CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-154

MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

MICROBIOLOGY REVIEW

NDA: 22-154 **SN:** 000 **DATE REVIEWED:** 10/16/08

Microbiology Reviewer: Sung S. Rhee, Ph.D.

NDA #: 22-154 **Serial #:** 000

Applicant Name and Address: Novartis Pharmaceuticals Corporation
1 Health Plaza
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Reviewer's Name(s): Sung S. Rhee, Ph.D.

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Amendments:

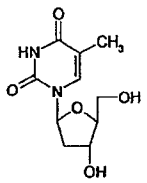
Related/Supporting Documents: IND 60459, NDA 22011

Product Name(s):

Proprietary: TYZEKA®
Non-Proprietary/USAN: Telbivudine (TBV)
Code Name/Number: LdT

Chemical Name: 1-(2-deoxy-β-L-ribofuranosyl)-5-methyluracil

Structural Formula:



Telbivudine (TBV)

Molecular Formula: $C_{10}H_{14}N_2O_5$
Molecular Weight: 242.23

Dosage Form(s): 600 mg Tablet; 20 mg/mL Solution
Route(s) of Administration: Oral
Indication(s): Treatment of chronic hepatitis B in adult patients with evidence of viral replication and either evidence of persistent elevations in serum aminotransferases (ALT or AST) or histologically active disease
Recommended Dosage: 600 mg once daily
Dispensed: Rx _X_ **OTC** ___ **(Discipline relevant)**

Abbreviations: ABC, abacavir; ADV, adefovir dipivoxil; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AZT, zidovudine; CC_{50}, 50% cytotoxic concentration; CMV, human cytomegalovirus; ddC, zalcitabine; ddI, didanosine; d4T, stavudine; EC_{50}, 50% effective concentration; ETV, entecavir; FIAU, fialuridine; FIC, fractional inhibitory

concentration; FTC, emtricitabine; HBeAg, HBV e antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; ITT, intent-to-treat; LAM, lamivudine; LdT, telbivudine; LdT-TP, telbivudine-5'-triphosphate; mtDNA, mitochondrial DNA; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PCR, polymerase chain reaction; PMEA, adefovir; PMPA, tenofovir; QD, once daily; rt, HBV reverse transcriptase; SKMC, human fetal skeletal muscle myoblast; TBV, telbivudine; TBV-TP, telbivudine-5'-triphosphate; TBV^r, telbivudine resistance; TDF, tenofovir disoproxil fumarate; TFV, tenofovir; YMDD, tyrosine-methionine-aspartate-aspartate; 3TC, lamivudine

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EXECUTIVE SUMMARY

Currently, 7 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) and pegylated recombinant human IFNα-2a (Pegasys), and the HBV nucleoside/nucleotide analog reverse transcriptase inhibitors (HBV NRTIs) adefovir dipivoxil (ADV), entecavir (ETV), lamivudine (LAM), telbivudine (TBV), and tenofovir disoproxil fumarate (TDF). 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.

Telbivudine (TBV, LdT, TYZEKA®), a synthetic thymidine nucleoside analog, exhibited inhibitory activity against hepatitis B virus (HBV) replication with EC_{50} and EC_{90} values of 0.19 μM and 0.98 μM, respectively, in the HBV stably-transfected human liver HepG2.2.15 cell line. Like other nucleoside analogs, TBV is phosphorylated by cellular kinases to the active triphosphate form (TBV-TP) that can be incorporated into HBV DNA by competing with the natural substrate, thymidine 5'-triphosphate. Incorporation of TBV-TP causes DNA chain termination, resulting in inhibition of DNA synthesis mediated by HBV reverse transcriptase (rt). TBV is an inhibitor of both HBV first strand (EC_{50} value = 1.3 ± 1.6 μM) and second strand synthesis (EC_{50} value = 0.2 ± 0.2 μM), while no inhibition was observed with human cellular DNA polymerases α, β, or γ in biochemical reactions at concentrations up to 100 μM. TBV-TP has an intracellular half-life ($t_{1/2}$) of 14 hours in stationary HepG2 cells. No appreciable mitochondrial toxicity was observed in HepG2 cells treated with TBV at concentrations up to 10 μM, and in primary human skeletal muscle cells and hepatocytes at concentrations as high as 10 times (152 μM) the mean C_{max} value in human plasma at a therapeutic dose. In cell-based 2-drug combination studies demonstrated that TBV exerted additive to weak synergistic anti-HBV activity when combined with entecavir (ETV). No evidence of cytotoxicity was observed when cells were exposed to drugs at the highest concentrations tested. In addition, no antagonistic effect of 7 FDA-approved HIV NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, and zidovudine) was detected on the anti-HBV activity of TBV.

TBV 600 mg QD for the oral tablet formulation was approved by FDA on October 25, 2006 (NDA 22-011) for the treatment of chronic hepatitis B in adult patients with evidence of viral replication and either evidence of persistent elevations in serum aminotransferases (ALT or AST) or histologically active disease, based on 1-year safety and efficacy data from the Phase 3 clinical study, Study NV-02B-007 (GLOBE study). This original NDA 22-154 contains 2-year safety and efficacy data updates from the 007 GLOBE study. Supporting analyses of the nonclinical virology data and the Year-1 clinical virology data can be found in the Clinical Microbiology review of the original NDA 22-011.

In an as-treated analysis of the 007 GLOBE study, 59% (252/430) of treatment-naïve HBeAg-positive and 89% (202/227) of treatment-naïve HBeAg-negative patients receiving TBV 600 mg QD achieved nondetectable serum HBV DNA levels (<300 copies/mL) by Week 52. Of those who continued treatment beyond Week 52, 58%

(243/418) and 85% (190/224) of HBeAg-positive and HBeAg-negative TBV recipients, respectively, had undetectable HBV DNA at Week 104 (or at the end of dosing in treatment Year 2).

In the paired amino acid sequence analysis of HBV reverse transcriptase (rt) from the pre-treatment and on-treatment samples of patients with evidence of virologic failure to TBV treatment (≥1,000 HBV DNA copies/mL at Week 48), the amino acid substitution rtM204I/V was identified to be primarily associated with virologic failure: the rtM204I/V substitution were detectable from the viruses of 46 patients (40%), out of the 115 evaluable TBV-treatment failure patients. Of those 46 patients with the rtM204I/V substitution, 44 patients had virus that carried additional amino acid substitutions in the HBV RT domain. In particular, amino acid substitutions rtL80I/V, rtL180M, and rtL229V/W were noted to frequently and/or exclusively accompany the rtM204I/V substitution.

In Year 2, the rtM204I/V substitutions emerged in additional 96 patients. The rtM204I/V substitution, thus, emerged in a total of 142 patients (78%) while on TBV (46 in Year 1 and 96 in Year 2), out of the 182 patients experiencing virologic failure with evaluable paired genotypic data. Thus, the cumulative probabilities of incurring genotypic TBV resistance (emergence of the rtM204I/V substitution) were calculated to be 7% and 21.9% in Years 1 and 2: 8.1% and 27.2% in HBeAg-positive patients and 4.8% and 12% in HBeAg-negative patients, respectively. Including rtL80I/V, rtL180M, and rtL229V/W already indentified as secondary substitutions that accompanied rtM204I/V in Year 1, a total of 12 amino acid substitutions were found, frequently and/or exclusively, in the same virus population harboring the primary TBV^r-associated rtM204I/V substitution: V27A, rtL80I/V, rtL82M, rtL91I, rtV173L, rtL180M, rtT184I/S, rtA200V, rtV207I/M, rtA222T, rtL229C/F/V/W, and rtR289K. Of the 143 patients with rtM204I/V (142 emerging on TBV and 1 pre-existing), these 12 secondary substitutions were detected in 131 patients (91.6%). The rtM204I/V + rtL80IV double substitutions were most frequently observed (n=74, 51.7%) with (n=32, 22.4%) or without (n=42, 29.4%) additional secondary substitutions.

Overall, the resistance profile for TBV appeared to be similar to that for LAM with the exception of rtM204V. Among the 142 TBV-treatment failure patients with the emergence of the rtM204I/V substitution, rtM204V HBV variants were detectable only in 3 patients: 2 patients had rtM204I/V mixed variants and 1 patient had a pure rtM204V mutant population in response to TBV therapy.

In addition to the primary TBV resistance (TBV^r)-associated substitution rtM204I, amino acid substitutions at position rtA181 (highly conserved among HBV isolates) were detected in 16 (8%) of the 201 TBV treatment-failure patients through Year 2 in the 007 GOBE study: 15 patients with no detectable rtM204I/V and 1 patient with detectable rtM204I in their virus population. In 11 of the 15 patients with no detectable rtM204I/V, rtA181S/T did not persist throughout TBV treatment, and 8 of those 11 patients showed evidence of emerging the rtM204I/V primary TBV^r-associated substitution in their later time-point isolates. Thus, the pre-existing rtA181 change emerged on TBV may have positive effect on the later development of other TBV resistance-associated substitutions. However, HBV genotype or context may be important with respect to the

rtA181T substitution and reductions in TBV susceptibility, since 3 patients developed rtA181T, but achieved virologic suppression (HBV DNA levels <1,000 copies/mL).

1. Recommendations

1.1. Recommendation and Conclusion on Approvability

Approval of this original NDA for TYZEKA® 600 mg for the oral 20 mg/mL solution formulation is recommended with respect to Clinical Microbiology for the treatment of chronic hepatitis B in adult patients with evidence of viral replication and either evidence of persistent elevations in serum aminotransferases (ALT or AST) or histologically active disease.

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

The review cycle for this NDA for TYZEKA® 600 mg for the oral 20 mg/mL solution formulation is complete. The applicant is receiving a complete response letter. Thus, no post-marketing recommendations are listed in this review.

2. Summary of OND Microbiology Assessments

2.1. Nonclinical Microbiology

Telbivudine (TBV, LdT, TYZEKA®) is a synthetic thymidine nucleoside analog and phosphorylated by cellular kinases to the active triphosphate form (TBV-TP). TBV-TP has a half-life of 14 h in stationary HepG2 cells and can be incorporated into HBV DNA by competing with the natural substrate, thymidine 5'-triphosphate. Incorporation of TBV-TP causes DNA chain termination, resulting in inhibition of HBV DNA synthesis. TBV-TP did not inhibit human cellular DNA polymerases α, β, or γ in biochemical reactions at concentrations up to 100 μM. TBV exhibited antiviral activity against HBV in stationary HepG2.2.15 cells, reducing extracellular HBV DNA with the EC_{50} value of 0.19 μM.

Cell-based 2-drug combination studies demonstrated that TBV exerted additive to weak synergistic anti-HBV activity when combined with entecavir (ETV). No evidence of cytotoxicity was observed when cells were exposed to drugs at the highest concentrations tested. In addit6ion, no antagonistic effect of 7 FDA-approved HIV NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, and zidovudine) was detected on the anti-HBV activity of TBV in cell-based 2-drug combination studies.

TBV appeared to have minimal effects on mitochondrial toxicity in primary human skeletal muscle cells after 6 days of treatment at concentrations as high as 10 times the mean C_{max} value in human plasma at a therapeutic dose. As expected, the positive control ddC reduced mtDNA content and increased lactic acid production in these cells. In primary human hepatocytes, TBV also exhibited minimal effects on mitochondrial toxicity after 6 days of treatment at concentrations as high as 10 times

the mean C_{max} value in human plasma at a therapeutic dose. The positive controls, AZT and ddC, produced no marked mitochondrial toxicity in these cells, either, in contrast to the applicant's previous findings in HepG2 cells (Study 02-CP-001A) where AZT at 50 μM was able to increase lactate production by 100% and ddC at 1 μM to decrease mtDNA content by 87%, compared to untreated control. Thus, conclusions based upon the results produced in primary hepatocytes under the current applicant's experimental conditions may not be valid.

In Year 1 (NDA 22-011, SN 000), evidence of emerging rtA181S/T substitutions was noted by Week 48 in patients with virologic failure (serum HBV DNA levels ≥1,000 copies/mL) to TBV treatment. In a standard cell culture anti-HBV drug susceptibility assay, the rtA181S and rtA181T substitutions conferred slightly reduced susceptibility (2.7 and 3.5 fold, respectively) to TBV.

Fourteen amino acid substitutions of highly conserved amino acid residues in HBV rt were infrequently found in the viruses of patients who experienced virologic failure at week 52 to TBV therapy: rtR22C, rtW58G, rtL69P, rtL82M, rtP99L, rtL180M, rtL209V, rtT240I, rtI254F, rtP261L, rtG295E, rtA307V, rtL331F, or rtA342T. To data, 6 of the 14 substitutions (rtR22C, rtL82M, rtL180M, rtL209V, rtT240I, rtA307V) were analyzed for its contribution to TBV resistance. The rtL180M substitution conferred 24.6-fold reduced susceptibility to TBV, while TBV was inactive against the primary TBV resistance-associated substitution, M204I (>1,391-fold reduction). In the 2 year-Phase 3 TBV GLOBE trial (NV-02B-007), rtL180M was detected in 18 (9.9%) of the 182 patients with virologic failure and virus samples of those 18 patients also harbored the primary rtM204I/V substitution. The 2.95 ± 2.50 fold-loss of TBV susceptibility was seen with HBV variants with rtA307V. In the TBV GLOBE trial, there was only 1 patient whose Week-48 isolates harbored rtA307V in the absence of detectable rtM204I/V substitution. The patient showed a further decline in viral load to achieve undetectable viremia at Week 100. The rtR22C, rtL82M, rtL209V, or rtT240I substitution-containing HBV variants did not exhibit any measurable reductions in susceptibility to TBV as indicated by fold-changes in EC_{50} values from wild-type HBV of <1.

2.2. Clinical Microbiology

In an as-treated analysis of the Phase 3 global registration trial (007 GLOBE study), 59% (252/430) of treatment-naïve HBeAg-positive and 89% (202/227) of treatment-naïve HBeAg-negative patients receiving TBV 600 mg QD achieved nondetectable serum HBV DNA levels (<300 copies/mL) by Week 52. Of those who continued treatment beyond Week 52, 58% (243/418) and 85% (190/224) of HBeAg-positive and HBeAg-negative TBV recipients, respectively, had undetectable HBV DNA at Week 104 (or at the end of dosing in treatment Year 2).

In Year 1, the amino acid substitution rtM204I/V was identified to be primarily associated with virologic failure (HBV DNA levels ≥1,000 copies/mL) at Week 52 or at the end of dosing within Year 1. Furthermore, amino acid substitutions rtL80I/V, rtL180M, and rtL229V/W were noted to accompany the rtM204I/V substitution.

In Year 2, the rtM204I/V substitutions emerged in additional 96 patients. The rtM204I/V substitution, thus, emerged in a total of 142 patients (78%) while on TBV (46 in Year 1 and 96 in Year 2), out of the 182 patients experiencing virologic failure with evaluable paired genotypic data. In the population nucleotide sequence analysis, the majority of the patients (n=139, 97.9% [139/142]) had rtM204I HBV variants in the treatment failure isolates, while rtM204I/V mixed variants were present in 2 patients. There was only 1 patient with the emergence of a pure rtM204V mutant population in response to TBV therapy.

The emergence of the rtM204I/V substitutions was highly associated not only with virologic failure (70.6%, 142/201) but also with virologic rebound (83.5%, 137/164; ≥1 $\log_{10}$ increase of HBV DNA from nadir while on therapy) to TBV therapy.

The cumulative probabilities of incurring genotypic TBV resistance (emergence of the rtM204I/V substitution) were 7% and 21.9% in Years 1 and 2: 8.1% and 27.2% in HBeAg-positive patients and 4.8% and 12% in HBeAg-negative patients, respectively.

Twelve amino acid substitutions, V27A, rtL80I/V, rtL82M, rtL91I, rtV173L, rtL180M, rtT184I/S, rtA200V, rtV207I/M, rtA222T, rtL229C/F/V/W, and rtR289K, were found, frequently and/or exclusively, in the same virus population harboring the primary TBV^r-associated rtM204I/V substitution. Of the 143 patients with rtM204I/V (142 emerging on TBV and 1 pre-existing), these 12 secondary substitutions were detected in 131 patients (91.6%). Overall, the resistance profile for TBV appeared to be similar to that for LAM with the exception of rtM204V. As observed in clinical isolates recovered from patients failing LAM therapy, the rtM204I/V-accompanying rtL80I/V, rtV173L, rtL180M, and/or rtV207I substitutions were also detected in the majority (n=86, 60.1%) of the 143 TBV-treatment failure isolates harboring rtM204I/V. The rtM204I/V + rtL80IV double substitutions were most frequently observed (n=74, 51.7%) with (n=32, 22.4%) or without (n=42, 29.4%) additional secondary substitutions.

Similar observations for TBV resistance were made in the China-specific clinical trial NV-02B-015. In an evaluable as-treated (≥16 weeks of treatment) analysis, 32.5% (54/166) of TBV recipients failed to suppress HBV DNA levels to <1,000 copies/mL by Week 104. Among the 54 treatment failures, 44 patients experienced virologic rebound. The primary TBV^r-associated substitution rtM204I emerged in a total of 46 patients (85.2% 46/54) while on TBV, including 41 patients experiencing virologic rebound (95.3%, 41/43). The rtM204V substitution was not detectable in these failure isolates. Genotypic resistance to TBV in Study 015 occurred slightly more frequently, compared to the 007 GLOBE study, by Year 2 in the overall TBV-treated population (27.7% [46/166] versus 21.6% [142/657]) and in the HBeAg-positive subpopulation (31.7% [44/139] versus 26.7% [115/430]), respectively. The probabilities of incurring genetic TBV resistance in HBeAg-negative patients were 7.4% (2/27) in Study 015 and11.9% (27/227) in Study 007.

Forty-six patients with emerging rtM204I substitution had all additional substitutions in HBV rt. Out of the 12 secondary substitutions identified in the 007 GOBE study, 10 substitutions, rtV27A, rtL80I/V, rtL82M, rtL91I, rtV173L, rtL180M, rtT184I, rtA200V, rtA222T, and rtL229F/V/W were exclusively found in the rtM204I substitution

harboring virus population. The rtM204I/V + rtL80IV double substitutions were also most frequently observed (n=30, 65.2%) with (n=19, 41.3%) or without (n=11, 23.9%) additional secondary substitutions.

In addition to the primary TBV resistance (TBV^r)-associated substitution rtM204I, amino acid substitutions at position rtA181 (highly conserved among HBV isolates) were detected in 16 (8%) of the 201 TBV treatment-failure patients through Year 2 in the 007 GOBE study: 15 patients with no detectable rtM204I/V and 1 patient with detectable rtM204I in their virus population. In 11 of the 15 patients with no detectable rtM204I/V, rtA181S/T did not persist throughout TBV treatment, and 8 of those 11 patients showed evidence of emerging the rtM204I/V primary TBV^r-associated substitution in their later time-point isolates. Thus, the pre-existing rtA181 change emerged on TBV may have positive effect on the later development of other TBV resistance-associated substitutions. However, HBV genotype or context may be important with respect to the rtA181T substitution and reductions in TBV susceptibility, since 3 patients developed rtA181T, but achieved virologic suppression (HBV DNA levels <1,000 copies/mL). There were 2 patients (3.7%), out of the 54 TBV treatment-failure patients through Year in Study 015, who developed the rtA181A/T substitution on TBV: 1 patient with no detectable rtM204I/V and 1 patient with detectable rtM204I in their virus population.

3. Administrative

3.1. Reviewer's Signature(s)

Sung S. Rhee, Ph.D.
Microbiologist

3.2. Concurrence

Date: _____

HFD-530/MicroTL/J. O'Rear

CC:
HFD-530/NDA # 22011
HFD-530/Division File
HFD-530/PM/K. Shade

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

MICROBIOLOGY REVIEW

NDA: 22-154 **SN:** 000 **DATE REVIEWED:** 10/16/08

Microbiology Reviewer: Sung S. Rhee, Ph.D.

OND MICROBIOLGY REVIEW

1. Introduction and Background

Telbivudine (TBV, LdT, TYZEKA®) is a synthetic nucleoside analogue with activity against hepatitis B virus (HBV) polymerase (reverse transcriptase, rt) that, like other nucleoside analogs, is phosphorylated to its active metabolite, telbivudine-5'-triphosphate (TBV-TP), by cellular kinases. TBV-TP inhibits HBV rt by competing with the natural substrate, deoxythymidine-5'-triphosphate (dTTP). Incorporation of TBV-TP into viral DNA causes DNA chain termination, resulting in inhibition of HBV replication.

TBV 600 mg QD for the oral tablet formulation was approved for the treatment of chronic hepatitis B in adult patients with evidence of viral replication and either evidence of persistent elevations in serum aminotransferases (ALT or AST) or histologically active disease by the FDA on October 25, 2006 (NDA 22-011) based on 1-year safety and efficacy data from the Phase 3 clinical study, Study NV-02B-007 (GLOBE study). TBV was also approved in more than 40 other countries, including Switzerland, China, South Korea, Canada, and countries of the EU, based on the 1-year data from the 007 GLOBE study.

The 007 GLOBE study demonstrated that antiviral efficacy of TBV at Week 52 was not inferior to lamivudine (LAM) in HBeAg-positive and HBeAg-negative patients. The rates of virologic failure (≥1,000 copies/mL at Week 52) and virologic rebound (≥1 $\log_{10}$ increase of serum HBV DNA from nadir while on therapy) were lower for TBV recipients at Week 52, compared to LAM recipients in both the HBeAg-positive and HBeAg-negative patient populations. For the HBeAg-positive patient population, virologic failure was observed in 33.7% (145/430) of TBV recipients versus 53.2% (233/438) of LAM recipients. In the HBeAg-negative patient group, 8.4% (19/227) of TBV recipients and 21.5% (48/223) of LAM recipients experienced virologic failure. Virologic rebound was observed in 7.9% (34/430) of TBV recipients, compared to 23.5% (233/455) of LAM recipients in the HBeAg-positive patient population, while in the HBeAg-negative patient population, it was observed in 4.9% (11/227) of TBV recipients and 16.6% (37/223) of LAM recipients.

In the paired sequence analysis of baseline and on-treatment samples from evaluable patients (n=115) with evidence of virologic failure, amino acid substitutions at position 204, rtM204I/V, were identified to be primarily associated with virologic failure and rebound, detectable in a total of 46 patients (46/115, 40%). Of 46 patients with the rtM204I substitution, 44 patients had virus that carried other amino acid substitutions in the HBV rt domain, including amino acid substitutions rtL80I/V, rtL180M, and rtL229W/V. In addition, amino acid substitutions at rtA181 developed in 16 of the 115 patients (13.9%): the mixed rtA181T/A and the pure rtA181T variants were detectable from the viruses of 8 (50%) and 7 patients (43.8%), respectively, and one patient had an rtA181S change. Of 16 patients, 1 patient also carried the rtM204I/M mutation and showed evidence of virologic rebound.

In this NDA, as basis for approval for the TYZEKA® oral 20 mg/mL solution formulation, the applicant is cross-referencing NDA 22-011, an original submission containing 1-year

safety and efficacy data from the Phase 3 clinical GLOBE study (NV-02B-007) and a supplemental submission (SE8-001) with 2-year data updates. The 007 GLOBE study is a randomized, double-blind trial of TBV 600 mg QD compared to LAM 100 mg QD for a treatment period of up to 104 weeks in adults chronically infected with HBV and having compensated liver disease but never treated with LAM or an investigational anti-HBV nucleoside or nucleotide analog. In addition, the applicant submitted 1- and 2-year data from the NV-02B-015 Phase 3 study conducted in China. Study 015 is a 104-week, randomized, double-blind trial of TBV (600 mg QD) versus LAM (100 mg QD) in Chinese adults with compensated chronic hepatitis B.

2. Materials and Methods

2.1. Quantification of Serum HBV DNA Levels

Serum HBV DNA levels were quantified using the COBAS Amplicor HBV Monitor™ PCR assay kit (Roche Laboratories) at [redacted] According to the manufacturer, the COBAS Amplicor HBV Monitor™ PCR assay gives a linear response from 300 (lower limit of quantification, 2.48 $\log_{10}$ copies/mL) HBV DNA copies/mL to at least 200,000 (upper limit of quantification, 5.3 $\log_{10}$ copies/mL) HBV DNA copies/mL in both EDTA-plasma and serum. For specimens suspected to contain high levels of HBV DNA (≥200,000 copies/mL), diluted samples were used for the assay. b(4)

The COBAS Amplicor HBV Monitor™ PCR assay gave equivalent results for HBV plasmid DNAs from genotypes A through E. However, the genotype F plasmid DNAs yielded significantly lower results than the nominal input and did not detect the isolate at input concentrations less than 150,000 HBV DNA copies/mL, due to several nucleotide mismatches between genotype F isolates and the sequence of one of the primers used in the assay. Therefore, genotype F will not be amplified with the same efficiency as the other genotypes and will result in a lower viral load result. There were 3 patients (1 in the LAM arm and 2 in the TBV arm) infected with HBV genotype F.

2.2. Nucleotide Sequence Analysis of the Reverse Transcriptase (rt) Domain of HBV Polymerase

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 (nucleotides 1045 to 2076, amino acid residues 349 to 692) were sequenced for all qualified patient samples (serum HBV DNA ≥1,000 copies/mL) in the [redacted] b(4)

HBV DNA was extracted from qualified patient serum samples by using the Total Nucleic Acid isolation kit (Roche Laboratories) and the rt domain of the HBV polymerase gene was amplified by PCR using the Expand High Fidelity kit (Roche Laboratories). For most samples, single-round (or standard) PCR amplification was achieved with primers that lay just outside the boundaries of the HBV rt domain. Alternatively, nested PCR amplification was achieved with distal primers followed by the standard PCR. The sensitivity of single-round PCR amplification is ~10,000 copies/mL, while the sensitivity of nested PCR amplification is ~300 copies/mL.

The PCR-amplified samples were then subjected to sequence analysis using a set of forward and reverse primers that were designed to generate overlapping rt sequences. Approximately 5-10% of the sequences have been partially or completely re-sequenced in order to obtain completely reliable data. The multiple overlapping sequence data were then assembled into a composite using the SeqScape software version 2.1.1. (Applied Biosystems).

3. Nonclinical Microbiology

Please refer to the Microbiology review NDA022011.000 for the nonclinical microbiology of TBV, including mechanism of action, antiviral activity in cell culture, cytotoxicity, and combination activity relationships and cross-resistance with for chronic HBV Infection with FDA-approved HBV nucleoside analogs for chronic HBV infection.

3.1. Anti-HBV combination activity relationships of LdT in cell culture with ETV

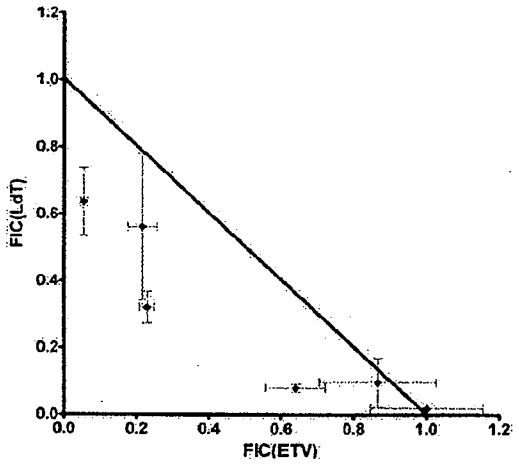
The applicant assessed the cell culture antiviral efficacy of LdT in combination with ETV against HBV. A stably transfected human hepatoma HepG2-derived cell line, WT3/C1, constitutively producing replication-competent wild-type HBV nucleocapsids and virions was utilized in this 2-drug combination study. A standard cell culture anti-HBV drug susceptibility assay was employed to determine the anti-HBV activity of each drug alone and in combinations with each other by measuring endogenous HBV polymerase activity. Results were submitted in Study Report IDIX-06-184 (NDA 22011, SN 039).

Five concentrations spanning the linear range of previously determined dose responses and the approximate EC_{50} values for each drug were tested: the final concentrations range from 2.5 to 0.0098 μM for LdT and from 0.02 to 0.000078 μM for ETV. Cells were cultured in the presence or absence of drugs for 10 days, and then cell lysates were prepared and subjected to endogenous polymerase assays as described previously (Seifer *et al.*, 1998). Antiviral activity data from 5 individual experiments were analyzed using both the Bliss independence drug interaction model with the MacSynergy II program (Prichard *et al.*, 1993) and the Loewe additivity model with the CombiTool program (Dressler *et al.*, 1999).

Results showed that 2-drug combinations of LdT with ETV exhibited additive (MacSynergy™ II and CombiTool analyses) to weak synergistic interaction (Isobologram analysis based on Loewe additivity). In addition, based on EC_{50} values of each drug in the absence and presence of the other compound, an isobologram was graphed to demonstrate the combination effects of the two tested compounds (Figure 1). Of note, Delaney *et al.*, (2004) reported that LdT exerted additive antiviral effects when combined with ADV in a stably transfected cell line, HepG2 49-29 with no evidence of cytotoxicity.

There was no evidence of cytotoxicity when HepG2 cells were exposed to drugs at the highest concentrations used in the antiviral combination experiments (2.5 μM LdT alone, 0.02 μM ETV alone, or 2.5 μM LdT plus 0.02 μM ETV) for 9 days (Table 1). Cell viability was determined by MTS staining using a CellTiter 96 Aqueous One Solution cell proliferation assay (Promega) according to the manufacturer's instructions.

Figure 1: Isobologram Analysis



The line intercepting both FIC (fractional inhibitory concentration) axes at a value of 1 represents dosewise additivity of the 2 drugs. Points above this line indicate antagonism, and points below the additivity line indicate synergy. The average D (observed deviation from dosewise additivity) value was -0.15967 (ranging from -0.09387 to -0.20771) with p-value of 0.0012. The value of D is negative when the drug combination is synergistic and positive for antagonistic combinations.

Table 1: Cytotoxicity of LdT and ETV alone and in combination in HepG2 Cells

Drug[1]	Cell Viability[2]
Untreated	100%
LdT (2.5 µM)	100%
ETV (0.02 µM)	100%
LdT (2.5 µM) +ETV (0.02 µM)	94%

[1]Drug containing cell culture medium was replaced every other day.
[2]Cell viability was determined by MTS staining. The assay was performed in triplicate.

3.2. Anti-HBV combination activity relationships of LdT in cell culture with HIV NRTIs

With approximately 10% of HIV-1 infected individuals also co-infected with HBV, LdT may be co-administered with antiretroviral therapies including HIV NRTIs. LdT and HIV NRTIs use the same cellular enzymes for phosphorylation to their active forms and thus their phosphorylation efficiency could be decreased upon co-administration, which in turn could decrease the efficacy of one or more drugs against HIV-1 and HBV.

Previously, the applicant conducted cell-based drug combination studies to show no antagonistic effect of HIV NRTIs on the anti-HBV activity of LdT (IDIX-05-115 [NDA 22-011, SN 000]): abacavir (ABC), didanosine (ddI), emtricitabine (FTC), lamivudine (LAM), stavudine (d4T), tenofovir (TNV, active moiety of ester prodrug TDF), or zidovudine (AZT) were tested. Zalcitabine (ddC) was not tested in this study. The Applicant did not provide data for the NRTIs alone so the conclusion with respect to antagonism was not validated.

In this current Study Report IDIX-07-123 (NDA 22-011, SN 034), the applicant repeated the experiments in a stably transfected HepG2-derived cell line, WT3/C1, constitutively producing HBV virion particles. Cells were treated for 10 days with each drug alone or in combination with LdT and one of the 7 HIV NRTIs (ABC, AZT, ddI, d4T, FTC, LAM, and TNV (active moiety of ester prodrug TDF), and endogenous HBV rt activity was

measured from cell lysates or intracellular HBV nucleocapsids. Each NRTI was tested at 2 fixed concentrations (low and high) that meet or exceed the maximum concentration found in the blood of patients treated with these drugs in the presence of a range of LdT concentrations (0.021 to 65 μM). Results indicating no negative interaction between LdT and HIV NRTIs are summarized below.

The mean EC_{50} values for the 7 HIV NRTIs ranged from 0.011 ± 0.002 μM (for LAM) to >50 μM (for ddI; Table 2). FTC, LAM, and TNV exhibited substantial anti-HBV activity, while ABC, AZT, ddI, and d4T showed only weak or no anti-HBV activity. None of the 7 HIV NRTIs alone exhibited measurable cytotoxicity in HepG2 cells following 4 days of drug exposure to up to 100 μM (Table 2). In addition, treatment of HepG2 cells with 2-drug combinations (10 μM LdT plus the "high" dose of each NRTI) did not significantly reduce cell viability (Table 2).

Table 2: Anti-HBV activity and cytotoxicity of HIV NRTIs

Drug	Mean EC_{50} (μM) plus SD	CC_{50} (μM) single NRTI	Cell Viability (% of no-drug control) LdT + NRTI
Lam	0.011 ± 0.002	>100	92
FTC	0.025 ± 0.012	>100	78
TNV	0.075 ± 0.081	>100	92
AZT	1.160 ± 0.170	>100	96
ABC	7.505 ± 2.369	>100	96
d4T	>30	>100	86
ddI	>50	>100	96
LdT	0.181 ± 0.037	>100	n/a

- CC_{50} and EC_{50} values were calculated from 2 independent experiments.
- SD, standard deviation
- Cell viability with 2-drug combinations was measured in the presence of 10 μM LdT plus high dose of each of HIV NRTIs.

Based on its anti-HBV activity (Table 2), the sponsor chose the 2 fixed concentrations (low and high) of HIV NRTIs for the cell-based drug combination assay (Table 3).

Table 3: Low and high concentrations of HIV NRTIs in μM

Drug	NRTI (low)	NRTI (high)
Lam	0.025	0.25
FTC	0.025	0.25
TNV	0.25	2.5
AZT	1	5
ABC	10	50
d4T	6	30
ddI	10	50

Low and high concentrations for LAM, FTC, and TNV were chosen based on single drug titration experiments, while those for AZT, ABC, d4T, and ddI were to be approximate C_{max} and 5X C_{max} concentrations, respectively, deduced from the published literature and drug package inserts.

None of the 7 HIV NRTI drugs exhibited measurable antagonistic interaction with LdT against HBV under the experimental conditions utilized in this study (Tables 4 and 5).

Table 4: Anti-HBV activity of LdT in the presence of HIV NRTIs

HIV NRTI	N	LdT EC_{50} (μM)	LdT + NRTI (low) EC_{50} (μM)	LdT + NRTI (high) EC_{50} (μM)
Lam	2	0.255 ± 0.115	<0.025 ± 0.007	<0.021 ± 0.000
FTC	2	0.385 ± 0.172	0.048 ± 0.005	<0.021 ± 0.000
TNV	2	0.329 ± 0.290	<0.185 ± 0.232	<0.021 ± 0.000
AZT	2	0.277 ± 0.119	0.034 ± 0.018	<0.021 ± 0.000
ABC	4	0.580 ± 0.201	0.248 ± 0.110	<0.021 ± 0.000
d4T	2	0.383 ± 0.331	0.391 ± 0.384	0.030 ± 0.012
ddI	2	0.343 ± 0.375	0.332 ± 0.300	0.382 ± 0.393

Table 5: Fold change in EC_{50} values for LdT in the presence of HIV NRTIs

Drug	LdT + NRTI (low)	LdT + NRTI (high)
Lam	<0.10 ± 0.02	<0.09 ± 0.04
FTC	0.13 ± 0.05	<0.06 ± 0.03
TNV	<0.41 ± 0.34	<0.10 ± 0.09
AZT	0.15 ± 0.13	<0.08 ± 0.04
ABC	0.57 ± 0.55	<0.04 ± 0.02
d4T	0.94 ± 0.19	0.10 ± 0.05
ddI	1.22 ± 0.48	1.21 ± 0.18

Fold change = EC_{50} values for the LdT + NRTI combination divided by the EC_{50} value for LdT alone

3.3. Susceptibility of HBV rtA181 Variants Harboring rtA181S, rtA181T, or rtA181V to LdT

In Year 1 (NDA 22-011, SN 000), evidence of emerging rtA181S/T substitutions was noted by Week 48 in patients with virologic failure to LdT treatment. In the paired sequence analysis of baseline and on-treatment samples, a total of 16 patients (13.9%, 16/115) had virus harboring the rtA181S/T variants. The rtA181T variant was detectable from the viruses of 15 patients and the rtA181S variant was of 1 patient. Interestingly, no variants with rtA181V were seen. The rtA181V mutation is known to confer resistance to ADV (Angus *et al.*, 2003). Previously, the applicant assessed the antiviral activity in cell culture of LdT against HBV harboring individually the ADV resistance-associated substitutions rtN236T and rtA181V, respectively, in Studies IDIX-04-185 and IDIX-06-101 (NDA 22-011, SN 000). In stably transfected HepG2 clonal cell lines harboring wild-type, rtN236T, or rtA181V mutant HBV genome (genotype D, subtype ayw), LdT was found to exhibit ~2-fold better antiviral activity against the rtN236T mutant compared to wild-type virus (fold changes in EC_{50} values of 0.5 ± 0.43) and 3 to 5 times less against the rtA181V mutant (fold changes of 3.72 ± 2.18).

In this current study (Study IDIX-07-173), the applicant evaluated the rtA181S or rtA181T single substitutions in HBV rt for reductions in susceptibility to LdT. Stable HepG2 cell lines were established to constitutively express wild-type, rtA181S, rtA181T, or rtA181V mutant HBV virus. Cells were exposed to drugs tested for 9 days and subjected to a standard cell culture anti-HBV drug susceptibility assay. Results from 3 independent experiments are summarized in Table 6.

The mean EC_{50} value against wild-type HBV was 1.45 ± 0.07 μM for LdT. The applicant noted that the mean EC_{50} value for LdT was approximately 2.2- to 8.5-fold higher in this study, compared to those previously observed (ranging from 0.17 to 0.65 μM). The rtA181S and rtA181T substitutions conferred slightly reduced susceptibility (2.7 and 3.5 fold, respectively) to LdT, while LdT as well as ADV (PMEA) retained wild-type phenotypic activity against the rtA181V substitution (Table 6). Of note, the applicant reported previously in Study IDIX-06-101 (NDA 22-011, SN 000) that the rtA181V substitution conferred 3- to 5-fold reduced susceptibility to LdT (mean fold changes of 3.72 ± 2.18) and 3.7-fold reduced susceptibility to ADV. Recently, Qi et al. (2007) also reported that the A181V substitution conferred reduced susceptibility to ADV (4.3-fold) and to TDF (3.2-fold).

Table 6: Susceptibility of HBV Variants Harboring rtA181 Substitutions to LdT (Fold Change in EC_{50} Values)

Cell line	Virus	Telbivudine	PMEA	Tenofovir	Entecavir
WT3/C1	WT	1	1	1	1
A181S/7C4G3	A181S	2.7 ± 1.6	2.1 ± 1.8	1.8 ± 0.8	1.4 ± 0.7
A181T/B4F2	A181T	3.5 ± 1.4	1.3 ± 0.6	2.3 ± 1.2	4.6 ± 2.3
A181V/2A3A	A181V	1.0 ± 0.4	0.5 ± 0.2	1.3 ± 1.1	0.6 ± 0.2

Fold change = EC_{50} values for HBV variants divided by the EC_{50} value for wild-type HBV

3.4. Susceptibility to LdT in Cell Culture of HBV Harboring Highly Conserved Amino Acid Substitutions

The applicant agreed to assess the cell culture anti-HBV activity of LdT against HBV variants the following substitutions of highly conserved amino acid residues in HBV rt: R22C, W58G, L69P, L82M, P99L, L180M, L209V, T240I, I254F, P261L, G295E, A307V, L331F, or A342T. These amino acid substitutions were found in the viruses of patients who experienced virologic failure (serum HBV DNA levels ≥1,000 copies/mL at Week 52) to LdT therapy. In Year 1, each substitution was found in only 1 patient with the exception of the rtL180M substitution that was found in 4 patients. By Year 2 (Week 104), the L82M, rt180M, and rtA342L/T/V substitutions were cumulatively found in 3, 18, 2 patients, while the occurrence of each of the other 11 substitutions remained unchanged.

To data, the applicant was able to analyze 6 of the 14 substitutions (rtR22C, rtL82M, rtL180M, rtL209V, rtT240I, rtA307V). Using site-directed mutagenesis (QuikChange kit, Stratagene), each substitution, in addition to the M204I substitution as a positive control, was introduced into the CMV promoter-driven HBV expression vector pCMVhbv in a genotype D background (provided by Dr. C. Seeger, Fox Chase Cancer Institute). Recombinant HBV expression plasmids were transiently transfected into HepG2 hepatoma cells, which were then cultured for 3 days in the presence of LdT. A standard cell culture anti-HBV drug susceptibility assay was employed to determine the anti-HBV efficacy of LdT against these HBV mutant variants. Results are summarized in Table 7 (IDIX-07-122).

The mean EC_{50} value against wild-type HBV was 0.71 μM for LdT, comparable to that

obtained in stable HBV cell lines (0.17 - 0.65 μM). LdT was inactive against the primary LdT resistance-associated substitution, M204I (>1,391-fold change in EC_{50} values; Table 7). In addition, the rtL180M substitution conferred 24.6-fold reduced susceptibility to LdT (Table 7). In the 2 year-Phase 3 LdT GLOBE trial (NV-02B-007), rtL180M was detected in 18 (9.9%) of the 182 patients with virologic failure and virus samples of those 18 patients also harbored the primary rtM204I/V substitution. Since L180M was not seen in the absence of M204I/V, the L180M substitution was considered as a secondary substitution associated with LdT resistance. Numerous LAM clinical studies (Allen *et al.*, 1998; Li *et al.*, 2005; Libbrecht *et al.*, 2007; Lok *et al.*, 2002; Roque-Alfonso *et al.*, 2003) also noted the rtL180M substitution was almost exclusively observed in conjunction with the rtM204I/V changes. In early cell culture studies, HBV variants with the rtM204I/V substitutions were shown to replicate less efficiently than wild-type HBV (Ling and Harrison, 1999; Melegari *et al.*, 1998), which could be partially or completely overcome by additional substitutions in HBV rt, particularly rtL180M (Fu and Cheng, 1998; Ono *et al.*, 2001), rtL80I/V (Warner *et al.*, 2007), rtV173L (Delaney *et al.*, 2003), and/or rtV207I (Zöllner *et al.*, 2005).

The rtR22C, rtL82M, rtL209V, or rtT240I substitution-containing HBV variants did not exhibit any measurable reductions in susceptibility to LdT as indicated by fold-changes in EC_{50} values from wild-type HBV of <1 (Table 7). The 2.95 ± 2.50 fold-loss of LdT susceptibility was seen with HBV variants with rtA307V (Table 7). In the LdT GLOBE trial, there was only 1 patient (ID 008-046) whose Week-48 isolates (3.6 log_{10} copies/mL) harbored rtA307V in the absence of detectable rtM204I/V substitution. Subject 008-046 showed a further decline in viral load to achieve undetectable viremia at Week 100.

Table 7: Susceptibility of HBV Variants Harboring Highly Conserved Amino Acid Substitutions to LdT (Fold Change in EC_{50} Values)

Virus	Telbivudine	Adefovir
WT	1	1
M204I*	>1,391	5.34 ± 1.79
R22C	0.48 ± 0.20	1.79 ± 1.08
L82M	0.73 ± 0.12	1.16 ± 0.08
L180M	24.60 ± 7.90	1.90 ± 1.70
L209V	0.43 ± 0.19	0.75 ± 0.27
T240I	0.80 ± 0.74	0.63 ± 0.04
A307V	2.95 ± 2.50	0.68 ± 0.24

- Fold change = EC_{50} values for HBV variants divided by the EC_{50} value for wild-type HBV
- Mean fold-resistance values were calculated from 2 individual experiments with the exception of the M204I variant whose mean fold-resistance value was from 6 individual experiments.

3.5. Mitochondrial Toxicity of LdT

Mitochondrial dysfunction has been associated with long-term toxicities of antiretroviral therapy, particularly with the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs; Brinkman *et al.*, 1998; Brinkman and Kakuda, 2000; Cossarizza and Moyle, 2004; Dagan *et al.*, 2002; Miller *et al.*, 2003; Safrin and Grunfeld, 1999). Clinical features included lactic acidosis, hepatomegaly with steatosis, peripheral neuropathy, myopathy, cardiomyopathy, pancreatitis, and lipodystrophy syndrome exhibiting

hyperlipidemia, glucose intolerance, and fat deposition (Brinkman *et al.*, 1999; Brinkman and Kakuda, 2000; Carr and Cooper, 2000; Chen *et al.*, 2002; Dalakas 2001; Falcó *et al.*, 2003; Guo and Fung, 2004; Moyle, 2000; Powderly, 2002). 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 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).

Clinical adverse effects due to NRTI-induced mitochondrial toxicity frequently occur in liver and muscle tissues (Brinkman *et al.*, 1998). Thus, the applicant assessed the potential of LdT to produce mitochondrial toxicity in human skeletal muscle cells and hepatocytes by measuring cell viability/proliferation, mtDNA content by real-time PCR, and lactic acid production using a lactic acid kit (Boehringer), and by determining changes in morphology of mitochondrial ultrastructure (e.g., loss of cristae, and matrix dissolution and swelling) and lipid droplet formation by transmission electron microscopy (Phillips 301A). Of note, a previously reported biochemical study (02-CP-001A) showed that human cellular DNA polymerases α, β, or γ were not inhibited by LdT-TP (telbivudine triphosphate) at concentrations up to 100 μM, approximately 100-fold higher than those required to inhibit HBV rt.

3.5.1. Mitochondrial Toxicity of LdT in Primary Human Skeletal Muscle Cells

Human fetal skeletal muscle myoblast (SKMC cells CC-2561; Lonza Walkersville) derived from the triceps muscle was used in this study (Study IDIX-07-116A). Cells were cultured for 6 days in the presence or absence of LdT. As positive controls, zidovudine (AZT) and zalcitabine (ddC) were included, and as a negative control, lamivudine (3TC) was included. Under normal culture conditions in the absence of drugs tested, SKMC cells grew linearly through approximately 10 population doublings during the duration of the experiments. Effects of ddC on mitochondria are well characterized. Thus, ddC was recently recommended by the European Medicines Agency (EMEA, 2007) to be included as a positive control in NRTI-induced mitochondrial toxicity studies to ensure the functionality of assays and methods and the general suitability of the cell culture model.

Cell viability/proliferation: No cytotoxicity was observed in cells exposed for 6 days to LdT at concentrations up to 1,000 μM (highest concentration tested; Table 8). AZT and 3TC inhibited cell proliferation by approximately 30% at 1,000 μM, and ddC was cytotoxic with a reported mean CC_{50} value of 148.1 ± 46.5 μM. Previously, Cihlar *et al.* (2002) reported that in SKMC cells treated with drugs for 6 days, ddC inhibited the cell growth with the CC_{50} value of 90 ± 38 μM, whereas AZT and 3TC showed a weak inhibition of the cell growth with CC_{50} values of 497 ± 19 and 1230 ± 331 μM, respectively.

Based upon the cell viability/proliferation results, the applicant chose 2 concentrations

for AZT (12 and 56 μM) and 3TC (30 and 90 μM), and 3 concentrations for LdT (12, 56, and 152 μM) to be studied for mitochondrial toxicity. The highest concentrations of AZT, 3TC, and LdT represent a 10-fold increase over the plasma C_{max} values in humans. Two concentrations of ddC (1 and 10 μM) were selected based upon the cell viability/proliferation results and published findings by Birkus *et al.* (2002) and Pan-Zhou *et al.* (2000). The lower concentration (1 μM) representing a 10-fold increase over the plasma C_{max} value in humans.

Table 8: Effects of LdT on Cell Viability and Proliferation in SKMCs

Compound	1st expt.	2nd expt.	3rd expt.	4th expt.	Mean ± SD
AZT	20% reduction @1000μM	40% reduction @1000μM	40% reduction @1000μM	NA*	35% reduction @1000μM
LdT	No tox @1000	No tox @1000	No tox @1000	NA	No tox @1000
3TC	31% reduction @1000μM	39% reduction @1000μM	25% reduction @1000μM	NA	32% reduction @1000μM
ddC CC_{50} (μM)	115.0	103.2	199.8	174.4	148.1±46.5

- Cell viability/proliferation was presented as a percent reduction in sulforhodamine B (SRB) absorption relative to the untreated control cells. In the event a 50% reduction in SRB absorption was observed, this was reported as the CC_{50} value for that compound.
- NA, not available

Mitochondrial DNA (mtDNA) content: No depletion of mtDNA was observed in proliferating SKMCs, following 6 days of treatment with LdT at concentrations up to 152 μM (Table 9). Relative mtDNA content remained unchanged in cells treated with LdT, AZT, or 3TC at the highest concentrations (10 times the C_{max} value in plasma), with the observed mean mtDNA levels corresponding to 102%, 114%, and 111 % of control values, respectively (Table 9). In contrast, ddC at 1 and 10 μM decreased mtDNA levels by 77% and 94%, respectively. Birkus *et al.* (2002) also observed no depletion of mtDNA when SKMCs were treated with AZT and 3TC at concentrations up to 300 μM for 9 days. In contrast, the authors observed pronounced effects of ddC in SKMCs, causing almost complete depletion of mtDNA at 3 μM.

Table 9: Effects of LdT on Mitochondrial DNA (mtDNA) Content and Production of Lactic Acid in SKMCs

Drug	Drug concentration (μM)	mtDNA (% of control)	Lactic acid production (% of control)
LdT	12	98.7 ± 4.5	93.4 ± 10.3
	56	109.9 ± 21.9	91.3 ± 12.2
	152	101.7 ± 11.1	100.3 ± 5.2
AZT	12	121.9 ± 16.9	98.3 ± 10.4
	56	113.7 ± 17.6	101.3 ± 11.0
3TC	30	100.1 ± 8.3	93.5 ± 11.8
	90	111.4 ± 10.0	101.5 ± 4.9
ddC	1	22.9 ± 4.6	129.2 ± 6.7
	10	5.9 ± 0.9	168.5 ± 6.5

- Data represent the mean of 3 independent experiments.
- The mtDNA content of the cells was expressed as a relative coxII mtDNA/β-actin nuclear DNA (nDNA) ratio and reported as a percentage relative to the untreated control cells.
- Lactic acid production was expressed as the ratio of milligrams of lactate normalized to cell number and reported as a percentage relative to the untreated control cells.

Lactic acid production: As summarized in Table 9 above, no increase in the level of lactic acid secreted in the medium was observed by LdT, AZT, or 3TC at any concentration tested. In contrast, ddC increased lactic acid production in a dose dependent manner (29% at 1 μM and 69% at 10 μM) in agreement with the published findings of 52% increase when SKMCs were treated for 6 days at 30 μM (Birkus *et al.* 2000) and of 60% increase when cells were treated for 10 days at 10 μM (Benbrik *et al.*, 1997). It should be noted that Birkus *et al.* (2002) observed a 38% increase in the levels of extracellular lactate production by AZT at 30 μM, whereas no increase was observed by the applicant at 56 μM. The applicant reasoned that observed differences may be due to methodological differences and/or donor cell variability. However, since the positive control ddC performed as anticipated, the applicant further reasoned that their results showing no effects of LdT on extracellular lactate production in SKMCs are valid.

Mitochondrial morphology: No enlarged mitochondria were observed in LdT-treated cells at any concentrations and mitochondrial cristae were observed normal (Table 10). Similarly, treatment with 3TC and ddC did not produce enlarged mitochondria. Abnormal mitochondria with disrupted mitochondrial cristae, observed by Pan-Zhou *et al.* (2000) in human hepatoma HepG2 cells following exposure to 10 μM ddC for 14 days, was not seen in this applicant's study with human skeletal muscle cells. Consistent with the findings in HepG2 cells (Pan-Zhou *et al.* 2000) where the authors noted slightly enlarged mitochondria in cells treated with 10 μM AZT for 14 days, the applicant also observed enlarged mitochondria, however infrequently, in only 1 cell (1.6%) of the 62 examined AZT-treated skeletal muscle cells. Lipids in cytoplasm were infrequently observed in LdT-treated cells at all 3 concentrations tested, as observed with AZT and ddC positive controls. According to the applicant, lipid droplets constituted less than 10% of the cell surface area and thus were not considered significant.

Table 10: Effects of LdT on Mitochondrial Morphology in SKMCs

Drugs	Conc. (μM)	Cells examined	Disrupted mitochondrial cristae	Large mitochondria	Lipids droplets *
No Drug	0	34	0	0	1 cell
No Drug	0	36	0	0	0
No Drug	0	36	0	0	0
LdT	12	28	0	0	1 cell
	56	40	0	0	1 cell
	152	33	0	0	1 cell
AZT	12	36	0	0	0
	56	26	0	1 cell	0
3TC	30	38	0	0	1 cells
	90	41	0	0	0
ddC	1	38	0	0	0
	10	36	0	0	1 cell

· Morphological evaluation was performed by visual assessment of approximately 5 pictures (magnification x2,500) containing approximately 30 cells for mitochondrial size and the presence of lipid droplets. Additional 2-3 micrographs (magnification x4,500 and x15,000) were taken to evaluate the mitochondrial cristae.
· Lipids in cytoplasm constituted <10% of the surface area in all cells where lipid droplets were observed.

In summary, LdT appeared to have minimal effects on mitochondrial toxicity in primary human skeletal muscle cells after 6 days of treatment at concentrations as high as 10 times the mean C_{max} value in human plasma at a therapeutic dose. As expected, the positive control ddC reduced mtDNA content and increased lactic acid production in these cells.

3.5.2. Mitochondrial Toxicity of LdT in Primary Human Hepatocytes

Previously, the applicant assessed the potential of LdT to produce mitochondrial toxicity in a human liver cell line HepG2 cells (Study 02-CP-001A). HepG2 cells have large amounts of mitochondria and mtDNA, and are highly sensitive for recognition of effects on mtDNA and mitochondrial function. Thus, HepG2 cells are considered to be a suitable model for the investigation of NRTI-induced mitochondrial toxicity (Pinti *et al.*, 2003; Höschele, 2006; Lund *et al.*, 2007). Briefly, the applicant observed that (1) exposure of HepG2 cells for 14 days to LdT at concentrations up to 10 μM had no effect on mtDNA content compared to an 87% reduction in the ddC-treated cells; (2) no differences were observed in lactic acid levels produced in LdT-treated cells after exposure for 4 days and untreated control cells, whereas lactic acid production in the AZT- and fialuridine (FIAU)-treated cells increased by 100% compared to untreated control; and (3) no discernible changes in cell architecture or mitochondrial morphology were detected in LdT-treated cells after exposure for 14 days, while AZT-treated cells showed typical swollen mitochondria and loss of cristae. Mitochrondrial morphology was also abnormal in the ddC- and FIAU-treated cells. The cells were proliferating and the cell doubling time was linear while cultured for 14 days in the presence of LdT.

In Study IDIX-07-116B, the potential mitochondrial toxicity of LdT was assessed in freshly isolated primary human hepatocytes. Cells were obtained from 5 donors (HIV/HCV/HBV-negative) in suspension from Cellzdirect (Durham, NC) where tissue samples were obtained from normal remnants of resected liver tissue removed due to the presence of metastatic tumors or from nontransplantable donor organs. As described above in Section 3.5.1. for primary human skeletal muscle cells, cells were cultured for 6 days in the presence or absence of LdT, positive controls (AZT or ddC), or 3TC (negative control). Of note, human hepatocytes in primary culture are quiescent.

Cell viability: At concentrations up to 1000 μM (highest concentration tested), LdT, AZT, ddC, and 3TC did not reduce ATP levels in primary human hepatocytes by 50% (Table 11), indicating that LdT and NRTIs tested are not toxic to primary human hepatocytes when cultured for 6 days in the presence of these drugs at concentrations up to 1 mM. Diclofenac, a known hepatotoxin included in this assay as a positive control, reduced ATP levels with a CC_{50} value of 230.4 ± 25.3 μM. In previous studies conducted in HepG2 cells by Pan-Zhou *et al.* (2000), AZT and ddC were shown to be cytotoxic with CC_{50} values of 14 and 20 μM, when treated for 6 days. The authors did not observe 3TC-induced cytotoxicity at concentrations up to 50 μM. It is not known whether the observed differences between HepG2 and primary hepatocytes are related to cell cycle status (proliferating versus quiescent).

Based upon the cell viability results, 2 concentrations for AZT (12 and 56 μM) and 3TC

(30 and 90 μM), 3 concentrations for LdT (12, 56, and 152 μM), and 4 concentrations for ddC (1, 10, 25, and 50 μM) were selected in the LdT mitochondrial toxicity studies. The highest concentrations of AZT, 3TC, and LdT represent a 10-fold increase over the plasma C_{max} values in humans, while the lowest concentration (1 μM) represents a 10-fold increase over the plasma C_{max} value.

Table 11: Effects of LdT on Cell Viability in Primary Human Hepatocytes

Compound	Hu0688	Hu0707	Hu0718	Hu0732	CC_{50}
AZT	No tox @1000	No tox @1000	No tox @1000	NA	>1000
LdT	No tox @1000	No tox @1000	44% reduction@1000	No tox @1000	>1000
3TC	No tox @1000	No tox @1000	No tox @1000	NA	>1000
ddC	No tox @1000	30% reduction@1000	No tox @1000	NA	>1000
Diclofenac	NA	209.6	223.1	258.6	230.4 ± 25.3

- Cell viability was presented as a percent reduction in ATP level relative to the untreated control cells using the CellTiter-Glo kit (Promega). In the event a 50% reduction in ATP was observed, this was reported as the CC_{50} value for that compound.
- NA, not available

Mitochondrial DNA (mtDNA) content: A modest decrease in mtDNA levels (approximately ≤20%) was observed in primary hepatocytes following a 6 day-exposure to LdT, AZT, or 3TC (Table 12). The observed mean mtDNA levels were 80%, 88%, and 85% of control values at LdT 12, 56, 152 μM, respectively. A positive control ddC decreased mtDNA levels by maximally 35% at 50 μM. Compared with HepG2 (Pan-Zhou *et al.* 2000) and with SKMC primary muscle cells (Study IDIX-07-116A; see Section 3.5.1. for details) where ddC at 1 μM decreased mtDNA levels by 85% and 77%, respectively, the effect of ddC on mtDNA content in primary hepatocytes appears to be limited.

Table 12: Effects of LdT on Mitochondrial DNA (mtDNA) Content and Production of Lactic Acid in Primary Human Hepatocytes

Drug	Drug concentration (μM)	mtDNA (% of control)	Lactic acid production (% of control)
LdT	12	79.7±10.1	95.3±11.1
	56	88.4±6.2	96.6±14.8
	152	85.0±18.8	91.2±3.8
AZT	12	86.7±4.7	120.0±24.8
	56	85.5±19.7	99.3±15.2
3TC	30	102.3±21.8	94.8±13.7
	90	93.7±19.4	91.9±5.9
ddC	1	81.1±10.0	105.8±23.5
	10	69.3±9.9	103.4±23.6
	25	71.0±11.1	113.0±24.5
	50	65.5±5.7	118.2±31.8

- Data represent the mean of 3 independent experiments (1 experiment per donor) except ddC at 50 μM where data represent the mean of 5 independent experiments (1 experiment per donor).
- The mtDNA content of the cells was expressed as a relative coxII mtDNA/β-actin nuclear DNA (nDNA) ratio and reported as a percentage relative to the untreated control cells.
- Lactic acid production was expressed as the ratio of milligrams of lactate normalized to cell number and reported as a percentage relative to the untreated control cells.

Lactic acid production: As summarized in Table 12 above, no significant increase in the level of lactic acid secreted in the medium was observed at any concentration tested for all compounds: ddC at 50 μM had lactic acid production of 118% of control. These data corresponded well with the observation that ATP levels in primary hepatocytes were not affected by treatment as demonstrated by the cell viability assay. It is known that, as mitochondrial function declines, cells respond to the loss of ATP production capacity by accelerating glycolysis, resulting in increasing production of lactate. Thus, serum lactic acidosis is a hallmark of mitochondrial impairment.

Mitochondrial morphology: Freshly isolated human hepatocytes from two donors (Hu0688 and Hu0718) were used for mitochondria morphological evaluation (Table 13). Following treatment with LdT, AZT, ddC, and 3TC for 6 days, 1-2 hepatocytes from control, 30 μM 3TC, and 50 μM ddC treated samples exhibited lipid accumulation, while no accumulation of lipid droplets was observed in LdT- and AZT-treated hepatocytes. Pan-Zhou *et al.* (2000) also noted in HepG2 cells no increase in lipid droplet formation compared with the untreated control following14-day exposure to AZT, ddC, and 3TC.

Table 13: Effects of LdT on Mitochondrial Morphology in Primary Human Hepatocytes

Donor	Drugs	Conc. (μM)	Cells examined	Disrupted mitochondrial cristae	Large mitochondria	Lipids droplets
Hu0688	Control-1	0	14	2	0	0
	Control-2	0	20	7	0	1
	LdT	12	19	0	0	0
		56	21	0	0	0
	AZT	12	20	0	2	0
		56	14	0	3	0
	3TC	30	17	0	13	1
	ddC	1	21	0	0	0
		10	18	0	0	0
		25	14	0	0	0
		50	23	0	1	2
Hu0718	Control-3	0	16	0	0	0
	LdT	152	16	4	3*	0
	3TC	90	14	10	13	0

Morphological evaluation was performed by visual assessment of approximately 5 pictures (magnification x2,500) containing approximately 14-23 cells for mitochondrial size and the presence of lipid droplets. Additional micrographs (magnification x4,500 and x15,000) were taken to evaluate the mitochondrial cristae.

No enlarged mitochondria were seen in the untreated control cells (50 cells examined) and in the LdT-treated hepatocytes (a total of 40 cells were examined) at 12 and 56 μM (Table 13). In contrast, 3 cells of the 16 examined cells treated with LdT at the highest concentration 152 μM contained a single enlarged mitochondrion per cell. As seen in HepG2 cells (Pan-Zhou *et al.* 2000), the applicant observed 5 enlarged mitochondria in a total of 34 examined cells treated with AZT at 12 or 56 μM. In addition, the applicant observed enlarged mitochondria in cells treated with 30 μM and 90 μM 3TC and 50 μM ddC. Of the 17 hepatocytes treated with 30 μM 3TC, 4 hepatocytes exhibited a total of 13 enlarged mitochondria. Similarly, 5 out of 14 hepatocytes treated with 90 μM 3TC had a total of 13 enlarged mitochondria. Out of 23 hepatocytes treated with 50 μM ddC,

1 enlarged mitochondrion was detected.

The 152 μM LdT- and 90 μM 3TC-treated cells displayed 4 and 10 abnormal mitochondria with disrupted mitochondrial cristae, respectively (Table 13). A few of abnormal cristae were also found in untreated control cells. In contrast, none were observed in the AZT- and ddC-treated cells. In HepG2 cells, Pan-Zhou *et al.* (2000) observed disrupted cristae when cells were exposed to 10 μM ddC for 14 days.

Overall, a few abnormal mitochondria (large size and/or disrupted cristae) were seen in primary hepatocytes treated with a high concentration LdT (152 μM). According to the applicant, an average of 100 mitochondria per cell were pictured in this study with a maximum 7 abnormal mitochondrial cristae observed in 1 cell and with a maximum 13 enlarged mitochondria observed in 5 cells. Thus, it is not clear whether the effects of these abnormal mitochondria among many normal mitochondria (average of 800 mitochondria per cell) are significant in hepatocytes.

In summary, as observed in primary human muscle cells, LdT appeared to have minimal effects on mitochondrial toxicity in primary human hepatocytes after 6 days of treatment at concentrations as high as 10 times the mean C_{max} value in human plasma at a therapeutic dose. Importantly, the positive controls, AZT and ddC, produced no marked mitochondrial toxicity in these cells, either, in contrast to the applicant's previous findings in HepG2 cells (Study 02-CP-001A) where AZT at 50 μM was able to increase lactate production by 100% and ddC at 1 μM to decrease mtDNA content by 87%, compared to untreated control. Thus, conclusions based upon the results produced in primary hepatocytes under the current applicant's experimental conditions may not be valid.

4. Clinical Resistance Analyses

In the Year-1 Phase 3 global registration trial (007 GLOBE study), the amino acid substitution rtM204I was identified to be primarily associated with virologic failure (HBV DNA levels ≥1,000 copies/mL) to telbivudine (TBV, TYZEKA®, LdT) therapy at Week 52 or at the end of dosing within Year 1 (NDA 22-011, 000). Of the 115 TBV-treated patients who were experiencing virologic failure and their paired baseline and on-treatment genotypes were submitted with the original NDA submission, 46 patients (40.0%) developed the rtM204I/V substitution. Furthermore, the rtM204I/V substitution, rtM204I variant in particular, were shown to be strongly associated with virologic rebound: 35 of the 46 patients with the rtM204I/V substitution failed TBV treatment due to virologic rebound. Treatment-emergent virologic rebound was defined as virologic breakthrough with ≥1 $\log_{10}$ increase of HBV DNA from nadir while on therapy. Amino acid substitutions rtL80I/V, rtL180M, and rtL229V/W accompanied the rtM204I/V substitution. In addition to the primary TBV resistance (TBV^r)-associated substitution rtM204I, the rtA181S/T substitution was frequently found in 16 (13.9%) of the 115 TBV treatment-failure patients. Please refer to the Microbiology review N022011.000 for a detailed resistance analysis of TBV in Year 1.

In order to obtain a better understanding of the TBV resistance profile, isolates from patients displaying virologic failure at end of dosing (up to 104 weeks) were analyzed for TBV treatment-emergent substitutions in the supplemental NDA submission (NDA 22-

011 SE8-001). In an evaluable as-treated analysis of the GLOBE study (Table 14), 75.2% (494/657) of TBV recipients were suppressed with serum HBV DNA <1,000 copies/mL (virologic suppression) as quantified by the COBAS Amplicor HBV Monitor PCR assay and maintained viral suppression at Week 52 (or at the end of dosing within Year 1). By Year 2, virologic suppression was achieved in 451 (70.2%) of 642 TBV recipients (Table 14). There were 65 patients (15 HBeAg-negative and 50 HBeAg-positive) who achieved virologic suppression at Week 52 but experience virologic failure in Year 2, largely due to virologic rebound (63 of the 65 patients; Table 15). Twenty-seven patients (2 HBeAg-negative and 25 HBeAg-positive) were classified as virologic failures with HBV DNA levels ≥1,000 copies/mL at Week 52 but eventually achieved virologic suppression during the 2nd year of TBV treatment (Table 15). The rate of virologic suppression was lower for the HBeAg-positive patient population, compared to that for the HBeAg-negative patient population: 66.3% versus 92.1% by Year 1 and 71.7% versus 86.2% by Year 2, respectively (Table 14). In the Year-1 antiviral efficacy analysis, higher rates of virologic failure and treatment-emergent virologic rebound were observed in the subgroup of patients with higher pretreatment HBV DNA levels in both HBeAg-positive and HBeAg-negative TBV-treated patients. Serum HBV DNA levels at Baseline was much higher in the HBeAg-positive population than the HBeAg-negative population. The difference in the means between the HBeAg-positive and HBeAg-negative patient groups was nearly 2 $\log_{10}$ copies/mL. Please refer to the reviews by Medical Officer Mary Singer, M.D. and Statistician Fraser Smith, Ph.D. for a detailed analysis of the efficacy of TBV.

Table 14: Cumulative Probability of Genotypic TBV^r-Associated Substitutions in Evaluable As-Treated Populations[1]

	Total		HBeAg-negative		HBeAg-positive	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Number of Subjects	657	642[2]	227	224[2]	430	418[2]
Virologic suppression	494 (75.2%)	451 (70.2%[3])	209 (92.1%)	193 (86.2%[3])	285 (66.3%)	258 (71.7%[3])
Emergence of rtM204I/V	46	96[4]	11	16[4]	35	80[4]
at risk	657	598[5]	227	213[5]	430	385[5]
Cumulative probability of emerging rtM204I/V substitution	7%	21.9%	4.8%	12%	8.1%	27.2%

[1]The evaluable as-treated population includes all randomized patients who received ≥16 weeks of treatment but excludes patients with missing or problematic data
[2]Fifteen patients prematurely discontinued during (or at the end of) the 1st year of TBV therapy: 3 patients were HBeAg-negative and 12 were HBeAg-positive (see Table 15 for details): rtM204I was detectable in 2 of the 10 treatment failure patients with submitted paired genotypic data.
[3]The proportions of patients who continued TBV treatment beyond Week 52 and achieved virologic suppression (HBV DNA levels <1,000 copies/mL) at the end of dosing (up to 104 weeks)
[4]Number of patients who developed the primary rtM204I/V TBV^r-associated substitution during the 2nd year of ETV treatment
[5]Forty-four patients (11 HBeAg-negative and 33 HBeAg-positive) were excluded from the at-risk cohort at Year 2, since the rtM204I/V TBV^r-associated substitution emerged in Year 1; Subjects "at risk" are those who were followed during Year (i) and did not develop genotypic TBV resistance during Year (i-1).

4.1. Primary Telbivudine Resistance (TBV^r)-associated Substitution rtM204I/V

The applicant submitted the complete HBV rt amino acid sequences of the screen isolates for all 1,367 patients (ITT population) in the GLOBE trial, and of the treatment-

failure isolates (n=229) from 182 TBV-treated patients who failed to achieve HBV DNA levels <1,000 copies/mL by Year 2. A total of 201 patients, including 10 patients who discontinued TBV treatment without achieving virologic suppression during (or at the end of) the 1st year of TBV therapy, showed evidence of virologic failure by Year 2. Thus, the paired (screen and on-treatment) genotypic analysis was conducted in 90.5% (182/201) of the 201 TBV failures.

The rtM204I/V substitution that was identified as a primary TBV^r-associated substitution (genotypic TBV resistance) in the Year-1 resistance analysis of TBV emerged in a total of 142 patients (78%) while on TBV (46 in Year 1 and 96 in Year 2; Table 14 and 15), out of the 182 patients experiencing virologic failure with evaluable paired genotypic data. In the population nucleotide sequence analysis, the majority of the patients (n=139, 97.9% [139/142]) had rtM204I HBV variants in the treatment failure isolates, while rtM204I/V mixed variants were present in 2 patients. There was only 1 patient with the emergence of a pure rtM204V mutant population in response to TBV therapy.

During the 1st year of TBV treatment, rtM204I/V emerged in 46 (including 35 with virologic rebound) of the 163 patients who failed to achieve virologic suppression (Table 15, highlighted in green). Among 11 subjects who developed rtM204I/V and suboptimally responded to TBV treatment by Year 1 (virologic failure without experiencing virologic rebound), virologic rebound was eventually observed in 8 patients in Year 2. During the 2nd year of TBV treatment, the rtM204I/V substitution emerged in 96 additional patients who all failed to suppress HBV DNA levels to <1,000 copies/mL by Week 104, and 90 of them (75 HBeAg-positive and 15 HBeAg-negative) experienced the 2nd year virologic rebound (Table 15, highlighted in yellow). Thus, the emergence of the rtM204I/V substitutions was highly associated not only with virologic TBV treatment failure (70.6%, 142/201) but also with virologic rebound (83.5%, 137/164). There were 47 patients whose on-treatment isolates were collected at 2 different time points for a longitudinal assessment of genetic changes. All 47 patients experienced virologic failure in Year 1 but the rtM204I/V substitution was not detectable in their 40 or 48-week isolates. When isolates collected at later time points (Weeks 68 to 104) were examined, rtM204I/V was detected in 29 of the 47 patients.

Table 15: Number of Patients Who Were Included in Evaluable As-Treated Populations and Their Virologic response to TBV Therapy

	by Year 1			by Year 2			rtM204I/V substitution developing during the 2nd year TBV therapy
	Virologic response		rtM204I/V Substitution	Virologic response		rtM204 Substitution	
HBeAg-positive (n=430)	Suppression (n=285)		ND[1]	Suppression (n=233)		ND[1]	ND[1]
				Failure (n=50)	Rebound (n=48)	90% (36/40)	36
					Non-Rebound[2] (n=2)	ND[1]	ND[1]
				Discontinued[3] (n=2)		ND[1]	ND[1]
	Failure (n=145)	[redacted]	[redacted]	Failure (n=31)		93.5% (29/31)	3
				Discontinued[3] (n=3)		1/3	0
		[redacted]	[redacted]	Suppression (n=25)		0/10	0

				Failure (n=79)	Rebound (n=52)	88.2% (45/51)	39
					Non-Rebound[2] (n=27)	4/22	2
				Discontinued[3] (n=7)		1/7	0
HBeAg-negative (n=227)	Suppression (n=209)		ND[1]	Suppression (n=191)		ND[1]	ND[1]
				Rebound (n=15)		100% (13/13)	13
				Discontinued[3] (n=3)		ND[1]	ND[1]
	Failure (n=18)	[redacted]	[redacted]	Failure (n=10)		100% (9/9)	1
		[redacted]	[redacted]	Suppression (n=2)		0/1	0
				Failure (n=6)	Rebound (n=5)	4/5	2
					Non-Rebound[2] (n=1)	1/1	0

[1]ND, not determined
[2]Non-Rebound, defined as virologic failure without experiencing virologic rebound
[3]Discontinued during (or at the end of) the 1st year of TBV therapy

Based on the results described above, the cumulative probability of patients incurring genotypic TBV resistance (emergence of the rtM204I/V substitution) was calculated using the formula previously described by Miller *et al.* (1981):

- $P_{Total} = 1 - (1 - P_{year\ 1}) \times (1 - P_{year\ 2})$
- $P_{year\ (i)}$ = Number of patients with the emergence of the rtM204I/V substitution at Year (i) / Number of patients at risk at Year (i), (i) =1, 2
- Patients "at risk" are those who were followed during Year (i) and did not develop genotypic TBV resistance during Year (i-1).

The cumulative probabilities of incurring genotypic TBV resistance were 7% and 21.9% in Years 1 and 2: 4.8% and 12% in HBeAg-negative patients, and 8.1% and 27.2% in HBeAg-positive patients, respectively (Table 14). Comparable or slightly higher genotypic resistance rates of lamivudine were reported to occur in nucleoside treatment-naïve patients, ranging 10 to 27% after 1 year and 37 to 48% after 2 years (Lai *et al.*, 2003). However, compared to ADV (Year 1, 0%; Year 2, 3% in HBeAg-negative HBV patients; Hadziyannis *et al.*, 2006) or entecavir (Year 1, 0.15%; Year 2, 0.51% in nucleoside treatment-naïve subjects; Microbiology Review for NDA 21-797.SE8-005), genotypic resistance to TBV occurred more frequently. Lower probabilities of patients incurring genotypic TBV resistance in HBeAg-negative patients could be explained by the observed higher rate of virologic suppression compared to that for HBeAg-positive patients (92.1% versus 66.3% in Year 1, and 86.2% versus 71.7% in Year 2; Table 14).

Of note, there was 1 patient (ID 116-025) in the TBV treatment group with a pre-existing rtM204I HBV variant (rtL80V) at Screen which persisted during TBV treatment. As expected, no decline in viral load was observed in patient 116-025 through 2 years of TBV treatment (Table 16).

Table 16: Patient with Pre-Existing rtM204 at Screen

Patient ID	HBV genotype	HBeAg	Virologic tesponse	Last visit (week)	Isolates	HBV DNA (log_{10} copies/mL)	Substitutions associated with TBV resistance
116-025	B	positive	Failure	104	Baseline	10	[redacted]

			with non-rebound[1]		Week 52	12.2	ND[2]
					Week 104	12.5	

[1]Failure with non-rebound, defined as virologic failure without experiencing virologic rebound
[2]ND, not determined

4.2. Amino Acid Substitutions Co-Selected in TBV-Treatment Failure Isolates Harboring the rtM204I/V Primary TBV^r-Associated Substitution

The paired sequence analyses of the 143 treatment-failure isolates (from 143 patients) harboring the rtM204I/V substitution (142 emerging on TBV and 1 pre-existing) revealed that the majority (97.2%, 139/143) had additional substitutions in HBV rt, ranging from 1 to 30 (median=2; mean=3.0 ± 3.6). Additional substitutions occurred at 114 amino acid positions in HBV rt (33.1% of the 344 resides comprising the entire rt domain of HBV polymerase; Appendix 1) and 71 residues of them were changed in 1 or 2 isolates out of the 143 examined failure isolates, unlikely to be of any statistical significance. Table 17 listed amino acids residues (n=19) changed in 5 or more patients (>3% of the 143 patients with rtM204I/V): rtI16, rtV27, rtS53, rtR55, rtL80, rtI91, rtS106, rtS109, rtT118, rtD134, rtS135, rtL180, rtA200, rtV207, rtT222, rtL229, rtQ267, rtI269, and rtC332

Five amino acids, rtV27, rtL80, rtL180, rtA200, and rtL229, are highly conserved in the viruses from all patients in the GLOBE 007 study at Screen (n=1367). Conserved was defined as naturally occurring genetic variations at each amino acid position in HBV rt that were not present in ≥2% of the 1367 pretreatment HBV isolates. All patients whose virus expressed detectable substitutions, rtL80I/V (74 patients), rtL180M (18 patients), rtA200V (11 patients), or rtV27A (5 patients), and 26 of the 27 patient isolates expressing the rtL229C/F/M/V/W change were found to have the rtM204I/V substitution (Table 17). These observations suggested that these 5 substitutions may not emerge independent of the rtM204I/V primary TBV^r-associated substitution. The rtL80I/V and rtL180M substitutions were previously identified as secondary substitutions that exclusively accompany rtM204I/V in clinical isolates recovered from patients who showed HBV breakthrough while on LAM treatment (Allen *et al.*, 1998; Li *et al.*, 2005; Lok *et al.*, 2002; Ogata *et al.*, 1999; Warner *et al.*, 2007). In cell culture studies, HBV variants with the rtM204I/V substitutions were shown to replicate less efficiently than wild-type HBV (Ling and Harrison, 1999; Melegari *et al.*, 1998), which could be partially or completely overcome by additional substitutions in HBV rt, particularly rtL180M (Fu and Cheng, 1998; Ono *et al.*, 2001), rtL80I/V (Warner *et al.*, 2007), rtV173L (Delaney *et al.*, 2003), and/or rtV207I (Fu and Cheng, 1998). Substitutions rtV173L (2 patients; conserved residue) and rtV207I (3 patients; polymorphic residue) were also detected in the rtM204I/V-harboring isolates in the TBV-treated patients who experienced virologic rebound (Table 17, highlighted in grey).

In addition, rtL82M (n=3), rtT184I/S (n=3), and rtR289K (n=4) substitutions were observed infrequently (<3% of the 143 patients with rtM204I/V) but exclusively in the rtM204I/V-containing isolates with evidence of virologic rebound (Table 17, highlighted in blue). Since no naturally occurring polymorphic variations were observed at these amino acid positions among the 1,376 pretreatment isolates collected in this GOLBE trial, the rtL82M, rtT184I/S, and rtR289K substitutions could be clinically relevant in the rtM204I/V-associated TBV resistance. Of note, the rtL82M substitution-containing HBV variants did not exhibit any measurable reduction in susceptibility to TBV in cell culture

(<1 fold-change in EC_{50} values from wild-type HBV), while rtM204I conferred a >1,391-fold reduction (see Section 3.4 for details).

Table 17: Amino Acid Changes Accompanying the rtM204I/V Substitution and Found in 5 or More Patients with Virologic Failure to TBV Treatment in Paired Sequences

Amino acid sequence Variation[1]		Baseline polymorphism found in patients (n=429) with HBV DNA levels <1,000 copies/mL by Week 104	TBV treatment-failure isolates	
			Isolates harboring the rtM204I/V substitution (n=143)	Total (n=229)
M204	Conserved	M (429)	143 M204I (140), M204V (1), M204I/V (2)	143 M204I (140), M204V (1), M204I/V (2)
L80	Conserved	L (428), V (1)	74 L80I (51), L80V (20), L80I/V (3)	74 L80I (51), L80V (20), L80I/V (3)
[redacted]	[redacted]	I (167), L (246), I/L (16)	[redacted]	L91I (32)
[redacted]	[redacted]	T (317), A (104), T/A (7), S (1)	[redacted]	A222T (26), T222A (1), T222S (1)
L229	Conserved	L (418), M (1), M/L (4), L/W/STOP (1), V (3), V/L (2)	26 L229F (7), L229M (1), L229V (12), L229W (5), L229C/F/W (1)	27 L229F (7), L229M (1), L229V (13), L229W (5), L229C/F/W (1)
L180	Conserved	L (429)	18 L180M (18)	18 L180M (18)
A200	Conserved	A (429)	11 A200V (11)	11 A200V (11)
R55	Polymorphic	H (189), H/R (8), K (8), Q (8), Q/H (13), Q/H/R (1), R (202)	8 H55R (6), R55H (1), Q/H55H/R (1)	11 H55R (7), R55H (3), Q/H55H/R (1)
[redacted]	[redacted]	V (419), I/M (2), L (4), M (2), M/I/V (1), M/V (1)	[redacted]	9 M207I (1), M207V (1), V207I (1), V207L (3), V207I/M (3)
I16	Polymorphic	I (298), T (127), I/T (4)	7 I16T (5), T16I (2)	8 I16T (6), T16I (2)
S53	Polymorphic	S (236), D (6), H (3), I (8), K (3), K/N (2), N (154), N/D (9), N/H (1), N/S (2), WS/I (3), T/N/S (1), V (1)	7 I53S(1), I53V(1), N53H(1), N53S(1), S53I (1), S53N (1), V53S (1)	9 I53S(1), I53V(1), N53H(1), N53S(1), S53I (1), S53N (2), S53R (1), V53S (1)
T118	Polymorphic	T (235), D (4), H (1), N (174), N/D (5), N/S (2), N/T (7), T/S (1)	7 D118N (1), N118T (4), T118N (2)	9 D118N (1), N118T (4), T118N (3), T118A (1)
S135	Polymorphic	S (407), A (1), F (1), P/S (1), S/F (3), T (4), Y (10), Y/F (2)	6 H/Y135N (1), H135Y (1), S135H/P/Y (1), S135Y (1), T135S (1), Y135F (1)	7 H/Y135N (1), H135Y (1), S135H/P/Y (1), S135Y (2), T135S (1), Y135F (1)
Q267	Polymorphic	L (109), F (1), H (48), M (3), Q (256), Q/H (2), Q/L (7), R (3)	6 L267Q (3), Q267H (2), Q267R (1)	8 L267Q (3), Q267L (2), Q267H (2), Q267R (1)
I269	Polymorphic	I (317), I/L (10), L (102)	6 L269I (6)	8 L269I (8)
V27	Conserved	V (428), I/V (1)	5 V27A (5)	5 V27A (5)
S106	Polymorphic	S (407), A 91), A/S (1), C (5), S/C (14), T (1)	5 S106C (5)	6 S106C (6)
S109	Polymorphic	P (206), P/S (1), Q/P (5), Q/P/S/STOP (1), S (216)	5 P109Q (1), P109S (4)	6 P109Q (1), P109S (5)
D134	Polymorphic	D (300), D/E (22). D/G (2), E (13), I (2), K/E (1), N (56), N/D (18), N/K (1), N/S (8), S (6)	5 E134D (1), N134D (3), N134S (1)	8 E134D (1), N134D (6), N134S (1)
C332	Polymorphic	C (280), N (2), R (27), R/C (1), S (98), S/R (13), unknown (1), Y (7)	5 C332S (1), C332Y (2), N332C/S/Y (1), S332R (1)	6 C332S (2), C332Y (2), N332C/S/Y (1), S332R (1)
[redacted]	[redacted]	R (429)	[redacted]	R289K (4)
[redacted]	[redacted]	L (429)	[redacted]	L82M (3)
[redacted]	[redacted]	T (429)	[redacted]	T184I (2), T184S (1)
[redacted]	[redacted]	V (429)	[redacted]	3 V173L (2), V173M (1)

S202	Conserved (0)	S (429)	2 S202G (2)	2 S202G (2)
M250	Conserved (0)	M (429)	1 M250L (1)	1 M250L (1)

[1]Conserved and polymorphic residue positions were identified by levels of amino acid sequence variations occurring in the general population (baseline genotype of 1367 patients in the GLOBE trial): frequencies of ≥2% were considered polymorphic. Conservative (0) represents no variations found among 1367 screen sequences. Naturally occurring genetic polymorphisms was observed at 72 residue positions (20.9% of the 344 resides comprising the HBV rt protein), comparable to 84 residue positions (24.4%) when 250 published wild-type HBV rt sequences (GenBank) were analyzed.

The other 13 amino acids rtI16, rtS53, rtR55, rtI91, rtS106, rtS109, rtT118, rtD134, rtS135, rtT222, rtQ267, rtI269, and rtC332 whose substitutions were frequently (>3% of the 143 patients) found in the failure isolates harboring rtM204I/V (Table 17) are variable between genotypes as well as within genotypes. The frequency of variants at these positions was 34.9 ± 17.1% (ranging from 6.1% to 59.6%) at Screen when compared to the reference sequence (genotype C). Individuals with these variants at Screen had the same response rate (HBV DNA <3 $\log_{10}$ copies/mL), 70.2 ± 5.5% (ranging from 60.5% to 84.6%), as the overall TBV-treated population by Year 2 (70.2%; Table 2). Thus, the substitutions at these positions *per se* are unlikely to contribute to reduced TBV susceptibility. However, frequent and disproportionate occurrences of these substitutions in isolates harboring rtM204I/V compared to those with no detectable rtM204I/V among TBV-treatment failure isolates, rtL91I and rtA222T in particular (31 and 26 out of the 32 and 28 total occurrences in 231 failure isolates, respectively; Table 17, highlighted in green), may suggest a possible role in the development of the rtM204I/V-associated resistance to TBV. The TBV-treatment emergent substitutions at rt91 and rt222 were further assessed in relationships to the baseline polymorphisms at these sites in Table 18.

Previously, Ciancio *et al.* (2004) noted 2 naturally-occurring DNA polymorphisms at amino acid positions rt91 (I or L) and rt256 (C or S) in HBV rt that were observed in the pretreatment serum samples and subsequently correlated with treatment failure to long-term LAM therapy (27 - 53 months) in 26 patients. HBV carrying a leucine at amino acid position 91 (rtL91) was detected in 16 patients of the 26 patients at Baseline and all these patients failed to suppress HBV DNA levels to <1,000 copies/mL (HBV Monitor, Roche). Similarly, 13 patients had rtC256 and all 13 patients experienced virologic failure. Since the genetic resistance profile for TBV (rtM204I/V substitution) is very similar to that for LAM, it was assessed whether the baseline polymorphism at these 2 amino acid positions can be associated with TBV treatment failure. In addition, amino acid changes at rt222 were included in this baseline polymorphism analysis.

As summarized in Table 18, the baseline polymorphisms (I or L at rt91, A or T at rt222, and C or S at rt256) at 3 amino acid positions were observed with comparable frequency in the ITT population of the GLOBE study (n=1,367) and in the TBV-treated population (n=657). In addition, 64.9% to 72.2% of the TBV-treated patients displaying each of the examined baseline polymorphisms achieved virologic suppression by Year 2 (Table 18), comparable to 70.2% of the overall TBV-treated population (Table 2). Similarly, comparable rates of virologic suppression were also observed by Year 1 between each of the examined polymorphic subpopulations (70 to 77%) and the overall TBV-treated population (75.2%). Thus, different from the previous findings by Ciancio *et al.* (2004) with LAM, polymorphisms at these 3 amino acid sites in the baseline isolates appeared

to have no effect on the response to TBV therapy.

No significant differences were observed in the emergence of the rtM204I/V substitution in patients with baseline polymorphism A or T at rt222 (20.1% [32/159] versus 22% [106/481]), or C or S at rt256 (23.6% [25/106] versus 21.3% [108/508]), respectively (Table 18). However, a slightly higher rate was noted in patients with baseline rtI91 (29.3% [76/259]) than in those with baseline rtL91 (16.8% [63/374]; Table 18). More importantly, of the 63 patients with baseline rtL91 who developed the rtM204I/V substitution on TBV (Table 18, highlighted in yellow), baseline leucine (L) was mutated to isoleucine (I) in the failure isolates from 31 patients (49.2%). In contrast, no I to L substitutions was found in isolates with rtM204I/V (Table 18, highlighted in green). These observations suggested that the isoleucine (I) residue at rt91 may play an advantageous role in the development of rtM204I/V-associated TBV resistance. Similarly, a high mutation rate was also observed in the subgroup of patients (n=32) with baseline rtA222: 26 patients (78.1%) developed the rtA222T substitution with the emergence of rtM204I/V (Table 18, highlighted in yellow). The rtT222A substitution was detected in only 1 patient out of the 106 patients with baseline rtT222 (Table 18, highlighted in green). Thus, these 2 polymorphic substitutions, rtL91I and rtA222T, appeared to be co-selected with the rtM204I/V primary TBV[r]-associated substitution under TBV drug pressure.

Of note, the rtI233V polymorphism that was known to affect response to ADV (Schildgen *et al.*, 2006) was rarely found in TBV-treated patients (2 patients) and in TBV-treatment failures (1 patient; Table 18).

Table 18: Baseline Polymorphisms and TBV Treatment-Emergent Substitutions at rt91, rt222, and rt256

Polymorphism at amino acid position		Screen isolate				Changes in failure isolate with the emergence of rtM204I/V
		Total (n=1367)	TBV-treatment population			
			TBV-treated (n=657)	Virologic suppression by Year 2 (n=456) (70.2% in the overall TBV-treated population)	rtM204I/V developed	
91	I	552 (40.4%)	259 (39.4%)	168 (64.9%)	[redacted]	[redacted]
	L	778 (56.9%)	374 (56.9%)	270 (72.2%)	63 (16.8%)	L91I (n=31)
	I/L	37	24	18	4	I/L91L (n=1)
						I/L91I (n=3)
222	A	297 (21.7%)	159 (24.2%)	112 (70.4%)	32 (20.1%)	A222T (n=25)
	T	1033 (75.6%)	481 (73.2%)	335 (69.6%)	[redacted]	[redacted]
	A/T	33	15	8	4	T/A222T (n=4)
	Others	4	2	1	1	ND[1]
256	C	250 (18.3%)	106 (16.1%)	71 (67%)	25 (23.6%)	C256S (n=2
	S	1018 (74.5%)	508 (77.3%)	356 (70.1%)	108 (21.3%)	S256C (n=4)
	C/S	33	11	5	5	S/C256S (n=3)
	Others	66	32	24	5	ND[1]
233	I	1359 (99.4%)	655 (99.7%)	455 (69.5%)	143 (21.8%)	I233V
	V	7 (0.5%)	2 (0.3%)	1	0	-
	I/V	1	0	0	-	-

[1] ND, not determined

In summary, 12 amino acid substitutions (highlighted in Table 17), V27A, rtL80I/V, rtL82M, rtL91I, rtV173L, rtL180M, rtT184I/S, rtA200V, rtV207I/M, rtA222T, rtL229C/F/V/W, and rtR289K, were found, frequently and/or exclusively, in the same virus population harboring the rtM204I/V substitution. Of the 143 patients with

rtM204I/V, these 12 substitutions were detected in 131 patients (91.6%; Table 19). Among the remaining 12 patients with rtM204I/V in the absence of detectable 12 additional substitutions, 4 patients (IDs 010-002, 039-005, 054-017, and 083-003; 2.8%) experienced virologic failure with the emergence of only 1 amino acid substitution at rtM204I in HBV rt, implying that the rtM204I substitution was sufficient to confer resistance to TBV. Thus, these 12 additional substitutions may be co-selected as a secondary substitution to enhance TBV resistance, possible by compensating for the reduced replication capability of HBV variants with the rtM204I/V substitutions (Ling and Harrison, 1999; Melegari *et al.*, 1998).

Previously, the rtL80I/V (Warner *et al.*, 2007), rtV173L (Delaney *et al.*, 2003), rtL180M (Fu and Cheng, 1998; Ono *et al.*, 2001), and/or rtV207I (Fu and Cheng, 1998) substitutions were reported in cell culture studies to enhance the replication of the rtM204I/V mutant HBV. Moreover, these substitutions accompanying rtM204I/V were detected in clinical isolates recovered from patients failing LAM therapy for HBV (Allen *et al.*, 1998; Li *et al.*, 2005; Lok *et al.*, 2002; Ogata *et al.*, 1999; Warner *et al.*, 2007). Similarly, these 4 substitutions were detected in 86 (60.1%) of the 143 TBV-treatment failure isolates harboring rtM204I/V (Table 19). The rtM204I/V + rtL80IV double substitutions were most frequently observed (n=74, 51.7%; Table 19) with (n=32, 22.4%) or without (n=42, 29.4%) additional secondary substitutions.

Table 19: Combinations of Secondary Substitutions Frequently Found in Patients with the rtM204I/V Substitution in Paired Sequences

Combination of primary and secondary Substitutions	007 GLOBE study (n=143)	Study 015 (n=46)
• rtM204I/V substitution without co-selected secondary substitutions[1]	12 (8.4%)	3 (6.5%)
• rtM204I/V substitution with co-selected secondary substitutions[1]	131 (91.6%)	43 (93.5%)
(1) with rtL80I/V, rtV173L, rtL180M, or rtV207I/M	86 (60.1%)	31 (67.4%)
rtL80I/V only	42	11
rtL80I/V + one or more additional secondary substitutions	32	19
rtV173L only	2	0
rtV173L + one or more additional secondary substitutions	0	1
rtL180M only	2	0
rtL180M + one or more additional secondary substitutions	16	10
rtV207I/M only	3	0
rtV207I/M + one or more additional secondary substitutions	5	1
(2) without rtL80I/V, rtV173L, rtL180M, or rtV207I/M	45 (31.5%)	12 (26.1%)
rtL229C/F/V/W only	11	4
rtL91I + rtA222T only	9	2
rtL91I + rtA222T + rtL229F/W only	5	0
rtA200V only	4	0
Others (≤2 occurrences)	16	6

[1]Secondary substitutions include rtV27A, rtL80I/V, rtL82M, rtL91I, rtV173L, rtL180M, rtT184I/S, rtA200V, rtV207I/M, rtA222T, rtL229C/F/V/W, and rtR289K

Overall, the resistance profile for TBV appeared to be similar to that for LAM with the exception of rtM204V. The rtM204V substitution was infrequently detected in TBV-treatment failure patients. The emergence of rtM204V was detected in 3 patients (IDs 003-085, 068-001, and 116-034) experiencing virologic rebound in conjunction with the rtL180M substitution that was previously reported to be strongly associated with LAM resistance developed through the M204V pathway (Gauthier *et al.*, 1999). Only 1 patient (003-085) had HBV variants harboring rtM204V single change at codon 204, while the

other 2 patients (068-001 and 116-034) had HBV mixed variants of rtM204M/I/V. These clinical findings are in good agreement with preclinical cell culture study results that TBV was inactive against the rtM204V + rtL180M double substitutions with the calculated fold changes in EC_{50} values of ≥1,360 ± 363 compared to that of wild-type reference HBV. In contrast, the rtM204V single substitution remained still susceptible to TBV (fold changes of 1.2 ± 0.4), thereby implying that HBV variants with the rtM204V single substitution could not be enriched under TBV drug section pressure. TBV was inactive against the rtM204I substitution without (fold changes of ≥1,360 ± 363) or with the compensatory rtL180M substitution (fold changes of ≥1,049 ± 226).

Similar observations for TBV resistance were made in the China-specific clinical trial NV-02B-015. Study 015 was a 104-week Phase 3, randomized, double-blind, multicenter, trial designed to compare the efficacy and safety of TBV (600 mg/day) to LAM (100 mg/day). In an evaluable as-treated (≥16 weeks of treatment) analysis, 32.5% (54/166) of TBV recipients failed to suppress HBV DNA levels to <1,000 copies/mL by Week 104. Among the 54 treatment failures, 44 patients experienced virologic rebound. The applicant submitted the complete HBV rt amino acid sequences of the screen isolates for all 166 patients, and of the treatment-failure isolates for 52 patients (including 43 with virologic rebound). Thus, the paired genotypic analysis was conducted in 96.3% (52/54) of the 54 TBV failures in Study 015.

The primary TBV-associated substitution rtM204I emerged in a total of 46 patients (85.2% 46/54) while on TBV, including 41 patients experiencing virologic rebound (95.3%, 41/43). The rtM204V substitution was not detectable in these failure isolates. These results confirmed the GLOBE study results that the emergence of the rtM204I/V substitutions was highly associated with virologic TBV treatment failure and rebound. However, genotypic resistance to TBV in Study 015 was observed to occur slightly more frequently by Year 2 in the overall TBV-treated population (27.7% [46/166] versus 21.6% [142/657]) and in the HBeAg-positive subpopulation (31.7% [44/139] versus 26.7% [115/430]), compared to the 007 GLOBE study, respectively. The probabilities of incurring genetic TBV resistance in HBeAg-negative patients were 7.4% (2/27) in Study 015 and11.9% (27/227) in Study 007.

Of note, there was 1 patient (ID 007-010) in the TBV treatment group with a pre-existing rtM204V HBV variant at Screen but in the absence of known compensatory substitutions rtL80I/V, rtV173L, rtL180M, and/or rtV207I/M. Slow decline in viral load was observed to achieve virologic suppression at Week 68. No on-treatment genotypic data were available to determine whether the rtM204V substitution persisted during TBV therapy. This finding supported previous observations that TBV is active against HBV harboring the rtM204V substitution alone.

All 46 isolates with rtM204I had additional substitutions in HBV rt that occurred at 62 amino acid positions and 53 of them occurred in 1 or 2 isolates (Appendix 1). Out of the 12 secondary substitutions identified in the 007 GOBE study, 10 substitutions (Table 20), rtV27A, rtL80I/V, rtL82M, rtL91I, rtV173L, rtL180M, rtT184I, rtA200V, rtA222T, and rtL229F/V/W were exclusively found in the rtM204I substitution harboring virus population. As noted in the GLOBE study, more than half of rtM204I/V isolates (67.4%; Table 19) had at least one of the 3 compensatory substitutions, rtL80I/V, rtV173L, or

rtL180M. The rtM204I/V + rtL80IV double substitutions were also most frequently observed (n=30, 65.2%; Table 19) with (n=19, 41.3%) or without (n=11, 23.9%) additional secondary substitutions.

Table 20: Frequencies of Secondary Substitutions Co-Selected with the Primary TBV^r-Associated rtM204I Substitution in Study 015

Secondary substitutions co-selected with rtM204I that were identified in Study 007	TBV treatment-failure isolates in Study 015	
	Isolates harboring the rtM204I substitution (n=46)	Total (n=52)
M204	46	46
L80	30	30
T222	12	12
I91	11	11
L180	10	10
L229	8	8
V27	5	5
A200	1	1
L82	1	1
T184	1	1
V173	1	1
V207	0	0
R289	0	0

4.3. TBV Treatment-Emergent rtA181 substitutions

Of the 182 patients with virologic failure to TBV treatment whose paired baseline and on-treatment genotypes were submitted with this application, 39 patients had HBV with no detectable rtM204I/V substitution in their TBV-treatment isolates (n=57): 18 patients of them had isolates collected at 2 different time points (at Week 48 and at Week 84 - 104). In addition, there were 29 isolates from 29 patients that harbored no detectable rtM204I/V at the time of virologic failure (Week 40 or 48) during the 1st year of TBV treatment. It should be noted that these 29 patients exhibited the rtM204I/V substitution in isolates collected at later time points (Weeks 68 to 104). Thus, a total of 86 isolates were analyzed to identify emerging TBV^r-associated substitutions in the absence of rtM204I/V. Of the 86 isolates, 39 isolates had no genetic changes in HBV rt. The remaining 47 isolates had substitutions, ranging from 1 to 21 (median=1; mean=2.1 ± 3.3). These substitutions occurred at 62 amino acid positions in HBV rt (18% of the 344 resides comprising the entire rt domain of HBV polymerase; Appendix 1) and all but rt181 residues were changed in ≤3 isolates. Some of these may be clinically significant but the low number of occurrences precludes making definitive conclusions.

The rtA181 residue is highly conserved among HBV isolates and located in one of the conserved domains (domain B), near the active site YMDD motive, of HBV RT: no naturally occurring polymorphic variations were observed at this amino acid position among the 1,698 pretreatment isolates collected in the 007 GOLBE (n=1376) and 015 china trials (n=331). The rtA181V/T (alone or in conjunction with rtN236T) substitution was reported to be one of the 2 primary substitutions (rtA181V/T and rtN236T) seen in ADV breakthrough subjects (Microbiology Review for NDA 21-449; Angus *et al.*, 2003): the rtN236T substitution occurs approximately 4 times more frequent than the rtA181V substitution (Locarnini *et al.*, 2005). The cumulative probability of ADV genotypic

resistance (rtA181V/T and/or rtN236T) was 0%, 3%, 11%, 19%, and 30% at Weeks 48, 96, 144, 192, and 240, respectively (Microbiology Review for NDA 21-499 SE008; Hadziyannis *et al.*, 2005). In addition to ADV, the rtA181 changes, rtA181T in particular, were seen in on-treatment failure isolates to clevudine (Yoo *et al.*, 2007), entecavir (Microbiology Reviews for NDA 21-449), and LAM (Yatsuji et al., 2006; Yeh et al., 2000) therapy at varying frequencies. Similarly, rtA181 changes were also detected in 17 TBV-treatment failure isolates from 16 HBeAg-positive patients (2.4%, 16/657; Table 21). However, HBV genotype or context may be important with respect to the rtA181T substitution and reductions in TBV susceptibility, since 3 patients (057-030, 057-067, and 104-001; Table 21, highlighted in grey) developed rtA181T in the virus samples isolated at Week 48 (HBV DNA levels >1,000 copies/mL), but achieved virologic suppression at Weeks 60, 84, and 68, respectively, and maintained suppression through Week 104.

As summarized in Table 21, the rtA181S/T substitutions emerged in 17 isolates collected from 15 patients with no detectable rtM204I/V and 1 patient (ID 116-059) who developed a rtA181T substitution at Week 48 in the same virus population harboring the primary rtM204I substitution. Ten of the 17 treatment emergent HBV variants had mixtures of T and A at codon 181 in place of the consensus rtA181, 6 variants had a pure rtA181T single change, and 1 had an rtA181S change. In Table 6, the applicant showed in HepG2 cell lines stably expressing HBV rtA181 variants that the rtA181S and rtA181T substitutions conferred slightly reduced susceptibility (2.7 and 3.5 fold, respectively) to TBV (Study IDIX-07-173). In 11 patients (Table 21, highlighted in blue), rtA181S/T appeared in the virologic failure samples isolated at earlier time point (Weeks 40 or 48) but was no longer detectable in their later time-point HBV isolates collected at Weeks 92 to 104, suggesting that rtA181S/T changes appeared to be not persistent while on TBV. Furthermore, 8 of the 11 later time-point HBV isolates showed evidence of emerging rtM204I/V primary TBV-associated substitution. Since the rtM204I/V substitution occurred frequently (66.7% [8/12]; Table 21) in patients whose virus population already harbored the rtA18S/T HBV variants, the possibility that the pre-existing rtA181 change emerged on TBV may have positive effect on the later development of other TBV resistance-associated substitutions cannot be ruled out.

Table 21: Patients (n=16) with the rtA181S/T Substitutions in Paired Sequences

Patient ID	HBeAg	Last study visit	Virologic Response	HBV DNA log_{10} copies/mL				Genotype of on-treatment failure isolates		
				Baseline	Week 24	Week 48	Week 104	Isolate (week)	# of genetic changes	Substitutions
[redacted]	+	104	Rebound in Year 2	8.9	4.1	3.9	6.5	48	2	S78S/T, A181T
								104	3	L180M, S202G, [redacted]
[redacted]	+	104	Rebound in Year 2	13.3	6.1	5.7	6.7	48	1	A181A/T
								104	3	L80I/V, [redacted] L269I
[redacted]	+	100	Failure with non-rebound[1]	9.1	3.8	3.8	4.1	48	21	E1D, Y9H, H13R, I16T, S53N, H55R, P109S, T118N, N121I, Y124H, G127R,D131N, L145M, F151Y, A181T, F221Y, T/A238H, T259S, R270K, Q271S, C332S

								100	31	E1D, Y9H, H13R, I16T, S53N, H55R, S78S/T, L80L/V, P109S, T118N, N121I, Y124H, G127R, D131N, S135S/H/P/Y, L145M, F151Y, V191V/I, [redacted], L220L/V, F221Y, T222T/A, I233I/V, T/A238H, S256S/C, T259S, R270K, Q271S, G258G/S, Q267Q/H, C332S
041-027	+	104	Failure with non-rebound[1]	9.7	5.8	4.5	3.8	48	1	A342A/T
								104	1	A181T
[redacted]	+	104	Rebound in Year 2	12.6	3.7	3.8	6.7	48	1	A181A/T
								104	3	A200V, [redacted], I214V
[redacted]	+	92	Rebound in Year 2	13.1	5.4	5.2	9.3	48	1	A181A/T
								92	4	L80V, L91I, [redacted], A222A/T
[redacted]	+	104	Failure with non-rebound[1]	12.1	4.9	4.4	3.2	48	1	A181A/T
								104	0	-
[redacted]	+	104	Rebound in Year 2	8.4	4.0	3.7	8.0	48	1	A181T
								104	1	S213S/T
079-008	+	104	Failure with non-rebound[1]	10.2	5.9	5.6	4.6	48	1	A181A/T
								104	1	A181T
080-009	+	104	Failure with non-rebound[1]	7.7	3.1	3.5	3.5	104	3	N122N/S, A181A/T, N337N/D
[redacted]	+	104	Rebound in Year 2	10.0	6.2	6.3	8.5	48	3	Y54Y/H, R110R/G, A181A/T
								100	3	L80I, L180L/V, [redacted]
[redacted]	+	104	Rebound in Year 1	10.3	4.3	5.5	10.1	40	1	A181S
								92	4	L91I, [redacted], A222T, M271M/L
116-059	+	104	Rebound in Year 2	12.2	5.0	4.5	9.0	48	5	S78S/T, S116S/A, A181A/T, [redacted], P261P/L,
[redacted]	+	104	Rebound in Year 2	10.1	4.3	3.8	10.0	48	1	A181T
								92	4	L180L/M, [redacted], V207L, L269I
[redacted]	+	104	Failure with non-rebound[1]	15.5	5.8	5.6	5.4	48	1	A181A/T
								104	1	F249F/S
126-004	+	104	Failure with non-rebound[1]	10.3	5.1	4.8	3.9	48	0	-
								104	1	A181A/T
[redacted]	-	104	Suppression	10.2	4.0	3.0	2.2	48	1	A181T
[redacted]	+	104	Suppression	8.7	3.9	3.8	2.5	48	3	H124Q, A181T, V214A
[redacted]	+	104	Suppression	10.2	4.8	4.6	2.2	48	2	A181T, P325P/S
Study 015										
002-011	+	104	Failure with non-rebound[1]	7.6	3.9	3.6	3.6	104	4	A181A/T, [redacted], L229L/W, C256C/S
016-019	+	100	Failure with non-rebound[1]	10	5	4.6	4.8	100	1	A181T

[1]Failure with non-rebound, defined as virologic failure without experiencing virologic rebound

5. Conclusion

Approval of this original NDA for TYZEKA® 600 mg for the oral 20 mg/mL solution formulation is recommended with respect to Clinical Microbiology for the treatment of chronic hepatitis B in adult patients with evidence of viral replication and either evidence of persistent elevations in serum aminotransferases (ALT or AST) or histologically active disease.

Minimal effects of TBV on mitochondrial toxicity in primary human skeletal muscle cells and hepatocytes were noted at concentrations as high as 10 times (152 μM) the mean C_{max} value in human plasma at a therapeutic dose. TBV exerted additive to weak synergistic anti-HBV activity when combined with entecavir in the cell-based 2-drug combination assays with no evidence of cytotoxicity. In addition, no antagonistic effect of 7 FDA-approved HIV NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, and zidovudine) was observed on the anti-HBV activity of TBV.

In an evaluable as-treated analysis of the Phase 3 GLOBE study (Study NV-02B-007), 70.2% (451/642) of TBV recipients who continued TBV treatment beyond Week 52 were suppressed with serum HBV DNA <1,000 copies/mL (virologic suppression) as quantified by the COBAS Amplicor HBV Monitor PCR assay: 71.1% (258/418) and 86.2% (193/224) of HBeAg-positive and HBeAg-negative patients, respectively.

TBV genotypic resistance was monitored for 2 years through the primary treatment-emergent substitutions at rtM204I/V. Genotypic analysis of paired baseline and on-treatment failure isolates was conducted in 90.5% (n=182) of the 201 patients who showed evidence of virologic failure (HBV DNA level ≥1,000 copies/mL) by Year 2. The rtM204I/V substitutions emerged in 46 (including 35 with virologic rebound) and 96 (including 90 experiencing virologic rebound) during the 1st and 2nd year of TBV treatment, respectively. Thus, the emergence of the rtM204I/V substitutions was highly associated not only with virologic TBV treatment failure (70.6%, 142/201) but also with virologic rebound (83.5%, 137/164). The cumulative probabilities of incurring genotypic TBV resistance were 7% and 21.9% in Years 1 and 2: 8.1% and 27.2% in HBeAg-positive patients and 4.8% and 12% in HBeAg-negative patients, respectively.

Twelve amino acid substitutions, V27A, rtL80I/V, rtL82M, rtL91I, rtV173L, rtL180M, rtT184I/S, rtA200V, rtV207I/M, rtA222T, rtL229C/F/V/W, and rtR289K, were found, frequently and/or exclusively, in the same virus population harboring the rtM204I/V substitution. Of the 143 patients with rtM204I/V (142 emerging on TBV and 1 pre-existing), these 12 substitutions were detected in 131 patients (91.6%). Overall, the resistance profile for TBV appeared to be similar to that for LAM with the exception of rtM204V. The rtM204V substitution was infrequently detected in TBV-treatment failure patients (n=3). Of note, the rtL180M substitution conferred 24.6-fold reduced susceptibility to TBV in cell culture (Table 7), while TBV was inactive against the primary TBV resistance-associated substitution, M204I (>1,391-fold reduction).

In addition, amino acid substitutions at position rtA181 (highly conserved among HBV isolates) were detected in 16 patients: 15 patients with no detectable rtM204I/V and 1 patient with detectable rtM204I in their virus population. However, in 11 of the 15

patients with no detectable rtM204I/V, rtA181S/T did not persist throughout TBV treatment. It appeared in the virologic failure samples isolated at earlier time point (Weeks 40 or 48) but was no longer detectable in their later time-point HBV isolates collected at Weeks 92 to 104. However, 8 of those 11 patients showed evidence of emerging the rtM204I/V primary TBV^r-associated substitution in their later time-point isolates. Thus, it is possible that the pre-existing rtA181 change emerged on TBV may have positive effect on the later development of other TBV resistance-associated substitutions.

6. Recommendations

- Determine the susceptibility to telbivudine and replication capability in cell culture of HBV harboring the following substitutions of conserved amino acid residues, individually and in combination with rtM204I, by site-directed mutagenesis: V27A, rtL80I/V, rtL82M, rtV173L, rtL180M, rtT184I/S, rtA200V, rtL229C/F/V/W, and rtR289K. Please include rtM204I as a positive control.
- Evaluate the contributions of the following polymorphisms to the rtM204I-associated resistance to telbivudine by site-directed mutagenesis: I or L at rt91, I, M, or V at rt207, or A or T at rt222.
- Determine the susceptibility to telbivudine and entecavir in cell culture of HBV harboring rtM204I in combination with rtT184I/S, with rtS202G, and with rtM250I by site-directed mutagenesis.

7. Microbiology Package Insert

The review cycle for this NDA for TYZEKA® 600 mg for the oral 20 mg/mL solution formulation is complete. The applicant is receiving a complete response letter. Thus, no post-marketing recommendations are listed in this review.

8. Appendices

Appendix 1: Identity and Frequency of Individual Amino Acid Changes in Paired Sequences of Samples from Patients with Virologic Failure to TBV treatment in Studies 007 and 015

Reference amino acid		TBV treatment-failure isolates					
Position	sequence Variation[1]	007 GLOBE study			Study 015		
		Total (n= 229)	with rtM204I/V change (n=143)	without rtM204I/V change (n=86)	Total (n=52)	with rtM204I/V change (n=46)	without rtM204I/V change (n=6)
E1	Polymorphic	5	3	2	1	1	0
W3	Conserved	1	1	0	0	0	0
T7	Polymorphic	3	3	0	0	0	0
E8	Conserved	0	0	0	1	1	0
H9	Polymorphic	4	3	1	1	1	0
E11	Conserved	2	2	0	0	0	0
H12	Conserved	1	1	0	0	0	0
H13	Polymorphic	6	4	2	1	1	0
I16	Polymorphic	8	7	1	1	1	0
R18	Polymorphic	3	3	0	0	0	0
A21	Polymorphic	2	2	0	0	0	0
R22	Conserved (0)	1	1	0	0	0	0
V23	Conserved	1	1	0	0	0	0
V27	Conserved	5	5	0	5	5	0

L29	Conserved (0)	2	1	1	0	0	0
H35	Conserved	3	3	0	2	2	0
T38	Polymorphic	3	3	0	0	0	0
L42	Conserved	1	1	0	0	0	0
V44	Conserved (0)	1	1	0	0	0	0
S53	Polymorphic	9	7	2	1	1	0
T54	Polymorphic	3	1	2	0	0	0
R55	Polymorphic	11	8	3	2	2	0
S57	Conserved	1	1	0	0	0	0
W58	Conserved (0)	1	0	1	0	0	0
L69	Conserved (0)	1	0	1	0	0	0
S75	Conserved	1	1	0	0	0	0
N76	Polymorphic	1	0	1	0	0	0
S78	polymorphic	5	3	2	0	0	0
L80	Conserved	74	74	0	30	30	0
L82	Conserved (0)	3	3	0	1	1	0
V84	Conserved	0	0	0	1	1	0
S85	Conserved	1	1	0	1	1	0
I91	Polymorphic	32	31	1	11	11	0
P99	Conserved (0)	1	1	0	0	0	0
V103	Polymorphic	1	1	0	0	0	0
S106	Polymorphic	6	5	1	0	0	0
S109	Polymorphic	6	5	1	1	1	0
R110	Polymorphic	6	4	2	1	1	0
V112	Conserved	0	0	0	1	1	0
S116	Conserved	1	1	0	0	0	0
T118	Polymorphic	9	7	2	1	1	0
R120	Conserved	1	0	1	0	0	0
I121	Polymorphic	5	4	1	2	2	0
I122	Polymorphic	5	4	1	1	1	0
Y124	Polymorphic	6	4	2	2	2	0
Q125	Polymorphic	1	1	0	0	0	0
H126	Polymorphic	1	1	0	0	0	0
G127	Polymorphic	3	2	1	1	1	0
N131	Polymorphic	4	2	2	2	2	0
L132	Conserved	1	0	1	1	1	0
D134	Polymorphic	8	5	3	2	2	0
S135	Polymorphic	7	6	1	2	2	0
S137	Conserved	3	0	3	0	0	0
R138	Polymorphic	2	2	0	0	0	0
N139	Polymorphic	4	4	0	1	1	0
Y141	Conserved	1	1	0	1	1	0
V142	Conserved	0	0	0	2	2	0
S143	Conserved	1	1	0	0	0	0
L144	Conserved	1	1	0	0	0	0
L145	Polymorphic	4	3	1	1	1	0
K149	Polymorphic	1	1	0	0	0	0
F151	Polymorphic	5	4	1	2	2	0
I163	Polymorphic	2	1	1	0	0	0
L164	Polymorphic	1	1	0	0	0	0
V173	Conserved	3	2	1	1	1	0
L180	Conserved	18	18	0	10	10	0
A181	Conserved (0)	17	1	16	2	1	1
T184	Conserved (0)	3	3	0	1	1	0
V191	Polymorphic	1	1	0	0	0	0
A200	Conserved	11	11	0	1	1	0
S202	Conserved (0)	2	2	0	0	0	0
M204	Conserved	143	143	0	46	46	0
V207	Polymorphic	9	8	1	0	0	0
V208	Conserved (0)	1	0	1	1	1	0
L209	Conserved (0)	1	1	0	0	0	0
A211	Conserved	0	0	0	1	1	0
S213	Polymorphic	2	1	1	1	1	0
V214	Polymorphic	1	1	0	0	0	0
Q215	Polymorphic	3	2	1	0	0	0
S219	Polymorphic	2	1	1	2	2	0
L220	Conserved	2	1	1	0	0	0
Y221	Polymorphic	6	3	3	3	3	0
T222	Polymorphic	28	26	2	12	12	0
A223	Polymorphic	2	2	0	1	1	0
V224	Polymorphic	1	1	0	1	1	0

N226	Polymorphic	2	1	1	0	0	0
L228	Conserved (0)	1	1	0	0	0	0
L229	Conserved	27	26	1	8	8	0
I233	Conserved	1	1	0	0	0	0
P237	Conserved	1	0	1	1	1	0
N238	Polymorphic	6	4	2	3	3	0
T240	Conserved (0)	1	1	0	0	0	0
R242	Conserved	1	1	0	0	0	0
G244	Conserved (0)	1	1	0	0	0	0
S246	Conserved	2	1	1	1	1	0
N248	Polymorphic	1	1	0	2	2	0
F249	Conserved (0)	1	0	1	0	0	0
M250	Conserved (0)	1	1	0	1	1	0
V253	Polymorphic	0	0	0	1	1	0
I254	Conserved (0)	1	1	0	0	0	0
S256	Polymorphic	7	4	3	2	2	0
G258	Conserved (0)	1	1	0	0	0	0
T259	Polymorphic	2	1	1	1	1	0
P261	Conserved (0)	1	1	0	0	0	0
E263	Polymorphic	0	0	0	1	1	0
V266	Polymorphic	1	0	1	0	0	0
Q267	Polymorphic	8	6	2	2	2	0
I269	Polymorphic	8	6	2	2	2	0
K270	Conserved	2	1	1	0	0	0
Q271	Polymorphic	5	4	1	1	1	0
K275	Conserved	1	0	1	0	0	0
P277	Conserved (0)	0	0	0	1	1	0
V278	Polymorphic	2	2	0	0	0	0
N279	Conserved	1	1	0	0	0	0
I282	Conserved	2	0	2	0	0	0
V286	Conserved	1	1	0	0	0	0
R289	Conserved (0)	4	4	0	0	0	0
V291	Conserved	2	2	0	0	0	0
G295	Conserved (0)	1	0	1	0	0	0
C303	Conserved	3	3	0	0	0	0
A307	Conserved (0)	1	0	1	0	0	0
M309	Polymorphic	2	2	0	0	0	0
A313	Conserved	1	2	0	0	0	0
C314	Conserved	1	0	1	0	0	0
A317	Polymorphic	1	1	0	1	1	0
K318	Conserved	1	1	0	0	0	0
L331	Conserved (0)	1	0	1	0	0	0
C332	Polymorphic	6	5	1	3	3	0
K333	Polymorphic	2	2	0	1	1	0
Y335	Conserved	1	1	0	0	0	0
L336	Polymorphic	1	1	0	0	0	0
N337	Polymorphic	4	3	1	0	0	0
L338	Conserved	1	1	0	0	0	0
Y339	Conserved (0)	1	1	0	1	1	0
P340	Conserved (0)	1	1	0	0	0	0
V341	Conserved (0)	1	1	0	0	0	0
A342	Conserved	2	1	1	0	0	0
R343	Conserved	1	1	0	0	0	0
Q344	Conserved	1	1	0	0	0	0

[1]Conserved and polymorphic residue positions were identified by levels of amino acid sequence variations occurring in the general population (baseline genotype of 1367 patients in the GLOBE trial): frequencies of ≥2% were considered polymorphic. Conservative (0) represents no variations found among 1367 screen sequences. Naturally occurring genetic polymorphisms was observed at 72 residue positions (20.9% of the 344 resides comprising the HBV rt protein), comparable to 84 residue positions (24.4%) when 250 published wild-type HBV rt sequences [redacted] were analyzed.

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