POL	Coverage POL	POL Change From BL
1176	E029705	C	KOR	Treatment Naive	TDF	Baseline	7/10/2014	6.07	HBV_GTA_X02763_POLRT	1-344 (344)	NA
1176	6600034225	C	KOR	Treatment Naive	TDF	Week 48	6/8/2015	2.33	Assay Failure	NA	NA
1339	F807645	D	IND	Treatment Naive	TDF	Baseline	10/1/2014	5.58	HBV_GTA_X02763_POLRT	1-344 (344)	NA
1339	F807865	D	IND	Treatment Naive	TDF	Follow-Up Week 4	3/18/2015	2.20	Assay Failure	NA	NA

IND = India; KOR = South Korea; NA = not applicable; TDF = tenofovir disoproxil fumarate

2. GS-US-320-0108 Resistance Analyses by NGS

Only one subject in this clinical trial qualified for resistance-analysis by deep sequencing, and that was Subject 06958-1318.

Table 10. GS-US-320-0108 Pol/rt change from consensus detected by NGS (Appendix 2, page 44, Integrated Virology Report).

Study	Subject ID	Accession	Visit Date	Visit	Genotype	POL Average Coverage	POL Deep Sequencing Mutation (change from Consensus)[a]	POL Deep Sequencing DRM[a]
GS-US-320-0108	1318	F346784	23-Sep-14	Baseline	D	31145.724	R120K(1065/35455=3.00) Y135N(1726/36261=4.76) C/S256C(17079/31966=53.43) C/S256S(14508/31966=45.39) M309K(4313/38226=11.28)	None
GS-US-320-0108	1318	6600211694	21-Aug-15	Week 48	D	43815.093	Q149K(5724/53296=10.74) R153Q(9264/60075=15.42) C/S256C(9078/50646=17.92) C/S256S(41507/50646=81.96)	None

3. Independent Assessment of GS-US-320-0108 Resistance Analyses by NGS by DAVP

DAVP detected R488Q which falls outside of the rt domain in the one subject who qualified for resistance testing by NGS (Table 11).

Table 11. DAVP NGS analysis (DAVP analysis). Type = polymorphism or emergent substitution, VARDET= variant detector used to detect the substitution. Polymorphisms were not detected by GSI as they used the baseline consensus sequence as their reference sequence for later timepoint analysis.

USUBJID	ARM	GT	AAPOS	Type	AAFREQ	SUB	VARDET
GS-US-320-0108-6958-1318	TAF	D	10	POLY	>40	K10R	LPQ
			17	POLY	>54	D17E	LPQ
			18	POLY	>45	T18A	LPQ
			45	POLY	100	N45D	LPQ
			46	POLY	>99	P46L	LPQ
			78	POLY	>99	T78F	LP
			90	POLY	>99	N90K	LPQ
			119	POLY	>92	F119V	LPQ
			149	**EMERGE**	**10.7**	**Q149K**	**GSI**
			153	**EMERGE**	**15.4**	**R153Q**	**GSI**
			178	POLY	>99	K178E	LPQ
			208	POLY	>98	Q208R	LPQ
			309	**EMERGE**	**11.3**	**K309M**	**GSI**
			488	**EMERGE**	**16.1**	**R488Q**	**LQ**

4. GS-US-320-0108 NGS Resistance Analyses Conclusions

The sponsor's NGS analysis identified three amino acid changes that could be associated with failure for this subject, including the following substitutions that met the SUBS10 criteria: Q149K, R153Q, and K309M. However, these substitutions were not detected by the three variant detection methods used by DAVP. DAVP identified treatment-emergent substitution R488Q with two variant detectors, but this substitution does not occur in the rt domain.

D. Review of GS-US-320-0110

In study GS-US-320-0110, 34 subjects were assessed for resistance, including 22 in the TAF group (Table 12) and 12 in the TDF group (Table 13). A total of 15 subjects in this clinical trial qualified for resistance-analysis by deep sequencing (see section below).

1. GS-US-320-0110 Overall Resistance Analyses

Below are the sponsor's tables for resistance analysis performed for the 34 subjects in study GS-US-320-0110 who were assessed for treatment-emergent resistance-associated substitutions.

Table 12. Changes in amino acids from Baseline to Post-Baseline for subjects in the TAF group (Study GS-US-320-0110 Virology Listing 2, page 334, Integrated Virology Study Report).

SUBJID	ACCSNNUM	Genotype	Country	Oral Antiviral Treatment Status	ARM	VISIT	Visit Date	HBV DNA (log_{10} IU/mL)	Ref_POL	Coverage POL	POL Change From BL
4510	Q746572	B	USA	Treatment Naive	TAF	Baseline	11/5/2013	7.86	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4510	Z211670	B	USA	Treatment Naive	TAF	Week 48	10/7/2014	2.05	HBV_GTA_X02763_POLRT	1-344 (344)	S/F/Y135S
4527	T877930	C	CAN	Treatment Naive	TAF	Baseline	1/9/2014	8.24	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4527	Y363708	C	CAN	Treatment Naive	TAF	Week 36	9/16/2014	2.31	HBV_GTA_X02763_POLRT	1-344 (344)	D134E V/I191V M309K
4558	J346585	D	RUS	Treatment Naive	TAF	Baseline	3/13/2014	8.58	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4558	6200006726	D	RUS	Treatment Naive	TAF	Week 48	2/10/2015	2.42	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
4565	J349814	D	RUS	Treatment Experienced	TAF	Baseline	3/20/2014	8.21	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4565	L355326	D	RUS	Treatment Experienced	TAF	Week 48	2/17/2015	2.61	HBV_GTA_X02763_POLRT	1-344 (344)	I80L/I I91I/L I204M/I E271A/E
4571	P475555	C	USA	Treatment Experienced	TAF	Baseline	3/31/2014	4.28	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4571	6500298488	C	USA	Treatment Experienced	TAF	Week 48	3/3/2015	3.43	Assay Failure	NA	NA
4594	H659334	C	HKG	Treatment Naive	TAF	Baseline	4/16/2014	8.27	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4594	E887554	C	HKG	Treatment Naive	TAF	Follow-Up Week 4	11/26/2014	2.23	Assay Failure	NA	NA
4689	A555439	C	JPN	Treatment Experienced	TAF	Baseline	5/30/2014	6.79	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4689	A812257	C	JPN	Treatment Experienced	TAF	Follow-Up Week 4	12/12/2014	6.97	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
4788	J071559	D	ROU	Treatment Experienced	TAF	Baseline	7/10/2014	8.03	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4788	6200872290	D	ROU	Treatment Experienced	TAF	Week 48	6/11/2015	4.00	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
4944	F419125	C	HKG	Treatment Naive	TAF	Baseline	8/22/2014	8.67	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4944	6600180275	C	HKG	Treatment Naive	TAF	Week 48	7/23/2015	5.99	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
4966	H552324	C	KOR	Treatment Naive	TAF	Baseline	8/28/2014	8.55	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4966	6600211730	C	KOR	Treatment Naive	TAF	Follow-Up Week 4	7/16/2015	3.07	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
5050	G154365	C	KOR	Treatment Experienced	TAF	Baseline	10/1/2014	4.63	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5050	G077474	C	KOR	Treatment Experienced	TAF	Follow-Up Week 4	3/19/2015	4.11	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
5071	F276285	D	IND	Treatment Naive	TAF	Baseline	10/8/2014	8.52	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5071	6600064411	D	IND	Treatment Naive	TAF	Week 28	4/29/2015	5.10	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
5132	E632305	D	IND	Treatment Naive	TAF	Baseline	10/31/2014	8.41	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5132	6600270423	D	IND	Treatment Naive	TAF	Week 48	10/1/2015	1.96	Assay Failure	NA	NA
5140	F800714	B	HKG	Treatment Naive	TAF	Baseline	11/3/2014	8.40	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5140	6600251212	B	HKG	Treatment Naive	TAF	Week 48	10/5/2015	5.04	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
5147	Y725209	C	USA	Treatment Experienced	TAF	Baseline	11/4/2014	4.62	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5147	6501317053	C	USA	Treatment Experienced	TAF	Week 48	10/6/2015	4.68	HBV_GTA_X02763_POLRT	1-344 (344)	S/C106S S256S/C
5170	D777175	A	IND	Treatment Naive	TAF	Baseline	11/8/2014	7.22	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5170	6600145821	A	IND	Treatment Naive	TAF	Week 36	7/16/2015	2.36	HBV_GTA_X02763_POLRT	1-344 (344)	W/R153W V/M207V A/S317A K/Q333K
5187	B032527	C	JPN	Treatment Experienced	TAF	Baseline	11/13/2014	4.94	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5187	6800085502	C	JPN	Treatment Experienced	TAF	Week 48	10/15/2015	2.21	HBV_GTA_X02763_POLRT	1-344 (344)	S13N/S R/H55H S117S/P W/R153W L267Q/L L269I/L
5188	F651045	D	IND	Treatment Naive	TAF	Baseline	11/13/2014	4.74	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5188	6600303514	D	IND	Treatment Naive	TAF	Week 48	10/28/2015	1.96	HBV_GTA_X02763_POLRT	1-344 (344)	V/E142E R153Q
5201	F351864	D	IND	Treatment Experienced	TAF	Baseline	11/17/2014	8.69	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5201	6600278439	D	IND	Treatment Experienced	TAF	Week 48	10/19/2015	5.22	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
5252	Z123512	D	CAN	Treatment Naive	TAF	Baseline	11/27/2014	8.72	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5252	6501324807	D	CAN	Treatment Naive	TAF	Week 48	10/26/2015	1.94	Assay Failure	NA	NA
5282	K914698	D	RUS	Treatment Naive	TAF	Baseline	12/5/2014	9.09	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5282	6201392925	D	RUS	Treatment Naive	TAF	Week 48	10/27/2015	3.16	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
5283	G770844	D	IND	Treatment Naive	TAF	Baseline	12/5/2014	8.80	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5283	6600126844	D	IND	Treatment Naive	TAF	Week 24	5/26/2015	5.70	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline

CAN = Canada, HKG = Hong Kong, IND = India, JPN = Japan, KOR = South Korea, NA = not applicable, ROU = Romania, RUS = Russia, TAF = tenofovir alafenamide, USA = United States

Table 13. Changes in amino acids from Baseline to Post-Baseline for subjects in the TDF group (Study GS-US-320-0110 Virology Listing 2, page 336, Integrated Virology Study Report).

SUBJID	ACCSNNUM	Genotype	Country	Oral Antiviral Treatment Status	ARM	VISIT	Visit Date	HBV DNA (log_{10} IU/mL)	Ref_POL	Coverage POL	POL Change From BL
4546	V855812	C	USA	Treatment Naive	TDF	Baseline	2/21/2014	8.07	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4546	F426708	C	USA	Treatment Naive	TDF	Week 48	1/23/2015	3.00	HBV_GTA_X02763_POLRT	1-344 (344)	D134D/E V214V/A A317A/S
4604	P191859	B	CAN	Treatment Experienced	TDF	Baseline	4/22/2014	7.72	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4604	6500362816	B	CAN	Treatment Experienced	TDF	Week 48	3/23/2015	2.58	Assay Failure	NA	NA
4707	H235984	C	KOR	Treatment Naive	TDF	Baseline	6/9/2014	6.54	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4707	6600050568	C	KOR	Treatment Naive	TDF	Week 48	5/11/2015	2.15	Assay Failure	NA	NA
4712	O800694	D	TUR	Treatment Naive	TDF	Baseline	6/10/2014	9.56	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4712	6200536865	D	TUR	Treatment Naive	TDF	Week 48	5/18/2015	7.94	HBV_GTA_X02763_POLRT	1-344 (344)	S/C256C
4721	I065357	D	ROU	Treatment Experienced	TDF	Baseline	6/12/2014	8.98	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4721	6200459018	D	ROU	Treatment Experienced	TDF	Week 48	5/14/2015	7.91	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
4790	J627327	D	TUR	Treatment Naive	TDF	Baseline	7/10/2014	9.48	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4790	6200536864	D	TUR	Treatment Naive	TDF	Week 48	6/11/2015	5.90	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
4845	A555778	C	JPN	Treatment Experienced	TDF	Baseline	7/24/2014	6.62	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4845	6800041555	C	JPN	Treatment Experienced	TDF	Week 48	6/25/2015	4.38	HBV_GTA_X02763_POLRT	1-344 (344)	R110R/G L269I/L
4873	M636175	A	ITA	Treatment Naive	TDF	Baseline	8/1/2014	8.84	HBV_GTA_X02763_POLRT	1-344 (344)	NA
4873	6200859879	A	ITA	Treatment Naive	TDF	Week 48	6/30/2015	1.97	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
5084	L240296	C	FRA	Treatment Experienced	TDF	Baseline	10/13/2014	8.48	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5084	6200953969	C	FRA	Treatment Experienced	TDF	Week 48	9/14/2015	2.33	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline
5131	E632325	D	IND	Treatment Naive	TDF	Baseline	10/30/2014	5.87	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5131	6600238004	D	IND	Treatment Naive	TDF	Week 48	10/1/2015	1.96	HBV_GTA_X02763_POLRT	1-344 (344)	Q288Q/*
5225	Y153235	B	USA	Treatment Naive	TDF	Baseline	11/20/2014	7.68	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5225	6501823135	B	USA	Treatment Naive	TDF	Week 48	10/20/2015	2.29	HBV_GTA_X02763_POLRT	1-344 (344)	Q67Q/H N118N/T N123N/D M207V/M
5374	M038845	D	TUR	Treatment Naive	TDF	Baseline	12/19/2014	5.34	HBV_GTA_X02763_POLRT	1-344 (344)	NA
5374	6201096100	D	TUR	Treatment Naive	TDF	Week 48	11/6/2015	2.66	HBV_GTA_X02763_POLRT	1-344 (344)	No change from baseline

CAN = Canada, FRA = France, IND = India, ITA = Italy, JPN = Japan, KOR = South Korea, NA = not applicable, ROU = Romania, TDF = tenofovir disoproxil fumarate, TUR = Turkey, USA = United States

2. GS-US-320-0108 Resistance Analyses by NGS

A total of 15 subjects in this clinical trial qualified for resistance-analysis by deep sequencing.

Table 14. GS-US-320-0110 Pol/rt change from consensus detected by NGS (Appendix 2, page 44-45, Integrated Virology Report).

Study	Subject ID	Accession	Visit Date	Visit	Genotype	POL Average Coverage	POL Deep Sequencing Mutation (change from Consensus)[a]	POL Deep Sequencing DRM[a]
GS-US-320-0110	4546	V855812	21-Feb-14	Baseline	C	45592.962	P109Q(4766/64640=7.37) D134E(8002/57942=13.81) R138K(3606/58576=6.16) N139K(1726/58211=2.97) K154Q(2197/61911=3.55) V214A(2810/45614=6.16)	None
GS-US-320-0110	4546	F426708	23-Jan-15	Week 48	C	41087.285	P109Q(8647/51932=16.65) D134E(9478/50825=18.65) K154Q(6791/52999=12.81) V214A(10620/46383=22.90) N236T(6652/45367=14.66) A317S(6260/30989=20.20)	N236T(6652/45367=14.66)
GS-US-320-0110	4689	A555439	30-May-14	Baseline	C	37779.875	S40A(340/8896=3.82) H124Y(4569/48277=9.46) L129M(1623/48975=3.31) G134D(2337/51385=4.55) S135T(1582/51174=3.09) D139N(3815/49549=7.70) I163V(2722/48183=5.65) N337H(483/22136=2.18)	None
GS-US-320-0110	4689	A812257	12-Dec-14	Follow-Up Week 4	C	40432.041	S40A(497/10756=4.62) S109P(1232/39315=3.13) H124D(1101/51650=2.13) H124Y(8907/51650=17.24) L129M(2162/51661=4.18) G134D(5863/52703=11.12) S135T(3195/52288=6.11) D139N(3902/52441=7.44) I163V(5390/52495=10.27) N337H(661/25712=2.57)	None
GS-US-320-0110	4712	O800694	10-Jun-14	Baseline	D	41860.456	I16T(616/16076=3.83) R18K(338/15862=2.13) C/S256C(22785/45246=50.36) C/S256S(22359/45246=49.42) M/K309K(14900/50320=29.61) M/K309M(35193/50320=69.94)	None
GS-US-320-0110	4712	6200536865	18-May-15	Week 48	D	31758.212	I16T(887/13146=6.75) C/S256C(32700/33677=97.10) C/S256S(932/33677=2.77) M/K309K(28776/39273=73.27) M/K309M(10361/39273=26.38)	None
GS-US-320-0110	4721	I065357	12-Jun-14	Baseline	D	47170.009	S78T(3029/47135=6.43) S213T(1708/53760=3.18)	None
GS-US-320-0110	4721	6200459018	14-May-15	Week 48	D	37683.828	S78T(3767/36227=10.40) R138K(934/43565=2.14) S213T(2070/43286=4.78)	None
GS-US-320-0110	4788	J071559	10-Jul-14	Baseline	D	41651.273	V214A(2373/50243=4.72) V231L(3933/47846=8.22)	None
GS-US-320-0110	4788	6200872290	11-Jun-15	Week 48	D	39879.701	V56M(732/28992=2.52) V231L(4055/42918=9.45) Q316H(1022/32427=3.15) C332Y(832/26322=3.16)	None
GS-US-320-0110	4790	J627327	10-Jul-14	Baseline	D	27187.834	Q149K(1688/35663=4.73)	None
GS-US-320-0110	4790	6200536864	11-Jun-15	Week 48	D	42581.026	Q149K(2893/53718=5.39)	None
GS-US-320-0110	4845	A555778	24-Jul-14	Baseline	C	37432.625	H55R(587/25837=2.27) Y124H(876/43308=2.02) T128N(2226/40434=5.51) D134E(1045/43467=2.40) L164F(1046/46075=2.27) A/T181A(23925/43777=54.65) A/T181T(19749/43777=45.11) V191I(7204/48062=14.99) T/N226N(13326/44803=29.74) T/N226T(31404/44803=70.09) L269I(2983/44081=6.77) H316Q(5247/37131=14.13) H337N(1246/23728=5.25)	A181T(19749/43777=45.11)
GS-US-320-0110	4845	6800041555	25-Jun-15	Week 48	C	48890.759	R110G(10238/46961=21.80) L164F(2512/62821=4.00) A/T181A(35977/59243=60.73) A/T181T(23235/59243=39.22) V191I(1653/64142=2.58) T/N226N(24726/57971=42.65) T/N226T(33151/57971=57.19) L269I(20473/52888=38.71) K275N(1554/58449=2.66) W284*(7067/61163=11.55) H316Q(1008/39507=2.55)	A181T(23235/59243=39.22)
GS-US-320-0110	4944	F419125	22-Aug-14	Baseline	C	53777.453	G/R110R(13005/45541=28.56) G/R110G(32400/45541=71.14)	None
GS-US-320-0110	4944	6600180275	23-Jul-15	Week 48	C	41244.968	G/R110R(9102/38656=23.55) G/R110G(29498/38656=76.31)	None
GS-US-320-0110	5084	L240296	13-Oct-14	Baseline	C	25380.712	No data at cutoff	None
GS-US-320-0110	5084	6200953969	14-Sep-15	Week 48	C	52896.968	R41*(450/20659=2.18) V207A(1085/53616=2.02) Q215R(1618/51875=3.12) K268N(1333/54065=2.47)	None
GS-US-320-0110	5140	F800714	3-Nov-14	Baseline	B	8697.288	R13L(213/4914=4.33) N/S134N(5553/10973=50.61) N/S134S(5385/10973=49.08) H234Q(1242/8685=14.30)	None
GS-US-320-0110	5140	6600251212	5-Oct-15	Week 48	B	31098.648	R13L(404/17529=2.30) N123D(1103/38779=2.84) N124D(902/38748=2.33) N/S134N(12624/36783=34.32) N/S134S(24102/36783=65.52) H234Q(1789/31746=5.64) A313V(1003/25874=3.88)	None
GS-US-320-0110	5147	Y725209	4-Nov-14	Baseline	C	38576.355	S106C(6440/46051=13.98) M129V(1997/47808=4.18) D131G(2422/48395=5.00) D134E(1177/49409=2.38) D134G(5307/49409=10.74) S/C256C(9689/38275=25.31) S/C256S(28359/38275=74.09) S317A(3805/31653=12.02)	None
GS-US-320-0110	5147	6501317053	6-Oct-15	Week 48	C	49829.968	S/C256S(37316/48960=76.22) S/C256C(11552/48960=23.59)	None
GS-US-320-0110	5201	F351864	17-Nov-14	Baseline	D	35168.913	No data at cutoff	None
GS-US-320-0110	5201	6600278439	19-Oct-15	Week 48	D	29807.698	No data at cutoff	None
GS-US-320-0110	5225	Y153235	20-Nov-14	Baseline	B	21085.177	N/T118N(17947/21383=83.93) N/T118T(3394/21383=15.87) N123D(898/20944=4.29) T/I128I(9099/22340=40.73) T/I128T(12867/22340=57.60) M129V(2629/22741=11.56) M207I(1210/17729=6.82) M207V(1335/17729=7.53) L220I(594/24404=2.43) L220V(740/24404=3.03) A317S(1873/20974=8.93) R332S(1515/18184=8.33) N333K(1285/17803=7.22)	None
GS-US-320-0110	5225	6501823135	20-Oct-15	Week 48	B	50785.837	Q67H(32185/48599=66.23) N/T118N(18536/60271=30.75) N/T118T(41692/60271=69.17) N123D(20332/59592=34.12) T/I128I(20209/64862=31.16) T/I128T(44431/64862=68.50) M207V(35294/49801=70.87) Q316R(1003/35797=2.80)	None
GS-US-320-0110	5283	G770844	5-Dec-14	Baseline	D	22883.343	V142E(3634/25060=14.50) V191I(2172/26682=8.14)	None
GS-US-320-0110	5283	6600126844	26-May-15	Week 24	D	24818.703	V142E(5053/28419=17.78) V191I(1852/29670=6.24)	None
GS-US-320-0110	5374	M038845	19-Dec-14	Baseline	D	39181.192	A38E(277/8885=3.12) A38T(221/8885=2.49) N53D(2204/25573=8.62) Y124D(3858/48433=7.97) Y124H(1977/48433=4.08) Q139H(1475/44601=3.31) Q149K(2808/51095=5.50) N238H(3687/54529=6.76) K333R(1134/20775=5.46) L338F(307/15067=2.04)	None
GS-US-320-0110	5374	6201096100	6-Nov-15	Week 48	D	35093.323	No data at cutoff	None

a number of reads/total reads = (% reads)
DRM = drug resistance mutations; POL = polymerase/reverse transcriptase

3. Independent Assessment of GS-US-320-0108 Resistance Analyses by NGS by DAVP

DAVP identified three treatment-emergent substitutions that were detected by >1 variant detectors among the 15 subjects: Q413H, A663S, and S613P/Q (Table 15); however, all of these substitutions occur outside of the rt domain.

Table 15. DAVP NGS analysis of 15 subjects from GS-US-320-0110 (DAVP analysis). This table only shows treatment-emergent substitutions that were detected by >1 variant detector used by DAVP or reported by the sponsor (GSI). VARDET= variant detector used to detect the substitution.

USUBJID	ARM	GT	AAPOS	AAFREQ	SUB	VARDET
GS-US-320-0110-05620-5225	TDF	B	67	66.2	Q67H	GSI
			413	>68	Q413H	PQ
GS-US-320-0110-01507-4546		C	236	14.7	N236T	GSI
			317	20.2	A317S	GSI
GS-US-320-0110-09035-4845			110	21.8	R110G	GSI
			181	39.2	A181T	GSI
			226	57.2	N226T	GSI
			284	11.6	W284*	GSI
GS-US-320-0110-1507-4546			663	>21.3	A663S	PQ
			613	>38.5	S613P/Q	LP

4. GS-US-320-0110 Resistance Analyses Conclusions

A total of 10 treatment-emergent substitutions were detected by GSI (n=7) or by >1 variant detector used by DAVP (n=3) among four subjects in GS-US-320-0110; however, none of these substitutions occurred in more than one subject. Furthermore, none of the variants identified by the sponsor were detected by the variant detectors used by DAVP. Based on these observations, we were unable to conclude that any of these treatment-emergent substitutions were associated with treatment failure.

E. Combined Resistance Analysis

To determine if substitutions that occurred in subjects in one clinical trial also occurred in the other, a combined resistance assessment was performed for all of the 16 subjects who qualified for NGS analysis from the pivotal phase 3 studies GS-US-320-0108 and GS-US-320-0110. Only substitutions that were detected by NGS that occurred in at least two subjects, regardless of clinical trial or viral genotype, were included in the analysis. Table 16 shows the results for the TAF group and Table 17 shows the results for the TDF group. Overall, there were no robust (detected by multiple variant detectors and in multiple subjects) resistance-associated substitutions in either group.

Table 15. DAVP NGS analysis of HBV from 8 subjects in the TAF group (DAVP analysis). This table shows treatment-emergent substitutions that were detected in more than one subject. TYPE= polymorphisms or Treatment-emergent or both; VARDET= variant detector used to detect the substitution. L, LFVD; P, PVD75; Q, QbVD; G, GSI. Positions in red are sites of interest for NrtI resistance.

ARM	SUBS	Position	TYPE	AAFREQ	NO.	VARDECT
TAF	T78F/S78F	78	POLY	>99	4	LP,Q,Q,Q
	H164R	164	POLY	>99	4	Q,Q,Q,Q
	N45D	45	POLY	>99	3	LPQ,Q,Q
	P208S/Q208R/S208R	208	POLY	>99	3	Q,LPQ,Q
	L265F	265	POLY	>99	3	Q,Q,Q
	T277S/A277S/T277S	277	POLY	>99	3	Q,Q,Q
	R153Q/Q153K	153	BOTH	E=15.4; P>99	2	G,Q
	L218F/F218L	218	POLY	>99	2	Q,Q
	V236I	236	POLY	>96	2	Q,Q
	S259N	259	POLY	>99	2	Q,Q
	T284A/Y284H	284	POLY	>32	2	Q,P
	P308S/S308P	308	POLY	>99	2	Q,Q
	F321L/L321F	321	POLY	>99	2	Q,Q

Table 17. DAVP NGS analysis of HBV from 8 subjects in the TAF group (DAVP analysis). This table shows treatment-emergent substitutions that were detected in more than one subject. TYPE= polymorphisms or Treatment-emergent or both; VARDET= variant detector used to detect the substitution. L, LFVD; P, PVD75; Q, QbVD; G, GSI. Positions in red are sites of interest for NrtI resistance.

ARM	SUBS	Position	TYPE	AAFREQ	NO.	VARDECT
TDF	H35R	35	POLY	>97	2	LPQ,Q
	T66S	66	POLY	>99	2	Q,Q
	S78F	78	POLY	>99	2	Q,Q
	H81D	81	POLY	>99	2	Q,Q
	Y95F	95	POLY	>99	2	Q,Q
	Y116F	116	POLY	>99	2	Q,Q
	F123C/G213C	123	POLY	>44	2	PQ,Q
	V185F	185	POLY	>99	2	Q,Q
	Q187K	187	POLY	>99	2	Q,Q
	C197F/V197F	197	POLY	>44	2	PQ,Q
	S208P	208	POLY	>99	2	Q,Q
	S2130	213	POLY	>99	2	Q,Q
	I214F/V214F	214	POLY	>99	2	Q,Q
	T228A/K228Q	228	**POLY**	**>31**	**2**	**LPQ,PQ**
	M232L	232	POLY	>99	2	Q,Q
	G261S/D261G	261	POLY	>97	2	Q,Q
	A265T/L265F	265	POLY	>99	2	Q,Q
	N267T	267	POLY	>99	2	Q,Q
	V302M	302	POLY	>99	2	Q,Q
	F307V/G307R	307	POLY	>99	2	Q,Q
	P308S	308	POLY	>99	2	Q,Q
	A317S/E317K	317	POLY	>21	2	GQ,Q
	S322P	322	POLY	>97	2	Q,Q
	N464T/N464A	464	BOTH	E=66; P>99	2	P,L
	S663A/A663S	663	BOTH	P>10; E>21	2	Q,PQ
	K841R	841	POLY	>13	2	Q,Q
	P206S/S206A	206	POLY	>99	3	LPQ,Q,Q
	S209P/P209S	209	POLY	>99	3	LPQ,Q,Q
	T272S/A272S	272	POLY	>99	3	Q,Q,Q
	W284*/T284A	284	POLY	>11	3	G,Q,Q
	S309P/P309S	309	POLY	>99	3	Q,Q,Q
	Q613S/L613S/S613P/Q	613	POLY	>38	3	L,L,LP
	D828V/A828V	828	POLY	>99	3	Q,Q,LPQ

1. Combined Resistance Analysis Conclusions

Overall, there were no robust (detected by multiple variant detectors and in multiple subjects) resistance-associated substitutions in either the TAF or TDF group among the 16 subjects who were analyzed for development of resistance using NGS.

V. METHODS

HBV Pol/rt Population Sequencing Assay

HBV polymerase is a large protein with three distinct functional domains: the terminal protein domain, the Pol/rt domain, and the RNase H domain. The Pol/rt domain (amino acids 1-344) was amplified and sequenced for all qualified subject samples (see Section 2.2.1). The primers used for amplification allow for sequence analysis to be performed for a 1032 base pair region of the HBV Pol/rt and also capture amino acids 1 through 226 of the small surface antigen (HBsAg). Serum samples were accessed from the specimen management system maintained by the central laboratory and shipped to [redacted] (b) (4) for

sequence analysis. In this assay, mixtures can be detected when present in a population at approximately 25%. The lower limit for amplification of the HBV Pol/rt is 69 IU/mL.

The Pol/rt population sequencing procedure comprises the following steps:

1. DNA isolation from 200 µL of serum using an automated MagNA Pure System (Roche Diagnostics Corporation, Indianapolis, IN)
2. Polymerase chain reaction (PCR) and nested PCR amplification of the HBV Pol/rt using the Expand High-Fidelity PCR System (Roche Diagnostics Corporation, Indianapolis, IN)
3. Population di-deoxy sequencing of the amplified product using the ABI Big Dye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA) employing a selection of forward and reverse primers
4. Analysis of the raw sequence data using the ABI SeqScape® Software v2.7 (Thermo Fisher Scientific, Waltham, MA)

HBV INNO-LiPA Multi-DR v2/v3 Hybridization Assay

The HBV INNO-LiPA Multi-DR v2/3 hybridization assay (Fujirebio, Ghent, Belgium) is a validated line probe hybridization assay that can detect specific substitutions in HBV Pol/rt that are present at levels below the 20-25% cut-off of most typical population based sequencing assays. The current version of the HBV Multi-DR v2/v3 assay is designed to detect substitutions at positions rtL80, rtV173, rtL180, rtA181, rtT184, rtA194, rtS202, rtM204, rtN236, and rtM250 when present at levels as low as 5% of the virus population. Serum samples were accessed from the specimen management system maintained by the central laboratory and shipped to (b) (4) for analysis. The assay has a limit of detection of 170 IU/mL.

The INNO-LiPA assay procedure comprises the following steps:

1. HBV DNA is isolated from clinical serum specimens and amplified using biotinylated primers.
2. The amplified DNA product is then subjected to reverse hybridization onto 2 strips coated with probes that can differentiate wild type vs. mutant at the previously listed positions.

HBV Pol/rt Population Sequencing Analysis

The amino acid sequence generated for all subjects at baseline was aligned to a reference sequence using the ClustalW2 software. The reference sequence utilized was a genotype A hepatitis B virus (Genbank X02763), previously identified as a HBV reference virus.

Results of the alignment are reported as a change from reference sequence. Determination of polymorphic and conserved sites in the HBV Pol/rt was defined by the baseline population analysis conducted in TDF Phase 3 Studies GS-US-174-0102 and GS-US-174-0103. Based on this analysis, conserved sites in the HBV Pol/rt were defined as those amino acid positions where only one variant is found at that position, or if frequency of a second minority amino acid variant was < 1%. All other positions within the HBV Pol/rt were considered polymorphic sites. Overall, 64% of amino acid positions were classified as conserved with the remaining 36% classified as polymorphic. The frequency distributions of baseline amino acid residues in Studies GS-US-174-0102 and GS-US-174-0103 are provided in Appendix 2.

On-treatment HBV Pol/rt sequences for subjects who qualified for testing were aligned to their respective baseline sequences. Differences between the baseline and on-treatment samples were categorized as polymorphic or conserved site substitutions. Substitutions from consensus by amino acid position (full substitution and mixtures) observed at on-treatment or post-switch visits were summarized. Mixtures of amino acids present at conserved positions in the baseline sample which resolve to a single amino acid were reported. Mixtures of amino acids present at polymorphic positions in the baseline sample which resolve to a single amino acid were not recorded. If the on-treatment sample contains a mixture of amino acids at a polymorphic or conserved site not present in the baseline sample, the development of a change was reported.

HBV INNO-LiPA Hybridization Analysis

The banding pattern obtained from each subject with the HBV INNO-LiPA Multi-DR v2/v3 assay was compared to a control strip that identifies whether a wild type or mutant amino acid variant is present at amino acid positions rtL80, rtV173, rtL180, rtA181, rtT184, rtA194, rtS202, rtM204, rtN236, and rtM250. Substitutions observed at any of these amino acid positions were then assigned to a resistance category as defined by the INNO-LiPA kit (Table 18). Occasionally, a polymorphism at or near an amino acid of interest may result in the absence of hybridization with any of the probes for that amino acid, resulting in a double blank. In these instances, the result is recorded as an unknown variant for that amino acid.

Table 18. Resistance categories defined by the INNO-LiPA assay (Table 2, page 13, Integrated Virology Study Report).

Mutation Category	Mutations
Adefovir Resistance	rtN236T
Lamivudine Resistance	rtM204V/I/S
Lamivudine/Adefovir Resistance	rtL180M, rtA181T/V
Entecavir Resistance	rtT184S/C/G/A/I/L/F/M, rtS202G/C/I, rtM250V/I/L
Tenofovir Predisposition	rtA194T
Compensatory Mutations	rtL80V/I, rtV173G/L

The substitutions detected in the HBV INNO-LiPA Multi-DR v2/v3 assay have been observed in patients failing chronic HBV infection treatments. However, only a subset of these substitutions confer reduced susceptibility to chronic HBV infection treatment in cell culture. Substitutions conferring reduced susceptibility were classified as primary resistance substitutions, with the remaining substitutions classified as other substitutions (Table 19). For the baseline analyses conducted in this report, subjects were assigned to a resistance category using the classifications defined in Table 19. Subjects who were classified as LAM-R+ADV-R harbored the rtM204V/I/S along with 1 or more of the ADV-R substitutions defined in Table 19.

Table 19. Resistance mutation categories defined by phenotypic analyses (Table 3, page 13, Integrated Virology Study Report).

Resistance Category		Mutations
Primary Resistance Mutations	Lamivudine Resistance[a]	rtM204V/I/S
	Adefovir Resistance[b]	rtA181T/V, rtN236T
	Entecavir Resistance[c]	rtM204V/I ± rtT184X[e] ± rtS202X ± rtM250X
Other Mutations[d]		rtL80V/I, rtV173L, rtL180M, rtT184X, rtA194T, rtS202X, rtM250X, unknown variants

Importantly, the HBV INNO-LiPA detected one substitution at baseline in subject 1507-4546 from the NGS data analysis cohort, and this substitution was not detected at baseline or Week 48 by population sequencing or by NGS. Moreover, in the population sequencing performed for 38 subjects (reviewed by the primary Virology Reviewer, Sung Rhee, Ph.D.) there were several discrepancies between the HBV INNO-LiPA assay and population sequencing. For the NGS analyses, the HBV INNO-LiPA assay results were not included in the analysis of baseline samples. For more information about the discrepancies between the HBV INNO-LiPA assay and population sequencing please see NDA208464 SDN 000.

In the two Phase 3 studies analyzed in this review, pre-treatment samples (baseline or screening) from 72 subjects also had sequencing *data* (population-based) that were compared with results generated by the *INNO-LiPA* assay: the concordance between the 2 assays for all 10 positions covered by LiPA probes was 91.7% (66/72). Discrepant results were observed in 8.3% [6/72] of the tested subjects: LiPA probes detected mixed wild-type and mutant virus populations in all 6 subjects' samples, but sequencing detected only wild-type sequences in 4 subjects and only mutant sequences in 2 subjects (see NDA208464 SDN 000).

VI. CONCLUSIONS

Overall, there were no robust (detected by multiple variant detectors and in multiple subjects) resistance-associated substitutions identified in either the TAF or TDF group among the 16 subjects who qualified for NGS analysis.

For complete labeling details, please see the review of NDA 208464 SDN 000 by Clinical Virology Reviewer Sung Rhee, Ph.D.

VII. POST MARKETING RECOMMENDATIONS

1. The baseline and Week 48 timepoints (b) (4) (by population sequencing or NGS) for all subjects who had HBV DNA >69 IU/mL.
2. Subjects 4296-4510, 5613-1163, and 9035-5187 had HBV DNA titers at the last PCR assessment that were >159 IU/mL, qualifying them for deep sequencing analysis. (b) (4) (b) (4)
3. (b) (4) for subjects 8006-5282 and 8600-4558 who had HBV DNA titers at the last PCR assessment that were >159 IU/mL. (b) (4) (b) (4)

VIII. ADMINISTRATIVE

A. Reviewer's Signature(s)

Eric F. Donaldson
Eric F. Donaldson, Ph.D.
Clinical Virology Reviewer

B. Concurrence(s)

Date: _____

HFD-530/Clin Micro TL/J O'Rear

cc:
HFD-530/NDA
HFD-530/Division File
HFD-530/RPM/Hong

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ERIC F DONALDSON
10/06/2016

SUNG S RHEE
10/06/2016

JULIAN J O REAR
10/06/2016